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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 Vancouver 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:

- Kocakuşak A, Yücel AF, Arıkan S. Karına nafiz delici kesici alet yaralanmalarında rutin abdominal eksplorasyon yönteminin retrospektif analizi. Van Tıp Dergisi 2006;13(3):90-6.
- Vikse BE, Aasard K, Bostad L, et al. Clinical prognostic factors in biopsyproven benign nephrosclerosis. Nephrol Dial Transplant 2003; 18:517-23.

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, Aasard K, Bostad L, et al. Clinical prognostic factors in biopsyproven benign nephrosclerosis. Nephrol Dial Transplant 2003;18:517-23.

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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.

Journal article published online ahead of print:
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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:
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When sharing patient photos, ensure that the identity is not recognized one-to-one, and images sufficient to identify the disease should be shared. For sharing images that reveal the patient's identity, a consent form in which the patient authorizes the sharing of the image is necessary.

Table 1. Limitations according to article types

Article Types	Word limitation of article	Word limitation of abstract	Limitation of references	Limitation of Tables	Limitation of Figures
Research Article	3500	300	50	6	6
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

Article Types

Selcuk Medical Journal publishes the types of articles briefly described below:

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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.

Case Presentations: Educational case reports on rare cases or conditions that pose difficulties in diagnosis and treatment, offer new treatments or reveal information not included in the literature are accepted for publication in our journal. The case report should include the subheadings Introduction, Case Presentation and Discussion and Coclusion. Interesting and unusual pictures are an advantage in the evaluation process.

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If patient identity is disclosed in patient-identifying pictures, patient consent must be provided to share the picture. Please see Table 1 for word count and specifications.

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OPEN ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

13 Year Retrospective Analysis of Tumors Metastasizing to Bone

Kemiğe Metastaz Yapan Tümörlerin 13 Yıllık Retrospektif Analizi

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ÖZET

Amaç: Kemik, onkolojik hastalarda primer tümörün metastazının sık görüldüğü bir dokudur. Çalışmamızda histopatolojik olarak kemik metastazı saptanan hastaların klinik ve patolojik özelliklerini, sağ kalım durumlarını değerlendirmek amaçlanmıştır.

Gereçler ve Yöntem: Patoloji anabilim dalımızda 2010-2023 yılları arasında kemikte metastatik tümör tanısı alan 107 hastaya ait materyallerin hematoksilen-eozin ve immünohistokimyasal boyalı preparatları retrospektif olarak değerlendirildi. Hastaların yaş, cinsiyet, sağ kalım, klinik sonuçları hastane kayıtlarından elde edildi. Primer odak, tümörün lokalizasyonu, tümörün histopatolojik özellikleri, hastaların sağ kalım durumu retrospektif olarak değerlendirildi.

Bulgular: 107 hastanın yaşları 36 ile 84 arasında değişmekte olup; 61'i erkek, 46'sı kadındı. Kemiğe en sık metastaz yapan tümör akciğer malignitesi (%32.7; n=35) olup onu meme malignitesinin (%22.4; n=24) takip ettiği izlendi. Hastaların % 35.5'inin (n=38)ilk tanısını metastaz ile aldığı tespit edildi. En çok femur kemiğine (%65.4) metastaz izlendi. Beş yıllık sağ kalım analizinde tükrük bezinin adenoid kistik karsinomunun metastazında sağkalımın en kısa (12 ay) olduğu, kolorektal, prostat ve tiroid tümörlerinde en uzun (60 ay) olduğu görüldü.

Sonuç: Kemikte en sık metastazın femurda görüldüğünü ve nadiren atipik bölgelerde de metastaz olabileceği, metastazın en sık adenokarsinom morfolojisinde olmakla birlikte karsinosarkom gibi farklı bir morfolojide de olabileceği saptandı. Hastaların bir kısmının ilk tanısını metastaz ile aldığı ve nadiren primer odağın belirlenemediği gözlendi. Tümör içeren kemik materyalleri değerlendirilirken, metastazların çok çeşitli lokalizasyon ve morfoloji ile karşımıza çıkabileceği akılda tutulmalıdır.

Anahtar Kelimeler: Kemik, metastaz, histopatoloji, malign tümör

ABSTRACT

Aim: Bone is a tissue in which metastasis of the primary tumor is common in oncological patients. In our study, we aimed to evaluate the clinical and pathological features and survival status of patients with histopathological bone metastases.

