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YAZARLARA BİLGİ/YAZIM KURALLARI

Selçuk Tıp Dergisi (Selcuk Med J), Necmettin Erbakan Üniversitesi'nin bilimsel, bağımsız, hakemli, açık erişimli yayın organıdır. Tıp doktorları, araştırmacılar ve bilim adamlarından oluşan geniş bir kitleye hitap eden disiplinli bir dergidir. Temel amaç Tıp/Sağlık alanında, tanı ve tedavideki güncel gelişmelerin, cerrahi yenilikler ve bilim dünyasına katkıda bulunacak çalışmaların ulusal ve uluslararası literatürde paylaşımının sağlanmasıdır.

Selçuk Tıp Dergisi, tıp bilimine ve akademik çalışmalara katkısı olan, klinik ve deneysel çalışmaları, editöryal yazıları, klinik olgu bildirimlerini, teknik ve eğitici derlemeleri, orijinal görüntü raporlarını ve editöre mektupları yayınlar. Anket/mülakat çalışmaları; Editörün ilk değerlendirmesi sonucunda çok değerli bir katkı sunuyorsa değerlendirmeye alınabilir.

Dergi gönderim kurallarına ve dergi kapsamına uygun görülen, editöryal çalışmalar hariç tüm yazılar alanında uzman hakemlere bilimsel değerlendirme için gönderilir. En az iki hakem kararı aranır. Yayımlanan tüm makaleler çift taraflı kör akran değerlendirmesi sürecine tabidir. Uygunluğunu tartışılan çalışmalarda yardımcı editörler hakemlerin yorumlarını dikkate alarak kendi değerlendirmelerini eklerler. Gönderilen tüm yazılar için nihai karar Baş Editör'e aittir. Bütün makaleler için süreçlerin editör ve yayın kurulu tarafından en geç üç ay içerisinde sonuçlandırılması hedeflenir. Fakat elde olmayan gecikmelerden dolayı bu süre uzayabilir.

Yayın kurulu kararları ile belirlenen bazı konular hakkındaki yazılar, yayın kurulu üyelerinin tamamının incelemesine sunulur. İncelemeler sonucu oy çokluğuna ulaşan çalışmaların dergideki süreçleri devam edecektir. Yayın kurulu kararları dergi web sitesinde yayınlanmaktadır.

Yayına kabul edilen yazıların her türlü yayın hakkı yazarlara ve Selçuk Tıp Dergisine aittir. Selçuk Tıp Dergisi, ilave olarak websitesinde bulunan telif hakları bildirim belgesinin de yazarlar tarafından onaylanarak imzalanmasını ve ıslak imzalı formun sisteme eklenmesini talep etmektedir. Dergi her yıl mart, haziran, eylül ve aralık aylarında olmak üzere dört sayı olarak yayımlanmaktadır. Derginin yayın dili İngilizcedir.

Gönderilen yazıların daha önce herhangi bir yerde/dergide yayınlanmamış olması ve yayın için başka bir dergiye gönderilmemiş olması gerekmektedir [Bilimsel kongrelerde sunulan sözlü bildiri ve posterler (özet ya da tam metin olabilir) bildirilmek kaydı ile hariçtir]. Dergide yayımlanan yazıların her türlü sorumluluğu (etik, bilimsel, yasal vb.) yazarlara aittir. Dergide yayımlanan yazılarda ifade edilen ifadeler veya görüşler yazarların görüşleri olup, editörlerin, yayın kurulu ve yayıncının görüşlerini yansıtmaz; editörler, yayın kurulu ve yayıncı, bu tür materyaller için herhangi bir sorumluluk veya yükümlülük kabul etmemektedir. Yazım kurallarına uygun olarak hazırlanmamış olan yazıların incelenmeye alınıp alınmaması Editör ve Editöryal Kurulun insiyatifindedir.

Tüm çalışmalarda etik kurul onayı ve bu onamın belgelendirilmesi gerekmektedir. Tüm çalışmalarda yazarların çalışmaya katkı düzeyi ve onayı bildirilmelidir. Çalışmada veri toplanması, deney aşaması, yazım ve dil düzenlemesi dahil olmak üzere herhangi bir aşamasında finansal çıkar çatışması olmadığı bildirilmelidir. Çalışmada varsa ticari sponsorluk bildirilmelidir. Selçuk Tıp Dergisi'nde intihal programı (iThendicate) kullanılmaktadır. Akademik atıf sınırını aşan benzerlik taşıyan makaleler ve yayın kurallarına uygun olarak hazırlanmamış makaleler değerlendirmeye alınmayacaktır. Dergi intihal tarama raporunu yazardan talep edeceği gibi kendisi de tarama yapabilir.

Derginin yayın politikası ve süreçleri Uluslararası Medikal Dergisi Editörleri Komitesi (International Committee of Medical Journal Editors-I**CMJE),** Dünya Tıbbi Editörler Derneği (World Association of Medical Editors-**WAME),** Bilim Editörleri Konseyi (Council of Science Editors-**CSE),** Avrupa Birliği Derneği Bilim Editörleri (European Association of Science Editors-**EASE)** ve Yayın Etiği Komitesi (Committee on Publication Ethics-**COPE)** ve Ulusal Bilgi Standartları Örgütü (National Information Standards Organization-**NISO)** yönergelerini takip eder. Dergimiz 'Şeffaflık ve Akademik Yayıncılık En İyi Uygulamalar İlkelerine' (Principles of Transparency and Best Practice in Scholarly Publishing) **(doaj.org/bestpractice)** uygundur. Yayın Kurulu, dergimize gönderilen çalışmalar hakkındaki intihal, atıf manipülasyonu ve veri sahteciliği iddia ve şüpheleri karşısında **COPE** kurallarına uygun olarak hareket edecektir.

Derginin Yayın Kurulu, itiraz ve şikâyet vakalarını, COPE rehberleri kapsamında işleme almaktadır. Yazarlar, itiraz ve şikayetleri için doğrudan baş editör veya editör/yayın kurulu ile temasa geçebilirler. İhtiyaç duyulduğunda Yayın Kurulu'nun kendi içinde çözemediği konular için tarafsız bir temsilci atanacaktır. İtiraz ve şikayetler için karar verme süreçlerinde nihai kararı Baş Editör verecektir. Yayıncı ve editör gerektiğinde düzeltmeler, açıklamalar, geri çekilmeler ve özürler yayınlamaya her zaman hazırdır.

Selçuk Tıp Dergisi ile ilgili tüm yazışmalar, makale gönderme, makalenin takibi, danışman raporları, düzeltmelerin yapılıp yüklenmesi, kabul yazısı gönderimi ve diğer tüm makale ile ilgili formların yüklenmesi **https://www. selcukmedj.org s**ayfasından yapılacaktır. Bu site üzerinden yüklenecek makaleler için kurallar aşağıda belirtilmiştir.

Selçuk Tıp Dergisi, ücretsiz, açık erişim politikası benimsemektedir. Bu bağlamda dergide yayınlanan tüm yazılar https://www. selcukmedj.org adresinden erişime açık olup yazarlardan hiçbir ek ücret talep edilmeyecektir.

Yazarlık

Selçuk Tıp Dergisi'ne gönderilen çalışmalarda yazar olarak listelenen herkesin ICMJE (www.icmje.org) tarafından önerilen yazarlık koşullarını karşılaması gerekmektedir. ICMJE, yazarların aşağıdaki 4 koşulu karşılamasını önermektedir:

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

Yazar olarak belirtilen her kişi yazarlığın dört koşulunu karşılamalıdır ve bu dört koşulu karşılayan her kişi yazar olarak tanımlanmalıdır. Yazar olarak atanan tüm kişiler yazarlık için hak kazanmalı ve hak kazanan herkes listelenmelidir. Dört kriterin hepsini karşılamayan kişilere makalenin başlık sayfasında teşekkür edilmelidir. Finansman alımı, veri toplanması ya da araştırma grubunun genel gözetimi, kendi başlarına, yazarlığı haklı çıkarmaz. Bir ya da daha fazla yazar, çalışma başlangıcından yayınlanmış makaleye kadar, bütün olarak çalışmanın bütünlüğünün sorumluluğunu üstlenmelidir.Çok merkezli çalışmalarda yazarlık bir gruba atfedilir. Yazar olarak adlandırılan grubun tüm üyeleri, yukarıdaki yazarlık kriterlerini tam olarak karşılamalıdır. Bu kriterleri karşılamayan grup üyeleri, onayları ile birlikte listelenmelidir. Mali ve maddi destek de kabul edilmelidir.

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Yazar Değişikliği Talepleri
Yazar listesindeki yazar isimlerinin eklenmesi, silinmesi veya yeniden düzenlenmesi ancak makale kabul edilmeden önce ve ancak dergi Editörü tarafından onaylandığı takdirde yapılabilir.
Böyle bir değişikliği talebi olursa Editör, sorumlu yazardan (a) yazar listesindeki değişikliğin nedeni ve (b) tüm yazarlardan eklemeyi kabul ettiklerine dair yazılı onay (e-posta), talep eder. Editör, yalnızca istisnai durumlarda, makale kabul edildikten sonra yazarların eklenmesini, silinmesini veya yeniden düzenlenmesini dikkate alacaktır.

Makale Yazımı
Orijinal araştırma makalesi kaleme alanlar, konuyu özgün bir şekilde ve nesnel bir tartışma ile ele almalıdır. Makale, başkalarının çalışmayı tekrarlamasına izin vermek için yeterli ayrıntı ve referansları içermelidir. Hileli veya bilerek yanlış beyanlar etik dışı davranış teşkil eder ve kabul edilemez.

Özgünlük
Yazar makalenin orijinal olduğu, daha önce başka bir yerde yayınlanmadığı ve başka bir yerde, başka bir dilde yayınlanmak üzere değerlendirmede olmadığı konusunda teminat sağlamalıdır. Makale yazımının yapay zekâ sistemleri kullanılarak yapıldığı çalışmalar kabul edilmemektedir. Yapay zekâ sistemleri, sadece yazıların dil düzenlemeleri için kullanılabilir.

Orijinal Kaynak Kullanımı ve Atıf Yapma
Yazarlar, tamamen özgün eserler yazdıklarından ve başkalarının eserlerini veya sözlerini kullanmışlarsa, bunun uygun şekilde alıntılandığından emin olmalıdır. Üçüncü taraflarla konuşma, yazışma veya tartışmalarda olduğu gibi özel olarak elde edilen bilgiler, kaynağın açık ve yazılı izni olmadan kullanılmamalıdır.

Veri Erişimi ve Muhafazası
Yazarlardan, editör incelemesi için makalelerini destekleyen araştırma verilerini sağlamaları ve/veya derginin açık veri gereksinimlerine uymaları istenebilir. Yazarlar, mümkünse, bu tür verilere kamu erişimi sağlamaya ve bu tür verileri yayınlandıktan sonra makul bir süre boyunca saklamaya hazır olmalıdır. Dergimiz, araştırma verilerinin TUBITAK'ın Aperta Portalı'na yüklenmesini tavsiye etmektedir.

Çoklu ve Eşzamanlı Yayın
Bir yazar aynı araştırmayı içeren makalesini birden fazla dergisinde yayımlamamalıdır. Aynı makalenin aynı anda birden fazla dergiye gönderilmesi etik dışı davranıştır. Bir yazar, özet şeklinde yayınlanmış olması dışında, daha önce yayınlanmış bir makaleyi başka bir dergide değerlendirilmek üzere sunmamalıdır.

Anket ve Mülakata Dayanan Çalışmaların Yayını ve Etik Kurul Onamları
Etik kurul izni gerektiren, tüm bilim dallarında yapılan araştırmalar için (etik kurul onayı alınmış olmalı, bu onay makalede belirtilmeli ve belgelendirilmelidir. Etik kurul izni gerektiren araştırmalarda, izinle ilgili bilgilere (kurul adı, tarih ve sayı no) yöntem bölümünde, ayrıca makalenin ilk/son sayfalarından birinde; olgu sunumlarında, bilgilendirilmiş gönüllü olur/onam formunun imzalatıldığına dair bilgiye makalede yer verilmelidir. Anket çalışmaları ve mülakata dayanan çalışmaların etik kurul onam belgeleri alınmış olmalı ve makale yüklenirken dergi sistemine eklenmelidir.

Çıkar Çatışması
Kişinin yaptığı işte çelişkiye düşmesine yol açacak, objektifliğini önemli oranda bozabilecek veya herhangi bir kişi ya da kuruluş lehine adil olmayan avantaj sağlayabilecek herhangi finansal ya da diğer tür çıkarlardır. Araştırmanın yürütülmesi ve makalenin hazırlanması sürecinde alınan tüm mali destek kaynakları ve sponsorların çalışmadaki rolü açıklanmalıdır. Finansman kaynağı yoksa bu da belirtilmelidir. Açıklanması gereken olası çıkar çatışması örnekleri arasında danışmanlıklar, maaş alımı, hibeler yer alır. Potansiyel çıkar çatışmaları mümkün olan en erken aşamada açıklanmalıdır.

Hata Bildirimi
Bir yazar yayınlanmış çalışmasında önemli bir hata veya yanlışlık fark ettiğinde, derhal dergiye bildirimde bulunmalıdır. Editör tarafından gerekli görüldüğü takdirde makaleyi geri çekmek veya düzeltmek için iş birliği yapmak da yazarın yükümlülüğüdür. Editör veya yayıncı, yayınlanan bir çalışmanın hata içerdiğini üçüncü bir şahıstan öğrenirse, yazarın konu hakkında editöre bilgi vermek de dahil olmak üzere editörle iş birliği yapması yazarın yükümlülüğüdür.

Görüntü Bütünlüğü
Bir görüntüde belirli bir özelliği geliştirmek, karartmak, taşımak, kaldırmak veya eklemek kabul edilemez. Yazarlar, dergi tarafından uygulanan grafik görseller için belirlenen politikaya uymalıdır.

Düzeltme ve Yayından Geri Çekme Talepleri
Selçuk Tıp Dergisi tarafından yayımlanan makaleler nihai versiyondur. Bu nedenle yayımlandıktan sonra düzeltme talepleri, Yayın Kurulu tarafından **COPE** yönergelerine göre değerlendirilir. Yayından geri çekme talepleri, makale kabulünden önce yapılmalıdır ve Editör Kurulu onayına tabidir. Makale kabulü sonrasında henüz yayınlanmadan önce bir geri çekme talebi olursa, gerekçesi ile birlikte baş editöre mail yolu ile ulaştırılmalıdır. Gerekçeler editör kurulu toplantısında değerlendirilerek nihai karar verilecek ve yazara mail yolu ile bildirilecektir. **Yayın aşamasına alınmış bir makalenin geri çekme talep başvuruları dikkate alınmayacaktır.** Yayımlanmadan önce çalışmasını geri çekme talebinde bulunmak isteyen yazar (lar), Geri çekme formunu doldurarak her bir yazarın ıslak imzası ile imzalanmış ve taratılmış halini editor@selcukmedj.org.tr adresi üzerinden e-posta aracılığıyla Baş Editör ve Editör kuruluna iletmekle yükümlüdür. Geri çekme formuna web sitemizin indirmeler sayfasından ulaşabilirsiniz(https://www.selcukmedj.org/tr-tr/indirmeler/). Editör Kurulu geri çekme bildirimini inceleyerek en geç 15 gün içerisinde dönüş sağlar.
Yazar isimleri, bağlantıları, makale başlıkları, özetler, anahtar kelimeler, herhangi bir bilgi yanlışı ve dijital nesne anımlayıcılardaki [digital object identifier (DOI)] yazım hataları, bir "erratum" ile düzeltilebilir.

Makale Değerlendirme Süreci
Dergiye gönderilen makalelerin hızlı bir şekilde değerlendirilmesi ve yayınlanması hedeflenmiştir. Tüm makaleler çift kör hakem değerlendirme sürecine tabidir. Makaleler, içerik, özgünlük, alandaki önem, istatistiksel analizin uygunluğu ve sonuçların çıkarılması için alanında uzman hakemler tarafından gözden geçirilecektir. En az iki hakem kararı aranacaktır. Hakemler arasında tutarsızlıklar olması durumunda, makale üçüncü ya da dördüncü bir hakeme gönderilebilecektir. Hakem kararları yardımcı editörler tarafından değerlendirilerek değerlendirme sonuçları baş editöre gönderilecektir. Gönderilen makalelerin kabulüne ilişkin nihai karar, baş editöre aittir.

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Hakemler tarafından bildirilen ve yazarlar için faydalı oldukları değerlendirilen yorum ve değerlendirmeler yazarlara gönderilir. Hakemler tarafından yapılan talimat, itiraz ve talepler kesinlikle yerine getirilmelidir. Hakem(ler)e cevap dosyası ayrıca bir Word belgesi halinde oluşturulmalıdır. Yazının gözden geçirilmiş şekliyle yazarlar, bu dosyada, hakemlerin taleplerine uygun olarak atılan her adımı açık ve net bir şekilde belirtmelidir. Yazar açıklama notları, hakemlerin değerlendirme sırasına göre numaralandırılmış olarak listelenmelidir. Ayrıca makale içerisinde de gerekli değişiklikleri yapmalı ve bunları makale içerisinde belirterek (boyayarak), revize edilmiş makale ve hakem önerilerine verilmiş yanıtları içeren formlar https://www. selcukmedj.org adresinden titizlikle yüklenmelidir.

Yazıların Gönderilmesi

Yazarlar Yayın Hakları Bildirim Formunu sisteme yüklemelidir. Tüm yazışmalar sorumlu yazara gönderilecektir. İlgili sorumlu yazarın, tüm diğer yazışmalar için bir e-posta adresi bildirilmelidir. Yazarlar makalelerininin alındığından kendisine verilen numara ile haberdar edilirler. Bildirilen makale numarası yapılan tüm yazışmalarda kullanılmalıdır. Yazarlara beyan edilir ki; editör ofisinin ilk değerlendirmesi sonucu okuyucunun menfaatine dönük olarak makalelerin içeriği dolayısıyla makalesi geri iade edilebilir. Bu hızlı reddetme süreci, yazarın başka bir yerde makalesini yayınlanmasına olanak sağlar.
Selçuk Tıp Dergisi'ne makale gönderilmesi, tüm yazarların, derginin yayın politikalarını ve yayın etiğini okuduğu ve kabul ettiği anlamına gelir. Makale gönderimi ve ilgili diğer tüm işlemler https://www. selcukmedj.org adresinden online olarak yapılacaktır.

Yazıların Hazırlanması

Yazarların, materyallerini göndermeden önce aşağıdaki kuralları okumaları ve makalelerini bu kurallara uygun halde sisteme yüklemeleri gerekmektedir:

Genel yazı biçimi: Tüm makaleler, her tarafta 2,5 cm genişliğinde kenar boşlukları bulunan standart A4 boyutunda bir word dosyası kullanılarak yazılmalı, kaynaklar, resim şekil ya da tablolar metinde geçiş sırasına göre numaralandırılmalıdır. Metin, sol hizalı ve heceli satır sonları olmayan 12 puntolu bir fontta çift boşluk kullanılarak ve Times New Roman karakterinde yazılmalıdır. Kelimeler arasında ve cümle noktası sonrasında tek boşluk bırakmaya özen gösterilmelidir. Paragraf için sol girintiyi sekme tuşu ile bir kez tıklayarak ayarlanmalıdır. Ölçüm birimleri için Uluslararası Birimler Sistemi (SI) kullanılmalıdır. Makalenin tüm sayfaları sayfa sonunda numaralandırılmalıdır. Tüm yazılar yazım kurallarına uymalı, noktalama işaretlerine uygun olmalıdır.

Tüm makalelerde; Kapak sayfası, Ön yazı (cover letter), makale dosyası, Etik kurul onay Belgesi (kurumdan alınan), intihal analiz raporu, Şekiller ve Resimler, Telif Hakları Devir Formu, ve gerekli ise hasta onam formu ayrı dosyalar olarak yüklenmelidir.
Kaynaklar makale dosyasında, makale biter bitmez değil ayrı bir sayfada başlamalıdır. Tablolar, tablo açıklamaları, resim/şekiller ve resim/şekil açıklamaları ayrıca makale ana dosyasına kaynakların ardından ayrı bir sayfada eklenmelidir. Tablo/Resim/şekil açıklamaları; Tablo/Resim/şekillerin hemen altlarında olmalıdır.

Makale bölümleri hakkında

1-Kapak Sayfası: Makalenin İngilizce tam başlığı ve 50'den fazla karakter içermeyen kısa bir başlık, tüm yazarların açık şekilde adları ve soyadları, ORCID numaraları, kurumları, sorumlu yazar ismi iş veya cep telefonu, e-posta ve yazışma adresi belirtilmelidir.Makale daha önce tebliğ olarak sunulmuş ise tebliğ yeri ve tarihi belirtilmelidir. Yazarlar ve kurumları hakkındaki bilgiler başlık sayfası haricinde ana metinde (materyal metot bölümü dahil), tablolarda, şekillerde ve video dokümanlarında yer almamalıdır. Herhangi bir hibe ya da diğer destek kaynaklarının detayları, makalenin hazırlanmasına katkıda bulunan ancak yazarlık kriterlerini karşılamayan bireylere teşekkür bölümü de kapak sayfasına eklenmelidir.

2-Ana makale dosyası; Ana makale dosyası, yazar isimleri ve kurumları gibi bilgiler içermemelidir. Ana makale dosyası:
1. Başlık, 2. Özet ve Anahtar Kelimeler, 3. Makale ana metni, 4. Kaynaklar, 5. Tablolar ve açıklamaları, 6. Resim ve Şekil açıklamaları ile birlikte resim ve şekiller, 7. Alt yazılar şeklinde dizilmelidir.
Başlık: Makale Word dosyasında en baş kısımda makalenin yazım dilinde tek uzun başlığı yer almalıdır.
Özet: Editöre Mektup haricinde tüm yazılar özet içermelidir. Orijinal araştırma makalelerinin özetleri Amaç, Gereçler ve Yöntem, Bulgular ve Sonuç alt başlıklarını içermelidir. Özetler; kaynak, şekil veya tablo numarası içermemelidir. Sözcük sayısı ve özellikler için Tablo 1'deki veriler dikkate alınmalıdır.
Anahtar sözcükler:Özelerin sonunda en az üç ile en fazla beş anahtar sözcük bildirilmelidir. Anahtar sözcükler kısaltmalar olmaksızın tam olarak listelenmeli birbirinden virgül ya da noktalı virgül kullanılarak ayrılmalıdır. Anahtar kelimeler, "Tıbbi Konu Başlıklarına **(MESH)"** uygun olmalıdır (Bakınız: www.nlm.nih.gov/mesh/MBrowser.html).
Kısaltmalar:Özetlerde ve başlıklarda kısaltmalar kullanılmamalıdır. Makalede kullanılacak kısaltmalar, mümkünse ulusal veya uluslararası kabul görmüş olmalı, ilk kullanıldığında metin içinde tanımlanmalı ve parantez içinde yazılmalıdır. Daha sonra metin boyunca o kısaltma kullanılmalıdır. Yaygın olarak kabul edilen kısaltmalar ve kullanım için lütfen "Bilimsel Stil ve Biçim"e bakınız. (https://www.scientificstyleandformat.org/Home.html). Ana metinde Bir ticari markalı ilaç, ürün, donanım veya yazılım programı ana metinde yer aldığında, ürün bilgisi, ürünün adını, ürünün imalatçısını ve şirket ile şirket merkezinin bulunduğu ülkeyi aşağıdaki biçimde parantez içinde verilmelidir: "Discovery St PET / CT tarayıcı (General Electric, Milwaukee, WI, ABD).
Makale ana metni:
Giriş: Konuyu ve çalışmanın amacını açıklayacak spesifik bilgilere yer verilir.
Gereçler ve Yöntem: Çalışmanın gerçekleştirildiği yer, zaman ve çalışmanın planlanması ile kullanılan elemanlar ve yöntemler bildirilmelidir. Verilerin derlenmesi, hasta ve bireylerin özellikleri, deneysel çalışmanın özellikleri ve istatistiksel metotlar detaylı olarak açıklanmalıdır. Çalışmaya alınanlar ve çalışmayı yürütmek için kullanılan tüm yöntemler ayrıntılı olarak açıklanmalıdır. Kullanılan yeni veya modifiye yöntemler ayrıntılı olarak açıklanmalı kaynak belirtilmelidir. İlaçların ve kimyasal ajanların dozları, konsantrasyonları, verilme yolları ve süresi belirtilmelidir. Elde edilen verileri özetlemek ve önerilen hipotezi test etmek için kullanılan tüm istatistiksel yöntemlerin kısa bir raporu, istatistiksel olarak anlamlı farklılık için belirlenen p değeri ölçütleri de dahil olmak üzere bir alt başlık altında sunulmalıdır. Yapılan istatistiksel değerlendirme ayrıntılı olarak açıklanmalıdır. Olabildiğince standart istatistiksel yöntemler kullanılmalıdır. Nadiren kullanılmış veya yeni istatistiksel yöntemler kullanılmışsa konuya ilişkin ilgili referanslar belirtilmelidir. Gerekirse, olağandışı, karmaşık veya yeni istatistiksel yöntemlerle ilgili daha ayrıntılı açıklamalar, çevrimiçi ek veri olarak okuyucular için ayrı dosyalarda verilmelidir.
Bulgular: Elde edilen veriler istatistiksel sonuçları ile beraber ayrıntılı olarak verilmelidir. Bulgular şekiller ve tablolar ile desteklenmelidir. Rakam ve tablolarda verilen bilgilerin gerekli olmadıkça metinde tekrarlanmamasına özen gösterilmelidir.
Tartışma: Çalışmanın sonuçları literatür verileri ile karşılaştırılarak değerlendirilmeli, yerel ve/veya uluslararası kaynaklarla desteklenmelidir. Yazıyla alakasız veya gereksiz genel bilgiler eklenmemeli, yazının amacına uygun yeterli uzunlukta olmalıdır.
Kaynaklar: Kaynaklar ayrı bir sayfaya yazılmalıdır. Kaynaklar APA 7 sistemine uygun olarak belirtilmelidir. Buna göre, kaynak numaraları cümle sonuna nokta konmadan () içinde verilmeli, nokta daha sonra konulmalıdır. Kaynak yazar isimleri cümle içinde kullanılıyorsa ismin geçtiği ilk yerden sonra () içinde kaynak verilmelidir. Birden fazla kaynak numarası veriliyorsa arasına ",", ikiden daha fazla ardışık kaynak numarası veriliyor ise rakamları arasına "-" konmalıdır [ör. (1,2), (1-4) gibi. Yazar sayısı 3 ve daha azsa tüm yazarların ismi olmalı, 3'dan daha fazla ise ilk3 yazar yazılıp diğerleri için et al. kullanılmalıdır. Kaynaklar metindeki kullanılış sırasına göre numaralandırılıp listelenmelidir. Atıf doğruluğu, yazarın sorumluluğundadır. Kaynaklar orijinal yazım, aksan, noktalama vb. ile tam olarak uyumlu olmalıdır. Metin içindeki tüm kaynaklar belirtilmelidir. Kaynak listesinde mükerrer yazım yapılmamalıdır. Farklı yayın türleri için kaynak stilleri aşağıdaki örneklerde sunulmuştur:

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Araştırma Makalesi:

- Mirza E, Oltulu R, Oltulu P, et al. Dry eye disease and ocular surface characteristics in patients with keratoconus. Saudi J Ophthalmol. 2022;36(1):117-21. doi: 10.4103/sjopt.sjopt_37_21.
- Vikse BE, Aasarød K, Bostad L, et al. Clinical prognostic factors in biopsy-proven benign nephrosclerosis. Nephrol Dial Transplant. 2003;18(3):517-23. doi: 10.1093/ndt/18.3.517.

Tek Yazarlı Kitaplar:

- Danovitch GM. Handbook of Kidney Transplantation. Boston: Little, Brown and Company (Inc.), 1996: 323-8.

Kitap Bölümü:

- Soysal Z, Albek E, Eke M. Fetüs hakları. Soysal Z, Çakalır C, ed. Adli Tıp, Cilt III,İstanbul Üniversitesi Cerrahpaşa Tıp Fakültesi Yayınları, İstanbul, 1999:1635-50.
- Davison AM, Cameron JS, Grünfeld JP, et al. Mesengiocapillary glomerulonephritis In: Williams G, ed. Oxford Textbook of Clinical Nephrology.New York:Oxford University Press, 1998: 591- 613.

Baskıdan önce çevrim içi olarak yayımlanan dergi makalesi:

- Doğan GM, Sığırcı A, Akyay A, Uğuralp S, Güvenç MN. A Rare Malignancy in an Adolescent: Desmoplastic Small Round Cell Tumor. Turkiye Klinikleri J Case Rep. 10.5336/caserep.2020-77722. Published online: 31 December 2020.
- Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging.DiagnIntervRadiol. 2016 Feb 24.doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Toplantı Raporları:

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

Bilimsel veya Teknik Rapor:

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

Tez:

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

Web sayfası ve Sosyal Medya araçları: Yazar. Başlık. Erişim linki: URL. Erişim tarihi ve yılı

3-Tablolar ve açıklamaları: Tablolar, ana makale metnine dahil edilmelidir, kaynak listesinden sonra sunulmalı ve ayrı bir sayfada olmalıdır. Ana metinde yer alan sıraya göre numaralandırılmalıdır. Her bir tablonun üzerine açıklayıcı bir başlık konulmalıdır. Tabloda kullanılan kısaltmalar, tablonun altında dipnotlarla tanımlanmalıdır (ana metin içerisinde tanımlanmış olsa bile). Tablolar kolay okunması için açık bir şekilde düzenlenmelidir. Tablolarda sunulan veriler, ana metinde sunulan verilerin tekrarı olmamalı, ancak ana metni desteklemelidir.

4-**Şekil ve Resimler:** Şekil, grafik ve resimler makale gönderim sistemi aracılığıyla ayrı dosyalar (TIFF veya JPEG formatında) halinde yüklenmeli ilaveten ana makale dosyasında ayrı bir sayfada tablolardan sonra ana metin içinde de gösterilmelidir. Sisteme ayrı olarak yüklenmeyen sadece makale içerisinde geçen resimler kabul edilmeyecektir. Şekil ve resimler mutlaka isimlendirilmeli ve numaralandırılmalı, metin içinde sıralamaya dikkat edilerek belirtilmelidir. Ana metine eklenecek resim, şekil ve grafik altına açıklamaları da eklenmelidir. Resimler minimum 300 dots per inch (dpi) çözünürlüğünde ve net olmalıdır. Şekil ve resim altlarında kısaltmalar kullanılmış ise, kısaltmaların açılımı alfabetik sıraya göre alt yazının altında belirtilmelidir. Mikroskobik resimlerde büyütme oranı ve tekniği açıklanmalıdır. Yayın kurulu, yazının özünü değiştirmeden gerekli gördüğü değişiklikleri yapabilir. Şekil alt birimleri olduğunda, alt birimler tek bir görüntü oluşturmak için birleştirilebilir. Şekiller, alt birimleri göstermek için işaretlenmeli ve her birinin açıklamaları (a, b, c, vb.) yazılmalıdır. Şekilleri desteklemek için kalın ve ince oklar, ok uçları, yıldızlar, yıldız işaretleri ve benzer işaretler kullanılabilir. Makale içeriği gibi şekiller de kör olmalıdır. Bir birey ya da kurumu tanımlayabilecek resimlerdeki olası bilgiler anonimleştirilmelidir. Hasta fotoğrafı paylaşımlarında kimliğin birebir tanınmamasına özen göstermeli, hastalığı belirlemeye yetecek yeterlilikte görüntü paylaşılmalıdır. Hastanın kimliğini açık eden resim paylaşımları için, hastanın resminin paylaşımına izin verdiği onam formu şarttır.