Materials and Methods: Hematoxylin-eosin and immunohistochemical stained preparations of materials belonging to 107 patients diagnosed with metastatic tumors in bone between 2010 and 2023 in our pathology department were evaluated retrospectively. Age, gender, survival, and clinical outcomes of the patients were obtained from hospital records. Primary focus, localization of the tumor, histopathological features of the tumor, and survival status of the patients were evaluated retrospectively.

Results: The ages of 107 patients ranged from 36 to 84; 61 of them were men and 46 were women. The tumor that most frequently metastasized to the bone was lung malignancy (32.7%; n=35), followed by breast malignancy (22.4%; n=24). It was determined that 35.5% of the patients (n = 38) were initially diagnosed with metastasis. Metastasis was mostly observed in the femur bone (65.4%). In the five-year survival analysis, survival was observed to be shortest (12 months) in metastasis of adenoid cystic carcinoma of the salivary gland and longest (60 months) in colorectal, prostate and thyroid tumors.

Conclusion: It was determined that the most common metastasis in bone was in the femur, and rarely in atypical areas. Although metastasis was most common in adenocarcinoma morphology, it could also be in a different morphology such as carcinosarcoma. It was observed that some of the patients were initially diagnosed with metastasis and rarely the primary focus could not be determined. When evaluating tumor-containing bone materials, it should be kept in mind that metastases can present with a wide variety of localizations and morphologies.

Keywords: Bone, metastases, histopathology, malignant tumor

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INTRODUCTION

One of the most frequent locations for primary tumor metastases in cancer patients is the bone. Metastatic bone cancers are more common than primary bone tumors. Every year, at least 100,000 people are diagnosed with bone metastases. However, the precise number is unknown (1, 2). Ninety percent of cancer-related fatalities globally are attributable to metastasis, with over 1.5 million people experiencing bone metastases (3). Additionally, in cases with unknown primary cancers, it may be identified as the sole finding (4). The prognosis is mainly determined by the histological characteristics of the initial tumor; however, the presence of bone metastases is a poor prognostic indication (4).

Similar to other organs, bone metastasis is a complex situation where tumor cells travel to the surrounding tissues singly or in groups before entering the circulatory or lymphatic system and eventually finding their way to the bone (5).

Bone metastases are more typically seen in the latter stages of breast cancer in women and prostate cancer in men. Epithelial malignancies often spread to the bone (5). Lung, thyroid cancers, melanoma, and kidney tumors also frequently spread to the bone (2, 6). Hematological cancers like lymphoma and myeloma can spread to the bone (2). The lung, liver and bone are the first three areas that should be considered in cases of metastatic cancer. Commonly affected areas are the femur, humerus, skull, vertebrae, ribs and pelvis (7).

Our study aimed to evaluate the clinical findings, histopathological features and survival status of patients with tumors that metastasized to the bone.

MATERIALS AND METHODS

The study included patients who were histopathologically diagnosed with metastatic tumors in the bone within 13 years and came to our Department of Medical Pathology between 2010 and 2023 and whose bone materials were available. The research excluded patients with hematolymphoid malignancies and primary bone cancers. The ethics committee at our university gave its approval for this retrospective study (21.06.2023/20.478.486/1904).

The hospital automation system provided information on the patients' ages, genders, presenting complaints, the bone or bones the tumor metastasized to, other metastatic foci, and their survival status. The patient's bone tissue samples were cut to 3–4 micrometers thick, fixed with 10% buffered formalin, and then stored in a 10% nitric acid solution for decalcification. Slides were then stained with standard hematoxylin and eosin and any additional immunohistochemically stained preparations that were thought necessary or desirable were retrospectively collected based on clinicopathological findings.

Statistical analysis

The normality of continuous data distribution was tested using the Kolmogorov-Smirnov test or the Shapiro-Wilk test. Continuous data were compared using the independent sample t test or the Mann-Whitney U test. The SPSS ver. 21 software package (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The measurement of overall survival (OS) was conducted from the initiation of primary therapy to the occurrence of death or the last follow-up. The analysis of associations was conducted using either Chi-square or Fisher's exact test. The Kaplan-Meier method was employed to estimate survival curves, and the log-rank test was utilized to calculate p-values. The study also included Cox proportional hazards regression models to investigate the association between survival and variables in both univariate and multivariate models. Significance was attributed to values of $p < 0.05$.