Tablo 1. Makale türlerine göre sınırlamalar

Makale türü	Sözcük sınırı	Özet sınırı	Kaynak sınırı	Tablo sınırı	Şekil sınırı
Araştırma makalesi	3500	300	50	6	6
Derleme	5000	300	80	6	10
Olgu sunumu	1500	200	15	3	5
Editöre mektup	1000	Özet yok	8	Tablo içermez	Şekil içermez
Editöryal	1000	Özet yok	20	3	3
Orijinal görüntü raporu	200	Özet yok	5	1	3

Makale Türleri

Selçuk Tıp Dergisi'nde aşağıda kısaca açıklanan makale türleri yayınlamaktadır:

Araştırma Makaleleri: Orijinal araştırmalara dayanan yeni sonuçlar sağlayan en önemli makale türüdür. Orijinal makalelerin ana metni Giriş, Yöntemler, Bulgular, Tartışma, Sonuç ve Kaynaklar alt başlıklarıyla yapılandırılmalıdır. Sözcük sayısı ve özellikler için lütfen Tablo 1'e bakınız. İstatistiksel analiz genellikle sonuçları desteklemek için gereklidir. İstatistiksel analizler uluslararası istatistik raporlama standartlarına uygun olarak yapılmalıdır (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983:7;1489–93). İstatistiksel analizler hakkında bilgi Materyaller ve Yöntemler bölümünde ayrı bir alt başlık ile sağlanmalı ve süreç boyunca kullanılan istatistiksel yazılım belirtilmelidir. Birimler Uluslararası Birimler Sistemine (SI) uygun olarak hazırlanmalıdır.
Makalenin kısıtlılıkları, sakıncalar ve eksik yönler, sonuç paragrafından önce Tartışma bölümünde belirtilmelidir.

Derleme Makaleleri: Yeterli sayıda bilimsel makaleyi tarayıp, konuyu bugünkü bilgi ve teknoloji düzeyinde özetleyen, değerlendirme yapan ve bulguları karşılaştırarak yorumlayan yazılar olmalıdır. Temel ve uygulamalı bilim alanlarında tüm gelişmeleri ile birlikte son bilimsel çalışmalardaki teknik ve uygulamalar değerlendirilir. Belirli bir alan hakkında kapsamlı bilgi sahibi olan ve bilimsel geçmişi yüksek atıf potansiyeli olan yazarlar tarafından hazırlanan derlemeler dergimiz tarafından kabul edilecektir. Bu yazarlardan makale kabul şekli davet yöntemiyle de olabilir. Ana metin Giriş, Klinik ve Araştırma Sonuçları ve Sonuç bölümlerini içermelidir. Sözcük sayısı ve özellikler için lütfen Tablo 1'e bakınız.

Olgu Sunumları: Tanı ve tedavide zorluk teşkil eden, yeni tedaviler sunan veya literatürde yer almayan bilgileri ortaya koyan nadir olgu veya durumlar hakkında eğitici olgu sunumları dergimizde yayınlanmak için kabul edilir. Olgu sunumu, Giriş, Olgu Sunumu ve Tartışma ve Sonuç alt başlıklarını içermelidir.

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İlginç ve sıra dışı resimler değerlendirme sürecinde bir avantajdır. Hasta tanımlayıcı resimlerde hasta kimliği açık ediliyorsa resmin paylaşımına izin veren hasta onamı mutlaka olmalıdır. Sözcük sayısı ve özellikler için lütfen Tablo 1'e bakınız.

Editöre Mektuplar: Bu yazı türü, daha önce yayınlanmış bir makalenin önemli kısımlarını, gözden kaçan yönlerini veya eksik kısımlarını tartışır. Derginin dikkatini çekebilecek konular başta olmak üzere, okuyucuların dikkatini çekebilecek konular hakkında makaleler, özellikle eğitici konularda Editöre Mektup şeklinde sunulabilir. Okuyucular, yayınlanmış yazılar hakkındaki yorumlarını Editöre Mektup olarak da sunabilirler. Özet, Anahtar Sözcükler ve Tablolar, Şekiller, Görüntüler ve diğer medya eklenmemelidir. Metin alt başlıkları içermemelidir. Sözcük sayısı ve özellikler için lütfen Tablo 1'e bakınız.

Editöryal: Tıbbın herhangi bir alanında bir görüşün açıklandığı ya da başkalarının görüşlerinin yayınlandığı, kısa makalelerdir. Normal bir dergi makalesine göre daha yaratıcı yazabilme olanağı sağlar. Dergide yakın zamanda yayınlanmış bir makale tartışılabilir, Tarihi materyal, Halk sağlığına dair konular, Sağlık politikaları, Tıp Eğitimi ve Tıpta teknolojik gelişmeler hakkındaki yazılar bu bölümde değerlendirilebilir. Tam bir derleme olamayacak bir konuda kısa derleme bu başlık altında değerlendirilebilir. Dergi editörü; okuyuculara kişisel mesaj iletmek, aynı sayıdaki bir makale ile ilgili yorum yapmak, okuyucunun dikkatini yeni gelişmelere çekmek isterse bu bölüme yazabilir. Bilimsel makalelerin tipik yazım bölümlerini içermez. Temel mesaj bir cümlede özetlenebilmelidir. Bu cümleyi editoryali yazmaya başlamadan belirlemek yazımı kolaylaştırır. Bu mesaj konusunda okuyucuyu ikna etmek için mantıklı bir tartışma yürütmelidir. Şekiller, Görüntüler ve diğer medya eklenebilir. Sözcük sayısı ve özellikler için lütfen Tablo 1'e bakınız.

Orijinal Görüntü Raporu: Tıbbın her alanına ait ilgi çekici, tanımlayıcı veya eğitici görüntüler (ilustrasyon ya da Fotoğraf) birkaç satırlık açıklayıcı metin ve referanslarla birlikte bu bölümde değerlendirmeye sunulabilir. Makale uzunluğu 200 kelimeyi geçmemelidir. Özet, tartışma veya sonuca gerek yoktur ancak kısa bir başlık eklenmelidir. Sözcük sayısı ve özellikler için lütfen Tablo 1'e bakınız.

Hakem Raporu Sonrasında Değerlendirme

Yazarlar hakem raporunda belirtilen düzeltme istenen konuları maddelendirerek bir cevap olarak kendilerine ayrılan cevap bölümüne yazmalıdırlar ve hakem (ler)e cevap başlıklı ek bir dosya şeklinde https://www. selcukmedj.org adresinden yüklenmelidir. Ayrıca makale içerisinde de gerekli değişiklikleri yapmalı ve bunları makale içerisinde belirterek (boyayarak) online olarak tekrar gönderilmelidir.

Son Kontrol

1. Yayın hakkı devir ve yazarlarla ilgili bildirilmesi gereken konular formu gereğince doldurulup imzalanmış,
2. Ana makale metni ve özetlerde gerekli kelime sayıları aşılmamış
3. Yeterli sayıda anahtar kelime eklenmiş,
4. Başlık uzun ve kısa başlık şeklinde yazılmış,
5. Kaynaklar kurallara uygun olarak yazılmış,
6. Tablo, resim ve şekillerde bütün kısaltmalar açıklanmış olmalıdır.

Online Yükleme Basamakları, https://www. selcukmedj.org sayfasında;

1. Makale türü *
2. Uzun Başlık *
3. Kısa başlık *
4. Özet*
5. Anahtar kelimeler *
6. Yüklenmesi gerekli bölümler (Kapak sayfası, Ön yazı (cover letter), makale dosyası, Etik kurul onay Belgesi (kurumdan alınan), intihal analiz raporu, Şekiller ve Resimler, Telif Hakları Bildirim Formu ve gerekli ise hasta onam formu şeklinde tamamlanmalıdır.

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- Vikse BE, Aasarød K, Bostad L, et al. Clinical prognostic factors in biopsy-proven benign nephrosclerosis. Nephrol Dial Transplant. 2003;18(3):517-23. doi: 10.1093/ndt/18.3.517.

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Single Author Books:
- Danovitch GM. Handbook of Kidney Transplantation. Boston: Little, Brown and Company (Inc.), 1996: 323-8.

Book Chapter:
- Soysal Z, Albek E, Eke M. Fetüs hakları. Soysal Z, Çakalır C, ed. Adli Tıp, Cilt III,İstanbul Üniversitesi Cerrahpaşa Tıp Fakültesi Yayınları, İstanbul, 1999:1635-50.
- Davison AM, Cameron JS, Grünfeld JP, et al. Oxford Textbook of Clinical Nephrology. In: Williams G, ed. Mesengiocapillary glomerulonephritis. New York: Oxford University Press, 1998: 591- 613.

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

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

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

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Review	5000	300	80	6	10
Case Presentations	1500	200	15	3	5
Letters to the Editor	1000	(-)	8	(-)	(-)
Editorial	1000	(-)	20	3	3
Original Image Report	200	(-)	5	1	3

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Review Articles: Review articles should be articles that review a sufficient number of scientific articles, summarize the subject at the current level of knowledge and technology, evaluate and interpret the findings by comparing them. Techniques and applications in the latest scientific studies with all the developments in basic and applied sciences are evaluated. Reviews prepared by authors who have extensive knowledge of a particular field and have a scientific background with high citation potential will be accepted by our journal. Acceptance of manuscripts from these authors can also be by invitation. The main text should include Introduction, Clinical and Research Results and Conclusion. Please see Table 1 for word count and specifications.

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OPEN ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Evaluation of Denosumab Efficacy after Bisphosphonate Use in Patients with Osteoporosis: A Single Center Experience

Osteoporozlu Hastalarda Bisfosfonat Kullanımı Sonrası Denosumab Etkinliğinin Değerlendirilmesi: Tek Merkez Deneyimi

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ÖZET

Amaç: Osteoporoz kemiklerdeki mineral yoğunluğunun azalması sonucu kemik kütle ve yapısının zayıflayarak kemiklerin kırılgan hale geldiği bir hastalıktır. Tedavide amaç kemik kırıklarının engellenmesidir. Osteoporoz tedavisinde yeterli kalsiyum ve D vitamini kullanılmasının yanı sıra kemik rezorbsiyonunu önleyici ve kemik yapımını arttırıcı ilaçlar kullanılmaktadır. Düşme riskini azaltıcı yaşam tarzı önlemlerinin alınması da gereklidir. Bu çalışmada osteoporozu olan Türk popülasyonunda bisfosfonat kullanımı sonrası denosumabın etkinlik ve güvenilirliğinin değerlendirilmesi amaçlandı.

Gereçler ve Yöntem: 2018-2022 yılları arasında osteoporoz tanısı alan ve denosumab kullanan hastalar çalışmaya dahil edildi. Hastaların demografik, klinik, kemik mineral yoğunluğu ve tedavi özellikleri kaydedildi. Hastaların denosumab kullanımından sonraki kemik mineral yoğunluğu değerleri tedavi öncesi başlangıç değerleri ile karşılaştırıldı.

Bulgular: Çalışmanın analizi 55 hastanın verileri ile yapıldı. Çalışmaya alınan hastaların tümü kadın olup ortanca yaş 69 (46-90)'du. Osteoporozun en sık nedeni postmenopozal (%56,4) iken, sekonder nedenler arasında en sık görülen neden ise primer hiperparatiroidizm (%14,5) idi. On dört (%25,5) hastada kırık öyküsü mevcuttu. Denosumab tedavisi sonrası hastaların fosfor (p=0,022) ve alkalen fosfataz (p<0,001) düzeylerinde istatistiksel olarak anlamlı düşüş saptandı. Denosumab tedavisi ile L1-L4 (0,887'den 0,933'e), femur boynu (0,693'ten 0,727'ye) ve total kalça (0,762'den 0,782'ye) bölgelerinde BMD (gr/cm^2) değerlerinde iyileşme gözlendi. Benzer şekilde, hastaların BMD Z skorları ve T skorlarında da iyileşme saptandı. Ayrıca denosumab ile ilişkili advers olay gözlenmedi.

Sonuç: Bu çalışmada osteoporozlu hastaların tedavi özellikleri geriye dönük olarak incelendi. Hastalarda denosumab tedavisi iyi tolere edildi ve tedavi ile ilişkili ciddi bir yan etki saptanmadı. Bisfosfonat kullanımından sonra denosumabın osteoporoz tedavisinde etkili ve güvenli bir tedavi seçeneği olduğu görüldü.

Anahtar Kelimeler: Osteoporoz, bisfosfonat, denosumab

ABSTRACT

Objective: Osteoporosis is a disease in which the bone mass and structure are weakened as a result of the decrease in the mineral density in the bones, and the bones become brittle. In addition to the use of adequate calcium and vitamin D in the treatment of osteoporosis, drugs that prevent bone resorption and increase bone formation are used. The aim of this study was to evaluate the efficacy and safety of denosumab after the use of bisphosphonates in the Turkish population with osteoporosis.

Materials and Methods: Patients diagnosed with osteoporosis and using denosumab between 2018-2022 were involved in the study. Demographic, clinical, bone mineral density, and treatment characteristics of the patients were recorded. The patient's bone mineral density values after denosumab were compared with the baseline values.

Results: Analysis of the study was performed with data from 55 patients. All patients involved in the study were female, and the median age was 69 (46-90). The most common cause of osteoporosis was postmenopausal (56.4%), and the most common cause among secondary causes was primary hyperparathyroidism (14.5%). Fourteen (25.5%) patients had a history of fracture. After denosumab treatment, a statistically significant decrease was detected in the phosphorus (p=0.022) and alkaline phosphatase (p<0.001) levels of the patients. Improvement was observed in BMD (gr/cm^2) values of L1-L4 (from 0.887 to 0.933), femoral neck (from 0.693 to 0.727), and total hip (from 0.762 to 0.782) regions with denosumab treatment. Similarly, the patients' BMD Z scores and T scores were improved. Also, the denosumab-related adverse event was not observed.

Conclusions: In this study, treatment characteristics of osteoporosis patients were retrospectively examined. Denosumab was well tolerated in patients, and no serious treatment-related side effects were detected. After bisphosphonate use, denosumab was shown to be an effective and safe treatment option in the treatment of osteoporosis.

Keywords: Osteoporosis, bisphosphonate, denosumab

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INTRODUCTION

Osteoporosis is a bone disease with decreased bone mineral density, microarchitectural disruption, and an increased risk of bone fracture. Many risk factors have been identified for the development of osteoporosis, including drug use, endocrine diseases, nutritional disorders, gastrointestinal absorption disorders, and genetic diseases. In order to find the cause of osteoporosis, a differential diagnosis should be made with past medical history and biochemical values such as calcium, phosphorus, alkaline phosphatase, and vitamin D levels. Osteoporosis is asymptomatic, and there is no pain in the patients until developing to the deformity associated with a bone fracture. The diagnosis of osteoporosis is made by increasing the fragility of the bones or by determining the T score below -2.5 by bone mineral density (BMD) measurement by dual-energy x-ray absorptiometry (DEXA) (1). Many lifestyle-related factors, such as calcium/vitamin D replacement, diet, exercise, and smoking cessation are effective in improving bone mineral density in the treatment of osteoporosis (2-5).

Osteoporosis treatment with pharmacological agents is recommended in postmenopausal women with a T-score of ≤-2.5 in BMD measurement or a history of fragility fracture (6). In addition to calcium/vitamin D replacement, the most commonly used treatment agents in the treatment of osteoporosis are bisphosphonate group drugs that inhibit bone resorption, such as zoledronic acid, risedronate, and alendronate (7). Denosumab can be used in the treatment of osteoporosis, especially in older people with a high risk of fracture, in patients with a contraindication to the use of bisphosphonates or who do not benefit sufficiently from bisphosphonate therapy. Denosumab is a monoclonal antibody that inhibits nuclear factor kappa-B ligand (RANKL) and thus inhibits bone resorption by inhibiting osteoclast formation and activation (8). Denosumab is not generally recommended for first-line therapy in patients with osteoporosis and in premenopausal patients. This study aimed to evaluate the efficacy and safety of denosumab treatment in patients with osteoporosis who had previously used bisphosphonates and did not benefit enough in the Turkish population.

MATERIAL AND METHODS

Patients and data collection

A retrospective observational design was used to create this study. Prior to conducting the study, approval from the ethics committee was obtained, and good clinical practice principles were followed. Patients diagnosed and treated in the single tertiary endocrinology outpatient clinic between 2018 and 2022 were involved in the study. The patients included in the study were identified by scanning the patients who received denosumab with the diagnosis of osteoporosis via the hospital data processing system. Inclusion criteria were determined as 1- being female, 2- having a diagnosis of osteoporosis, and 3- using bisphosphonates before denosumab. All patients had used bisphosphonates regularly and had not responded to treatment. Patients with insufficient follow-up data for analysis and male patients were excluded from the study. The diagnosis of osteoporosis was accepted as a BMD T score of ≤-2.5, which is also accepted by the World Health Organization. Demographic characteristics, medical histories, menopause status, and treatment characteristics of the patients were recorded. Biochemical parameters such as parathormone (PTH), creatine, calcium, phosphorus, albumin, vitamin D, alanine transaminase (ALT), and alkaline phosphatase (ALP) levels were noted.

The patients used denosumab (PROLIA®, Amgen, USA) 60 mg every six months. Each patient was given vitamin D 800 IU and calcium 1200-1500 mg (except for patients with hyperparathyroidism) daily. In all patients, lumbar vertebrae 1 (L1), lumbar vertebrae 2 (L2), lumbar vertebrae 3 (L3), lumbar vertebrae 4 (L4), lumbar vertebrae 1-4 (L1-L4), lumbar vertebrae 2-4 (L2-L4), femoral neck, and total hip BMD measurements were made with DEXA at baseline and after denosumab treatment. Baseline BMD (pre-denosumab) and 1st year BMD (post-denosumab) T scores, Z scores, and bone densities (gr/cm2) were compared, and improvement under denosumab was evaluated. In addition, treatment-related side effects were recorded.

Statistical analysis

The statistics of the study were performed via SPSS 29 (IBM, Armonk, NY, USA). Continuous variables in the study were represented by median (as well as minimum and maximum values) value numbers and percentages, while categorical variables were described by numbers and percentages. Paired sample t-test was done to assess pre-and post-BMD outcomes and biochemical values. When the p-value was less than 0.05, results were deemed statistically significant, and the probability ratio was calculated.

RESULTS

Patient characteristic

Sixty-three patients using denosumab with the diagnosis of osteoporosis were identified, and statistical analyses were performed with the data of 55 patients who met the study criteria. All patients included in the study were women. The median age of the patients was 69 (46-90), and the median follow-up period was 14 (12-36) months. All patients included in the study were postmenopausal. The most common comorbidities in the patients were hypertension (56.4%), diabetes mellitus (27.3%), and hypothyroidism (30.9%). Fourteen (25.5%) patients had a history of fracture. The most common cause of osteoporosis was postmenopausal (56.4%), and the most common cause among secondary causes was primary hyperparathyroidism (14.5%). The general features of the patients are shown in Table 1.

Treatment modality and results

All patients had used bisphosphonates (median 24 months) before denosumab treatment. All patients received concurrent vitamin D and calcium replacement with denosumab therapy. No hypocalcemia or any adverse events associated with the use of denosumab were observed. When the basal BMD characteristics of the patients were examined, the median L1-L4 T score was -2.34 before the treatment, and the median value

Table 1. Patients Characteristics

	Number of Patients (Total:55)	(%)
Age at diagnosis		
65<	14	25.5
65 ≥	41	74.5
Medical history of endocrine disease		
Hypertension	31	56.3
Diabetes Mellitus	15	27.3
Chronic Kidney Disease	8	14.5
Hypothyroidism	17	30.9
Hyperthyroidism	4	7.3
Fracture history		
Yes	14	25.5
No	41	74.5
Osteoporosis type		
Senile	4	7.3
Postmenopausal	31	56.4
Secondary	20	36.4
Secondary causes of osteoporosis		
No	35	63.6
Hyperparathyroidism	8	14.5
Hypogonadism	4	7.3
Steroid use	4	7.3
Other	4	7.3

of the total hip T score was -2.53. Improvement was observed in BMD (gr/cm2) values of L1-L4 (from 0.887 to 0.933), femoral neck (from 0.693 to 0.727), and total hip (from 0.762 to 0.782) regions with denosumab treatment. The basal BMD values of the patients by region are shown in Table 2. When the changes in biochemical values were examined, it was determined that the mean vitamin D level of the patients increased by 10.8 ng/mL compared to before denosumab in the post-denosumab period. In addition, it was observed that the serum phosphorus level (p=0.022) and ALP level (p<0.001) decreased statistically significantly (Table 3). After denosumab treatment, in BMD measurement, L1-L4 (p=0.003), L2-L4 (p=0.001), femur neck (p=0.017), and femur total (p<0.001) T scores were improved (Table 4). In the evaluation made in terms of the BMD Z score, there was an additional improvement in the L2 (p=0.006) Z score, and the improvement in the femoral neck (p=0.068) Z score not remained within the statistical limit (Table 5). In addition, bone densities showed a general statistically

Table 2. BMD characteristics of patients pre- and post-denosumab treatment

	T Scores				Z scores				BMD (gr/cm²) levels			
	Mean	N	SD	SEM	Mean	N	SD	SEM	Mean	N	SD	SEM
L1 Post-denosumab	-2.407	40	1.682	0.266	-0.877	39	1.905	0.305	0.840	40	0.201	0.031
Pre-denosumab	-2.418	40	1.517	0.239	-1.049	39	1.661	0.266	0.845	40	0.179	0.028
L2 Post-denosumab	-2.600	36	2.432	0.405	-0.979	39	1.584	0.253	0.907	40	0.180	0.028
Pre-denosumab	-2.625	36	1.497	0.249	-1.495	39	1.626	0.260	0.868	40	0.185	0.029
L3 Post-denosumab	-1.711	38	1.599	0.259	-0.586	36	1.468	0.244	0.999	40	0.219	0.034
Pre-denosumab	-2.213	38	1.346	0.218	-0.981	36	1.525	0.254	0.955	40	0.211	0.033
L4 Post-denosumab	-1.672	39	1.489	0.238	-0.314	37	1.505	0.247	1.011	40	0.219	0.034
Pre-denosumab	-2.082	39	1.397	0.223	-0.689	37	1.642	0.270	0.970	40	0.197	0.031
L1-L4												
Post-denosumab	-2.010	52	1.540	0.213	-0.518	39	1.625	0.260	0.933	52	0.185	0.025
Pre-denosumab	-2.344	52	1.422	0.197	-0.941	39	1.546	0.247	0.887	52	0.179	0.024
L2-L4												
Post-denosumab	-1.934	47	1.393	0.203	-0.464	36	1.512	0.252	0.975	49	0.191	0.027
Pre-denosumab	-2.353	47	1.351	0.197	-0.856	36	1.522	0.253	0.915	49	0.190	0.027
Femure Neck												
Post-denosumab	-2.352	54	0.684	0.093	-0.585	41	0.768	0.120	0.727	54	0.086	0.011
Pre-denosumab	-2.539	54	0.687	0.093	-0.815	41	0.825	0.128	0.693	54	0.076	0.010
Total Hip												
Post-denosumab	-1.806	54	0.874	0.119	-0.407	41	0.864	0.135	0.782	53	0.091	0.012
Pre-denosumab	-2.539	54	0.687	0.093	-0.707	41	0.815	0.127	0.762	53	0.084	0.011

N: Number, SD: Standart deviation, SEM: Standart Error Mean, CI: Confidence Interval

Table 3. Comparison of patients' pre- and post-denosumab biochemical values

Paired differences							
				95% CI of the difference		T-test value	Sig (two-tailed)
	Mean	SD	SEM	Lower	Upper		
Parathormone (pg/mL) Post- Parathormone (pg/mL) Pre	-9.217	65.321	13.620	-37.464	19.029	-0.677	0.506
Creatine (mg/dL) Post- Creatine (mg/dL) Pre	-0.238	2.005	0.280	-0.802	0.325	-0.850	0.399
Calcium (mg/dL) Post- Calcium (mg/dL) Pre	0.005	0.540	0.075	-0.146	0.1580	0.078	0.938
Phosphorus (mg/dL) Post- Phosphorus (mg/dL) Pre	-0.214	0.582	0.089	-0.395	-0.032	-2.386	0.022
Albumin (g/dL) Post- Albumin (g/dL) Pre	0.596	3.622	0.528	-0.468	1.659	1.128	0.265
Vitamin D level (ng/mL) Post- Vitamin D level (ng/mL) Pre	10.820	49.561	7.080	-3.415	25.056	1.528	0.133
ALT (U/L) Post – ALT (U/L) Pre	-1.092	7.867	1.112	-3.328	1.143	-0.982	0.331
ALP (U/L) Post – ALP (U/L) Pre	-17.905	18.743	4.090	-26.436	-9.373	-4.378	<0.001

SD: Standart deviation, SEM: Standart Error Mean, CI: Confidence Interval

Table 4. Comparison of patients' pre- and post-denosumab BMD T scores

	Paired differences						
				95% CI of the difference		T-test value	Sig (two-tailed)
	Mean	SD	SEM	Lower	Upper		
L1 Post- L1 Pre	0.010	1.126	0.178	-0.350	0.370	0.056	0.956
L2 Post- L2 Pre	0.025	2.356	0.392	-0.772	0.822	0.064	0.950
L3 Post- L3 Pre	0.502	1.043	0.169	0.159	0.845	2.969	0.005
L4 Post- L4 Pre	0.410	1.097	0.175	0.054	0.765	2.335	0.025
L1-4 Post- L1-4 Pre	0.334	0.767	0.106	0.121	0.548	3.144	0.003
L2-4 Post- L2-4 Pre	0.419	0.819	0.119	0.178	0.659	3.508	0.001
Femoral Neck Post-Femoral Neck Pre	0.187	0.557	0.075	0.034	0.339	2.466	0.017
Total Hip Post- Total Hip Pre	0.733	0.792	0.107	0.517	0.949	6.803	<0.001

SD: Standart deviation, SEM: Standart Error Mean, CI: Confidence Interval

Table 5. Comparison of patients' pre- and post-denosumab BMD Z scores

	Paired differences						
				95% CI of the difference		T-test value	Sig (two-tailed)
	Mean	SD	SEM	Lower	Upper		
L1 Post- L1 Pre	0.171	1.058	0.169	-0.171	0.514	1.014	0.317
L2 Post- L2 Pre	0.515	1.115	0.178	0.153	0.877	2.885	0.006
L3 Post- L3 Pre	0.394	0.763	0.127	0.136	0.652	3.099	0.004
L4 Post- L4 Pre	0.375	1.032	0.169	0.031	0.720	2.213	0.033
L1-4 Post- L1-4 Pre	0.423	0.881	0.141	0.137	0.708	2.997	0.005
L2-4 Post- L2-4 Pre	0.391	0.707	0.117	0.152	0.631	3.321	0.002
Femoral Neck Post-Femoral Neck Pre	0.229	0.781	0.122	-0.017	0.475	1.879	0.068
Total Hip Post- Total Hip Pre	0.300	0.413	0.064	0.169	0.430	4.645	0<0.001

SD: Standart deviation, SEM: Standart Error Mean, CI: Confidence Interval

Table 6. Comparison of patients' pre- and post-denosumab BMD levels

	Paired differences					T-test value	Sig (two-tailed)
				95% CI of the difference			
	Mean	SD	SEM	Lower	Upper		
L1 Post- L1 Pre	-0.004	0.133	0.021	-0.047	0.038	-0.211	0.834
L2 Post- L2 Pre	0.038	0.126	0.019	-0.001	0.078	1.932	0.061
L3 Post- L3 Pre	0.044	0.176	0.027	-0.012	0.100	1.581	0.122
L4 Post- L4 Pre	0.041	0.135	0.021	-0.001	0.084	1.933	0.060
L1-4 Post- L1-4 Pre	0.046	0.096	0.013	0.019	0.072	3.428	0.001
L2-4 Post- L2-4 Pre	0.060	0.099	0.014	0.031	0.088	4.233	<0.001
Femoral Neck Post-Femoral Neck Pre	0.033	0.073	0.009	0.013	0.053	3.401	0.001
Total Hip Post-Total Hip Pre	0.020	0.054	0.007	0.005	0.035	2.784	0.007

SD: Standart deviation, SEM: Standart Error Mean, CI: Confidence Interval

significant improvement except for L1 (Table 6). During the follow-up period, no patients had any symptomatic fractures detected.

DISCUSSION

Osteoporosis is a common public health problem in postmenopausal women, especially in the elderly. Morbidity due to osteoporosis-related bone fractures can cause severe social and psychogenic difficulties for patients. Although it is tried to prevent the development of osteoporosis with lifestyle changes and calcium and vitamin D supplementation, in some cases, pharmacological treatments are needed. This study showed real-life outcomes of denosumab after bisphosphonate use in patients with osteoporosis at a single endocrinology center. Denosumab was found effective and safe in the treatment of osteoporosis in this study. In the FREEDOM study, patients with postmenopausal osteoporosis were evaluated, and after three years of follow-up, it was shown that denosumab treatment reduced the risk of new vertebral (2.3 vs. 7.2 percent), total hip (0.7 vs. 1.2 percent) and nonvertebral (6.5 vs. 8.5 percent) fractures compared to placebo (9). In addition, in the FREEDOM study, the BMD density of the patients increased, and bone turnover markers levels decreased. In a phase 3 study comparing the efficacy of denosumab and alendronate in postmenopausal women with osteoporosis, it was found that denosumab increased BMD in all bone regions measured and significantly decreased bone turnover markers compared to alendronate (10). In another study, patients who used alendronate for at least six months and continued alendronate were compared with patients who were switched to denosumab; a 1.90% increase in total hip BMD was found in patients in the denosumab arm, and also a statistically significant improvement in the lumbar spine, femoral neck, and 1/3 radius regions was achieved (11). After the use of bisphosphonate, denosumab provides an improvement in at least one of the DEXA T or Z scores, and BMD measurements in other regions except for the L1 region in this study. The lack of statistically significant improvement in the L1 vertebra in this study may be explained by the limited number of patients and the duration of treatment. The number of studies comparing denosumab with bisphosphonates in clinical practice is limited, and a comparative study with an endpoint of fracture risk reduction is not available in the literature. Therefore, there are some controversial issues with the optimal use of denosumab therapy. Due to the ease of oral use of bisphosphonates and their low cost, bisphosphonates are primarily used in the first series in the treatment of osteoporosis in clinical practice, and denosumab is used after the use of bisphosphonates. Terminating denosumab treatment in a short time may increase the risk of multiple fractures in the vertebral bones (12-14). Therefore, patients who will be started on denosumab should be evaluated in terms of treatment compliance.

Denosumab is generally well tolerated, and side effects are rare. The risk of denosumab-associated hypocalcemia is less than 1% and is especially seen in patients with hyperparathyroidism, malabsorption syndrome, and chronic kidney disease (15). Since denosumab impairs bone remodeling, long-term side effects such as jaw necrosis and atypical fractures can be seen rarely (16). It has also been shown that denosumab-related bone healing may be delayed, and this may affect other wound-related complications (17). In this study, no adverse events were observed in the patient group under denosumab treatment. This may be explained by the limited number of patients involved in the study and the rare occurrence of denosumab-related side effects. As a result of being retrospective, this study had some limitations. The patients number was relatively limited, and some patients' data were missing. The patient group involved in the study was heterogeneous.

CONCLUSIONS

In this study, it showed that using denosumab after bisphosphonate use in patients with osteoporosis in the Turkish population is effective and safe. Also, it was detected the osteoporotic patients profile used denosumab after bisphosphonate in the Turkish population. This study contributes to the literature in terms of demonstrating the effectiveness of denosumab in the Turkish population with osteoporosis. Osteoporosis is a bone disease that is affected by many environmental factors. In the future, a better

understanding of the development processes of osteoporosis will shed light on the development of new advances in terms of both prevention and treatment of osteoporosis.

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OPEN ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Monocyte to High-Density Lipoprotein Cholesterol Ratio in Patients with Retinal Artery Occlusion

Retinal Arter Oklüzyonu Olan Hastalarda Monosit/Yüksek Dansiteli Lipoprotein Kolesterol Oranı

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ÖZET

Amaç: Retinal arter oklüzyonu (RAO), oftalmolojik hastalıkların en önemli acil durumlarından biridir. Tromboembolizm RAO'nun ana etiyolojik faktörüdür. Monosit/yüksek dansiteli lipoprotein kolesterol (HDL) oranını (MHO), çeşitli inflamatuar bozukluklarda, kardiyovasküler ve serebrovasküler hastalıklarda yeni bir prognostik biyobelirteçtir. Bu çalışmanın amacı RAO geçirmiş hastalarda MHO incelemektir.

Gereç ve Yöntemler: Retrospektif olarak yapılan bu çalışmada, Ocak 2015 ile Mayıs 2019 tarihleri arasında muayene olan hastaların dosya kayıtları değerlendirildi. Çalışmaya toplam 76 hasta dahil edildi. Hastalar iki gruba ayrıldı. RAO tanısı alan 38 hasta grup 1, 38 katılımcı ise grup 2 (kontrol grubu) olarak kabul edildi. Grup 1'de 23 hasta santral retinal arter oklüzyonu (SRAO), 15 hasta ise retinal arter dal oklüzyonu (RADO) olarak sınıflandırıldı.

Bulgular: Gruplar arasında cinsiyet ve yaş açısından fark yoktu (p=0,231 ve p=0,685). Gruplar kardiyovasküler hastalıklar, sistemik hipertansiyon ve diyabet açısından benzerdi (p=0,341, p=0,427, p=0,554, sırasıyla). Ortalama MHO, RAO grubunda kontrol grubuna göre anlamlı olarak daha yüksekti (14.9±5.5'e karşı 7.9±1.9, p <0.001). Alıcı çalışma karakteristikleri analizinde, MHO için eğri altındaki alan 0.908 olup MHO > 9.95 değeri % 89.5 duyarlılık ve % 84.2 özgüllük ile RAO' yu öngörmüştür. Tek değişkenli lojistik regresyonda, MHO'nın, RAO' nun bağımsız bir prediktörü olduğu görülmüştür (OR = 1.892; % 95 CI = 1.398-2.561; p <0.001).

Sonuç: MHO'nın sistemik inflamasyon ve vasküler-tıkayıcı hastalıklar için basit, kullanışlı ve ucuz bir öngörücü biyobelirteç olduğu gösterilmiştir. Çalışmamız, artmış MHO'nın RAO ile önemli ölçüde ilişkili olduğunu göstermiştir. Ayrıca, MHO'nın hem SRAO hem de RADO'da daha yüksek olduğunu ortaya koymuştur. Bu nedenle MHO, riskli hastalarda RAO' nun gelişmesi açısından öngörücü bir biyobelirteç olabilir.

Anahtar Kelimeler: Monosit/yüksek yoğunluklu lipoprotein kolesterol oranı, santral retinal arter oklüzyonu, retinal arter dal oklüzyonu, ateroskleroz, inflamasyon

ABSTRACT

Aim: Retinal artery occlusion (RAO) is one of the most important emergencies of ophthalmologic diseases. Thromboembolism is the main etiological factor in RAO. Monocyte to HDL cholesterol ratio (MHR) is a novel prognostic biomarker in several inflammatory disorders, various cardiovascular and cerebrovascular diseases. In this study, it was aimed to investigate MHR in RAO patients.

Materials and Methods: In this retrospective study, the record files of subjects who were examined between January 2015 and May 2019 were reviewed. Seventy-six subjects were enrolled in the groups. Thirty-eight patients with RAO were considered as group 1, 38 participants were considered as group 2 (control group). In group 1, 23 patients were classified as central retinal artery occlusion (CRAO), and 15 patients were classified as branch retinal artery occlusion (BRAO).

Results: There was no difference in terms of gender and age among the groups (p=0.231 and p=0.685). As well, the groups were similar in terms of cardiovascular diseases, systemic hypertension, and diabetes mellitus (p=0.341, p=0.427, p=0.554, respectively). The mean MHR was higher in group 1 compared to the group 2, significantly (14.9±5.5 vs 7.9±1.9, p<0.001). The area under the curve for MHR was 0.908 in receiver operating characteristics analysis and an MHR of > 9.95 predicted RAO with a specificity of 84.2% and sensitivity of 89.5%. In univariate logistic regression, MHR was found to be an independent predictor of RAO (OR=1,892; 95% CI=1,398-2,561; p<0,001).

Conclusions: MHR has been shown to be a simple, useful and inexpensive predictive biomarker for systemic inflammation and vascular-occlusive diseases. Our study showed that increased MHR was significantly associated with RAO. Also, we have researched the level of MHR in patients with RAO and presented that MHR is higher in both CRAO and BRAO. Therefore, MHR may be a predictive biomarker for the emergence of RAO in risky patients.

Keywords: Monocyte to high-density lipoprotein cholesterol ratio, central retinal artery occlusion, branch retinal artery occlusion, atherosclerosis, inflammation

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INTRODUCTION

Retinal artery occlusion (RAO) is one of the most important emergencies of ophthalmologic diseases (1). Painless, sudden unilateral partial/total loss of vision that develops within minutes or hours is the symptoms of RAO. The incidence of RAO varies between 1 and 3 in 100 000 (2,3). Although this disease can be seen in the first and ninth decades, it occurs most often in the sixth decade and bilateral involvement was observed in 1-2% of the cases (2). In general, RAO is categorized as branch retinal artery occlusion (BRAO) and central retinal artery occlusion (CRAO) due to the placement of the occluded artery. CRAO is more common than BRAO but BRAO is more than CRAO when only young patients are considered (4).

Thromboembolism is the main etiological factor both in BRAO and CRAO (5). Major risk factors in elderly patients are systemic hypertension, diabetes mellitus, atherosclerosis, cardiovascular and cerebrovascular diseases (5). Embolic events due to vasculitis, congenital vascular or cardiac anomalies, hyperhomocysteinemia, antiphospholipid antibody syndrome, and hypercoagulability are more prominent in young patients (6,7). Particularly, atherosclerosis is the major risk factor of many vascular-occlusive disorders such as atrial fibrillation, acute myocardial infarction (AMI), stroke, and retinal vein occlusion (8,9). In addition, increased inflammation and accumulation of lipids are the main features of atherosclerosis (10,11).

It is known that monocytes have a major mission in the pro-inflammatory cascades, pro-oxidant reactions, and development of atherosclerosis. Besides, high-density lipoprotein cholesterol (HDL-c) has reverse effects against monocytes (12,13). Recently, it has been shown that monocyte to HDL cholesterol ratio (MHR) is a novel prognostic biomarker in several inflammatory disorders, various cardiovascular and cerebrovascular diseases (14-17). From this point of view, it was aimed in this study to analyze the MHR level in RAO patients.

MATERIALS AND METHODS

This retrospective study adhered to the principles of the Declaration of Helsinki and was conducted in the Department of Ophthalmology at Necmettin Erbakan University, Faculty of Medicine with approval from the local ethics committee. The record files of subjects who were examined between January 2015 and May 2019 were reviewed and 38 patients (CRAO=23 patients, BRAO=15 patients) who were diagnosed with signs of an acute RAO were participated in this study.

Basically, subjects who had an anamnesis of sudden vision loss in one eye were diagnosed with CRAO in case of albescent retina with cherry red spot due to the retinal ischemia by dilated fundus examination. In addition, BRAO diagnosis was based on due to visualization of retinal opacity in the region of occluded branch retinal artery or visualization of emboli located at occluded branch retinal artery in patients with an anamnesis of sudden visual deterioration in one eye. Also, spectral-domain optical coherence tomography (SD-OCT) was done at first visit to all patients to detect acute retinal edema.

After the subjects had fasted for at least 12 hours, venous blood was drawn into K2-EDTA tubes. An automated hematology analyzer (XN-1000, Sysmex America, Inc.) was used to count the monocytes in each subject, and a chemistry analyzer (ARCHITECT c16000, Abbott, Illinois, USA) was used to measure the HDL levels. By dividing each subject's monocyte count by their HDL level, the MHR was determined.

All subjects with inadequate information or doubtful diagnosis and RAO patients presenting later than 5 days of symptom onset were excluded. Also, the patients who have anamnesis of any ocular diseases, of vasculitis, of blood dyscrasias, of hepatic disorders, of renal failure, of autoimmune diseases, of acute systemic infections; and the patients with a history of surgery recently, with a history of any systemic/topical medication usage such as non-steroid/steroid drugs, anti-hyperlipidemic medications, anticoagulant medications, and alcohol consumption were not included in the groups. In addition, the control group was created from subjects with senile cataracts.

The statistical analyses were done by SPSS 20.0 software (SPSS Inc., Chicago, IL). Number and percentage values were used to express the categorical variables. Numerical variables with a normal distribution were displayed as mean±standard deviation (SD). To analyze categorical data, the Pearson chi-square test was employed. For non-parametric values between groups, the Kruskal-Wallis test was employed, and for parametric values between groups, the one-way ANOVA test was utilized. In order to predict the sensitivity and specificity for RAO, the best cut-off value for MHR was determined using receiver-operating-characteristic (ROC) curves. A measure of

Table 1. Demographic characteristics and hematologic parameters of the groups.

	Mean ± SD		p
	RAO Group	Control Group	
Gender (Female/Male)	11/27	16/22	0.231
Age (years)	60.7±16.1	61.2±6.1	0.685
Monocyte (×109/L)	634.4±165.1	397.5±107.6	<0.001
HDL (mg/dL)	44.5±8.4	51.21±10.1	0.002
MHR	14.9±5.5	7.9±1.9	<0.001
Cardiovascular disease (n)	10	6	0.341
Hypertension (n)	11	8	0.427
Diabetes mellitus (n)	8	6	0.554

HDL: High-density lipoprotein MHR: Monocyte/HDL ratio

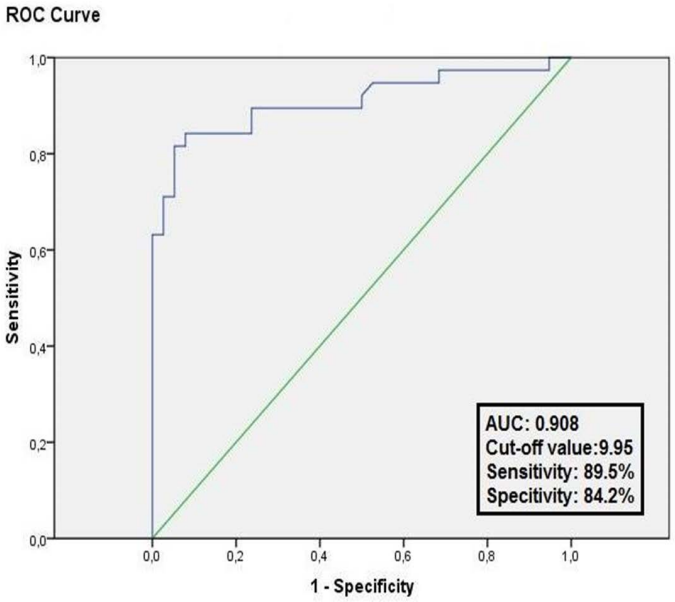

Figure 1. The receiver-operating characteristic curve analysis for MHR in RAO.

the test's accuracy called the area under the curve (AUC) was computed. A univariate logistic regression model was used to calculate the adjusted odds ratio (OR) and 95% confidence interval (CI) for the independent association between MHR and RAO. At $P < 0.05$, statistical significance was established.

RESULTS

The demographic characteristics and laboratory parameters were shown in Table 1. The groups did not differ in terms of gender or age (p=0.231 and p=0.685). As well, the groups' rates of diabetes mellitus, systemic hypertension, and cardiovascular diseases were comparable (p=0.341, p=0.427, p=0.554, respectively).

When laboratory parameters were compared between the two groups, it was found that the RAO patients had a considerably higher mean MHR (p <0.001). Also, the optimal cut-off value of MHR for RAO was calculated as 9.95 with 84.2% specificity and 89.5% sensitivity and AUC was 0.908 (Figure 1). Furthermore, an investigation of univariate logistic regression revealed that MHR was a separate predictor of RAO (OR=1,892; 95% CI=1,398 -2,561; p<0,001).

Moreover, there were no differences in gender, age, the mean monocyte count, HDL, and MHR values between the patients with BRAO and CRAO (p=0.791, p=0.695, p=0.767, p=0.156, p=0.083).

DISCUSSION

Actually, inflammation is a defense mechanism which is a body response in case of acute stress but in many systemic disorders such as cardiovascular diseases, cancer, and atherosclerosis there is a subclinical inflammation due to the persistent elevation of inflammatory biomarkers (18-22). Basically, monocytes, smooth muscle cells, lymphocytes infiltration and lipid accumulation into the arterial wall which starts a continuous inflammatory reaction is the hallmarks of atherosclerosis (23). In this inflammatory cascade, monocytes and macrophages have a pivotal role (24). They are the main source of certain proinflammatory cytokines and reactive oxygen species (24). As a result, the accumulation of these substances develops atherosclerotic plaque. When vulnerable plaques rupture, thromboembolic events arise and acute ischemia occurs due to the complete occlusion of the blood vessel in the affected tissue (25,26). Indeed, the eye is one of the target organs in thromboembolic events.

As far as is known, the etiology of RAO is multifactorial. Many disorders may be the reason for this ophthalmic emergency. Carotid artery dissection, cardiogenic embolism, giant cell arteritis, Susac syndrome, Fabry disease, sickle cell disease are the possible causes of RAO but it is known that 70% cause of BRAO or CRAO is ipsilateral internal carotid artery (ICA) atherosclerosis (27-30). The first branch of the ICA is ophthalmic artery and because of this reason it is an easy path for thromboembolic plaque to cause occlusions in the eye, primarily. The source of thromboembolism is usually fibrin or cholesterol plaque from the atherosclerotic background and particularly, thromboembolic plaque can cause blockages at the distal side to the bifurcation of retinal arteries (29). These thromboembolic plaques may be visible at admission in approximately 40% of patients (31). Thus, the absence of a thromboembolic plaque does not exclude the possibility of embolic occlusion. The possible causes of and risk fac¬tors for RAO are similar to those of cerebrovascular ischemic events, coronary artery disease (CAD), ischemic heart disease and it is known that RAO is associated with systemic hypertension, diabetes mellitus, cardiovascular diseases (32). Moreover, many studies have revealed that there is a co-occurrence of ischemic stroke (IS), transient ischemic attack or AMI with RAO (30,33,34). Avery and colleagues have reported that ocular ischemic syndrome, BRAO, and CRAO are significantly associated with stroke and Park et al. showed that AMI and IS significantly increased in the first month after CRAO occurrence especially in the first 7 days (35,36).

Monocytes are the responsible cells in the first steps of prooxidant and proinflammatory and reactions in atherosclerosis (12). Besides; HDL-c protects cells from inflammatory reactions by inhibiting the oxidation of low-density lipoprotein cholesterol, reducing macrophage migration, and monocyte activation (13). More recently, MHR was proposed as a novel marker in many inflammatory disorders, cardiovascular and cerebrovascular diseases (14-17). This is because MHR shows the equalization of pro- and anti-inflammatory reactions. Bolayir et al. published that MHR level is a predictor of 30-day mortality in patients with acute IS (17). As well, Korkmaz et al. reported that increased MHR values were independently associated with functionally

significant coronary artery lesions (37). Cetin and colleagues have demonstrated that MHR is an independent predictor of severity of CAD (38).

In this article, we have researched the level of MHR in patients with RAO and presented that MHR is higher in both CRAO and BRAO. It is important to state that MHR, particularly in subjects with thromboembolic events, may be a useful marker for predicting the risk of RAO. The limitations of this study are evidently a small sample size, retrospective design, and single-center study methodology.

CONCLUSIONS

As discussed above, it has been shown that MHR is a simple, useful, and inexpensive predictive biomarker of systemic inflammation and vascular-occlusive diseases. In addition, patients with thromboembolic events have a higher risk of RAO due to possible similar etiology. In this regard, MHR value may be a practical biomarker for evaluation of RAO development in risky subjects.

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Effects of Valproate and Carbamazepine Monotherapy on Leukocyte Subsets and Neutrophil-Lymphocyte Ratio in Children

Çocuklarda Valproat ve Karbamazepin Monoterapisinin Lökosit Alt Tipleri ve Nötrofil-Lenfosit Oranı Üzerine Etkileri

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ÖZET

Amaç: Epilepsili hastalarda kullanılan nöbet önleyici ilaçların lökopeni ve nötropeni gibi yan etkileri yakından bilinmekle birlikte, lökosit alt tipleri üzerine etkileri iyi bilinmemektedir. Lökosit alt tiplerindeki değişiklikler, vücudun enflamasyon durumunu değerlendirmek için de kullanılmaktadır. Nötrofilin lenfosite oranı (NLR), bu amaçla son yıllarda yaygın kullanılan indekslerdendir. Bu çalışma, iyi bilinen nöbet önleyici ilaçlardan valproat (VPA) ve karbamazepinin (KBZ) monoterapisinin lökosit alt tipleri ve NLR üzerine etkileri ve bu etkinin serum ilaç düzeyleriyle ilişkisini değerlendirmeyi amaçlamıştır.

Gereç ve Yöntemler: İdiopatik epilepsili, KBZ (n=25) veya VPA monoterapisi (n=62) başlanan, tedavi öncesi ve sonrasındaki ilk 1- 6 ay (T1) ve 9-18 ayda (T2) tam kan sayımı incelemesi ve en az bir kez eş zamanlı serum ilaç düzeyi değerlendirilmiş olan hastaların verileri, retrospektif olarak incelendi. Veriler, lökosit, nötrofil, lenfosit ve monosit sayı ve yüzdeleri ve eşzamanlı bakılan serum anti-nöbet ilaç düzeylerini içerdi. Serum ilaç düzeylerinin değişkenlerle ilişkisi, VPA grubunda 130, KBZ grubunda 30 eş zamanlı ölçümle değerlendirildi.

Bulgular: VPA grubunda, tedavi öncesine göre, T1 döneminde, nötrofil sayısı ve NLR'de azalma saptandı (p=0.026, p=0.038). Buna karşın lenfosit ve monosit oranında artış oldu (p=0.015, p<0.001). KBZ grubunda anlamlı değişiklik yoktu. Serum VPA düzeyleri monosit sayısı ile (r=0.2, p=0.022) pozitif korelasyon gösterdi. Serum KBZ düzeyleri nötrofil sayısı (r=-0.574, p=0.001) ve NLR ile (r=-0.413, p=0.023) negatif korelasyon gösterdi.

Sonuç: VPA monoterapisinin NLR oranında anlamlı baskılanmaya neden olması, antienflamatuar etki gösterdiğini desteklemektedir. Serum KBZ düzeylerinin NLR ile negatif korelasyonu, KBZ'nin NLR üzerine dozla ilişkili olarak benzer etkisinin olabileceğini düşündürmüştür. Bu etkilerin dikkate alınması, epilepsili çocuklarda uygun ilaç seçiminde fayda sağlayabilir.

Anahtar Kelimeler: Karbamazepin, nötrofil-lenfosit oranı, lökosit alt grupları, valproat

ABSTRACT

Objective: Although the adverse effects of anti-seizure medications (ASMs), like leukopenia and neutropenia, are widely recognized, little is known about how they affect leukocyte subsets. Changes in leukocyte subsets are also used to assess the inflammation status during diseases. The neutrophil-to-lymphocyte ratio (NLR) is widely used for this purpose. This study aims to evaluate the effects of valproate (VPA) and carbamazepine (CBZ) monotherapy on leukocyte subsets and NLR and to determine the relationship of these effects with serum drug levels.

Material and Methods: The data of children with idiopathic epilepsy who began CBZ (n=25) or VPA monotherapy (n=62) and underwent complete blood count examinations before treatment and at 1–6 months (T1) and 9–18 months (T2) after treatment, with at least one concurrent serum ASM level, were collected retrospectively. The data included the number and percentages of leukocytes, neutrophils, lymphocytes, and monocytes, as well as simultaneously measured serum ASM levels. The relationships between serum ASM levels and variables were evaluated with 130 simultaneous measurements in the VPA and 30 in the CBZ groups.

Results: In the VPA group, when compared to baseline, neutrophil count and NLR decreased (p=0.026, p=0.038, respectively), and lymphocyte and monocyte percentages increased (p=0.015, p<0.001, respectively). There were no significant changes in the CBZ group. Serum VPA levels were positively correlated with monocyte counts (r=0.2, p=0.022). Serum CBZ levels were negatively correlated with neutrophil counts (r= -0.574, p=0.001) and NLR (r= -0.413, p=0.023).

Conclusion: VPA monotherapy significantly decreased NLR, supporting its anti-inflammatory effects. The negative correlation of serum CBZ levels with NLR suggested that CBZ may have a dose-related effect on NLR. Consideration of these results may be beneficial in choosing an appropriate ASM for patients with epilepsy.

Keywords: Carbamazepine, leukocyte subsets, neutrophil-lymphocyte ratio, valproate

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INTRODUCTION

Epilepsy is a common disease characterized by spontaneous recurrent seizures, with a prevalence of 0.05-1% in the general pediatric population (1,2). The side effects associated with these drugs should be closely monitored due to the long-term use of anti-seizure medications (ASMs) in these patients. The hematological side effects show a wide spectrum, including leukopenia and neutropenia (3,4). Among the commonly used ASMs, valproate (VPA) and carbamazepine (CBZ) have the potential to cause leukopenia and neutropenia (5). In a study, VPA caused changes in the leukocyte subset distribution without causing neutropenia (6), suggesting that the effects of these drugs on leukocyte subsets may be more extensive than previously recognized. However, these effects are not well known.

In recent years, assessing alterations in leukocyte subsets has gained popularity for evaluating the inflammatory state and determining whether a disease is in its active phase, in addition to being cost-effective. For this purpose, the ratio of the neutrophil count to the lymphocyte count (NLR) is widely used, and its high level is associated with an increase in the inflammatory status (7). Considering the strong evidence that inflammation accompanies both epilepsy and febrile seizures (7,8), understanding the effects of ASMs on leukocyte subsets and NLR could offer valuable insights for choosing appropriate ASM for patients. However, few studies have evaluated ASM-related changes in leukocyte subsets (6,9,10), and the effect on NLR has been evaluated in only one study in adults. This study found no significant differences, although a decrease in NLR levels was observed with VPA (11). In addition, previous studies exhibit certain methodological limitations, such as a small patient sample size (9) and the inclusion of other ASM groups or healthy individuals as control groups (6,11). Epilepsy itself may alter leukocyte subsets and NLR levels, making a healthy control group insufficient for evaluating the effects of ASMs on epileptic patients. Some studies indicate that epileptic patients have higher NLR levels compared to the control group, particularly in the acute and subacute phases following seizures (12). Therefore, this study aimed to determine the temporal effects of VPA and CBZ on leukocyte subsets and NLR in pediatric epilepsy patients and the relationship between these effects and serum ASM levels.

MATERIALS AND METHODS

The records of consecutive epileptic patients who applied to the Pediatric Neurology clinic were retrospectively reviewed after local ethics committee approval (no: 2017/219). The study included children with idiopathic epilepsy who were started on VPA or CBZ as monotherapy between 2013-2018. These children had complete blood count and serum drug levels measured simultaneously during at least one of two periods: 1-6 months after treatment (T1 period) and 9-18 months after treatment (T2 period). Those with additional systemic diseases, chronic drug use, and infection during the examination were excluded from the study.

Collected data included the patients' sex, age, starting time of treatment, counts, and percentages of the leukocytes, neutrophils, lymphocytes, and monocytes measured at pre-treatment, T1 and T2 periods, and simultaneous serum ASM levels. The ratio of neutrophils to lymphocytes (NLR) was calculated for each patient. Complete blood count was evaluated using cell counter, and serum ASM levels were evaluated using spectrophotometric method. Therapeutic ranges for VPA and CBZ were established as 50–100 µg/mL and 4–12 µg/mL, respectively. Leukopenia was defined as a leukocyte count of less than 4000/mL, neutropenia as a neutrophil count of less than 1500/mL, and lymphopenia as a lymphocyte count of less than 1500/mL.

Statistical analysis

Continuous data were expressed as mean ± standard deviation (SD). The groups' distribution was assessed for normality using the Kolmogorov-Smirnov test. Friedman two-way analysis of variance and post-hoc Wilcoxon test were used to compare the data in the ASM groups at baseline, T1, and T2 periods. Student T or Mann Whitney U tests were used to compare ASM groups based on the normality of variable distribution; a Pearson's chi-square test was used to compare sex distribution across the ASM groups. The association between serum ASM levels and other variables was evaluated using Spearman or Pearson's correlation analysis, depending on the normality of the data. The statistical significance level was accepted as $p<0.05$.

RESULTS

General findings:

The study included 62 patients in the VPA group and 25 in the CBZ group. There were 33 males and 29 females in the VPA group, mean age was 8.1±3.5 years. There were 11 males and 14 females in the CBZ group, with a mean age of 8.5±2.5 years. The distribution of age and sex did not differ between ASM groups (p=0.312, p=0.436, respectively, Table 1).

Thirty measurements of serum ASM levels were taken simultaneously in the CBZ group and 130 in the VPA group. No leukopenia, neutropenia, or lymphopenia was observed in any patient after treatment with either drug.

Comparison of the values in the pre-treatment, T1, and T2 periods:

Compared to pre-treatment values, VPA treatment reduced neutrophil counts and NLR (p=0.026, p=0.038, respectively), while increasing the percentages of lymphocytes and monocytes (p=0.015, p<0.001). The lymphocyte count was the only value higher in the T2 period when comparing the T1 and T2 periods (p=0.042, Wilcoxon test). Although serum VPA levels were lower in the T2 period compared to the T1 period (p=0.028, Wilcoxon test), other findings were similar (Table 2, Figure 1).

Comparison of pre-treatment values with values in the T1 and T2 periods did not show a significant difference in the CBZ group (Table 1).

Comparison of drug groups:

Pre-treatment values did not show significant differences between the two ASM groups. In the T1 period, the VPA

Table 1. Comparison of ASM Groups (VPA and CBZ)

Variables (mean±SD)	VPA group	CBZ group	p-value
Age at the treatment starting (years)	8.1±3.5	8.5±2.5	0.312
Sex (M/F)	33/29	11/14	0.436*
Pre-treatment			
Leukocyte count (x10³/µL)	8.9±2.97	8.1±2.2	0.262
Neutrophil count (x10³/µL)	4.97±2.62	4.67±2.31	0.840
Neutrophil (%)	52.2±15	56.1±13.2	0.153
Lymphocyte count (x10³/µL)	3±1.15	2.6±0.9	0.088**
Lymphocyte (%)	36.4±12.9	33.5±11.4	0.216
NLR	2.14±2.29	2.3±2.1	0.234
Monocyte count (x10³/µL)	0.7±0.3	0.6±0.2	0.476
Monocyte (%)	7.9±3	7.6±2.5	0.485
T1 period			
Leukocyte count (x10³/µL)	8.3±3.2	7.7±1.8	0.991
Neutrophil count (x10³/µL)	3.9±2.3	3.9±1.6	0.196
Neutrophil (%)	44.4±12.5	50.5±11.1	0.049**
Lymphocyte count (x10³/µL)	3.3±1.3	2.8±0.8	0.024
Lymphocyte (%)	42.1±10.9	37.2±10	0.069**
NLR	1.24±0.73	1.55±0.94	0.087
Monocyte count (x10³/µL)	0.7±0.2	0.6±0.2	0.008
Monocyte (%)	9.6±2.6	8±2.3	0.017**
T2 period			
Leukocyte count (x10³/µL)	8.4±5.5	7.1±1.9	0.156
Neutrophil count (x10³/µL)	3.4±1.2	3.7±1.7	0.421**
Neutrophil (%)	42±9.7	49.8±11.2	0.007
Lymphocyte count (x10³/µL)	3.4±1.1	2.6±0.5	0.001
Lymphocyte (%)	44.5±8.7	38.3±9.6	0.015**
NLR	1.04±0.43	1.45±0.66	0.01
Monocyte count (x10³/µL)	0.7±0.2	0.7±0.4	0.035
Monocyte (%)	9.5±2.7	8.1±1.5	0.025**

T1 period: 1-6 months after treatment initiation, T2 period: 9-18 months after treatment initiation. Statistical tests: * Pearson's chi-square test, ** Student-T test, others used Mann-Whitney U test. ASM, anti-seizure medication; CBZ, carbamazepine; F, female; M, male; SD, standard deviation; VPA, valproate.