RESULTS

The ages of the patients diagnosed with metastases in a total of 107 bones ranged between 36 and 84 years (median 62). The male/female ratio of the patients, 61 of whom were male and 46 of whom were female, was 1.32. The distribution of patients according to gender of primary focus is given in Table 1. The youngest patient was a 36-year-old female patient diagnosed with ductal adenocarcinoma metastasis of the breast. The oldest patient was an 84-year-old man with cutaneous squamous cell carcinoma metastases. It was determined that most of the patients (84, 78.5%) applied

Table 1. Metastatic Tumor Distribution by Main Focus and Gender

Primary Tumor Focus	Male, n(%)	Female, n(%)	Total n(%)
Lung	30 (85.7)	5 (14.3)	35 (32.7)
Breast	0 (0)	24(100)	24 (22.4)
Prostate	9 (100)	0(0)	9(8.4)
Not found	6 (66.7)	3 (33.3)	9 (8.4)
Kidney	5 (55.6)	4 (44.4)	9 (8.4)
Upper gastrointestinal	5 (71.4)	2 (28.6)	7 (6.5)
Thyroid	0 (0)	4 (100)	4 (3.7)
Cutaneous	1 (33.3)	2 (66.7)	3 (2.8)
Hepatopancreaticobiliary	2 (66.7)	1 (33.3)	3 (2.8)
Colorectal	1 (100)	0 (0)	1 (0.9)
Bladder	1 (100)	0 (0)	1 (0.9)
Uterus	0 (0)	1 (100)	1 (0.9)
Salivary gland	1 (100)	0 (0)	1 (0.9)
Total	61 (57)	46 (43)	107 (100)

to the hospital with complaints of pain. It was observed that the most frequently metastasized cancer was lung adenocarcinoma, followed by ductal adenocarcinoma of the breast. Macroscopic images of the patients materials are presented in figure 1. The following macroscopic images show colon adenocarcinoma metastases to the hand's distal phalanx (A), Lung adenocarcinoma metastases to the tibia bone (B), Squamous cell carcinoma of the lung metastases to the fibula bone (C), Breast ductal adenocarcinoma metastases to the femur bone (D), Lung adenocarcinoma metastasis to the femur bone (E), Clear cell renal cell carcinoma metastases to the humerus bone (F), Metastatic prostate cancer to the femur bone (G), Metastatic thyroid cancer to the femur bone (H), Metastasis of lung squamous cell carcinoma to the humerus bone (I)

The most commonly involved bone in metastasis is the femur (n=71, 60.5%), followed by the humerus (n=13, 15.8%), vertebrae (n=8, 7.4%), tibia (n=6, 5.6%) and pelvis (n=6, 5.6%), forearm (n=2, 1.8%), scapula (n=1, 0.9%) and distal phalanx of the hand (n=1, 0.9%).

Most of primary tumors metastasizing to the femur 81.7% (n=58) have adenocarcinoma morphology, followed by squamous cell carcinoma 7% (n=5), malignant epithelial tumor (Unable to determine specific subtypes) 7% (n=5), and small cell carcinoma of the lung n=3 (4.2%). It was noted