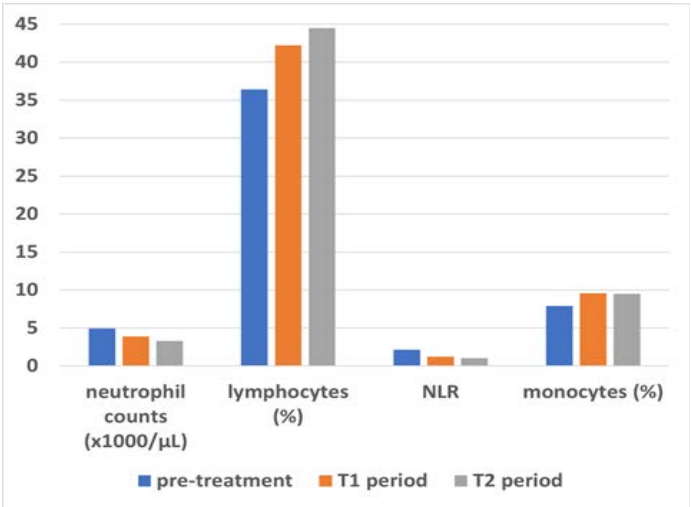

Figure 1. Variables Showing Significant Differences Over Time in the VPA Group

Following treatment, the percentages of lymphocytes and monocytes increased, while the neutrophil count and NLRs decreased. NLR, neutrophil to lymphocyte ratio; VPA, valproate

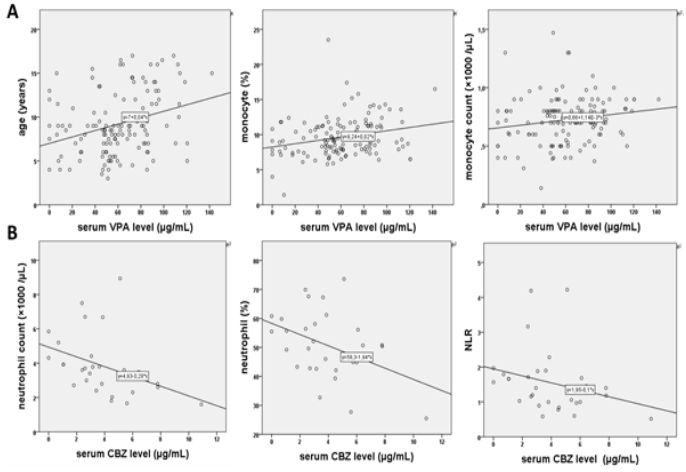

Figure 2. Variables Showing Significant Correlations with Serum ASM Levels

(A) Serum VPA levels were positively correlated with age (r=0.296, p=0.001) and counts and percentages of monocytes (r=0.2, p=0.022 and r=0.238, p=0.006, respectively). (B) Serum CBZ levels were negatively correlated with NLR (r= -0.413, p=0.023) and counts and percentages of neutrophils (r= -0.574, p=0.001 and r= -0.414, p=0.023, respectively). ASM, anti-seizure medication; CBZ, carbamazepine; NLR, neutrophil to lymphocyte ratio; VPA, valproate

Table 2. Statistical Comparison of the Leukocyte Count, Leukocyte Subset Counts and Percentages, Serum ASM Concentrations, and NLRs at the Pre-treatment, T1, and T2 Periods

Variables (mean±SD)	Pre-treatment	T1 period	T2 period	p-value
VPA group				
Serum VPA level (µg/mL)	-	65±4.4	50±4.7	0.028
Leukocyte count (x10^3/µL)	8.87±2.98	8.28±3.2	8.36±5.48	0.135
Neutrophil count (x10^3/µL)	4.97±2.8	3.9±2.3	3.3±1.2	0.026
Neutrophil (%)	52.2±15	44.4±12.45	42±9.7	0.059
Lymphocyte count (x10^3/µL)	3±1.15	3.3±1.25	3.4±1.07	0.062
Lymphocyte (%)	36.4±12.9	42.2±10.9	44.5±8.7	0.015
NLR	2.14±2.29	1.24±0.7	1.04±0.43	0.038
Monocyte count (x10^3/µL)	0.69±0.3	0.75±0.2	0.72±0.2	0.07
Monocyte (%)	7.9±3	9.6±2.6	9.5±2.7	<0.001
CBZ group				
Serum CBZ level (µg/mL)	-	3.6±2.4	4.2±3	0.249
Leukocyte count (x10^3/µL)	8.06±2.17	7.7±1.8	7.07±1.9	0.526
Neutrophil count (x10^3/µL)	4.7±2.3	3.9±1.6	3.7±1.7	0.807
Neutrophil (%)	56.1±13.2	50.5±11.1	49.8±11.2	0.931
Lymphocyte count (x10^3/µL)	2.6±0.9	2.8±0.8	2.6±0.47	0.807
Lymphocyte (%)	33.5±11.4	37.2±10	38.3±9.6	0.931
NLR	2.3±2.1	1.55±0.94	1.45±0.66	0.931
Monocyte count (x10^3/µL)	0.59±0.18	0.62±0.19	0.65±0.35	1.00
Monocyte (%)	7.6±2.5	8±2.3	8±1.5	0.492

T1 period: 1-6 months after treatment initiation, T2 period, 9-18 months after treatment initiation. Statistical tests: Friedman's two-way analysis of variance was used. ASM, anti-seizure medication; CBZ, carbamazepine; NLR, neutrophil to lymphocyte ratio; SD, standard deviation; VPA, valproate.

group had higher monocyte counts and percentages as well as lymphocyte counts (p=0.008, p=0.017, and p=0.024, respectively), and lower neutrophil percentages (p=0.049) than the CBZ group. Alongside these changes, there were also lower NLRs and higher lymphocyte percentages (p=0.01 and p=0.015, respectively) in the T2 period (Table 1).

Correlation analysis results:

Serum VPA levels showed statistically significant positive correlations with age (r=0.296, p=0.001), monocyte counts (r=0.2, p=0.022), and monocyte percentages (r=0.238, p=0.006) (Figure 2).

There was a strong negative correlation between serum CBZ levels and neutrophil counts (r=-0.574, p=0.001). Serum CBZ levels also showed statistically significant negative correlations with neutrophil percentages and NLRs (r=-0.414 and r=-0.413, respectively, p=0.023, for both) (Figure 2).

DISCUSSION

This study demonstrated that VPA significantly reduced the neutrophil count and NLR while increasing the percentage of lymphocytes and monocytes, even in the first six months of the treatment. CBZ did not affect leukocyte subsets and NLR compared to the pre-treatment period. However, correlation analysis results suggested that as serum CBZ levels increased, the counts and percentages of neutrophils and NLR levels decreased.

To our knowledge, this is the first study to assess the temporal effects of VPA and CBZ on NLR, particularly in pediatric patients. In the only study we could find evaluating the effects of ASMs on NLR, adult patients receiving VPA, CBZ, and levetiracetam were compared to healthy controls, and changes in NLR levels were found statistically insignificant (11). The results of that study may differ from the current study due to the following factors: comparison with healthy controls, evaluation of the adult age group, failure to consider temporal NLR changes, and the smaller number of patients in the VPA and CBZ groups (21 and 17, respectively).

The results of this study indicate that VPA may improve the anti-inflammatory response even in the first six months following treatment initiation because VPA suppresses neutrophil count and NLR. Even though serum VPA levels were significantly lower in the T2 period than in the T1 period, those effects of VPA persisted, demonstrating that they are not affected by dosing or drug levels. However, the strong positive correlation between serum VPA levels and monocyte count and percentage suggests that VPA has a dose-dependent effect on monocyte activation. In the study by Bartels et al., serum VPA levels did not correlate with monocyte activation but showed a negative correlation with neutrophil percentage (6). That study did not account for treatment duration, which could be why the current study results differed from theirs. However, the effects of VPA on monocytes are supported by evidence. Some studies suggest that VPA induces the differentiation of myeloid hematopoietic progenitor cells into monocytic lineages. This effect is attributed to the increased acetylation of histones H3 and H4 and the increased expression of p21, which is crucial for monocyte development (13,14).

Similar to this study, several studies have addressed the suppressive effect of VPA on neutrophil counts. In a study involving pediatric and adult patients, VPA significantly

decreased the neutrophil counts and increased the lymphocyte counts compared to patients using phenytoin and CBZ (6). In 15 adult patients evaluated before and three months after VPA treatment, decreases were observed in leukocytes, neutrophil counts, and neutrophil percentage after treatment (9). Those studies showed no significant difference in monocyte percentages, possibly due to adult patient evaluation, comparison with other ASMs, and small patient groups. In a study including a larger number of patients hospitalized due to COVID-19, a comparison of 165 adult patients with epilepsy using VPA and 330 control patients without epilepsy showed higher lymphocyte and monocyte counts, fewer lung infiltrates, and fewer intensive care unit admissions in the VPA group (15). In another study, 50 epileptic children who were started on VPA were followed up for nine weeks after treatment; no significant change was found in leukocyte counts, but leukocyte subsets were not evaluated (16).

Valproate is a histone deacetylase (HDAC) inhibitor, which may have a role in its effects on leukocyte subsets. In human hematopoietic stem cells, lymphoid and more limitedly myeloid-related genes are associated with acetylated histones H3 and H4 (17). Histones, crucial in nucleosome formation, have been recognized as essential components of epigenetic regulation in recent years. Histone modifications influence gene expression patterns by either facilitating or hindering access to transcription factors or regulatory proteins, leading to the genetic activation or silencing of genes (14,18). VPA inhibits HDACs classified as class Ia, Ib, and IIa, leading to increased acetylation of histones H2, H3, and H4. This modification alters gene expression associated with apoptosis, the cell cycle, differentiation, and the protection against tumor cells (14). Reports have indicated that HDAC inhibitors influence the regulation of normal hematopoiesis, with VPA showing effectiveness in cell fate determination, particularly in myeloid development. VPA inhibits the differentiation of myeloid cells towards the granulocyte/macrophage series and blocks neutrophil differentiation at increasing doses (19). In bone marrow mesenchymal stromal cells, VPA increases the secretion of several trophic factors, the ability to prevent oxidative damage, and migration capacity (20). The effects of VPA treatment on lymphocyte development are poorly understood; however in vitro and in vivo studies have shown that VPA and other HDAC inhibitors increase the number and function of regulatory T cells (6,21).

This study showed that CBZ did not significantly affect leukocyte counts or subset distribution. However, the strong inverse relationship between serum CBZ levels and neutrophil count, percentage, and NLR implies that CBZ may suppress neutrophil activation and NLR in a dose-dependent manner. In another study comparing patients taking CBZ and those taking folic acid in addition to CBZ, the results showed that, one year later, the leukocyte and neutrophil counts of the CBZ-only group were significantly lower (22). In a study involving ten epileptic patients, a decrease in leukocyte count was observed at one month following CBZ treatment, but T and B lymphocyte percentages remained unaffected (10). In a pediatric study, CBZ monotherapy reduced the number of lymphocytes, compared to the control group (23). The inconsistent results in these studies are likely due to variations in control groups, age group inclusion, and the number of patients included. The mechanism of action of CBZ on neutrophils is not clear; however, an invitro study showed that CBZ inhibited neutrophil chemotaxis via peripheral benzodiazepine receptors (pBZrs) in a dose-dependent manner and increased pBZrs expression in neutrophils, which could potentially affect neutrophil count (24). CBZ-associated neutropenia may be caused by its dose-dependent inhibition of granulopoiesis (25). Another proposed mechanism for the hematological effects of CBZ is that it is metabolized by the myeloperoxidase enzyme contained by neutrophil precursors in the bone marrow. This leads to the formation of intermediate metabolites that cause covalent binding to neutrophils and may also lead to bone marrow-related side effects (26).

In this study, VPA and CBZ did not cause pathological alterations in the quantity of leukocytes and their subsets. According to some studies, children using VPA may have leukopenia as high as with a rate of 12.5% (27). Mild and transient neutropenia with VPA occurs only in isolated pediatric cases, usually during the first few weeks of treatment, and regresses within a few days when the drug is discontinued (5). VPA-related hematological changes may occur immediately after starting the drug therapy or after prolonged use but are most frequently seen when the serum drug level exceeds 100 µg/mL (6,28). Transient leukopenia occurs in children receiving CBZ, but significant leukopenia and neutropenia are rare, reversible, and asymptomatic. Benign leukopenia is seen in 10–12% of adults and children using CBZ and is not thought to be related to aplastic anemia (29). Leukopenia was the most common abnormality in a study involving 200 children with epilepsy receiving CBZ. However, it resolved without discontinuing CBZ treatment, and only four patients experienced persistent or recurrent leukopenia (30). Although there have been reports of CBZ-related lymphopenia (31,32), agranulocytosis is uncommon (4).

One limitation of the study is its retrospective design. However, evaluating the temporal effects of VPA and CBZ is a strengthening factor. Another limitation is that, although examinations during the infection period were excluded, the retrospective review may not have completely excluded them. Therefore, additional comparisons of drug groups aimed to eliminate this problem and supported the effects of VPA. The study's limitations include a lack of control for confounding variables like seizure types and recurrences. A recent seizure before the blood test may impact NLR levels. A study found that NLR levels in the first 24 hours after a seizure in epilepsy patients were higher than those measured 5-14 days later, and these levels were associated with seizure severity and recurrence (33). Therefore, lower NLR levels may also result from the seizure control that ASMs provide. This issue is partially resolved by the study's comparison of the VPA and CBZ groups. Another limitation of the study is its single-center design, limiting the generalizability of study results. However,

a multicenter study may also have disadvantages, such as the inability to standardize laboratory data in a retrospective study.

CONCLUSION

In conclusion, this study found that VPA caused changes in leukocyte subsets and decreased NLR, suggesting an anti-inflammatory effect regardless of serum VPA levels. The study's findings provide evidence that, depending on serum drug levels, CBZ may affect NLR similarly. In epilepsy cases accompanied by inflammation, it may be beneficial to consider these features in ASM selection.

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OPEN ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

The Dose-Dependent Effects of Duloxetine on the Mechanical Muscle Activities of Rat Diaphragms

Duloksetinin Sıçan Diyafram Kası Mekanik Aktiviteleri Üzerine Doz Bağımlı Etkileri

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ÖZET

Amaç: Duloksetin çok geniş bir hastalık yelpazesinde hastalara reçete edilen bir etken maddedir. Duloksetin içeren ilaçların prospektüsünde farklı kas dokuları üzerindeki birçok yan etkisinden bahsediliyor olsa da diyafram kası üzerindeki muhtemel etkileri hakkında kısıtlı bilgi bulunmaktadır. Diyafram ise solunum fonksiyonları için önemli role sahip bir iskelet kasıdır. Bu çalışmada farklı dozlardaki duloksetinin in vitro diyafram kası mekanik aktiviteleri üzerindeki olası etkileri incelendi. Ayrıca diyafram kasına ait bu aktivitelerdeki etkilenimler duloksetin reçete edilen hastaların solunum fonksiyonları açısından tartışıldı.

Gereçler ve Yöntem: 16 adet 24 haftalık erişkin Wistar-Albino sıçanlar rastgele seçimle duloksetin (DLXT) ve çözücü (VHC) olarak iki gruba ayrıldı. Her iki gruba ait sıçanlardan anestezi altında diyafram kasları izole edildi ve 5mL hacimli izole organ banyosuna aktarılan kas şeritleri 2g'lik ön gerime ayarlandı. DLXT grubuna ait sıçanlardan izole edilen diyafram kaslarına kümülatif olarak 1, 10, 20, 30, ve 40 µg/mL olacak şekilde duloksetin, VHC grubuna ise aynı hacimde çözücü uygulandı. Kas preparatlarının kasılması 0.5 Hz frekansta, 1 ms süreli kare uyaranlar kullanılarak oluşturulan elektrik alan stimülasyonu ile sağlandı.

Bulgular: Kasılma eğrilerinden kasılma kuvveti ($g.mg^{-1}$), kasılma süresi (s), gevşeme süresi (s), eğri altında kalan alan ($g.mg^{-1}.s$), $+dF/dt_{max}$ ($g.mg^{-1}.s^{-1}$) ve $-dF/dt_{max}$ ($g.mg^{-1}.s^{-1}$) verileri elde edildi. DLXT grubunda gevşeme süresi, eğri altında kalan alan, $+dF/dt_{max}$ ve $-dF/dt_{max}$ parametreleri 10 µg/mL duloksetin uygulaması ile VHC grubuna kıyasla anlamlı fark oluşturdu. 30 µg/mL duloksetin uygulamasında ise benzer bir farklılık kasılma kuvveti ve kasılma süresinde gözlendi.

Sonuç: Sonuç olarak, duloksetinin bu çalışmada ortaya çıkan diyafram kası kasılma parametreleri üzerindeki etkileri, solunum fonksiyonlarını olumsuz yönde etkileyebileceğini düşündürmektedir. Bu yüzden duloksetin reçete edilen hastalarda solunum fonksiyonlarının takip edilmesi önemli olacaktır.

Anahtar Kelimeler: Antidepresanlar, diyafram, duloksetin, izometrik kasılma, solunum

ABSTRACT

Aim: Duloxetine is an active ingredient prescribed to patients for a wide range of conditions. Although the information with purchased drugs containing duloxetine mention various side effects on different muscle tissues, there is limited research available on the potential effects on the skeletal muscle of the diaphragm, which plays an important role in respiratory functions. In this study, the potential effects of different doses of duloxetine on in vitro mechanical activities of the diaphragm muscle were examined. Additionally, the impact of these activities on the diaphragm muscle was discussed in terms of the respiratory functions of patients prescribed duloxetine.

Materials and Methods: Sixteen adult Wistar-Albino rats, each 24 weeks old, were randomly divided into two groups: duloxetine(DLXT) and vehicle(VHC). Diaphragm muscles were isolated from rats in both groups under anesthesia and transferred to a 5mL volume isolated organ bath, in which muscle strips were adjusted to a 2g preload. For DLXT group, the diaphragm muscles were cumulatively treated with duloxetine at concentrations of 1, 10, 20, 30, and 40 µg/mL, while VHC group received the same volume of the vehicle. Contractions were induced using square pulses of 0.5 Hz frequency and 1 ms duration.

Results: From the contraction curves, data was obtained on contraction force($g.mg^{-1}$), contraction time(s), relaxation time(s), under area curve($g.mg^{-1}.s$), $+dF/dt_{max}$($g.mg^{-1}.s^{-1}$), and $-dF/dt_{max}$($g.mg^{-1}.s^{-1}$). In DLXT group, relaxation time, area under the curve, $+dF/dt_{max}$, and $-dF/dt_{max}$ parameters showed significant differences compared to the VHC group with the application of 10 µg/mL duloxetine. Similar difference was observed in contraction force and contraction time with the application of 30 µg/mL duloxetine.

Conclusions: The effects of duloxetine on diaphragm muscle contraction parameters observed in this study suggest that duloxetine may adversely affect respiratory functions. It is therefore important that the respiratory functions in patients prescribed duloxetine be closely monitored.

Keywords: Antidepressants, diaphragm, duloxetine, isometric contraction, respiratory

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INTRODUCTION

Duloxetine is a selective serotonin-norepinephrine reuptake inhibitor (SNRI) used to treat urinary incontinence, diabetic neuropathic pain, depression, and anxiety (1). The side effects on muscles listed in the information commercially supplied with duloxetine include muscle pain, cramps, stiffness, and trismus (2). The diaphragm, a skeletal muscle, has been the main instrument of many studies because it plays an important role in respiration. When the diaphragm contracts, the thoracic volume increases and pleural pressure decreases, thus facilitating the flow of air into the lungs while breathing (3).

Respiratory dysfunctions are often caused by respiratory conditions such as asthma and chronic obstructive pulmonary disease. However, they can also occur in patients without any known respiratory system disorders (4). Although limited information is available about the relationship between psychological conditions and respiratory symptoms, some studies have suggested that psychological symptoms may be associated with a higher risk of developing asthma (5). Additionally, psychological stress that accompanies respiratory disorders can exacerbate respiratory problems, making treatment for depression essential for healthy breathing in these individuals (6). Respiratory disorders, a metabolic disease that affects the entire body and is reported in diabetes, are considered an important comorbidity that can affect the course of Type 2 diabetes (7).

Consequently, duloxetine is currently prescribed to patients who may experience secondary respiratory problems. The aim of this study is to investigate whether duloxetine has a detrimental effect on the mechanical activities of the diaphragm muscle in rats, thus impacting respiratory functions. The study was conducted in vitro by applying different concentrations of duloxetine to an isolated diaphragm muscle in an organ bath.

MATERIALS AND METHODS

Animals and Groups

Experiments were performed upon 16 adult (24 weeks old) Wistar-Albino rats with body weights ranging from 250 to 320 grams. The rats, who had unrestricted access to food and water, were housed in conditions which were exposed to 12 hours of light and then 12 hours of darkness. The subjects were randomly divided into two groups, one being labeled as the vehicle (VHC; n=8), and the other as the duloxetine (DLXT; n=8) group. Diaphragm muscles from both groups were isolated, transferred to an organ bath, and the effects of duloxetine on contraction parameters were examined. Duloxetine was dissolved in a vehicle consisting of 10% ethanol (32221, Honeywell International Inc., Germany) and 90% saline (23414134, OSEL Drug A.Ş., Türkiye). The procedures employed in this study were approved on February 08, 2024 (Approval number 2024-07) by the Local Ethics Committee for Animal Experiments at Necmettin Erbakan University, Experimental Medical Application and Research Center.

Isolation of Diaphragm Muscle and Transfer to the Organ Bath

Since isolated fresh diaphragm muscle was required in the study, rats were dissected by cervical dislocation under anesthesia (80 mg/kg ketamine and 10 mg/kg xylazine). The diaphragm muscle was accessed through thoracotomy and the preparation was isolated. The excised diaphragm tissue was placed in a modified Krebs solution (in mM: 15 $NaHCO_3$, 5 KCl, 1 $MgCl_2$, 135 NaCl, 2 $CaCl_2$, 1 Na_2HPO_4, 11 glucose at pH 7.4, gassed with a mixture of 95% O_2 and 5% CO_2), and muscle strips of dimensions 20x5 mm were obtained (8). The muscle strips were tied together with 4-0 silk thread and placed in a 5 ml isolated organ bath. The costal side of the strips was attached to a force transducer (MAY FDT 05, Commat Ltd., Ankara, Türkiye) and the other side was connected to a micromanipulator. The muscle preparations, adjusted to a pre-load of 2g, were subjected to a 30-minute rest period, during which the Krebs solution was refreshed every 10 minutes. During this time, the muscle was stimulated using a (BSLSTM100, BIOPAC Systems Inc., USA) custom-designed electric field electrode stimulator, which delivered 1 ms duration square pulses at a frequency of 0.5 Hz. The stimulation voltage started at 5 Volts and was gradually increased to determine the supramaximal stimulus voltage (9).

In Vitro Drug Application

The stock solution was prepared by adding the vehicle to 120 mg of duloxetine to attain a total volume of 10 mL. In the DLXT group, cumulative doses of duloxetine were applied in the isolated organ bath at concentrations of 1, 10, 20, 30, and 40 µg/mL in order to determine the potential effects of the vehicle on the results in the DLXT group. The procedures applied are schematically summarized in Figure 1.

Data Obtained from Isometric Contraction Recordings

The following parameters were determined from the contraction traces: Contraction force ($g.mg^{-1}$), contraction time (s), relaxation time (s), area under the curve ($g.mg^{-1}.s$), maximum rate of force development ($+dF/dt_{max}$; $g.mg^{-1}.s^{-1}$), and maximum rate of force decline ($-dF/dt_{max}$; $g.mg^{-1}.s^{-1}$). Contraction force, area under the curve, $+dF/dt_{max}$, and $-dF/dt_{max}$ data were normalized by dividing the weight of the muscle in mg. The data for each application in all subjects was

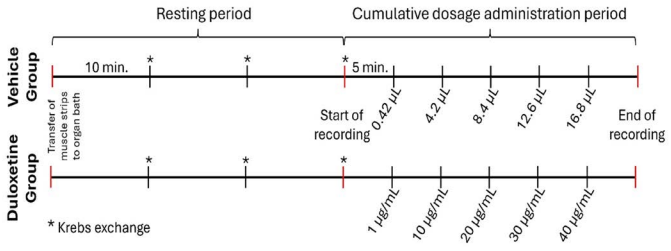

Figure 1. Procedures applied to the experimental groups.

determined by calculating the average of 10 different values (10).

Statistical Analyses

All data was presented as mean ± standard deviation (SD) and normal data distribution was tested with Kolmogorov-Smirnov. In order to analyze the effects of different doses applied to the same group, one-way ANOVA, followed by the Tukey post-hoc test was used, and a nonparametric t-test was used to determine the significance between the VHC and DLXT groups. $p<0.05$ was considered statistically significant.

RESULTS

In this study, which investigated the potential adverse effects of duloxetine prescribed to patient groups at risk of secondary respiratory problems on the mechanical activities of the diaphragm muscle, concentration-dependent results were obtained. The initial preloads set to 2g were reassessed after a rest period and were found to be 1.66±0.29g in the VHC group and 1.62±0.26g in the DLXT group. No statistically significant difference was found between the preloads of the groups ($p=0.9613$, non-parametric t-test). While no difference was found in contraction forces between the groups before the administration of duloxetine and vehicle, cumulatively applied duloxetine showed a dose-dependent inhibition effect (Figure 2).

In the VHC group, no statistically significant difference was determined in contraction force, contraction time, relaxation time, and the area under the curve data, compared to the previous measurements at different doses. In the DLXT group, a 30 µg/ml duloxetine concentration produced a statistically significant inhibitory effect on contraction force and contraction time data compared to the VHC group. This inhibitory effect was observed at a 10 µg/ml duloxetine concentration for relaxation time and the area under the curve data (Figure 3).

In the DLXT group, compared to previous measurements, the maximum value of force changes during contraction and relaxation decreased at a 10 µg/ml dose of duloxetine. When

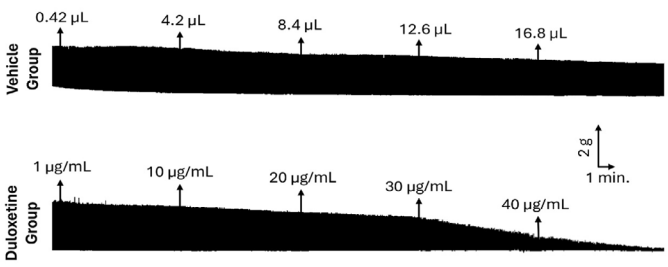

Figure 2. An example of contraction records obtained from cumulative dose administration.

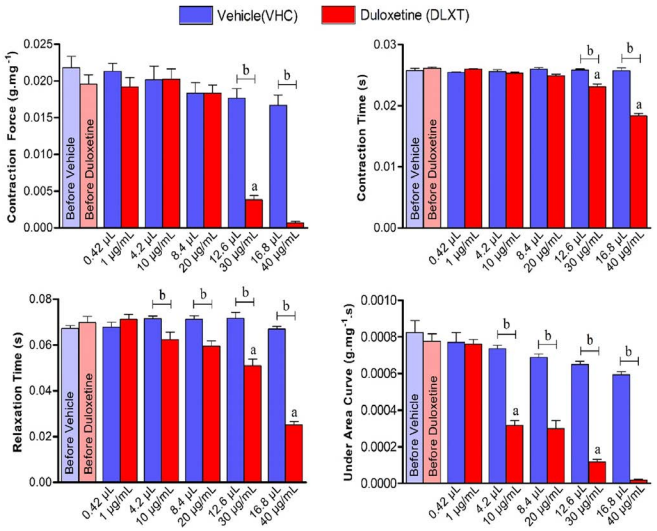

Figure 3. Data obtained from contraction curves at five different doses and control contractions before dose administration, including contraction force, contraction time, relaxation time, and area under the curve. The data is presented as mean ± standard deviation (SD). The letter 'a' indicates the significance of an ANOVA test with $p<0.05$ between consecutive measurements within the same group, while 'b', indicates the significance of a nonparametric t-test with $p<0.05$ between the two groups for measurements at the same dose.

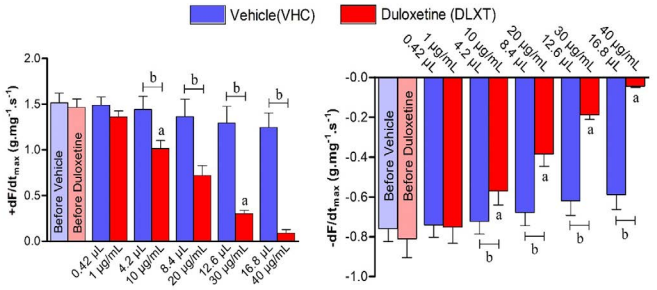

Figure 4. Data obtained from contraction curves at five different doses and control contractions before dose administration, including $+dF/dt_{max}$, $-dF/dt_{max}$. The data is presented as mean ± standard deviation (SD). The letter 'a' indicates the significance of an ANOVA test with $p<0.05$ between consecutive measurements within the same group, while 'b' indicates the significance of a nonparametric t-test with $p<0.05$ between the two groups for measurements at the same dose.

comparing the DLXT and VHC groups, the $+dF/dt_{max}$ and $-dF/dt_{max}$ values decreased at the same dose (Figure 4).

DISCUSSION

Serotonin and norepinephrine are two of the brain's primary neurotransmitters. As duloxetine, through its function as an SNRI, is an inhibitor of the reuptake of these neurotransmitters, thus increasing their availability in the brain, duloxetine is widely used in the treatment of many conditions, including anxiety, obsessive-compulsive disorder, depression, urinary incontinence, and diabetic neuropathic pain, due to its ability to regulate synaptic transmission between brain cells (11,12). While the side effects of duloxetine on muscles are frequently mentioned in the research; the effects of the drug on the diaphragm muscle are not widely elucidated. Although the use of the drug is generally considered safe with tolerable side effects, reports have indicated toxic effects and rarely reported cases of death due to overdose (13,14).

There is the potential for patients who have been prescribed duloxetine to experience respiratory difficulties as a secondary condition due to their existing illnesses. The diaphragm is a skeletal muscle that plays a crucial role in respiration by contracting to reduce pleural pressure and thus facilitate breathing (10). Therefore, duloxetine has the potential to both impair diaphragm muscle function through an exacerbation of existing respiratory problems, or to actually be a direct cause of respiratory issues.