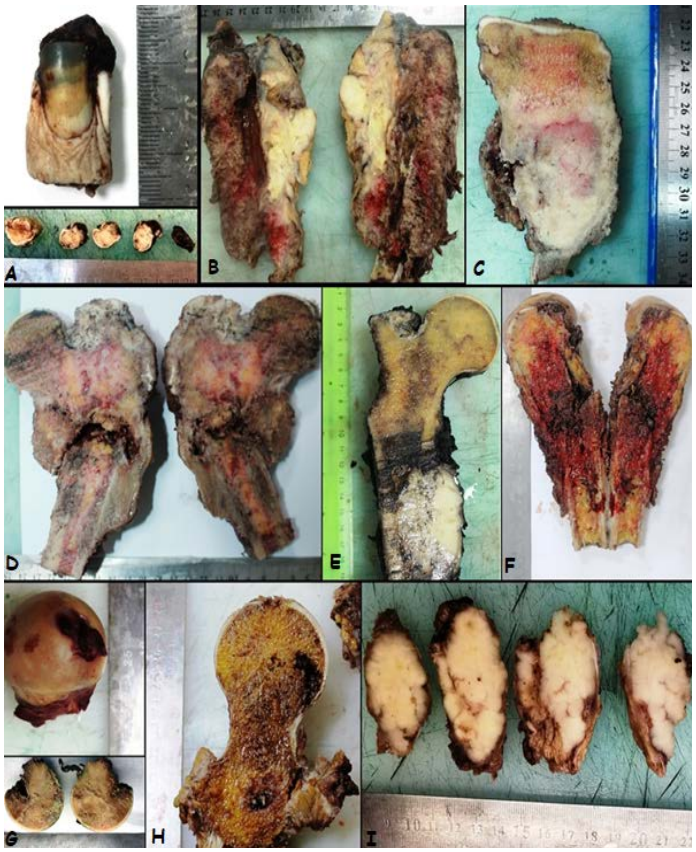

Figure 1. Macroscopic images of the patients materials

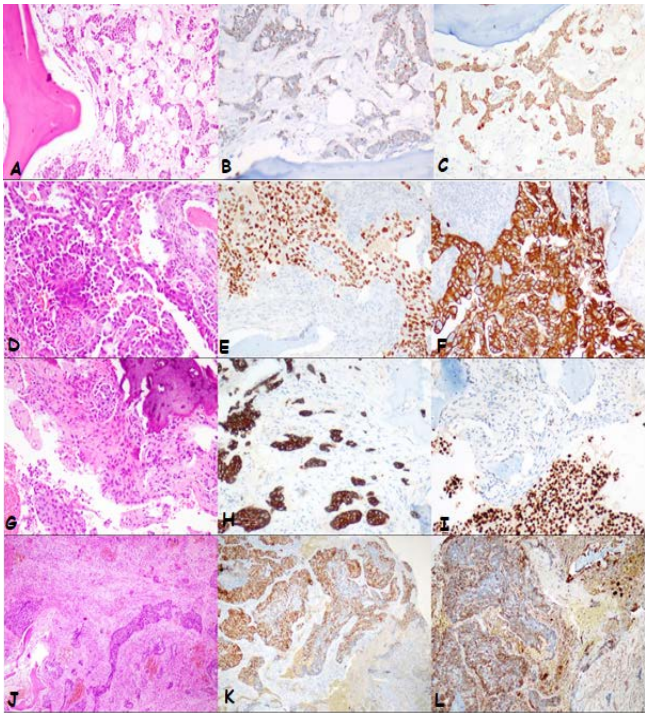

Figure 2. Microscopic findings of the metastatic tumors

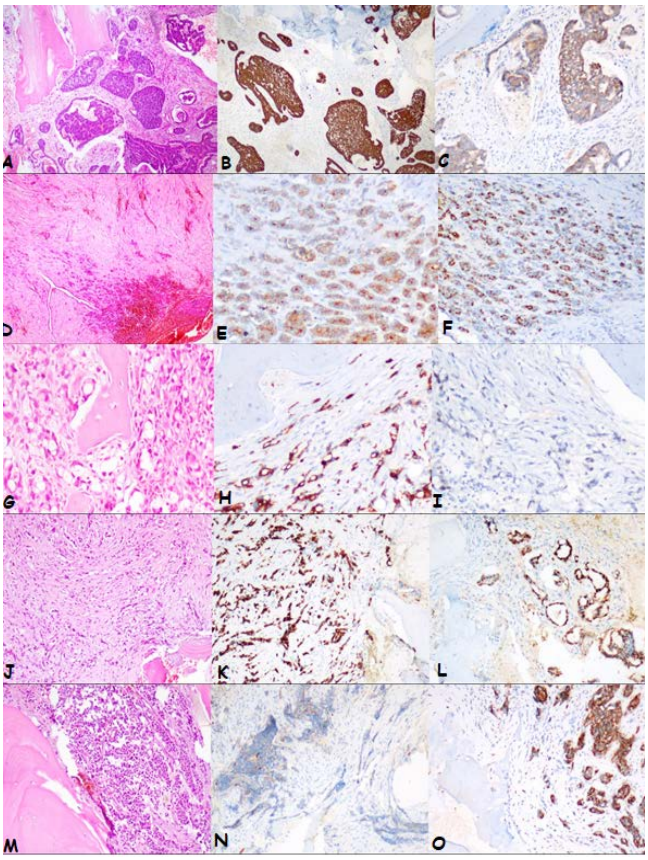

Figure 3. Microscopic findings of the metastatic tumors

Table 2. Tumor histopathological subtypes of metastatic patient

Tumor Histopathology	n	%	Total (%)
Adenocarcinoma	85	86.9	77.6
Squamous cell carcinoma	10	9.3	86.9
Malignant epithelial tumor (Unable to determine specific subtypes)	7	6.5	93.5
Small cell carcinoma	4	3.7	99.1
Carcinosarcoma	1	0.9	100.0
Total n (%)	107	100.0	100.0

that 32.4% (n=23) of primary tumors that metastasized to the femur were diagnosed with metastasis. The most common primary focus is lung in 29.6% (n=21), breast in 22.5% (n=16), renal cell carcinoma of the kidney in 11.3% (n=8), prostate in 9.9% (n=7), upper gastrointestinal (GI) system in 8.5% (n=7). =6), thyroid 4.2% (n=3), not found 7% (n=5), pancreaticobiliary 2.8% (n=2), salivary gland 1.4% (n=1) (Table 2). Microscopic findings of the cases are shown in figure 2 and 3. Metastatic prostate adenocarcinoma: (A) Epithelial island-like tumor cell groups following bone trabeculae (H&E x100); (B) Positivity of tumor cells with PSAP (x100), (C) Positivity of tumor cells with PanCK (x100). Metastatic lung adenocarcinoma: (D) Infiltration of epithelial cells in the papillary architecture adjacent to the bone trabeculae (H&E x100), (E) Positivity of tumor cells with TTF-1 (x100), (F) Positivity of tumor cells with CK7 (x100), Metastatic urothelial carcinoma: (G) Epithelial cell groups adjacent to bone trabeculae (H&E x100), (H) Positivity of tumor cells with CK7 (x100), (I) Positivity of tumor cells with GATA3 (x100), Metastatic carcinosarcoma: (J) Metastatic foci where epithelial and mesenchymal components are observed together between bone trabeculae (H&E x40), (K) Positivity of tumor cells with estrogen receptor (x40), (L) Positivity of tumor cells with vimentin (x40) and Metastatic adenoid cystic carcinoma: (A) Epithelial tumor islands adjacent to bone trabeculae (H&E x40), (B) Positivity of tumor cells with CK7 (x40), (C) Positivity of tumor cells with CD117 (x100), Metastatic thyroid carcinoma: (D) Epithelial tumor cell groups in a relatively follicular pattern with occasional overlap between bleeding areas adjacent to millimetric bone trabeculae (H&E x40), (E) Positivity of tumor cells with thyroglobulin (x200), (F) Positivity of tumor cells with TTF-1 (x100), Metastatic gastric signet ring cell carcinoma: (G) Intracytoplasmic mucin accumulation between bone trabeculae, sometimes glandular, sometimes scattered individual malignant epithelial cell infiltration (H&E x200), (H) Pale focal positivity of tumor cells with CDX2 (x200), (I) Positivity of tumor cells with CK7 (x200), Metastatic breast ductal adenocarcinoma: (J) Epithelial cell infiltration adjacent to bone trabeculae (H&E x100), (K) CK7 positivity of tumor cells (x100), (L) GATA 3 positivity of tumor cells (x100), Metastatic small cell neuroendocrine carcinoma: (M) Tumor cell infiltration adjacent to bone trabeculae (H&E x100), (N) Pale punctate positivity of tumor cells with PanCK (x100), (O) Positivity of tumor cells with chromogranin (x100)

Of the primary tumors that metastasized to the humerus, 30.8% (n=4) were lung and breast, 7.7% (n=1) could not be found, and 1 (7.7%) patient each had metastasis from the uterus and thyroid. The first diagnosis of malignancy were reported 46.2% (n=6) of the metastatic patients. It was observed that most of the metastases, 69.2% (n=9), had adenocarcinoma morphology. It was noted that 1 (7.7%) patient had carcinosarcoma. Among these metastatic tumors, one of them (12.5%) was originated from prostate and 37.5% (n=3) of them from breast.