There are a number of studies which suggest that significant side effects can occur in patients treated with different doses of duloxetine in clinical applications. While Müller et al. reported that approximately 70% of patients who were treated for depression with duloxetine experienced a reduction of symptoms, they also experienced low cardiovascular levels and sexual dysfunction (15). Polychroniou et al. stated that about 50% of patients discontinued the medication due to side effects (16). Thase et al. found a significant decrease in PR and QRS intervals in the electrocardiogram data of patients receiving 120 mg/day duloxetine (17). In a case report by Eyal and Yaeger, attention was drawn to a newborn mother who had been taking 90 mg/day of duloxetine, and who exhibited weak crying, low muscle tone, respiratory distress, and a low Apgar score at birth (18). Finsterer and Habitzl describe a patient who used duloxetine and aripiprazole and experienced progressive generalized weakness, myalgia, and muscle stiffness, which lead to difficulties in walking and moving (2). Sahan and Parlakkaya Yıldız published a case report indicating that duloxetine (30 mg/day) prescribed to a 45-year-old patient who complained of headaches, anxiety, and fear resulted in hyponatremia after one week of use (19). In a study conducted by Wang et al., (20) it was found that duloxetine blocks voltage-dependent Na^+ channels, and this blocking property was associated with the drug's analgesic effect.

In a rat's diaphragm muscle, an increase in Na+ permeability depolarizes the membrane, resulting in the release of Ca^{++} from the sarcoplasmic reticulum and a subsequent trigger of a series of biochemical events that lead to muscle contraction (21). Possible disturbances in Na^+ homeostasis affect action potential kinetics, which in turn impact contraction performance (22). It is believed that the mechanical activity disorders observed in our findings may be due to the adverse effects of duloxetine on ionic activities.

Since this study was conducted only on adult female rats, possible age and sex-dependent differences could not be elucidated. Additionally, as a fundamental research study, the effects of duloxetine on respiratory functions were investigated solely through the mechanical activity of the diaphragm muscle. The finding of this study should therefore be supported by future studies that examine underlying molecular and ionic mechanisms.

CONCLUSION

In conclusion, the adverse effects of duloxetine on diaphragm muscle contraction parameters revealed in this study suggest that the drug may negatively impact respiratory functions. It is therefore recommended that care should be taken to monitor the respiratory functions of patients who are prescribed duloxetine.

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OPEN ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

The Predictive Role of Neutrophil Percentage to Albumin Ratio (NPAR) and Systemic Inflammatory Markers in Methotrexate Treatment Outcomes for Ectopic Pregnancy

Metotreksat Tedavisi Almış Ektopik Gebeliklerde Nötrofil Yüzdesi Albümin Oranı (NPAR) ve Diğer Sistemik İnflamatuvar Markerların Prediktif Değeri

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ÖZET

Amaç: Bu çalışma, ektopik gebelikte (EP) metotreksat (MTX) tedavi başarısını öngörmede Nötrofil Yüzdesi Albümin Oranı (NPAR), Platelet-Lenfosit Oranı (PLR) ve Sistemik İmmün-İnflamasyon İndeksi'nin (SII) prediktif rolünü incelemeyi amaçlamaktadır. MTX tedavisinin başarısını önceden tahmin edebilecek biyobelirteçlerin tanımlanması, klinik uygulamada daha doğru ve etkin kararlar alınmasını sağlayabilir.

Gereçler ve Yöntemler: Bu retrospektif çalışma, 1 Ocak 2020 ile 1 Mayıs 2024 tarihleri arasında üçüncü basamak bir hastanede MTX ile tedavi edilen 166 ektopik gebelik hastasını kapsamaktadır. Hastalar tedavi sonuçlarına göre iki gruba ayrılmıştır: MTX tedavisiyle başarıya ulaşanlar ve ek dozlar veya cerrahi müdahale gerektiren tedavi başarısızlığı yaşayanlar. Çalışmada, hastaların başlangıç kan parametrelerinden NPAR, PLR ve SII değerleri hesaplanmış ve bu markerların tedavi sonuçlarını öngörme potansiyeli değerlendirilmiştir. İstatistiksel analizler, lojistik regresyon ve ROC eğrisi analizlerini içermektedir. Optimal eşik değerler, klinik karar verme süreçlerini desteklemek için belirlenmiştir.

Bulgular: NPAR ve PLR değerleri, MTX tedavi sonuçlarının anlamlı prediktörleri olarak bulunmuş ve yüksek değerlerin MTX başarısızlığı ile ilişkili olduğu gösterilmiştir ($p < 0.05$). ROC analizi, NPAR ve PLR'nin sırasıyla 0.777 (95% CI: 0.690-0.864) ve 0.659 (95% CI: 0.548-0.770) AUC değerlerine sahip olduğunu göstermiştir. Buna karşın, SII değerleri ile MTX tedavi başarısı arasında anlamlı bir ilişki bulunamamıştır. NPAR ve PLR'nin yüksekliği, tedavi başarısızlığı ile güçlü bir şekilde ilişkilendirilmiştir.

Sonuç: NPAR ve PLR, ektopik gebelikte MTX tedavisinin başarısını öngörmede etkili biyobelirteçler olarak öne çıkmaktadır. Bu markerlar, risk taşıyan hastaların erken dönemde belirlenmesine olanak sağlayarak, daha kişiselleştirilmiş ve etkili bir tedavi yönetimi sunabilir. Ancak, bu bulguların farklı popülasyonlarda daha geniş kapsamlı çalışmalarda doğrulanması gerekmektedir. Ayrıca, ek biyobelirteçlerin değerlendirilmesi ve bu markerların klinik algoritmalara entegrasyonu tedavi süreçlerini daha da iyileştirebilir.

Anahtar Kelimeler: Ektopik gebelik, metotreksat tedavisi, nötrofil yüzdesi albümin oranı (NPAR), platelet-lenfosit oranı (PLR), sistemik inflamatuvar markerlar

ABSTRACT

Objective: This study investigates the predictive role of the Neutrophil Percentage to Albumin Ratio (NPAR), Platelet-to-Lymphocyte Ratio (PLR), and Systemic Immune-Inflammation Index (SII) in determining methotrexate (MTX) treatment outcomes in patients with ectopic pregnancy (EP).

Materials and Methods: We conducted a retrospective analysis of 166 patients with ectopic pregnancy treated with MTX at a tertiary hospital between January 1, 2020, and May 1, 2024. Patients were categorized into two groups: those achieving successful MTX treatment and those experiencing treatment failure, necessitating additional MTX doses or surgical intervention. NPAR, PLR, and SII values were calculated from baseline blood parameters and analyzed to assess their predictive value. Statistical analyses included logistic regression and ROC curve analysis to determine optimal cutoff values.

Results: NPAR and PLR were significant predictors of MTX treatment outcomes, with higher values correlating with increased likelihood of MTX failure ($p < 0.05$). In contrast, SII did not show a significant association with treatment outcomes. ROC analysis showed that NPAR and PLR had satisfactory predictive performance, with AUC values of 0.777 (95% CI: 0.690-0.864) and 0.659 AUC (95% CI: 0.548-0.770), respectively. Optimal cutoff values for NPAR and PLR were determined to guide clinical decision-making.

Conclusion: This study identified NPAR and PLR as valuable markers for predicting MTX treatment outcomes in ectopic pregnancy, whereas SII was not predictive in this context. NPAR and PLR may aid in early identification of patients at risk for MTX treatment failure, enabling more personalized and effective management of ectopic pregnancy. Further research is needed to validate these findings in larger, prospective studies.

Keywords: Ectopic pregnancy, methotrexate treatment, neutrophil percentage to albumin ratio (NPAR), platelet-to-lymphocyte ratio (PLR), systemic Inflammatory markers

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INTRODUCTION

Ectopic pregnancy (EP), defined as the implantation of a fertilized ovum outside the uterine cavity, remains a significant cause of morbidity and, in severe cases, mortality in reproductive-aged women. Accounting for approximately 1-2% of all pregnancies, ectopic pregnancies often require timely intervention to prevent life-threatening complications, such as tubal rupture and hemorrhage (1). Methotrexate (MTX) therapy, a non-surgical treatment option that selectively targets trophoblastic tissue, has become an established approach for managing unruptured ectopic pregnancies in clinically stable patients. By inhibiting DNA synthesis, MTX effectively halts the proliferation of trophoblasts, allowing the EP to resolve without the need for invasive surgery (2,3).

Despite the clinical efficacy of MTX in many cases, a subset of patients does not respond adequately to this therapy, requiring additional MTX doses or eventual surgical intervention. Predicting MTX treatment outcomes early in the management process could improve clinical decision-making, optimize treatment strategies, and potentially reduce the need for repeat interventions. Recently, systemic inflammatory markers have emerged as potential predictors of various clinical outcomes, including treatment response in ectopic pregnancy. These markers are accessible, cost-effective, and easily derived from standard blood tests, making them appealing candidates for predictive analysis (4,5).

The Neutrophil Percentage to Albumin Ratio (NPAR), although unstudied in the context of ectopic pregnancy, has shown promise in prognosticating other medical conditions. Elevated NPAR levels are often indicative of systemic inflammation and immune activation, factors that could impact MTX treatment efficacy. In addition to NPAR, other indices such as the Systemic Inflammatory Index (SII), Neutrophil-to-Lymphocyte Ratio (NLR), and Platelet-to-Lymphocyte Ratio (PLR) have been linked to clinical outcomes in other systemic diseases (6,7). However, no previous studies have explored the predictive role of NPAR specifically in EP.

This study is the first to evaluate the potential of NPAR as a predictive marker for MTX treatment outcomes in EP. We hypothesize that higher baseline levels of NPAR, as well as elevated SII, NLR, and PLR, may correlate with an increased likelihood of MTX treatment failure, necessitating either repeat MTX doses or surgical intervention.

MATERIALS AND METHODS

Study Design and Population

This retrospective study was conducted in a tertiary care hospital between 1 January 2020 and 1 May 2024 and included 166 patients with EP receiving MTX treatment. Ectopic pregnancy was confirmed by a combination of transvaginal ultrasound findings and serum beta-hCG levels, interpreted by experienced obstetricians. Patients were categorised according to their response to MTX treatment as follows:

1. Successful MTX treatment: 136 patients who achieved treatment success with MTX without further intervention. (A beta hCG drop of at least 15% between day 4 and day 7 of treatment was considered successful MTX treatment) (8).

2. Unsuccessful MTX treatment: 30 patients requiring additional MTX doses or surgical intervention due to treatment failure. (A beta hCG decline of less than 15% between day 4 and day 7 of treatment was considered failed MTX therapy)

Inclusion and Exclusion Criteria

Patients were included if they met the following criteria: Confirmed diagnosis of EP (no intrauterine GS and GS in the right or left tuba uterina, with or without embryo), all patients had an ectopic pregnancy diagnosed by an obstetrician and gynaecologist with more than 10 years of experience using transvaginal ultrasound. Haemodynamic stability, eligibility for MTX therapy according to clinical guidelines (8) and complete medical and laboratory records. Exclusion criteria included cases of ruptured EP, first surgical treatment, concomitant inflammatory conditions or infections that may confound inflammatory marker levels.

Data Collection

Data collected from electronic medical records included patient age, clinical parameters and laboratory results. Systemic inflammatory indices were calculated using baseline and MTX treatment day laboratory values:

- NPAR: Percent neutrophils to albumin ratio.
- NLR: Neutrophil/lymphocyte ratio.
- PLR: Platelet-to-lymphocyte ratio.
- SII: Systemic Immune-Inflammation Index calculated as (Neutrophils × Platelet /Lymphocyte count
- SIRI: Systemic Inflammation Response Index (Neutrophils × monocytes/lymphocytes)
- MLR: Monocyte/lymphocyte ratio.
- Aggregate Index: Neutrophils × platelets × monocytes/lymphocytes).

Outcome Measures

The primary outcome was MTX treatment success or failure. Success was defined as complete resolution of the EP with MTX treatment alone, whereas failure was defined as the need for additional doses of MTX or surgical intervention.

Ethical approval was obtained from the Ethics Committee of the local Institutional Ethics Committee and the study adhered to the principles outlined in the Declaration of Helsinki.

Statistical analysis

SPSS 26 was used for statistical analysis. Normality of data distribution was assessed using Kolmogorov-Smirnov, Shapiro-Wilk tests and histograms. Independent t-test was used to compare between independent groups for normally distributed data, and results were presented as mean ± standard deviation (mean ± SD). The Mann-Whitney U test was used for non-normally distributed data, and results are presented as median (minimum-maximum). Logistic regression analysis was performed to determine the independent risk factors for predicting MTX treatment failure. As a result of the analysis, NPAR and PLR were found to be independent risk factors. ROC analysis was performed to evaluate the diagnostic performance of NPAR and PLR in predicting MTX treatment failure. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under the curve

(AUC) values were reported with 95% confidence intervals. Cut-off values for NPAR and PLR were determined according to Youden index. A two-sided p-value of 0.05 was considered statistically significant.

RESULTS

A total of 166 patients were included in this study, including 136 patients in the successful treatment group and 30 patients in the unsuccessful treatment group. When clinical and laboratory parameters were compared, age, hCG, haemoglobin, Monocyte, Lymphocyte, Platelet, Immature Granulocyte at admission did not differ significantly between the groups (p>0.05). However, MTX day hCG levels (1887.65 ± 1636.15 vs. 2640.47 ± 1864.65, p=0.028), WBC count (9.18 ± 2.57 vs. 10.30 ± 2.27, p=0.029) and neutrophil levels (6.20 ± 2.09 vs. 7.32 ± 1.72, p=0.006) were significantly higher in the failed group. Albumin levels were lower in the failed group (45.00 (26.4-51.0) vs. 43.50 (24.0-51.0), p=0.025) (Table 1).

In Table 2, inflammatory and immune response indices were significantly elevated in the failed group. NPAR (1.47

Table 1. Comparison of Clinical and Laboratory Parameters According to the Success of Methotrexate Treatment in Ectopic Pregnancy

Variable	Successful Group (n=136)	Failed Group (n=30)	p-value
Age	31.79 ± 6.08	31.96 ± 7.33	0.893[α]
Admission hCG	1810.56 ± 1620.71	1948.23 ± 1461.56	0.669[α]
Mtx day hCG	1887.65 ± 1636.15	2640.47 ± 1864.65	0.028[α]
WBC	9.18 ± 2.57	10.30 ± 2.27	0.029[α]
Hemoglobin	12.85 (9.0-15.0)	12.70 (9.0-15.1)	0.515[β]
Neutrophil	6.20 ± 2.09	7.32 ± 1.72	0.006[α]
Monocyte	0.64 ± 0.59	0.62 ± 0.22	0.894[α]
Lymphocyte	2.88 ± 2.06	2.25 ± 0.77	0.103[α]
Platelet	259.97 ± 80.00	287.84 ± 80.55	0.086[α]
IG	0.30 (0.1-0.8)	0.30 (0.1-0.8)	0.740[β]
Albumin	45.00 (26.4-51.0)	43.50 (24.0-51.0)	0.025[β]

WBC: White Blood Cells, IG: Immature Granulocyte α:independet t test (Mean ± SD), β:Mann Whitney U test (Median(Min-Max)

Table 2. Comparison of Inflammatory and Immune Response Indices Between Successful and Failed Methotrexate Treatment in Ectopic Pregnancy

Variable	Successful Group (n=136)	Failed Group (n=30)	p-value
NPAR	1.47 (1.00-1.93)	1.68 (1.29-3.87)	0.001
NLR	2.33 (0.99-4.65)	3.13 (2.25-9.51)	0.001
PLR	108.52 (11.64-297.58)	138.64 (67.60-279.33)	0.007
MLR	0.25 (0.04-0.86)	0.29 (0.09-0.86)	0.030
SII	573.04 (98.49-3827.34)	979.88 (254.73-3827.34)	0.001
SIRI	1.20 (0.40-9.68)	2.30 (0.53-7.47)	0.001
Agregate index	358.45 (90.69-2440.82)	601.50 (146.64-1640.86)	0.001

All comparisons were conducted using the Mann-Whitney U test. NPAR: Neutrophil % -to- Albumin Ratio, NLR: Neutrophil-to-Lymphocyte Ratio, PLR: Platelet-to-Lymphocyte Ratio, MLR: Monocyte-to-Lymphocyte Ratio, SII: Systemic Immune-Inflammation Index, SIRI: Systemic Inflammation Response Index

Table 3. Binary Logistic Regression Analysis to Predict Failure of Methotrexate Treatment in Ectopic Pregnancy

Predictor	Estimate	SE	Z	p-value	Odds Ratio	95% CI Lower	95% CI Upper
Intercept	-11.43	2.65	-4.31	0.001			
NPAR	3.4	1.14	2.99	0.003	30.02	3.23	279.07
NLR	0.62	0.39	1.56	0.118	1.85	0.85	4.01
PLR	0.02	0.01	2.08	0.038	1.01	1.00	1.03
MLR	-0.44	6.38	-0.07	0.946	0.65	2.38	175376.42
SII	-0.0	0.0	-0.07	0.945	1.00	1.00	1.00
SIRI	0.86	0.79	1.09	0.276	2.36	0.50	11.13
Agregate index	-0.0	0.0	-0.93	0.354	1.00	0.99	1.00
IG	-1.3	1.94	-0.67	0.504	0.27	0.01	12.31
Admission hCG	-0.0	0.0	-1.03	0.304	1.00	1.00	1.00
Mtx day hCG	0.0	0.0	1.9	0.058	1.00	1.00	1.02

IG: Immature Granulocyte, NPAR: Neutrophil % -to- Albumin Ratio, NLR: Neutrophil-to-Lymphocyte Ratio, PLR: Platelet-to-Lymphocyte Ratio, MLR: Monocyte-to-Lymphocyte Ratio, SII: Systemic Immune-Inflammation Index, SIRI: Systemic Inflammation Response Index

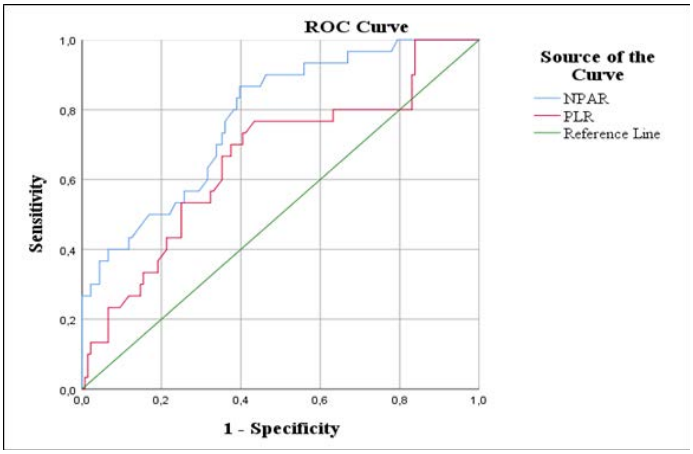

Figure 1. ROC Curve Analysis for NPAR and PLR in Predicting Methotrexate Treatment Failure in Ectopic Pregnancy

(1.00-1.93) vs. 1.68 (1.29-3.87), p=0.001), NLR (2.33 (0.99-4.65) vs. 3.13 (2.25-9.51), p=0.001), PLR (108.52 (11.64-297.58) vs. 138.64 (67.60-279.33), p=0.007), MLR (0.25 (0.04-0.86) vs. 0.29 (0.09-0. 86), p=0.030), SII (573.04 (98.49-3827.34) vs. 979.88 (254.73-3827.34), p=0.001), SIRI (1.20 (0.40-9.68) vs. 2.30 (0.53-7.47), p=0.001) and aggregate index (358.45 (90.69-2440.82) vs. 601.50 (146.64-1640.86), p=0.001).

There were independent risk factors identified by logistic regression analysis to predict failed MTX treatment, including NPAR with an odds ratio of 30.02 (p=0.003) and PLR with an odds ratio of 1.01 (p=0.038) (Table 3).

It demonstrates the diagnostic performance of NPAR and PLR in predicting MTX treatment failure. With a cut-off point of 1.5, NPAR achieved a sensitivity of 86.67%, specificity of 60.29% and AUC of 0.777 (95% CI: 0.690-0.864). With a cut-off point of 120.17, PLR achieved 76.67% sensitivity, 56.62% specificity and 0.659 AUC (95% CI: 0.548-0.770) (Table 4 & Figure 1).

Table 4. Diagnostic Performance of NPAR and PLR in Predicting Methotrexate Treatment Failure in Ectopic Pregnancy

Variables	Cutpoint	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (95 % CI)
NPAR	1.5	86.67	60.29	32.5	95.35	0.777 (0.690-0.864)
PLR	120.17	76.67	56.62	28.05	91.67	0.659 (0.548-0.770)

NPAR: Neutrophil % -to- Albumin Ratio, PLR: Platelet-to-Lymphocyte Ratio

DISCUSSION

This study demonstrates the significance of the NPAR and PLR as independent predictors of MTX treatment failure in EP. Both NPAR and PLR were significantly associated with treatment outcomes, while the Systemic Immune-Inflammation Index (SII) did not show a meaningful association in predicting MTX success. This finding refines our understanding of inflammatory markers in EP management, highlighting the value of NPAR and PLR over broader indices like SII.

The role of inflammatory markers in MTX outcomes aligns with findings by Dereli et al., who reported that higher SII and NLR values correlated with MTX treatment failure, likely due to increased inflammation and trophoblastic invasion (5). Although our study found SII to be less predictive, the importance of NLR and similar markers in EP prognosis is underscored by our significant findings for NPAR and PLR. This suggests that while broader indices like SII may capture general inflammatory status, specific ratios such as NPAR may be more sensitive to the nuances of EP pathophysiology.

Similarly, Dinc and Issın evaluated SII in predicting tubal rupture in EP and found a correlation between high SII levels and severe trophoblastic invasion (9). In contrast, our study found no significant link between SII and MTX treatment failure, suggesting that the role of SII may vary depending on the endpoint studied-rupture risk versus MTX responsiveness. In this context, our findings reinforce the specificity of NPAR and PLR as markers that directly inform MTX success rather than rupture risk, supporting the idea that these markers may reflect distinct inflammatory pathways.

Research by Reis et al. further supports the predictive utility of NLR and PLR in EP cases, where elevated levels were associated with increased rupture risk (10). Although their focus was on rupture, the association of PLR with treatment failure in our study is consistent with their findings, emphasizing PLR's role in identifying more complex or treatment-resistant EP cases. This aligns with the observed odds ratio of 1.01 for PLR in our logistic regression analysis, underlining its potential as a clinically useful parameter for MTX outcome prediction.

Seyfettinoglu and Adıguzel also highlighted NLR's importance as a predictor for EP rupture, linking higher levels with greater inflammatory activity and an aggressive EP course (4). By incorporating NPAR, our study builds on these insights and provides an alternative marker with potentially higher specificity in the context of MTX treatment, offering a new avenue for enhancing patient selection and monitoring.

Sarikaya et al. emphasized SII's role in distinguishing between medical and surgical treatments in EP, correlating elevated levels with the need for surgical intervention (11). However, our study did not find a significant association between SII and MTX outcomes, suggesting that while SII might be valuable in evaluating cases progressing to surgery, NPAR and PLR could offer more precise information when

assessing MTX responsiveness specifically. This discrepancy reinforces the need to tailor marker selection to the treatment endpoint and underscores the importance of NPAR and PLR as focused predictors of MTX treatment success.

This study has several limitations. First, as a retrospective analysis, it is subject to selection bias and relies on the accuracy of recorded data. Additionally, the relatively small sample size may limit the generalizability of our findings, and larger, prospective studies are needed to validate the predictive value of NPAR and PLR in diverse populations. Finally, while we focused on specific inflammatory markers, other factors influencing MTX response in ectopic pregnancy, such as hormonal levels and genetic markers, were not assessed. Future research incorporating these factors may provide a more comprehensive understanding of treatment outcomes.

CONCLUSION

In conclusion, our study identified NPAR and PLR as potential predictors of MTX treatment failure patients with EP. These findings suggest that integrating inflammatory markers into routine assessment could offer a valuable approach for anticipating treatment outcomes and tailoring clinical management. This study bridges a critical knowledge gap by introducing NPAR, alongside established markers like PLR, as a potential biomarker for predicting MTX treatment outcomes in EP. Further studies with larger cohorts and prospective designs are essential to validate the utility of NPAR and PLR in diverse clinical settings. Future research should explore the combination of these indices with other biochemical markers to develop a more robust predictive model, with the ultimate goal of enhancing patient care in EP management.

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OPEN ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Prognostic Risk Factors Affecting Survival in Patients with Metastatic Non-Small Cell Lung Cancer Receiving Nivolumab As Second-Line Therapy

Nivolumab Kullanan Metastatik Küçük Hücreli Dışı Akciğer Kanserli Hastalarda Sağkalımı Öngören Prognostik Risk Faktörleri

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ÖZET

Amaç: Küçük hücreli dışı akciğer kanseri (KHDAK), sık görülen ve ölümcül seyreden bir kanser türüdür. Bu çalışmanın amacı, platin bazlı tedavi sonrası ikinci basamak tedavi olarak nivolumab kullanılan metastatik KHDAK tanılı hastalarda, klinik, laboratuvar ve sistemik inflamatuar yanıt verilerinin hastalıksız sağkalım (PFS) ve genel sağkalım (OS) üzerindeki etkilerini incelemektir.

Gereçler ve Yöntemler: Çalışma retrospektif ve tek merkezli olarak yürütüldü. İkinci basamak tedavi olarak nivolumab kullanan 84 metastatik KHDAK tanılı hastaların demografik verileri, kanser tanısıyla ilişkili özellikleri ve nivolumab başlanmadan hemen öncesine ait hastaların laboratuvar parametreleri kaydedildi. C-reaktif protein/albumin oranı (CAR), aspartat aminotransaminaz/alanin aminotransaminaz (De Ritis) oranı, Glasgow prognostik skoru (GPS), nötrofil/lenfosit oranı (NLR), platelet/lenfosit oranı (PLR), prognostik nütrisyonel indeks (PNI) ve sistemik immün-inflamasyon indeksi (SII) parametreleri her hasta için ayrı ayrı hesaplandı.

Bulgular: Tüm katılımcılar için ortalama yaş 62.08±8.43 yıl idi. Medyan PFS ve OS süreleri sırasıyla 6.4 ve 13 ay olarak belirlendi. PFS açısından yapılan tek değişkenli risk analizinde, Doğu Kooperatif Onkoloji Grubu Performans Durumu (ECOG PS) (p=0.05) ve CAR (p=0.046) PFS açısından önemli birer prognostik risk faktörü olarak belirlendi. Ancak, çok değişkenli analiz bu iki parametrenin PFS ile ilişkisini desteklemedi. OS açısından yapılan analizde ise, tek değişkenli analizde ECOG PS (p=0.01), kemik metastazı (p=0.047), CAR (p=0.019) ve De Ritis oranı (p=0.051) prognostik risk faktörleri olarak belirlendi. Çok değişkenli analizde ise, ECOG PS (HR=0.45, %95 GA 0.22-0.94, P=0.035), kemik metastazı (HR=0.38, %95 GA 0.19-0.78, P=0.008) ve De Ritis oranı (HR=0.47, %95 GA 0.23-0.96, P=0.037) bağımsız risk faktörleri olarak tespit edildi.

Sonuç: Bu çalışmada, ikinci basamak tedavi olarak nivolumab kullanan metastatik KHDAK tanılı hastalarda, kötü ECOG performans durumu, kemik metastazı ve yüksek De Ritis oranı daha kısa OS süresiyle ilişkilendirilen birer bağımsız prognostik faktör olarak belirlendi. Bu faktörler, klinik pratikte hastaların prognozlarını değerlendirmede yardımcı olabilir.

Anahtar Kelimeler: Akciğer kanseri, nivolumab, prognostik faktörler, CAR, De ritis oranı

ABSTRACT

Aim: Non-small cell lung cancer (NSCLC) is a commonly occurring and potentially fatal type of cancer. The aim of this study is to investigate the effects of clinical, laboratory, and systemic inflammatory response data on progression-free survival (PFS) and overall survival (OS) in metastatic NSCLC patients who were treated with nivolumab as a second-line treatment following platinum-based therapy.

Materials and Methods: The sample of this retrospective single-center study consisted of 84 adult patients with metastatic NSCLC receiving nivolumab as second-line treatment. Demographic data, cancer diagnosis-related characteristics and laboratory parameters of the patients just before nivolumab was started were recorded. The C-reactive protein/albumin ratio (CAR), aspartate aminotransferase/alanine aminotransferase (De Ritis) ratio, Glasgow prognostic score (GPS), neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), prognostic nutritional index (PNI), and systemic immune-inflammation index (SII) parameters were calculated for each patient.

Results: The mean age for all participants was 62.08 ± 8.43 years. The median PFS and OS were 6.4 and 13 months, respectively. In the univariate risk analysis for PFS, Eastern Cooperative Oncology Group Performance Status (ECOG PS) (p=0.05), and CAR (p=0.046) were identified as significant prognostic risk factors for PFS. However, the multivariate analysis did not confirm these two parameters as prognostic factors for PFS. In the multivariate analysis, ECOG PS (HR=0.45, 95% CI 0.22–0.94, p=0.035), bone metastasis (HR=0.38, 95% CI 0.19–0.78, p=0.008), and the De Ritis ratio (HR=0.47, 95% CI 0.23–0.96, p=0.037) remained independent prognostic risk factors of OS.

Conclusion: In this study, in patients with metastatic NSCLC receiving nivolumab as second-line treatment, poor ECOG performance status, bone metastasis, and a high De Ritis ratio were identified as independent prognostic factors associated with shorter overall survival OS. These factors may help in evaluating patients' prognosis in clinical practice.

Keywords: Lung cancer, nivolumab, prognostic factors, CAR, Deritis ratio

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INTRODUCTION

Lung cancer is an important cause of morbidity and mortality worldwide and is the second most common cancer in both men and women (1). According to the Surveillance, Epidemiology, and End Results (SEER), an estimated 238,340 new lung cancer cases will occur in the USA in 2023 and 127,070 people will die from this disease (2). Non-small cell lung cancer (NSCLC), which includes lung adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, accounts for 80~85% of all primary lung cancer (3). In metastatic or inoperable patients, treatment is based on targeted agents or immune checkpoint inhibitors (ICIs) in combination with cytotoxic chemotherapy (4,5).

Nivolumab is a human recombinant monoclonal IgG4 antibody targeting programmed cell death protein-1 (PD-1), a key checkpoint molecule in T-cell regulation. Inhibition of PD-1 receptors on the surface of activated T cells by nivolumab increases T cell activation and proliferation, continuing T cell-mediated cytotoxic reactivity against cancer cells (6,7).

As second-line treatment, nivolumab monotherapy had better response rates and overall survival than docetaxel chemotherapy in patients with metastatic lung cancer with both squamous and non-squamous histology in randomized phase 3 clinical trials (8,9). Nivolumab efficacy was correlated with the tumor PD-1 level in patients with non-squamous lung cancer (8-10). Although some patients with lung cancer respond to ICI treatment, others develop resistance to treatment from the outset or via mechanisms acquired during treatment (11). Therefore, this study investigated the factors affecting progression-free (PFS) and overall (OS) survival in patients with metastatic NSCLC given nivolumab as second-line treatment after platinum-based therapy.

MATERIALS AND METHODS

Informed consent

The protocol for sample collection was approved by our Hospital Ethics Committee and was carried out according to the requirements of the Declaration of Helsinki.

Study population

This retrospective single-center study enrolled patients with de novo or recurrent metastatic NSCLC admitted to the Medical Oncology outpatient clinic between November 2021 and June 2023. All patients received platinum-based chemotherapy as first-line treatment, and all patients were started on nivolumab as second-line treatment. Patients were followed until disease progression or death while receiving nivolumab. The patients' demographic data at the time of diagnosis and characteristics related to cancer diagnosis (presence of metastasis, metastasis localization, palliative radiotherapy, chemotherapy regimen, and bisphosphonate use) were recorded.

Evaluated parameters

The patients' laboratory parameters just before nivolumab was started were recorded. The dates on which nivolumab was started, when progression under nivolumab occurred, and death or last follow-up were recorded. The C-reactive protein/albumin ratio (CAR), aspartate transaminase/alanine transaminase (De Ritis) ratio, Glasgow prognostic score (GPS), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), prognostic nutritional index (PNI), and systemic immune-inflammation index (SII) were calculated for each patient. Cytopenia ratios and immune-related side effects (pneumonitis, nephritis, thyroiditis, and hypophysitis) developing during nivolumab treatment were recorded.

The selection method of these patients and inclusion criteria were given as follows:

1. having de novo or recurrent metastatic disease,
2. being over 18,
3. agreeing to receive platinum-based therapy as first line treatment,
4. agreeing to receive nivolumab as part of the informed consent process,
5. having received nivolumab treatment for at least three months,
6. not having had surgery

On the other hand, the exclusion criteria of the study were determined as follows:

1. having a history of autoimmune disease,
2. having a history of active infection,
3. using an ICI other than nivolumab,
4. having been diagnosed with a second primary cancer,
5. lack of laboratory tests,
6. Lost to follow-up,
7. having high-grade anemia (Hemoglobin<8 g/dL), thrombocytopenia (Platelets<75,000x109 /L) or neutropenia (Neutrophils<1,000x109 /L),
8. having an Eastern Cooperative Oncology Group (ECOG) performance status of 3-4,
9. having any of epidermal growth factor receptor (EGFR) mutation, or anaplastic lymphoma kinase (ALK) or receptor tyrosine kinase 1 (ROS 1) rearrangement

In the end, a total of 84 non-small cell lung cancer patients, 76 males and 8 females, were included in the sample (Figure 1).

Statistical Analysis

Statistical analyses were performed using SPSS Statistics 27 (ver. 20.2.1.15749). Categorical variables are presented as

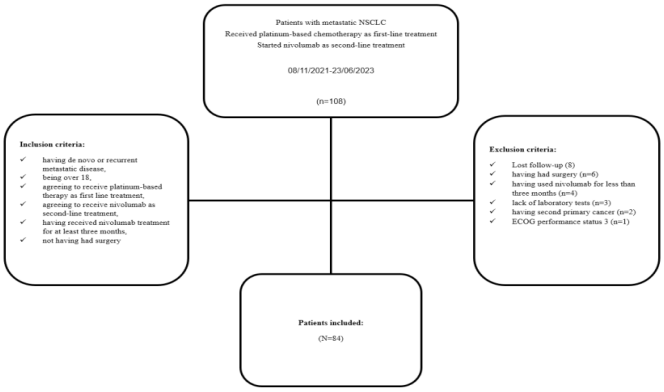

Figure 1. Patient selection flow diagram.

numbers and percentages and continuous measures as the mean and standard deviation. Median-based cutoff values for CAR, De Ritis ratio, GPS, NLR, PLR, PNI, SII, and other laboratory parameters were determined based on the median values and used to separate the 'low' and 'high' groups. Survival was analyzed using the Kaplan–Meier method and the log-rank test was used for group comparison. Univariate and multivariate analyses of factors affecting survival used Cox proportional hazards models. For multivariate analysis, the "Forward: LR" method was used. The hazard ratio (HR) was reported with the corresponding 95% confidence intervals (95% CI). The endpoint for PFS was defined as clinical or radiological disease progression after starting nivolumab, and the endpoint for OS was defined as death after starting nivolumab or the date of last follow-up. Statistical significance was accepted as $p < 0.05$.

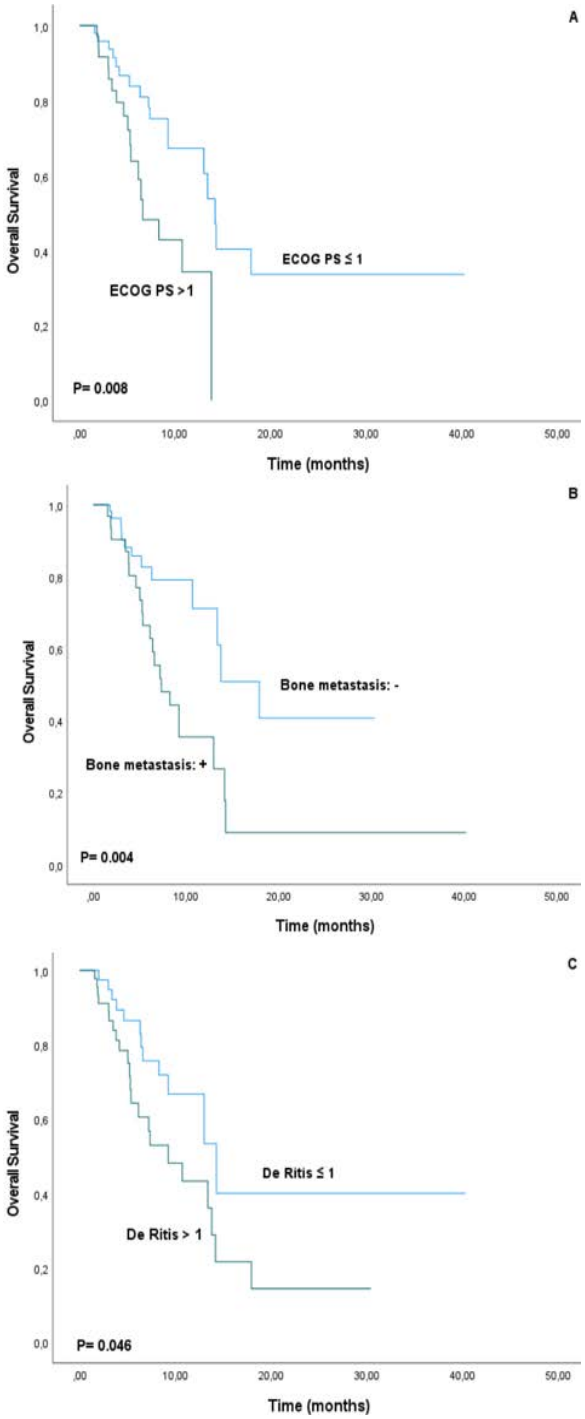

Figure 2. Kaplan Meier survival curves for Overall Survival according to ECOG PS (A), Bone metastasis (B) and De Ritis (C).

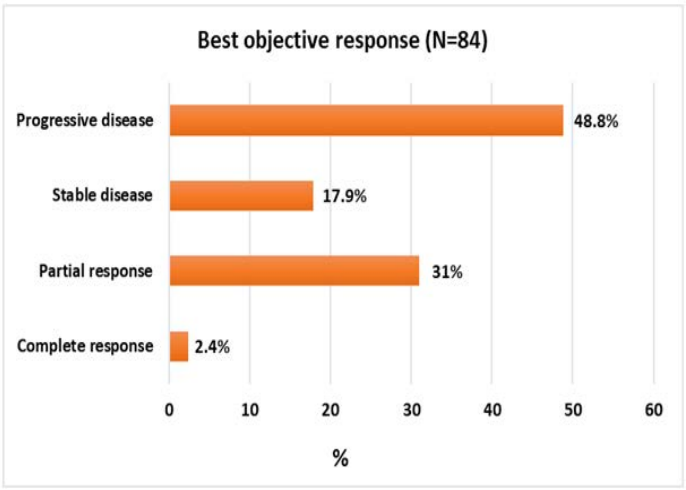

Figure 3. Best objective response.

RESULTS

Clinicopathological and laboratory parameters

The mean age of the 84 patients was 62.08 ± 8.43 years, and 90.5% of them were male. At the time of diagnosis, 57.1% of the patients had an Eastern cooperative oncology group performance status (ECOG PS) of 0 or 1. Programmed cell death ligand 1 (PD-L1) was positive in 58.3% of the patients and kirsten rat sarcoma viral oncogene (KRAS) was positive in 25%. In terms of cancer subtype, 64.3% of the patients were diagnosed with adenocarcinoma and 35.7% with squamous cell cancer. Of the patients, 30.9% had received palliative radiotherapy.

Using the median values, the optimum cutoff values were 5.73 for CAR, 1 for the De Ritis ratio, 1 for GPS, 3.74 for NLR, 223.5 for PLR, 46 for PNI, and 970.2 for SII. Table 1 gives details of the clinicopathological and laboratory parameters.

Risk Factors For Progression Free Survival

The median PFS of our patients was 6.4 months. In the univariate analysis risk assessments of the patients, age, gender, PD-L1, surgery history, metastasis status, brain metastasis, alcohol history, smoking history, BMI, bone metastasis, blood group, the De Ritis ratio, GPS, NLR, PLR, PNI, and SII were not associated with PFS. In the univariate risk analysis for PFS, ECOG PS (p=0.050), and CAR (p=0.046) were identified as

Table 1. Clinicopathological and laboratory parameters of the patients.

Clinical parameters	**N=84**	**%**
Age (years)	62.08*	8.43**
Gender (male)	76	90.5
Weight (kg)	71.60*	13.35**
Height (cm)	167.74*	18.14**
BMI (kg/m^2)	24.86*	3.96**
ECOG PS (≥2)	36	42.9
Smoker (yes)	55	65.5
Alcohol (yes)	16	19
PD-L1 (positive)	49	58.3
BRAF (positive)	0	0
KRAS (positive)	12	25
Cancer Subtypes (adenocarcinoma/squamous cell ca)	54/30	64.3/35.7
Metastasis site (CL lung/brain/liver/adrenal/bone/ENLM)	12/12/9/19/36/16	14.3/14.3/10.7/22.6/42.8/19
Blood group (0/A/B/AB//Rh+/Rh-)	32/30/13/9//75/9	38/35.7/15.5/10.7//89.3/10.7
First-line chemotherapy (yes)	84	100
Palliative radiotherapy (yes)	26	30.95
Bisphosphonate use (yes)	34	40.5
Laboratory Parameters	**Mean**	**SD**
Bun (mg/dl)	38.11	15.26
Kreatinin (mg/dl)	1.11	3.08
Sodium (MeQ/L)	138.71	2.77
Potassium (MeQ/L)	4.54	0.58
CRP (mg/dL)	34.05	38.53
Glucose (mg/dL)	115.62	44.06
Lactate dehydrogenase (U/L)	229.05	112.25
AST (IU/L)	17.74	10.21
ALT (IU/L)	21.85	26.12
Total bilirubin (mg/dL)	0.41	0.25
Total protein (g/dL)	68.06	11.95
Albumin (g/dL)	38.98	5.59
Hemoglobin (g/dL)	12.14	2.01
Platelets (109 /L)	309.23	108.55
Leukocyte (109 /L)	8.17	4.54
Neutrophil (109 /L)	5.42	3.61
Lymphocyte (109 /L)	1.43	0.87
Monocytes (109 /L)	0.7	0.41
Basophil (109 /L)	0.04	0.02
Eosinophils (109 /L)	0.13	0.13
Laboratory Indexes	**Median**	**Min-Max**
CAR	5.73	0.20-59.33
De-ritis	1.00	0.31-3.17
GPS	1.00	0.00-2.00
NLR	3.74	0.19-235.50
PLR	223.50	58.00-11250.00
PNI	46.00	23.00-66.00
SII	970.20	34.98-59223.08

*Mean** SD, Standard deviation. ALT, Alanine aminotransferase; AST, Aspartic transaminase; BRAF, V-raf murine sarcoma viral oncogene homolog B; BUN, Blood urea nitrogen; BMI, Body mass index; CAR, C-reactive protein/albumin ratio; CL, Contralateral; CRP, C-reactive protein; De Ritis, Aspartate transaminase/alanine transaminase ratio; ECOG PS, Eastern cooperative oncology group performance status; ENLM, Extranodal lymph node; GPS, Glasgow prognostic score; K, Potassium; KRAS, kirsten rat sarcoma viral oncogene; LDH, Lactate dehydrogenase; Na, Sodium; NLR, Neutrophil to lymphocyte ratio; PD-L1, Programmed cell death ligand 1; PLR, Platelet - lymphocyte ratio; PNI, Prognostic nutritional index; SD, standard deviation; SII, Systemic immune-inflammation index.

prognostic risk factors for PFS. However, the multivariate analysis did not support the univariate analysis finding that these two parameters were prognostic risk factors for PFS. Factors affecting PFS are shown in Table 2.

Risk Factors for Overall Survival

The median OS of our patients was 13 months. In the univariate risk assessment of the patients, age, gender, PD-L1, surgery history, metastasis status, brain metastasis, alcohol history, smoking history, BMI, blood group, GPS, NLR, PLR, PNI, and SII were not associated with OS. However, ECOG PS ($p = 0.010$), bone metastasis ($p = 0.047$), CAR ($p = 0.019$), and the De Ritis ratio ($p = 0.050$) were identified as prognostic risk factors of OS. In the multivariate analysis, ECOG performance status >1 (14.1 and 6.6 months, HR = 0.45, 95% CI 0.22–0.94, $p = 0.035$), the presence of bone metastases (17.9 and 7.3 months, HR = 0.38, 95% CI 0.19–0.78, $p = 0.008$), and the De Ritis ratio > 1

Table 2. Univariate and multivariate analysis of characteristic parameters and laboratory indices related to progression free survival.

PFS		Univariate Analysis		Multivariate Analysis	
Characteristics	**Category**	**HR(95% CI)**	**P**	**HR(95% CI)**	**P**
Age	<65 vs ≥65	0.95(0.54-1.67)	0.863		
Sex	Female vs male	1.04(0.47-2.30)	0.928		
ECOG PS	≤1 vs >1	1.74(1.00-3.02)	0.050	1.68(0.96-2.91)	0.067
Sub type	SCC vs AC	1.12(0.65-1.9)	0.686		
PD-L1	Negative vs positive	0.88(0.47-1.62)	0.654		
Surgery history	Negative vs positive	0.98(0.54-1.84)	0.992		
Metastasis status	Denovo vs recurrence	0.92(0.53-1.60)	0.756		
Brain metastasis	Negative vs positive	1.07(0.50-2.23)	0.867		
Alcohol history	Yes vs /no	0.64(0.31-1.30)	0.215		
Smoking history	Yes vs no	1.01(0.99-1.00)	0.898		
BMI	<25 vs ≥25	0.63(0.37-1.06)	0.083		
Bone metastasis	Negative vs positive	1.16(0.69-1.97)	0.574		
Blood group	O/A/B/AB	0.99(0.0.76-1.30)	0.950		
	RH- vs RH+	0.64(0.28-1.46)	0.290		
CAR	≤5.73 vs >5.73	0.58(0.34-0.99)	0.046	1.57(0.93-2.66)	0.093
De-ritis	≤1 vs >1	0.66(0.39-1.12)	0.124		
GPS	≤1 vs >1	1.04(0.60-1.80)	0.895		
NLR	≤3.74 vs >3.74	1.02(0.60-1.72)	0.946		
PLR	≤223.5 vs >223.5	1.09(0.65-1.84)	0.747		
PNI	≤46 vs >46	0.97(0.58-1.62)	0.895		
SII	≤970.2 vs >970.2	1.03(0.61-1.73)	0.926		

AC, Adenocarcinoma; BMI, Body mass index; CAR, C-reactive protein/albumin ratio; CI, Confidence interval; De Ritis, Aspartate transaminase/alanine transaminase ratio; ECOG PS, Eastern cooperative oncology group performance status; GPS, Glasgow prognostic score; HR, Hzard ratio; NLR, Neutrophil to lymphocyte ratio; PFS, progression free survival; PLR, Platelet - lymphocyte ratio; PNI, Prognostic nutritional index; SII, Systemic immune-inflammation index; SCC,Squamous cell carcinoma.

Table 3. Univariate and multivariate analysis of characteristic parameters and laboratory indices related to overall survival.

OS		Univariate Analysis		Multivariate Analysis	
Characteristics	**Category**	**HR(95% CI)**	**P**	**HR(95% CI)**	**P**
Age	<65 vs ≥65	1.08(0.52-2.23)	0.840		
Sex	Female vs male	1.04(0.37-2.96)	0.943		
ECOG PS	≤1 vs >1	2.62(1.26-5.44)	0.010	0.45(0.22-0.94)	0.035
Sub type	SCC vs AC	1.13(0.54-2.4)	0.744		
PD-L1	Negative vs positive	1.09(0.54-2.20)	0.803		
Surgery history	Negative vs positive	1.05(0.36-3.10)	0.925		
Metastasis status	Denovo vs recurrence	0.88(0.25-3.12)	0.841		
Brain metastasis	Negative vs positive	1.10(0.42-2.88)	0.849		
Alcohol history	Yes vs /no	0.55(0.20-1.50)	0.242		
Smoking history	Yes vs no	1.32(0.54-3.20)	0.540		
BMI	<25 vs ≥25	0.46(0.21-1.00)	0.051		
Bone metastasis	Negative vs positive	2.95(1.02-8.58)	0.047	0.38(0.19-0.78)	0.008
Blood group	O/A/B/AB	1.06(0.75-1.50)	0.732		
	RH- vs RH+	0.64(0.28-1.46)	0.290		
CAR	≤5.73 vs >5.73	0.43(0.21-0.87)	0.019	1.58(0.72-3.47)	0.253
De-ritis	≤1 vs >1	2.02(0.99-4.11)	0.050	0.47(0.23-0.96)	0.037
GPS	≤1 vs >1	1.65(0.75-3.64)	0.217		
NLR	≤3.74 vs >3.74	0.85(0.43-1.68)	0.644		
PLR	≤223.5 vs >223.5	1.09(0.55-2.15)	0.806		
PNI	≤46 vs >46	0.95(0.48-1.88)	0.890		
SII	≤970.2 vs >970.2	1.07(0.54-2.10)	0.846		

AC, Adenocarcinoma; BMI, Body mass index; CAR, C-reactive protein/albumin ratio; CI, Confidence interval; De Ritis, Aspartate transaminase/alanine transaminase ratio; ECOG PS, Eastern cooperative oncology group performance status; GPS, Glasgow prognostic score; HR, Hzard ratio; NLR, Neutrophil to lymphocyte ratio; PFS, progression free survival; PLR, Platelet - lymphocyte ratio; PNI, Prognostic nutritional index; SII, Systemic immune-inflammation index; SCC,Squamous cell carcinoma.

Table 4. Adverse event associated with nivolumab.

Events	N	%
Anemia	37	44.0
Thrombocytopenia	7	8.3
Thyroiditis	4	4.7
Neutropenia	3	3.5
Pneumonitis	2	2.3
Nephritis	1	1.2
Hypophysitis	1	1.2
Febrile neutropenia	0	0

(14.2 and 9.2 months, HR = 0.47, 95% CI 0.23-0.96, p = 0.037) were identified as risk factors for shorter OS (Figure 2). Table 3 shows the factors affecting OS.

Best Objective Response Rate

In our patients, 2.4% had a complete response, 31% had a partial response, 17.9% had stable disease, and 48.8% had progressive disease (Figure 3).

Adverse Events

During nivolumab treatment, 44.0% of the patients had anemia, 8.3% had thrombocytopenia, 4.7% had thyroiditis, 3.5% had neutropenia, 2.3% had pneumonitis, 1.2% had nephritis, and 1.2% had hypophysitis. Table 4 gives details of side effects.

DISCUSSION

This study examined the relationship between prognostic factors and survival in patients with NSCLC receiving nivolumab as second-line therapy. Studies of the use of nivolumab as second-line treatment in patients diagnosed with NSCLC have generally focused on patients with ECOG PS ≤ 1 (8,9). A study evaluating the treatment effectiveness of nivolumab, including patients with ECOG PS > 1, found that patients with an ECOG PS > 1 had a similar OS advantage to patients with ECOG PS ≤ 1 (12). However, meta-analyses and studies have shown that patients with ECOG PS ≤ 1 have better PFS and OS than patients with ECOG PS > 1 (13,14). We observed that patients with ECOG PS ≤ 1 had better PFS and OS than patients with ECOG PS > 1.

The presence of bone metastases in patients diagnosed with NSCLC is not only a poor prognostic factor but is also associated with a lower ICI treatment response (15,16). Similarly, in our patients, the presence of bone metastases was associated with a shorter OS.

Many recent studies have addressed the prognostic significance of different inflammatory markers in different types of cancer, such as GPS, NLR, PLR, PNI, SII, CAR, and the De Ritis ratio (17-20). In patients with NSCLC, a high CAR was found to be associated with earlier recurrence, worse local control, and shorter PFS and OS (21-26). In our cohort, a higher CAR was also associated with a shorter PFS and OS in univariate analyses, but not in the multivariate analysis.

A high De Ritis ratio has prognostic importance for many tumors, especially colon, pancreas, and renal cell cancers. Although the De Ritis ratio has been studied in colon, pancreatic, and renal cancers, its role in NSCLC remains underexplored (20,27,28). In our patients, a high De Ritis ratio was associated with a shorter OS.

The limitations of our study are that it was single-center, retrospective, and enrolled a small number of patients. ICI-related side effects may develop up to 12 months after discontinuing ICI treatment and therefore some side effects may not have been evaluated. Its strengths are that it is the first study to evaluate the prognostic value of the De Ritis ratio in patients with NSCLC using ICI.

CONCLUSION

In our study, a poor ECOG PS, the presence of bone metastases, and a De Ritis ratio > 1 were found to be prognostic risk factors for poor survival in patients with NSCLC using ICI. These risk factors, especially the De Ritis ratio, need to be evaluated in more comprehensive, prospective, and randomized controlled studies.

Ethical Statement

This study was conducted in accordance with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee (KAEK/2023.12.652). Written informed consent was obtained from all patients before the conduct of the study.

Author Contribution

TK and NB conducted the literature review and designed the study. GUE wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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OPEN ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Protective Effects of Melatonin and Alpha Lipoic Acid Against Cisplatin-Induced Ototoxicity

Cisplatinin İndüklediği Ototoksisite Üzerine Melatonin Ve Alfa Lipoik Asitin Etkileri

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ÖZET

Amaç: Bu çalışmada, sıçan modelinde cisplatine bağlı ototoksisiteye karşı melatonin ve alfa-lipoik asidin potansiyel koruyucu etkilerini beyin sapı işitsel uyarılmış potansiyelleri kullanarak araştırmak amaçlanmıştır.

Gereçler ve Yöntem: Altmış Sprague-Dawley sıçanı rastgele altı gruba ayrıldı: kontrol, cisplatin, melatonin, alfa-lipoik asit, cisplatin+melatonin ve cisplatin+alfa-lipoik asit. Cisplatin tek doz intraperitoneal enjeksiyon (10 mg/kg) şeklinde uygulandı. Melatonin (4 mg/kg) ve alfa-lipoik asit (100 mg/kg), cisplatin uygulamasından bir gün önce başlayarak sekiz gün boyunca günlük olarak verildi. Beyin Sapı İşitsel Uyarılmış Potansiyelleri başlangıçta ve 3., 7. ve 15. günlerde ölçüldü. Dalga latansları, interpik latanslar, işitme eşikleri ve dalga formu morfolojisi analiz edildi.

Bulgular: Cisplatin uygulaması, V. dalga latansında anlamlı uzama ve işitme eşiklerinde artışa neden oldu. Melatonin tedavisi, sadece cisplatin grubuna kıyasla V. dalga latansı ve işitme eşiklerindeki cisplatine bağlı değişiklikleri anlamlı ölçüde azalttı ($p<0.05$). Alfa-lipoik asit, cisplatine bağlı değişikliklere karşı anlamlı bir koruma göstermedi. Hem melatonin hem de alfa-lipoik asit grupları, tek başına uygulandıklarında dalga latanslarında değişiklikler gösterdi. Dalga formu bozulmaları en çok cisplatin grubunda görülürken, melatonin ve alfa-lipoik asit tedavi gruplarında daha az sıklıkta gözlendi.

Sonuç: Melatonin tedavisi, sıçanlarda cisplatine bağlı ototoksisiteyi anlamlı ölçüde azaltmaktadır ve bu muhtemelen güçlü antioksidan özelliklerinden kaynaklanmaktadır. Bu bulgu, cisplatin kemoterapisi alan hastalarda işitme kaybını önlemek veya en aza indirmek için melatoninin potansiyel bir terapötik strateji olabileceğini desteklemektedir. Bununla birlikte, alfa-lipoik asit bu çalışmada anlamlı bir koruma göstermemiştir ve bu konuda daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: Cisplatin, ototoksisite, melatonin, alfa-lipoik asit, antioksidan

ABSTRACT

Aim: To investigate the potential protective effects of melatonin and alpha-lipoic acid against cisplatin-induced ototoxicity in a rat model using brainstem auditory evoked potentials.

Materials and Methods: Sixty Sprague-Dawley rats were randomly divided into six groups: control, cisplatin-only, melatonin-only, alpha-lipoic acid-only, cisplatin+melatonin, and cisplatin+alpha-lipoic acid. Cisplatin was administered as a single intraperitoneal injection (10 mg/kg). Melatonin (4 mg/kg) and alpha-lipoic acid (100 mg/kg) were administered daily for eight days, starting one day before cisplatin. Brainstem Auditory Evoked Potentials were measured at baseline and on days 3, 7, and 15. Wave latencies, interpeak latencies, hearing thresholds, and waveform morphology were analyzed.

Results: Cisplatin administration resulted in significant prolongation of wave V latency and increased hearing thresholds. Melatonin treatment significantly mitigated cisplatin-induced changes in wave V latency and hearing thresholds compared to cisplatin alone ($p<0.05$). Alpha-lipoic acid did not demonstrate significant protection against cisplatin-induced changes. Both melatonin and alpha-lipoic acid groups showed alterations in wave latencies when administered alone. Waveform distortions were most prevalent in the cisplatin group, with lower incidence in melatonin and alpha-lipoic acid treatment groups.

Conclusions: Treatment with melatonin significantly mitigates cisplatin-induced ototoxicity in rats, likely due to its potent antioxidant properties. This finding supports the potential of melatonin as a therapeutic strategy to prevent or minimize hearing loss in patients receiving cisplatin chemotherapy. However, alpha-lipoic acid did not exhibit significant protection in this study, warranting further investigation.

Keywords: Cisplatin, ototoxicity, melatonin, alpha-lipoic acid, antioxidants

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INTRODUCTION

Cisplatin, a cornerstone of cancer chemotherapy, frequently induces ototoxicity, characterized by irreversible inner ear damage and subsequent hearing loss. This debilitating side effect necessitates strategies to mitigate ototoxicity without compromising cisplatin's anti-cancer efficacy. Oxidative stress, driven by free radical generation, is a key mechanism underlying cisplatin-induced cochlear damage. Antioxidants, capable of neutralizing free radicals, present a promising therapeutic avenue (1). This study investigates the otoprotective potential of two such antioxidants: melatonin and alpha lipoic acid (ALA). Melatonin, a hormone with established antioxidant and anti-inflammatory properties (2-4), scavenges reactive oxygen and nitrogen species and modulates cytokine production. ALA, a potent antioxidant and mitochondrial cofactor (5), directly neutralizes free radicals and regenerates endogenous antioxidants like vitamins C and E, glutathione, and coenzyme Q10 (6). Furthermore, ALA modulates inflammatory signaling pathways (5). We hypothesize that melatonin and ALA will attenuate cisplatin-induced ototoxicity. This research aims to elucidate their protective mechanisms and inform the development of therapeutic strategies to improve the quality of life for patients receiving cisplatin.

MATERIALS AND METHODS

This study was conducted at the Department of Otorhinolaryngology, Selçuk University Meram Faculty of Medicine, utilizing rats obtained from the Selçuk University Experimental Medicine Research and Application Center. A total of 60 healthy adult female Sprague-Dawley rats, approximately three months old, were used. The weight of the rats ranged from 180 to 220 grams, with an average weight of 200 grams. Throughout the study, the rats were maintained in a controlled environment with a 12-hour light/dark cycle, temperature maintained at 20±2°C, and relative humidity at 50±10%, with air changed 15 times per hour. Food and water were provided ad libitum. The rats were housed in groups of five in polycarbonate cages with a floor area of 1820 cm2 (Tecniplast Company, Italy). Approval from the Ethics Committee of the Faculty of Medicine at Selçuk University was obtained prior to the commencement of the study (number: 2007/18).

The rats were divided into six groups:

- Group 1 (Control Group -C-; n = 10): This group received intraperitoneal (i.p.) injections of 1 mg/kg physiological serum for 8 days to counteract the stress caused by injections in other groups.
- Group 2 (Cisplatin Group -CP-; n = 10): A single i.p. injection of 10 mg/kg cisplatin (Cis-Diammineplatinum II chloride) was administered.
- Group 3 (Melatonin Group -Mel-; n = 10): Melatonin (Melatonine for synthesis C13H15N2O2, M: 232.28 g/mol, Merck, Germany) was administered i.p. at a dose of 4 mg/kg for 8 days.
- Group 4 (Alpha-Lipoic Acid Group -ALA-; n = 10): Alpha-lipoic acid (DL-α-lipoic acid > 98.0% HPLC, Fluka) was administered i.p. at a dose of 100 mg/kg for 8 days.
- Group 5 (Cisplatin + Melatonin Group -CP+Mel-; n = 10): A single i.p. injection of 10 mg/kg cisplatin was administered, followed by i.p. injections of 4 mg/kg melatonin starting one day before the cisplatin injection and continuing for 8 days.
- Group 6 (Cisplatin + Alpha-Lipoic Acid Group -CP+ALA-; n = 10): A single i.p. injection of 10 mg/kg cisplatin was administered, followed by i.p. injections of 100 mg/kg alpha-lipoic acid starting one day before the cisplatin injection and continuing for 8 days.

In all groups, brainstem auditory evoked potentials (BAEPs) were measured 30 minutes before the initial injections. The day of the first cisplatin injections was designated as day 0. For groups other than the control group, BAEPs were measured 4 times in total, on days 0, 3, 7, and 15. For the control group, BAEPs were measured 3 times, on days 0, 7 and 15.

Brainstem Auditory Evoked Potential (BAEP) Measurements

Prior to recording evoked potentials, animals were anesthetized with intramuscular injections of ketamine hydrochloride (50 mg/kg) and xylazine (10 mg/kg). The animals were then placed in a sound-attenuated and electrically isolated environment. BAEP measurements were performed using Oxford Instruments MedelecSynergy EMG and EP Systems. TDH-49p headphones and Viasys Healthcare TECA Needles subdermal needle electrodes were used. Active electrodes were placed in the retroauricular region, the reference electrode at the vertex along the midline, and the neutral electrode between the eyes on the median line (7,8).

Auditory stimuli were delivered alternately to each ear using click stimuli, with the non-stimulated ear masked with white noise. Stimuli were delivered at a frequency of 15 Hz, with an analysis time of 10 ms (9). To determine the threshold, stimulus intensity was reduced in increments of 10 dB from above-threshold levels and in 5 dB steps near the threshold. Each test presented 1000-2000 click stimuli. I., III., and V. wave formations were observed on the monitor. Latencies of I., III., and V. waves, as well as I-III, III-V, and I-V interpeak latencies (IpLs), were recorded. BAEP recordings were assessed based on the wave latency-intensity functions. Threshold values were determined and any variations in wave formation were documented. The single dose cisplatin injection (10 mg/kg) method was utilized as previously described in studies demonstrating ototoxicity (10,11).

Statistical Analysis:

Data were analyzed using SPSS 13.0 software. Descriptive statistics were reported as mean ± standard deviation. Variance analysis was conducted for group comparisons across repeated measurements, with post-hoc Tukey tests identifying specific group differences. Within-group repeated measurements were analyzed using the Wilcoxon signed-rank test, while between-group comparisons were performed using the Mann-Whitney U test. Categorical variables were compared using the chi-square test. Statistical significance was set at $p<0.05$. Results were visualized through graphs and tables.

Table 1. Latencies of waves I, III, and V of Brainstem Auditory Evoked Potentials (BAEPs) in all experimental and control groups at days 0, 3, 7, and 15.

	Day 0. (Mean±sd)	Day 3. (Mean±sd)	Day 7. (Mean±sd)	Day 15. (Mean±sd)	p
Wave I					
Control	1.75±0.17		1.80±1.12	1.78±0.13	
CP	1.75±0.18	1.79±0.13	1.72±0.14	1.72±0.11	
ALA	1.73±0.15	1.71±0.14*	1.71±0.09	1.71±0.12*	0.013 / 0.047
Mel	1.83±0.13	1.79±0.13	1.84±0.10	1.79±0.15	
CP+ALA	1.74±0.17	1.79±0.12*	1.82±0.07*	1.71±0.12	0.001 / <0.001
CP+Mel	1.67±0.18	1.76±0.10*	1.76±0.14*	1.72±0.12*	<0.001 /<0.001/ 0.005
Wave III					
Control	3.78±0.18		3.70±0.17	3.65±0.17	
CP	3.78±0.27	3.75±0.12	3.80±0.21	3.73±0.12	
ALA	3.84±0.36	3.83±0.10	3.84±0.09	3.79±0.10	
Mel	3.71±0.18	3.79±0.09*	3.84±0.11*	3.71±0.09	<0.001 / 0.002
CP+ALA	3.90±0.29	3.74±0.15*	3.79±0.12*	3.69±0.15*	<0.001 /<0.001/ <0.001
CP+Mel	3.90±0.21	3.83±0.17*	3.76±0.13*	3.75±0.11*	0.005 /<0.001/ <0.001
Wave V					
Control	5.76±0.23		5.82±0.10	5.70±0.14	
CP	5.71±0.14	5.73±0.42*	5.71±0.26	5.73±0.10	0.020
ALA	5.74±0.16	5.75±0.09	5.79±0.12	5.82±0.28	
Mel	5.76±0.14	5.79±0.14	5.76±0.28	5.82±0.05	
CP+ALA	5.71±0.13	5.75±0.11*	5.85±0.07*	5.76±0.08*	0.009 /<0.001/ 0.003
CP+Mel	5.81±0.15	5.81±0.11	5.86±0.05*	5.74±0.10*	<0.001 / <0.001

CP: Cisplatin ALA: Alpha-Lipoic Acid Mel: Melatonin *: Indicates a statistically significant difference compared to Day 0 (P < .05).

RESULTS

Three rats in the melatonin group and two rats in the ALA group were lost due to peritonitis on days 6 and 8, respectively.

Wave Latencies

Table 1 presents the latencies of waves I, III, and V of BAEPs in all experimental and control groups at different time points (days 0, 3, 7, and 15).

Cisplatin administration significantly prolonged wave V latency at day 3 compared to baseline ($p<0.05$). Alpha-lipoic acid (ALA) alone shortened wave I latency at days 3 and 15 ($p<0.05$), while melatonin alone prolonged wave III latency at days 3 and 7 ($p<0.05$). In the combined treatment groups, both cisplatin + ALA and cisplatin + melatonin induced significant changes in wave I, III, and V latencies across various time points compared to baseline ($p<0.05$). Crucially, cisplatin + melatonin mitigated the cisplatin-induced prolongation of wave V, demonstrating a significant difference between these groups ($p<0.05$). Similarly, the cisplatin + melatonin group exhibited significantly shorter wave V latencies compared to the cisplatin + ALA group ($p<0.05$). ALA and cisplatin + melatonin groups showed altered wave III latencies compared to the control ($p<0.05$).

Interpeak Latencies (IPLs)

Table 2 illustrates the IPLs for I-III, I-V, and III-V in all experimental and control groups at different time points (days 0, 3, 7, and 15). Analysis of IPLs revealed that cisplatin significantly increased III-V IPL at days 3 and 7 ($p<0.05$). While both ALA and melatonin alone affected IPLs, the most notable finding was the significant reduction in I-V and I-III IPLs between the cisplatin and cisplatin + melatonin groups ($p<0.05$), indicating a protective effect of melatonin. Additionally, the cisplatin + melatonin group showed significantly different I-V and I-III IPLs compared to the cisplatin + ALA group ($p<0.05$).

Hearing Thresholds

Table 3 shows the hearing thresholds in all experimental and control groups at different time points (days 0, 3, 7, and 15). Cisplatin significantly elevated hearing thresholds at days 3, 7, and 15 ($p<0.05$). ALA and melatonin alone also induced changes in hearing thresholds. However, the cisplatin + melatonin group demonstrated significantly lower thresholds compared to the cisplatin-only group ($p<0.05$, Figure 1), confirming melatonin's protective effect. No significant difference was observed between the cisplatin and cisplatin + ALA groups. Consistent with the wave V latency findings, the cisplatin + melatonin group exhibited significantly lower hearing thresholds compared to the cisplatin + ALA group ($p<0.05$).

Waveform Morphology

Waveform morphology analysis revealed transient distortions in all treatment groups. The highest incidence was observed in the cisplatin group, while the combination treatment groups, particularly cisplatin + melatonin, showed a tendency toward recovery by day 15.

DISCUSSION

Cisplatin is a highly effective chemotherapeutic agent

Table 2. Interpeak Latencies (IPLs) for I-III, I-V, and III-V in all experimental and control groups at days 0, 3, 7, and 15.

	Day 0. (Mean±sd)	Day 3. (Mean±sd)	Day 7. (Mean±sd)	Day 15. (Mean±sd)	p
I-V IpL					
Control	4.01±0.27		4.00±0.16	3.92±0.21	
CP	3.95±0.25	4.02±0.15	3.97±0.31	4.00±0.14	
ALA	3.99±0.21	4.05±0.12	4.10±0.12	4.10±0.30*	0.002
Mel	3.94±0.15	3.98±0.21	3.91±0.23	4.03±0.16*	0.017
CP+ALA	3.98±0.22	3.95±0.15	4.02±0.10	4.04±0.14*	<0.001
CP+Mel	4.14±0.20	4.02±0.22*	4.11±0.16	4.03±0.15*	<0.001/<0.001
I-III IpL					
Control	2.03±0.29		1.89±0.19	1.86±0.21	
CP	2.05±0.33	1.94±0.20	2.05±0.23	2.00±0.15	
ALA	2.10±0.43	2.11±0.18	2.13±0.12	2.08±0.14	
Mel	1.88±0.23	2.00±0.12*	2.00±0.11*	1.91±0.18	0.007 / 0.050
CP+ALA	2.16±0.30	1.95±0.17*	1.96±0.12*	1.97±0.13*	<0.001/<0.001/<0.001
CP+Mel	2.19±0.24	2.07±0.21*	2.00±0.17*	2.03±0.16*	<0.001/<0.001/<0.001
III-V IpL					
Control	1.97±0.22		2.12±0.18	2.05±0.17	
CP	1.90±0.27	2.07±0.15*	1.98±0.24*	2.00±0.12	<0.001 / 0.049
ALA	1.90±0.35	1.93±0.13	1.95±0.12	2.03±0.28	
Mel	2.04±0.14	1.97±0.16*	1.91±0.28*	2.11±0.09*	0.001/0.002/0.001
CP+ALA	1.82±0.35	2.00±0.18*	2.07±0.14*	2.08±0.17*	<0.001/ <0.001/ <0.001
CP+Mel	1.91±0.22	2.01±0.16*	2.10±0.13*	1.99±0.13*	<0.001/ <0.001/ 0.001

CP: Cisplatin ALA: Alpha-Lipoic Acid Mel: Melatonin *: Indicates a statistically significant difference compared to Day 0 (P < .05).

Table 3. Hearing thresholds in all experimental and control groups at days 0, 3, 7, and 15.

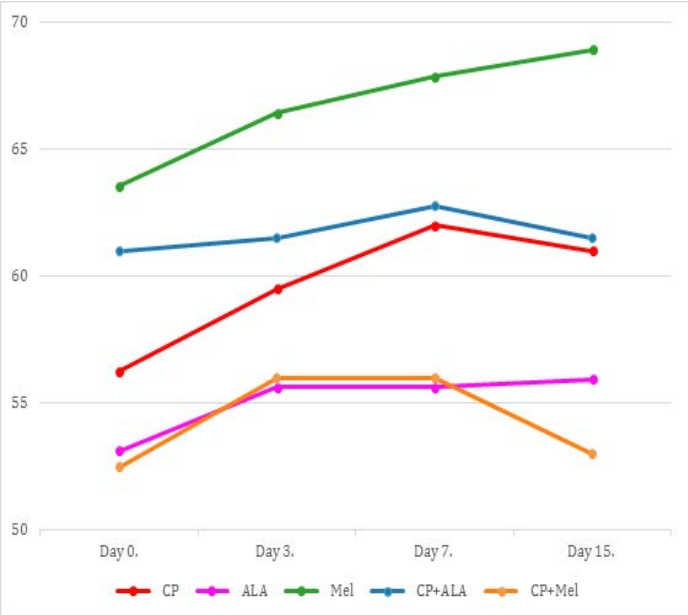
	Day 0. (Mean±sd)	Day 3. (Mean±sd)	Day 7. (Mean±sd)	Day 15. (Mean±sd)	p
Control	56.25±6.46	56.75±4.94	57.00±5.48		
CP	56.75±11.80	59.50±11.54*	62.00±12.24*	61.00±10.69*	0.001/<0.001/<0.001
ALA	53.12±5.31	55.62±5.00*	55.62±4.67*	55.94±4.44*	<0.001/<0.001/<0.001
Mel	63.57±8.22	66.43±9.83*	67.86±9.31*	68.93±10.51*	<0.001/<0.001/<0.001
CP+ALA	61.00±10.95	61.50±11.12	62.75±12.94*	61.50±10.18	<0.001
CP+Mel	52.50±7.36	56.00±7.21*	56.00±8.79*	53.00±6.24	<0.001/<0.001

CP: Cisplatin ALA: Alpha-Lipoic Acid Mel: Melatonin *: Indicates a statistically significant difference compared to Day 0 (P < .05).

used to treat a wide variety of malignancies (12). However, its clinical efficacy is often limited by dose-dependent and often irreversible ototoxicity, primarily manifesting as sensorineural hearing loss (13). This study investigated the potential protective effects of melatonin and alpha-lipoic acid, two potent antioxidants, against CP-induced ototoxicity in a rat model using BAEPs.

Our findings demonstrate that a single i.p. injection of 10 mg/kg CP resulted in a significant increase in BAEP wave V latency at day 3, a commonly used indicator of cochlear and auditory nerve dysfunction (14). This observation aligns with previous studies that have established the ototoxic nature of CP, particularly targeting the outer hair cells of the cochlea's basal turn, leading to high-frequency hearing loss that can progress to lower frequencies over time (15,16). The prolonged latency observed in our study indicates a delay in the transmission of auditory signals through the auditory pathway, likely due to CP-induced damage to cochlear structures and potentially the auditory nerve.

Interestingly, the groups receiving melatonin or ALA alone exhibited significant changes in BAEP wave latencies compared to the control group. ALA administration resulted in a statistically significant shortening of wave I latency on days 3 and 15, while melatonin administration led to a significant prolongation of wave III latency on days 3 and 7. These findings suggest that both melatonin and ALA, when administered independently, may have an effect on auditory signal processing within the brainstem. However, the exact mechanisms underlying these observed changes warrant further investigation. For instance, ALA's ability to shorten wave I latency could indicate an enhanced excitability of the auditory nerve, potentially through its known neuroprotective



CP: Cisplatin ALA: Alpha-Lipoic Acid Mel: Melatonin

Figure 1. Comparison of hearing thresholds between all experimental groups at days 0, 3, 7, and 15

effects (2). Conversely, the prolongation of wave III latency with melatonin could be attributed to its modulation of neurotransmitter systems in the brainstem, a well-documented effect of melatonin (17,18).

Importantly, our study provides evidence for the protective effect of melatonin against CP-induced ototoxicity. Rats receiving melatonin concomitantly with CP exhibited significantly shorter wave V latencies compared to the CP-only group, particularly at days 7 and 15. This suggests that melatonin pre-treatment may mitigate the delayed signal transmission caused by CP, thus preserving auditory function. These results are consistent with previous research highlighting melatonin's potent antioxidant properties and its ability to protect against oxidative stress-mediated damage in various tissues, including the cochlea (19). Melatonin's multiple mechanisms of action, including direct scavenging of reactive oxygen species, upregulation of antioxidant enzymes, and inhibition of pro-oxidant enzymes, likely contribute to its protective effects against CP-induced ototoxicity (20).

Surprisingly, ALA, despite its well-established antioxidant properties (12), did not demonstrate significant protection against CP-induced changes in wave V latency in our study. This unexpected finding suggests that the complex interplay between CP's ototoxic mechanisms and ALA's antioxidant and metabolic effects might contribute to this result. It is plausible that ALA's pro-oxidant potential under certain conditions, particularly in the presence of free iron (21), might have played a role in negating its protective effects against CP-induced ototoxicity. Further investigations are needed to clarify the specific interactions between CP and ALA in the context of cochlear damage.

Moreover, the study revealed significant differences in BAEP hearing thresholds between the groups. The CP-only group demonstrated a progressive increase in hearing thresholds over the 15-day observation period, confirming the development of SNHL. Notably, the group receiving CP and melatonin had significantly lower hearing thresholds compared to the CP-only group, further supporting the protective effect of melatonin against CP-induced ototoxicity. However, the CP+ALA group did not exhibit a significant difference in hearing thresholds compared to the CP-only group, further reinforcing the lack of protective effect of ALA in our study model.

Analysis of BAEP waveform morphology revealed transient distortions in a proportion of ears across different groups, with a higher incidence observed in the CP-only group. These distortions, often characterized by reduced wave amplitudes and altered peak latencies, provide additional evidence of CP-induced alterations in auditory signal processing within the brainstem (22). The lower incidence of waveform distortions in the melatonin and ALA pre-treatment groups, especially the normalization observed in some ears over time, further suggests a potential for these antioxidants to attenuate the severity of CP-induced ototoxicity.

This study has several limitations that should be considered. First, the study used a single dose of CP, limiting the ability to draw conclusions about the effects of cumulative CP doses, which are commonly used in clinical settings. Future studies using multiple CP doses are needed to further assess the long-term protective effects of melatonin and ALA. Second, the study only evaluated BAEPs and hearing thresholds as measures of ototoxicity. Future research incorporating histological analysis of cochlear structures would provide more detailed insights into the specific cellular and molecular mechanisms underlying the protective effects of melatonin and ALA. Finally, the relatively small sample size of each group, particularly after the loss of some rats due to peritonitis, may have limited the statistical power to detect subtle differences between the treatment groups.

Despite these limitations, this study provides valuable insights into the potential of melatonin and ALA as protective agents against CP-induced ototoxicity.

CONCLUSION

Our findings suggest that melatonin pre-treatment may effectively reduce CP-induced hearing loss in rats, potentially through its potent antioxidant properties. This finding has important implications for clinical practice, as it highlights a possible strategy to mitigate the debilitating side effects of CP treatment, improving the quality of life for cancer patients. The unexpected lack of protective effect of ALA in our study model necessitates further investigations to elucidate the complex interactions between CP and ALA in the context of cochlear damage. Future research should focus on optimizing dosing regimens, exploring combination therapies, and investigating the underlying molecular mechanisms of action to translate

these pre-clinical findings into effective clinical interventions for the prevention and management of CP-induced ototoxicity.

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OPEN ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Peripheral Biomarkers That May Be Associated with Mild Cognitive Impairment in Geriatric Patients

Geriatrik Hastalarda Hafif Bilişsel Bozuklukla İlişkili Olabilecek Periferik Biyobelirteçler

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ÖZET

Amaç: Hafif bilişsel bozukluk (HBB), bilişsel fonksiyonlarda azalmanın olduğu, erken müdahale edilmediği takdirde işlevsellikte kayıpların yaşandığı bir bozukluktur. Bu çalışmadaki amacımız HBB ile kontrol grubu arasında, nötrofil/yüksek- yoğunluklu lipoprotein kolesterol (HDLc) düzeyi oranı (NHO), monosit/HDLc oranı (MHO), ürik asit/HDLc oranı (UHO)'nı kıyaslamak ve bu parametrelerin HBB için prediktör bir biyobelirteç olarak kullanılıp kullanılamayacağını araştırmaktır.
Gereç ve Yöntemler: Kesitsel olan bu çalışmada 65 yaş üstü kişilerden ICD-10'a göre HBB tanısı konulan 68 kişi hasta grubunu oluşturdu. Kontrol grubu 65 yaş üzeri mental yeterlilik değerlendirmesi sonucu HBB olmayan bireylerden seçildi.
Bulgular: Monosit, UHO ve MHO seviyelerinin HBB grubunda daha yüksek olduğu görüldü (sırasıyla; p= 0.036, p= 0.007, p= 0.004). Mini-Mental Durum Muayene (MMSE) skoru ile HDLc seviyeleri arasında pozitif (p= 0.032), UHO arasında negatif yönlü ilişki (p= 0.019) bulundu. Tanı durumunu tahmin etmede UHO cut-off değeri= 0.1285 alındığında duyarlılığın %41.9 ve özgüllüğün %80.3 olduğu bulundu. MHO için cut-off değeri= 0.125 alındığında duyarlılığın %40.3 ve özgüllüğün %78.9 olduğu görüldü.
Sonuç: Düşük duyarlılık ve özgüllükte olsa da HBB tanısını tahmin etmede UHO ve MHO anlamlı bulundu. MMSE skoru ile HDLc seviyeleri arasındaki pozitif yönlü ilişki bulunması HDLc'nin bilişsel fonksiyonlara karşı koruyucu olabileceğini göstermektedir. UHO arttıkça MMSE skorunun düşmesi, UHO'nun prognozda kullanılabileceğine işaret edebilir. Çalışma sonuçlarımızın ileriye dönük çalışmalarla desteklenmesi gerekir.

Anahtar Kelimeler: Geriatri, mini-mental durum, bilişsel işlev, monosit/HDLc oranı, ürik asit/HDLc oranı

ABSTRACT

Aim: Mild cognitive impairment (MCI) is a disorder in which there is a decrease in cognitive functions and a loss of functionality is experienced unless early intervention is made. Our aim in this study was to compare neutrophil/high-density lipoprotein cholesterol (HDLc) levels ratio (NHR), monocyte/HDLc ratio (MHR), uric acid/HDLc ratio (UHR) between MCI and the control group and to investigate whether these parameters can be used as a predictive biomarker for MCI.
Materials and Methods: In this cross-sectional study, 68 people over the age of 65 who were diagnosed with MCI according to ICD-10 constituted the patient group. The control group was selected from individuals over the age of 65 who underwent the mental competence assessment and did not have MCI as a result of this assessment.
Results: Monocyte, UHR, and MHR were found to be higher in the MCI group (p= 0.036, p= 0.007, p= 0.004; respectively). A positive relationship was found between the Mini-Mental State Examination (MMSE) score and HDLc (p= 0.032) and a negative relationship was seen between UHR (p= 0.019). When the UHR cut-off value of 0.1285 was taken as a predictor of the diagnostic status, the sensitivity was found to be 41.9% and the specificity was 80.3%. When the cut-off value for MHR was taken as 0.125, the sensitivity was found to be 40.3% and the specificity was 78.9%.
Conclusion: Although they had low sensitivity and specificity, UHR and MHR were found to be significant in predicting the diagnosis of MCI. The positive relationship between the MMSE score and HDLc levels shows that HDLc may be protective against any deterioration in cognitive functions. The decrease in MMSE score while UHR increases may indicate that UHR can be used in prognosis. Our study results should be supported by prospective studies.

Keywords: Geriatrics, mini-mental state, cognitive function, monocyte/HDLc ratio, uric acid/HDLc ratio

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INTRODUCTION

Mild Cognitive Impairment (MCI) is a disorder that lies somewhere between normal aging and Alzheimer's Disease (AD), characterized by more significant memory loss and neuronal loss compared to normal aging, making individuals more susceptible to AD (1). According to the World Health Organization, there are over 50 million people globally with dementia (2). The majority of these dementia cases are due to AD (3). In the elderly, there can be a natural mild decline in neurocognitive functionality with advancing age. These mild declines can rapidly progress to AD in some older adults. A study reported that 32% of MCI patients progressed to AD within two years (4). AD can reduce the quality of life and lead to accidents. Therefore, early diagnosis and treatment of MCI before it progresses to AD is crucial. Considering the pathophysiology of cognitive functions, a direct examination of the brain is necessary for a definitive diagnosis of cognitive impairment (5). This is quite costly and requires long waits, even until after death, for examination.

Cognitive impairment typically manifests itself in the later stages, leading to functional decline and the clinical presentation of AD, resulting in delays in diagnosing MCI (6). To detect cognitive function disorders early enough, various survey-based test batteries have been developed. Currently, AD diagnosis relies on clinical evaluation and neurocognitive tests like the Mini-Mental State Examination (MMSE) (7). In fact, the MMSE is not a direct diagnostic tool. Because it is closely related to the person's intellectual background, its diagnostic value is low. However, it can be used for screening. These questionnaire-based tests assess neurocognitive functions, including attention/working memory, executive-motor functions, processing speed, learning, and recollection. By their subjective nature, these tests have the potential for false positive and negative results. Therefore, MCI diagnosis is often supported with brain imaging (8). Given the expense and time-consuming nature of brain imaging, discovering simple, inexpensive, and quick peripheral biochemical parameters is important (9).

In the pathophysiology of AD, the increase in amyloid-beta (Aβ) in the brain parenchyma is known to play a role (10). Additionally, recent studies suggest that systemic and local mild chronic inflammation may contribute to the onset of neurodegeneration observed in AD (11). Individuals with elevated inflammatory markers have been found to have a higher risk of developing dementia (12, 13). Despite numerous studies, there is still no peripheral biochemical parameter with high validity and reliability that can predict, indicate prognosis, and definitively diagnose MCI.

The Neutrophil/High-Density Lipoprotein Cholesterol Ratio (NHR) is calculated by dividing the peripheral neutrophil count by the HDLc level. Similarly, the Monocyte/HDLc Ratio (MHR) is determined by dividing the monocyte count in peripheral blood by the HDLc level. NHR and MHR are emerging markers considered predictors of systemic inflammation and vascular diseases (14, 15). The close association of MCI with inflammation and vascular events suggests that these biomarkers could be related to MCI. Although the roles of NHR and MHR as new inflammatory markers have been investigated in various psychiatric disorders, there are no studies examining their relationship with MCI (16). The Uric Acid to High-Density Lipoprotein Cholesterol Ratio (UHR) has been studied in many chronic diseases potentially related to neurocognitive disorders in recent years, no literature exists yet on the association between UHR and MCI (17, 18).

The primary aim of this study is to compare levels of neutrophils, monocytes, uric acid, and HDLc, as well as the ratios of NHR, MHR, and UHR between patients diagnosed with the MCI and the control group. We seek to investigate whether these parameters can be used as biomarkers for the differential diagnosis of MCI. The secondary aim is to explore any potential correlation between MMSE scores and these biochemical parameters.

MATERIALS AND METHODS

In this cross-sectional study, the patient and control groups were selected from individuals over 65 years old who visited the outpatient psychiatry clinic at Karaman Training and Research Hospital between 2015 and 2023. The patient group included 68 individuals who met the MCI diagnosis criteria based on the clinical evaluation and MMSE results and were diagnosed with MCI according to ICD-10. The control group was selected from individuals who wanted to perform transactions at the notary or land registry office and were sent to the psychiatry polyclinic for a mental capacity report. In Turkey, people over the age of 65 are generally required to obtain a mental capacity report before they can perform transactions at the notary or land registry office. Individuals who applied for this report and had sufficient mental capacity designed the control group. Participants with chronic inflammatory diseases, those using anti-inflammatory drugs, those with hematological diseases, those in an acute infection period, those with a history of psychiatric disorders, and those using alcohol or narcotic substances were excluded from both the patient and control groups. The ones with hypertension and diabetes mellitus, common in this age group, were not excluded.

After obtaining the necessary permissions, the MMSE scores of the patients diagnosed with MCI were recorded from their hospital electronic records. The biochemical data from laboratory tests conducted at the time of their visit were also recorded. The control group was selected in reverse order from individuals over 65 years old, ensuring gender matching. Similarly, the MMSE values and biochemical data of the control group at that time were recorded. First, the biochemical data of the patient and control groups were compared. Secondly, the relationship between total MMSE scores and biochemical data was examined.

Ethics committee approval for the research was received from the Ethics Committee of Karamanoğlu Mehmetbey University from the Medical Faculty (Ethics Committee Decision No. 01-2024/2, dated 27.02.2024). This study was conducted in accordance with the guidelines stated in the principles of the Declaration of Helsinki.

Mini-Mental State Examination (MMSE)

The Mini-Mental State Examination (MMSE) was developed to evaluate cognitive function (19). In this study, the Turkish version of this test was used (20). The maximum score obtainable on this test is 30. It consists of five sections: 5 questions about time orientation, 5 questions on orientation to place, 3 on memory registration, 5 on attention and calculation, 3 on memory recall, 8 on language, and 1 on visual construction. The cut-off point for 'normal' cognitive function on the MMSE is 24. Any score of 23 or below indicates possible cognitive impairment. Scores between 19 and 23 are considered indicative of MCI. It has been reported that individuals scoring low on certain subscales of the MMSE have a higher risk of progressing to AD (21).

Statistical analysis

The SPSS 25.0 program was used to analyze the data. To ensure suitability of continuous variables to normal distribution, the normality test, q-q graph, skewness and kurtosis were taken into account. In these data, HDLc, uric acid, neutrophils, UHR, NHR showed normal distribution, while monocytes and MHR did not show normal distribution. Those with normal distribution were compared using the Independent T test, and those with non-normal distribution were compared through the Mann-Whitney U test. The Chi-square test was used to evaluate categorical data. Categorical data are reported as counts (n) and percentages (%), while continuous data are reported as means (M) and standard deviations (SD). Pearson Correlation test was used to evaluate the correlation. ROC analysis was used to estimate cut-off values for UHR and MHR. The AUC index value range is $0.5 \leq AUC \leq 1$. (For all analysis results, the cases in which the significance level was $p < 0.05$ were used.

RESULTS

The patient group was made up of 27.9% males (n= 19) and 72.1% females (n= 49), while the control group comprised 34.2% males (n= 26) and 65.8% females (n= 50). The mean age of the control group was 71.89±6.59, whereas that of the patient group was 68.76±5.74 (Table 1).

There was no significant difference between the groups in terms of HDLc levels, uric acid levels, neutrophil counts, and NHR values (p> 0.05). However, monocyte counts, UHR, and MHR values were found to be high in the patient group (p= 0.036, p= 0.007, p= 0.004; respectively), (Table 2).

A positive correlation was observed between MMSE score and HDLc levels (p= 0.032), while a negative correlation was found between MMSE score and UHR (p= 0.019), (Table 3).

In order to predict the diagnostic status, a cut-off value of

Table 1. Comparison of sociodemographic data in mild cognitive impairment and control groups

	Control (n=76)	MCI (n=68)	t/ χ^2	p
Age, Mean±SD	71.89±6.59	68.76±5.74	-3.02	0.003
Male, n(%)	26(34.2)	19(27.9)	0.657	0.418
Female, n(%)	50(65.8)	49(72.1)		
DM, n(%)	31(40.7)	26(38.2)	0.0979	0.754
HT, n(%)	34(44.7)	30(44.1)	0.0056	0.940

MCI: Mild cognitive impairment, DM: Diabetes mellitus, HT: Hypertension

Table 2. Comparison of biochemical parameters in mild cognitive impairment and control groups

	Control (n=76) Mean±SD	MCI (n=68) Mean± SD	t/Z	df	p
HDLc	52.44±12.20	48.42±14.53	-1.784	139	0.077
Uric acid	5.05±1.40	5.28±1.42	0.953	134	0.342
Neutrophil	4.69±1.55	4.53±1.92	-0.554	142	0.580
Monocyte	0.48±0.14	0.54±0.17	2061	142	0.036
UHR	0.10±0.04	0.12±0.06	2.752	132	0.007
MHR	0.009±0.0039	0.012±0.0059	1800	139	0.005
NHR	0.09±0.04	0.10±0.05	0.948	130	0.345

MCI: Mild cognitive impairment, HDLc: High density lipoprotein cholesterol, UHR: Uric acid/High density lipoprotein cholesterol ratio. MHR: Monocyte/High density lipoprotein cholesterol ratio, NHR: Neutrophil/High density lipoprotein cholesterol ratio. Data are presented as mean and standard deviation.

Table 3. MMSE Score Correlation Analysis with other variables

		HDLc	Uric acid	Neutrophil	Monocyte	UHR	MHR	NHR
MMSE Score	r	0.181*	-0.047	0.032	-0.105	-0.203*	-.194	-0.091

*: p< 0.05, Pearson Correlation Test was used.
MMSE: Mini-Mental State Examination Test, HDLc: High density lipoprotein cholesterol, UHR: Uric acid/High density lipoprotein cholesterol ratio, MHR: Monocyte/High density lipoprotein cholesterol ratio, NHR: Neutrophil/High density lipoprotein cholesterol ratio.

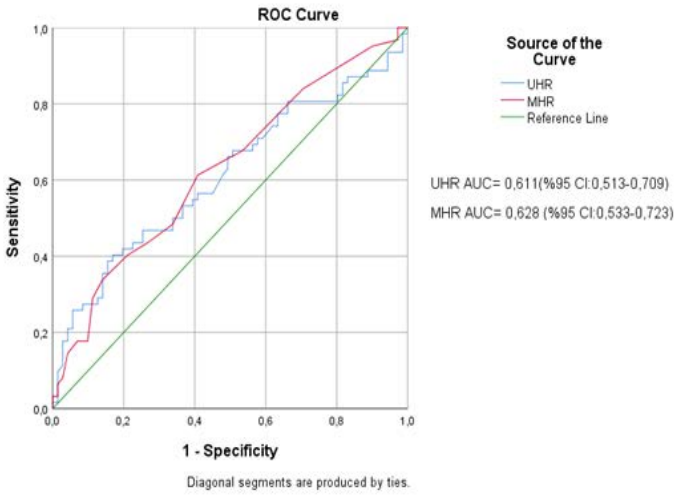

Figure 1. ROC Curve of UHR and MHR for Predicting Patients' Diagnosis

0.1285 was determined for UHR, with a sensitivity of 41.9% and a specificity of 80.3%. The area under the ROC curve was statistically significant at 0.611 (p= 0.027). For MHR, a cut-off value of 0.125 resulted in a sensitivity of 40.3% and a specificity of 78.9%. The area under the ROC curve was statistically significant at 0.628, (p= 0.011), (Figure 1).

DISCUSSION

In our study, when we compared the serum uric acid levels, neutrophil counts, monocyte counts and HDLc levels, UHR, MHR, and NHR rates of the MCI and control groups, it was seen that only monocyte counts, UHR and MHR were different between the two groups, and they were higher in the MCI group. UHR and MHR were found to be significant in predicting MCI diagnosis. MMSE was only associated with HDLc levels and UHR when correlated with biochemical parameters. There was a positive relationship between MMSE score and HDLc levels, and a negative relationship between MMSE score and UHR.

In our study, there was no significant difference in serum uric acid levels between the two groups. There are various studies on the relationship between uric acid and MCI. The results of these studies are conflicting. While some studies suggest that low uric acid levels are a risk factor for MCI (22), others have reported the opposite, indicating that high uric acid levels increase oxidation and lead to poor cognitive performance (23). A recent meta-analysis, similar to our study, concluded that there was no association between uric acid and cognitive impairment (24). These differences in the literature may be related to the stage of neurocognitive impairment. Uric acid can act as an antioxidant by inhibiting nitrite-mediated nitration, but it can also act as a pro-oxidant by generating radicals in reactions with various oxidants associated with inflammation (25, 26). Therefore, it seems that uric acid plays a dual role. Further prospective studies are needed to investigate the relationship between uric acid and cognition.

In this study, the monocyte count was found to be increased in the MCI group. There are few studies in the literature comparing monocyte counts between the MCI and control groups, with most studies focusing on the AD group. In a study, unlike our study, a significantly lower peripheral monocyte count was found in the AD group compared to the control group (27). Monocytes may reduce the development of AD by mediating the phagocytosis of amyloid-beta (Aβ) and tau, which play a role in AD pathophysiology (28). Therefore, a decrease in monocyte count may contribute to cognitive impairment by leading to the increase of amyloid-beta (Aβ) and tau. The difference in monocyte counts in studies may be related to the stage of neurocognitive impairment. The higher monocyte count found in our study may indicate a secondary increase for protective purposes against the disease (29). Further longitudinal studies evaluating monocytes in MCI patients who progress to AD are needed for definitive evidence.

In our study, unlike the data in the literature, no difference was found in terms of neutrophils between the two groups. Most studies in the literature on MCI report an increase in neutrophils (30), which has also been reported in AD patients. A meta-analysis reported increased neutrophil activation in MCI patients (31). According to another meta-analysis, neutrophils may have contributed to the progression of MCI patients to AD, suggesting that modulating neutrophils and their activities may be beneficial in preventing cognitive decline (32). Studies in the literature suggest that systemic inflammation may contribute to cognitive impairment by leading to neuroinflammation. Prospective follow-up of MCI patients and monitoring longitudinal changes in neutrophils would provide more accurate information for definitive evidence.

There is a wealth of data suggesting that HDLc levels have a positive effect on cognition. In a prospective study, it was reported that individuals with high HDLc levels in mid-life had a lower risk of developing MCI in old age after a 19-year follow-up, compared to those with low HDLc levels (33). However, in our study, no significant difference was found in serum HDLc levels between the MCI and control groups. The lack of difference between the two groups may be related to the early stage of MCI. Monitoring changes in serum HDLc levels in the same group of patients after MCI diagnosis as they age could provide more accurate information about the relationship between HDLc and cognitive function. The increase in MMSE scores with increasing HDLc levels in our study may suggest a protective effect of HDLc on cognitive function. Consistent with our findings, a study reported that MMSE scores showed a positive correlation with HDLc and a negative correlation with other lipid parameters in dementia patients (34). HDLc is believed to be protective against cognitive impairment by mediating antioxidant and anti-inflammatory systems (35). Large-scale prospective studies are needed for definitive evidence.

There was no significant difference between the MCI and control groups in terms of NHR values. A high NHR is indicative of an increase in neutrophils and a decrease in HDLc. There are no studies in the literature investigating NHR in the MCI patients. Most studies on NHR are related to cerebrovascular and cardiovascular diseases. This ratio reflects inflammatory activity and abnormal lipid metabolism, both of which impair vascular structure. A study indicated that NHR is associated with coronary artery stenosis and predicts the disease (36). An increase in this ratio has been reported to worsen the prognosis in cardiovascular diseases and may be a marker of mortality (37). Another study concluded that it may be associated with acute ischemic stroke (38). Considering the relationship between MCI and inflammatory events, cerebrovascular, and cardiovascular diseases, an elevation in NHR in MCI patients would be expected (39, 40). The lack of difference in NHR between the MCI and control in our study may be related to the early stage of the disease. Further studies with longitudinal follow-ups are needed to understand the relationship.

In our study, in comparison, MHR was found to be higher in the MCI group than in the control group. There are no studies in the literature investigating MHR in MCI patients. Most studies on MHR have been conducted in cerebrovascular and cardiovascular diseases. In these studies, a high MHR has been reported to predict the risk of stroke in ischemic patients and may be a marker of mortality in these patients (41). MHR has been found to negatively affect the course of cardiovascular diseases and be significantly associated with mortality (42). A high MHR is associated with an increase in monocyte count and a decrease in HDLc. It has been reported that HDLc may have an anti-inflammatory effect by inhibiting the expression of monocyte adhesion molecules such as CD11b and preventing the accumulation of monocytes in the vascular endothelium (43). A high MHR indicates that the balance between inflammatory and anti-inflammatory factors shifts towards inflammation in MCI patients. The fact that MHR can be a new inflammatory biomarker for cerebrovascular events and is closely related to prognosis in cardiovascular diseases suggests that it could be an important predictor for MCI patients.

In our study, UHR was found to be higher in the MCI group than in the control group. There are no studies in the literature investigating UHR in MCI patients. Most studies on UHR have been conducted in metabolic syndrome, diabetes, and thyroid patients, and UHR has been proposed as a new inflammatory marker (18, 44, 45). The inconsistent information between cognitive impairment and uric acid in the literature suggests that more consistent results could be obtained in MCI patients when uric acid is evaluated not alone but together with HDLc. The imbalance between the inflammatory effect of uric acid and the anti-inflammatory effect of HDLc may have impaired cognitive function.

Limitations

Our study has some limitations. Firstly, since our study is a cross-sectional study, we cannot know how these parameters will change as cognitive impairment progresses. Secondly, because hypertension and diabetes mellitus are commonly found in this age group, the effect of these diseases has not been excluded. Studies that exclude these limitations may provide more accurate results, as uncontrolled diabetes and hypertension can affect biochemical parameters. In our study, the proportion of those with hypertension and diabetes in the patient and control groups was equalized to reduce the effect of this limitation. Despite these limitations, our study may contribute to the literature as the first study to evaluate NHR, MHR, and UHR in MCI patients. It is expected that prospective, multicenter, and controlled studies will shed more light on this issue.

CONCLUSION

In this study, we found that monocyte count, UHR, and MHR were higher in the MCI patients compared to the control group. Although they have low sensitivity and specificity, UHR and MHR were found to be significant in predicting MCI diagnosis. The positive correlation between MMSE score and HDLc suggests that HDLc may be protective against impairment in cognitive functions. The decrease in MMSE score with increasing UHR may indicate the potential use of UHR in prognosis. Inflammation may play a role in the pathophysiology of MCI by affecting various pathways such as neurodegenerative processes, oxidative stress, and immune response. Therefore, more studies are needed that include more parameters of inflammation, oxidative stress, and immune response. Data from this study need to be supported by forthcoming prospective studies with the larger number of patients.

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OLGU SUNUMU / CASE REPORT

Infectious Keratitis After Small-Incision Lenticule Extraction: First Reported Case From Türkiye

KKLE Sonrası Enfeksiyöz Keratit: Türkiye'den Bildirilen İlk Olgu

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ÖZET

Amaç: Türkiye'de küçük kesiden lentikül ekstraksiyonu (KKLE) sonrası gelişen ilk enfeksiyöz keratit olgusunu bildirmektir. KKLE, femtosaniye lazer ile korneada stromal bir lentikül oluşturularak ve bu lentikülün küçük bir kesiden çıkarılmasıyla yapılan bir lazer refraktif prosedürdür.

Olgu: 22 yaşında erkek bir hasta, KKLE'den 15 gün sonra sağ gözünde keratit gelişmesi nedeniyle kliniğimize yönlendirilmiştir. Hasta, sağ gözünde şiddetli ağrı, görme kaybı ve ışığa hassasiyet (fotofobi) şikayetleri ile başvurmuştur. Muayenesinde konjonktival enjeksiyon, yaygın korneal ödem ve santral ile parasantral bölgede yoğun infiltratif lezyonlar saptanmıştır. Güçlendirilmiş antibiyotiklerle ampirik tedaviye rağmen durumun ilerlemesi üzerine tedaviye antifungal ajanlar eklenmiştir. Revize edilen tedavi sonrasında hastanın semptomlarında belirgin bir iyileşme görülmüş ve bir aylık takip sürecinde korneal ödem ile epitel defektleri tamamen düzelmiştir. Alınan kültür örneklerinin sonuçları negatif olmasına rağmen, antifungal tedaviye yanıt verilmesi fungal bir etiyoloji olasılığını düşündürmüştür.

Sonuç: Bu olgu, KKLE sonrası kültür negatif enfeksiyöz keratit vakalarında fungal patojenlerin göz önünde bulundurulması gerektiğini vurgulamaktadır. Benzer vakalarda hızlı müdahale ve tedavinin revizyonu önemli bir rol oynamaktadır.

Anahtar Kelimeler: Refraktif cerrahi, küçük kesiden lentikül ekstraksiyonu, enfeksiyöz keratit

ABSTRACT

Objective: To report the first documented case of infectious keratitis following small-incision lenticule extraction (SMILE) surgery in Türkiye. SMILE is a laser refractive procedure designed to correct refractive errors by creating a stromal lenticule with a femtosecond laser, which is then removed through a small incision.

Case: A 22-year-old male patient presented to our clinic with keratitis in the right eye, which developed 15 days after undergoing SMILE surgery. The patient reported severe pain, decreased vision, and sensitivity to light (photophobia) in the affected eye. Examination findings included conjunctival injection, diffuse corneal edema, and dense infiltrative lesions in both the central and paracentral cornea. Initial empirical therapy with fortified antibiotics was ineffective as the condition progressed, leading us to add antifungal agents to the treatment regimen.: Following the revised treatment approach, the patient experienced significant relief from symptoms, with a marked resolution of corneal edema and epithelial defects within one month. Although culture results from corneal scrapings were negative, the patient's favorable response to antifungal treatment suggested a possible fungal etiology.

Conclusion: This case highlights the importance of considering fungal pathogens as potential culprits in culture-negative infectious keratitis following SMILE surgery. Prompt intervention and careful adjustment of empirical therapy play a critical role in managing similar cases effectively.

Keywords: Refractive surgery, small incision lenticule extraction, infectious keratitis

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INTRODUCTION

Small-incision lenticule extraction (SMILE) is a laser refractive procedure designed to correct refractive errors by creating a stromal lenticule with a femtosecond laser, which is then removed through a small incision. SMILE is increasingly recognized worldwide as a safe alternative to existing corneal refractive surgery techniques. Infectious keratitis is a rare complication of the SMILE procedure, with an incidence rate of 0.3% reported in the literatüre (1). Only five cases with microbiological evidence have been documented (2-6). This report presents a case of culture-negative infectious keratitis following SMILE.

CASE

A 22-year-old male patient was referred to our clinic with keratitis in the right eye, which developed 15 days after undergoing SMILE. The patient reported severe pain, decreased vision, and photophobia in the affected eye. He had no history of systemic illness or contact lens use but resided in a rural area, which could

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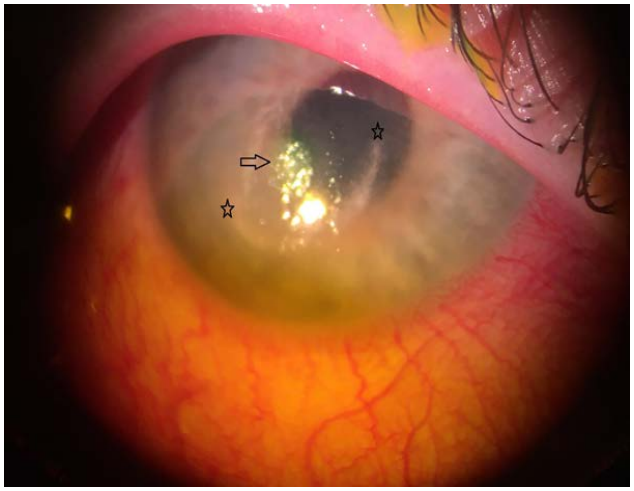

Figure 1. Shows the lesions at the time of presentation. The arrow indicates the central lesion, while the stars mark the starting and ending points of the arc-shaped lesion.

be considered a risk factor for infection. Postoperatively, the patient had been using ofloxacin eye drops (4x1), dexamethasone eye drops (4x1), and artificial tears (4x1).

On examination, visual acuity in the right eye was limited to hand movements. Slit-lamp examination revealed conjunctival injection, diffuse corneal edema, and two dense infiltrative lesions in the central cornea, measuring 2x1.5 mm and extending in an arc from the central to the paracentral area (Figure 1). Additionally, there were multiple punctate infiltrates and epithelial defects surrounding the lesions. A 4+ anterior chamber reaction was observed, without hypopyon. Corneal scrapings were collected under sterile conditions from

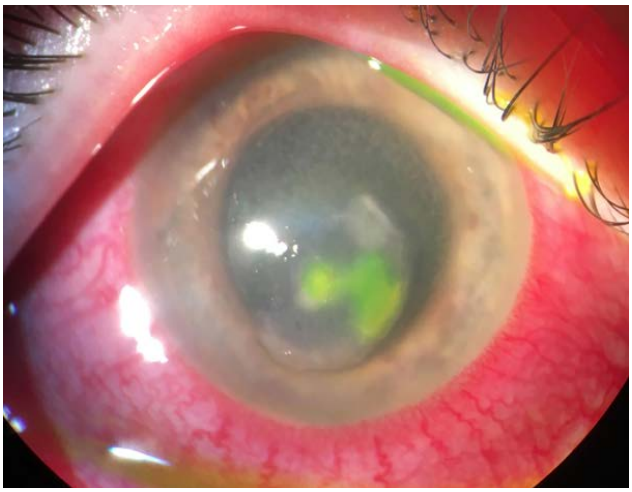

Figure 3. Shows the anterior segment photograph one day after the addition of fortified antifungal treatment.

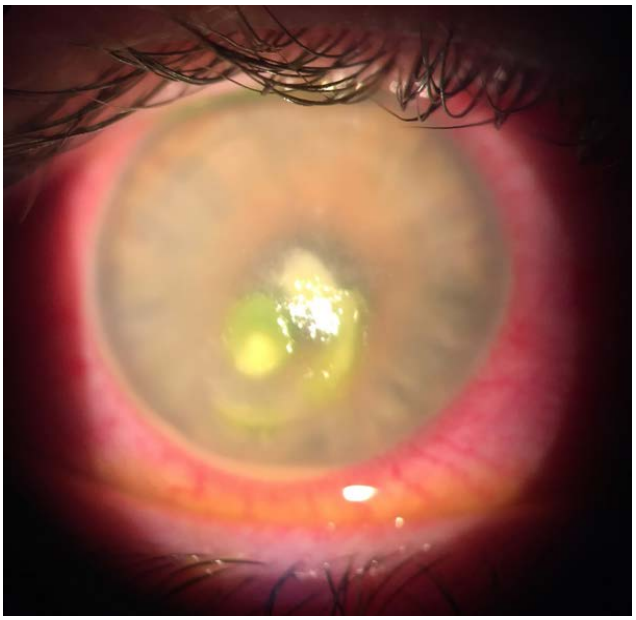

Figure 2. Shows the enlargement of keratitis and the presence of hypopyon on the first day after treatment.

the interface rather than the corneal surface for culture and direct microscopic examination, followed by irrigation with fortified antibiotics (amikacin, clarithromycin, vancomycin). The patient's previous medications were discontinued, and empirical therapy with fortified amikacin (40 mg/ml), clarithromycin (10 mg/ml), vancomycin (50 mg/ml), and cyclopentolate eye drops (2x1) was initiated.

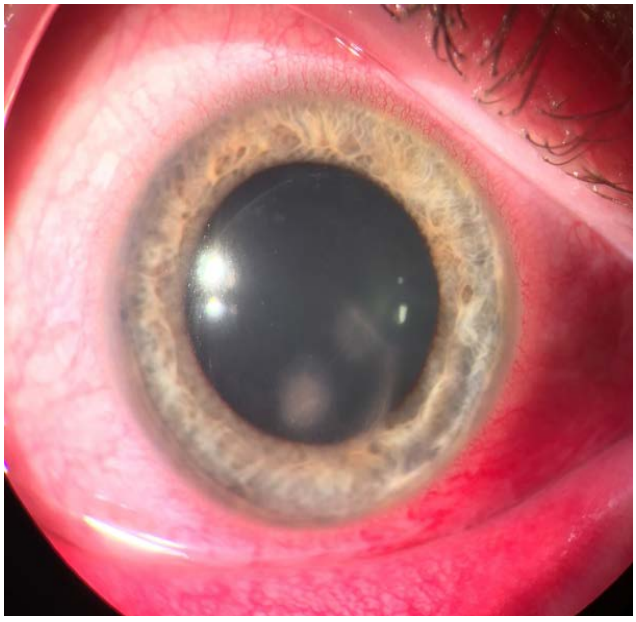

Figure 4. Shows the healing of the lesions with scarring at the follow-up one week later.

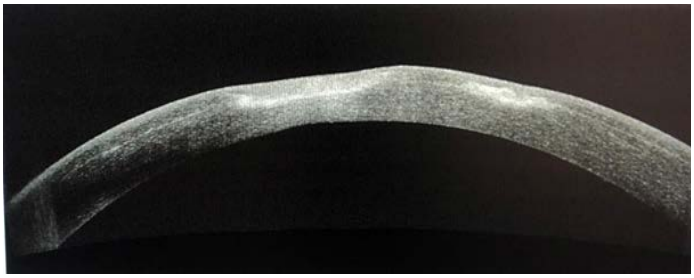

Figure 5. Shows the depth of the scar on anterior segment OCT taken at the first month.

On the first day of follow-up, hypopyon was detected, and the size of the lesions had increased (Figure 2). Due to the progression of keratitis, the treatment regimen was modified to include fortified liposomal amphotericin-B (5 mg/ml) and voriconazole (10 mg/ml) eye drops every hour. On the subsequent day, the hypopyon had resolved, and there was a reduction in corneal edema, infiltrate density, and epithelial defect size (Figure 3). The patient reported significant relief from symptoms. Fortified amikacin, clarithromycin, and vancomycin drops were discontinued, and daily follow-up continued.

By the end of the first week, peripheral punctate infiltrates and corneal edema had resolved, with a significant reduction in the epithelial defect size at the site of the keratitis focus. The keratitis areas were healing with scarring (Figure 4), and uncorrected visual acuity improved to 0.2. There were no signs of active keratitis. Culture results from the samples taken before empirical therapy remained negative. The current treatment continued without cyclopentolate drops. At the 1-month follow-up, the corneal edema and epithelial defect had fully resolved, uncorrected visual acuity improved to 0.6, and a stromal scar was observed. Figure 5 shows an anterior segment OCT image depicting the depth of the scar.

DISCUSSION

Infectious keratitis is a rare but vision-threatening complication following refractive surgery. Various causative agents have been identified in post-LASIK infectious keratitis, with early infections primarily attributed to gram-positive bacteria, and late-onset infections often involving atypical mycobacteria and fungi (7). Predisposing factors include excessive surgical manipulation, disruption of the epithelial barrier/delayed epithelial healing postoperatively, and intraoperative contamination (8). SMILE is the latest addition to the field of refractive surgery. Only a few cases of culture-positive infectious keratitis after SMILE have been reported in the literature, with only one case attributed to a fungal pathogen (5). To our knowledge, no cases, either culture-positive or culture-negative, have been reported from Türkiye. The management of infectious keratitis following SMILE poses challenges due to the difficulty in obtaining adequate corneal scrapings from deep-seated infiltrates. Rapid diagnosis and appropriate treatment require a high degree of suspicion regarding potential causative agents. Our case is the first report of infectious keratitis after SMILE in Türkiye. Based on the literature, empirical antibiotic therapy was initiated, but treatment was quickly adjusted following progression, leading to successful infection control. Although cultures were negative, the dramatic response to antifungal therapy suggests that fungal pathogens should always be considered in similar cases.

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OLGU SUNUMU / CASE REPORT

Coexistence of Celiac Disease, Autism Spectrum Disorder and Duchenne Muscular Dystrophy: A Rare Case Report

Duchenne Kas Distrofisi, Otizm Spektrum Bozukluğu ve Çölyak Hastalığı Birlikteliği: Nadir Bir Olgu Sunumu

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ÖZET

Çölyak hastalığı, genetik yatkınlığı olan bireylerde diyetsel glütene karşı gelişen otoimmün reaksiyonlarla karakterize bir enteropatik hastalıktır. Türkiye'de hastalığın prevalansının % 0,49 ile % 0,97 arasında olduğu bildirilmiştir. Duchenne kas distrofisi, distrofin genindeki mutasyonlardan kaynaklanan, genetik olarak iletilen bir nöromusküler hastalıktır. Hastalık X bağlı resesif olduğundan, erkeklerdeki insidans 1/ 3 500 ile 5 000 arasında değişmektedir. Otizm spektrum bozukluğu, sosyal iletişimdeki eksiklikler ve sınırlı, tekrarlayan davranışlarla karakterize edilen klinik olarak tanımlanmış bir nörogelişimsel bozukluktur. Türkiye'deki mevcut hastalık prevalansı kesin olarak belirlenmemiştir. Literatürde Duchenne kas distrofisi ve çölyak hastalığının birlikte görüldüğü iki vaka bildirilmiştir. Ancak, Duchenne kas distrofisi, çölyak hastalığı ve otizm spektrum bozukluğunun bir arada görüldüğü bir vaka literatürde bulunmamaktadır. Bu yazıda, çölyak hastalığı, Duchenne kas distrofisi ve otizm spektrum bozukluğu bulunan iki yaşındaki erkek bir hasta sunulmaktadır. Genetik temele dayanan bu üç hastalığın birbiriyle ilişkisi, mevcut literatür ışığında incelenmiştir.

Anahtar Kelimeler: Otizm spektrum bozukluğu, Çölyak Hastalığı, Duchenne Kas Distrofisi

ABSTRACT

Celiac disease is an enteropathy characterised by a series of autoimmune reactions against dietary gluten in genetically predisposed individuals. The disease is reported to have a prevalence of between 0.49 % and 0.97 % in Turkiye. Duchenne muscular dystrophy is a genetically transmitted neuromuscular disease resulting from mutations in the dystrophin gene. Since the disease is X-linked recessive, the incidence in boys varies between 1/ 3 500 –5 000. Autism spectrum disorder is a clinically defined neurodevelopmental disorder that is characterised by deficits in social communication and restricted, repetitive behaviours. The current prevalence of the disease in Turkiye is not definitively established. Two cases showing duchenne muscular dystrophy-celiac disease coexistence have been reported in the literature. However, there is no case in the literature in which duchenne muscular dystrophy, celiac disease and autism spectrum disorder coexist. In this article, a two-year-old male patient with celiac disease, duchenne muscular dystrophy and autism spectrum disorder is presented. The interrelationship between these three diseases with a genetic basis is examined in the context of existing literature.

Keywords: Autism spectrum disorder, Celiac Disease, Duchenne Muscular Dystrophy

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INTRODUCTION

Celiac disease (CD) is an enteropathy characterized by a series of autoimmune reactions to dietary gluten in genetically predisposed individuals. The disease is reported to have a prevalence of between 0.49% and 0.97% in Turkiye (1, 2). Duchenne muscular dystrophy (DMD) is a genetically transmitted neuromuscular disease resulting from mutations in the dystrophin gene. Since the disease is X-linked recessive, the incidence in boys varies between 1/3500 -5000 (3).

Autism spectrum disorder (ASD) is a clinically defined neurodevelopmental disorder that is characterised by deficits in social communication and restricted, repetitive behaviours (4). The current prevalence of this disease in Turkiye has not yet been definitively established. Two cases of coexisting DMD-CD have been reported in the literature (5,6). However, there are no cases in the literature in which DMD, CD, and ASD coexist. In this article, a two-year-old male patient with CD, DMD and ASD is presented. The interrelationship between these three genetically based

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diseases is analysed in the context of the existing literature.

CASE REPORT

A two-year-old male patient was admitted with complaints of constipation, abdominal distension and growth retardation. His medical history showed that she was able to hold her head upright at three months, sit with support at seven months, sit unsupported at ten months, crawl at thirteen months and walk at fifteen months. He started spelling at the age of eight months and started speaking with two-syllable words at the age of two years. However, it was learned that the patient was followed up by our child psychiatry outpatient clinic with a diagnosis of ASD since the age of one year due to limited eye contact and repetitive movements since infancy. The patient was diagnosed with DMD P034: Exon 46,47,48,49,50 hemizygous deletion after an aetiological work-up in which a creatine kinase (CK) elevation (>17000 U/L, normal range: 39-308 U/L) was detected 3 months prior to admission. There was no family history of celiac disease or muscle disease. There was no consanguinity between the parents.

On physical examination, body weight was 12 kg (10-25p), height was 95 cm (50-75p) and head circumference was 49 cm (25-50p). Abdominal examination revealed diffuse tenderness in the periumbilical region. There was no defense or rebound. There was no hepatosplenomegaly. On neurological examination, muscle strength was 5/5 in the upper and lower extremities, and there was no Govers deficit. There was no pseudohypertrophy in the legs. Other neurological and systemic examination findings were normal. Hemoglobin 12.3 mg/dl, white blood cell 12 100 /mm^3, platelets 389 000 mm^3, aspartate aminotransferase: 283 U/L (normal range: 0- 56 U/L): 283 U/L (normal range: 0- 56 U/L), alanine aminotransferase: 325 U/L (normal range: 0- 39 U/L) and creatine kinase: 9739 U/L (normal range: 39 to 308 U/L). Other biochemical tests were normal. Serum immunoglobulin A (IgA) and serum immunoglobulin G (IgG) levels were normal for his age. Tissue transglutaminase IgA: 19.6 U/mL (normal range: 0- 10 U/mL) and tissue transglutaminase IgG: 151.6 U/mL (normal range: 0-10 U/mL) were found to be elevated. Upper gastrointestinal endoscopy performed with a prediagnosis of CD an revealed edematous appearance and prominent scalloping in the duodenum. Histopathologic evaluation revealed nearly complete villus atrophy and increased intraepithelial lymphocytes in the duodenum, consistent with modified Marsh type 3C. As a result of these findings, the patient was diagnosed with CD and a gluten-free diet was started. The patient continued to be followed up in our Pediatric Gastroenterology clinic and her gastrointestinal complaints decreased significantly at the 6th month of gluten-free diet.

DISCUSSION

Celiac Disease can be seen with different presentation findings as typical, atypical and silent disease (7). Autoimmune diseases such as selective Ig A deficiency, chromosome anomalies, type 1 diabetes mellitus may accompany CD or CD may be observed in the course of these diseases (8).

Neurological symptoms associated with CD are rare in children. Antibody cross-reactions, immune complex accumulation, neurotoxicity and vitamin or nutrient deficiency and gluten-mediated reactions play a role in the pathophysiology of neurological involvement in CD. Cerebellar ataxia, peripheral neuropathy, epileptic seizures, headaches, mild cognitive disorders, depressive disorders, bipolar disorders, schizophrenia, attention deficit/hyperactivity disorders and autism spectrum disorders may be observed (9). Our patient also exhibited features of both celiac disease and autism spectrum disorder.

Deletions in the dystrophin gene are responsible for DMD pathogenesis (10). Studies have shown that patients with DMD have varying degrees of cognitive impairment. Furthermore, the frequency of ASD and attention deficit/hyperactivity disorder has been found to be higher in DMD compared to the general population (10,11). Gastrointestinal findings in DMD are observed in the progressive stages of the disease and often occur due to smooth muscle involvement. Gastroparesis, decreased intestinal motility, and intestinal pseudo-obstruction may be observed in these patients (8). Nevertheless, the association of CD has only been documented on a few occasions. L. Stenhammar et al. (5) recorded a case of a 13-month-old male patient with DMD associated with CD. Another reported case was a 7-year-old male patient reported by Sharawat et al. (6).

Studies have reported that CD is more common in ASD patients. This is attributed to increased permeability of the intestinal barrier in ASD patients and the different intestinal microbiota compared to healthy individuals (12,13). In our case, the association of CD and DMD was also accompanied by ASD. No cases of CD, DMD, or ASD have been reported in the literature. We aim that our case will contribute to the literature in this respect and we think that more comprehensive studies are needed in this direction.

In conclusion, CD can be observed in DMD and ASD patients. As these patients are frequently disadvantaged and unable to adequately express their symptoms, it is imperative that they be questioned in terms of gastrointestinal symptoms. CD should be considered as a differential diagnosis, and morbidities that may develop should be prevented by ensuring an early diagnosis of CD in patients.

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