Adenocarcinoma was the most prevalent metastatic tumor (77.6%; n = 83), with lung metastases accounting for 26.9% of cases (n = 7). Of the patients, 70 (65.4%) had multiple bone metastases. Hematoxylin and eosin-stained sections were used to identify the significant focus in 79 (73.8%) of the tumors, coupled with further immunohistochemistry slide targeted at the primary focus. Based on histopathological analysis, adenocarcinomas accounted for 23 (65.7%) of the tumors that metastasized from the lung to the bone, squamous cell carcinomas for 9 (25.7%), and small cell carcinomas for 3 (8.6%). Diagnoses were established by immunohistochemistry using the following done: p63, synaptophysin, chromogranin, CDX-2, CK7, TTF-1, HMWCK, CD56, CK19, EMA, NAPSIN A, PanCK, and synaptophysin. The most common site of metastasis in bone was the femur (n=21).

According to histopathology, the tumor that spread from the colorectal area to the bone had an adenocarcinoma-like appearance. It was determined that CK7, CDX-2, CK20, CEA, CK19, SATB2 positive or negative slides were used immunohistochemically to make the diagnosis. If the 24 tumors that spread from the breast to the bone, 22 (91.6%) had ductal adenocarcinoma-like histopathology, whereas the remaining 2 (8.4%) had lobular adenocarcinoma-like morphology. Immunohistochemistry was used to identify GCDFP15, GATA3, Mamaglobin, CK7, Estrogen, Progesterone, and e-cadherin slides as positive or negative. A tumor exhibiting carcinosarcoma morphology on histopathology metastasized from the uterus to the bone. It was discovered that the vimentin, PanCK, EMA, and CK5/6 slides that were immunohistochemically positive or negative were used to make the diagnosis. Noted was metastasis to the humerus bone.

Nine tumors with an acinar-type adenocarcinoma morphology were found to have metastasized from the prostate to the bone. By employing immunohistochemistry, it was discovered that the diagnosis was achieved in cases of positive or negative findings using the slides PSA, HMWCK, NKX3.1, AMACR, CK7, CK20, and PSAP. The most prevalent locations for bone metastases were seven femurs (77.7%), one pelvic (11.1%), and one vertebral (11.1%). Histopathologically, four tumors with a follicular pattern papillary carcinoma

Table 3. Primary foci of tumors that metastasize to bone and their diagnosis based on metastasis

Primary Tumor Focus	Diagnosed with Known, n(%)	Diagnosed with Metastasis, n(%)	Total Percentage n(%)
Colorectal	1 (100)	0(0)	1 (0.9)
Lung	23 (65.7)	12 (34.3)	35 (32.6)
Upper gastrointestinal	3 (42.9)	4 (57.1)	7 (6.4)
Breast	22 (91.7)	2 (8.3)	24 (22.4)
Cutaneous	2 (66.7)	1 (33.3)	3 (2.7)
Hepatopancreaticobiliary system	2 (66.7)	1 (33.3)	3 (2.7)
Kidney	5 (55.6)	4 (44.4)	9 (8.4)
Prostate	8 (88.9)	1 (11.1)	9 (8.4)
Thyroid	2 (50)	2 (50)	4 (3.7)
Not found	0 (100)	9 (100)	9 (8.4)
Bladder	0(0)	1 (100)	1 (0.9)
Uterus	1 (100)	0 (0)	1 (0.9)
Salivary gland	1 (100)	0 (0)	1 (0.9)
Total	70 (62.6)	37 (37.4)	107 (100)

Table 4. Univariate and Multivariate analysis results of tumors according to histopathological types

	Univariate		Multivariate	
Tumor Histopathology	**OR (95% CI)**	**p value**	**OR (95% CI)**	**p value**
Adenocarcinoma	Reference		Reference	
Squamous cell carcinoma	2.65 (1.33-5.27)	0.005	2.60 (1.28-5.30)	0.008
Malignant epithelial tumor (Unable to determine specific subtypes)	0.71 (0.30-1.66)	0.431	0.75 (0.28-1.98)	0.564
Small cell carcinoma	1.99 (00.61-6.47)	0.260	1.90 (0.56-6.43)	0.302
Carsinosarcoma	9.64 (1.24-74.78)	0.030	9.88 (1.24-78.60)	0.030
Diagnosis with metastasis				
Yes	Reference		Reference	
No	1.12 (0.72-1.74)	0.625	1.18 (0.71-1.98)	0.492
Number of metastatic focus				
Soliter	Reference		Reference	
Multible	1.28 (0.82-2.00)	0.275	1.27 (0.78-2.08)	0.335
Gender				
Male	Reference		Reference	
Female	0.72 (0.47-1.12)	0.147	0.93 (0.58-1.50)	0.778

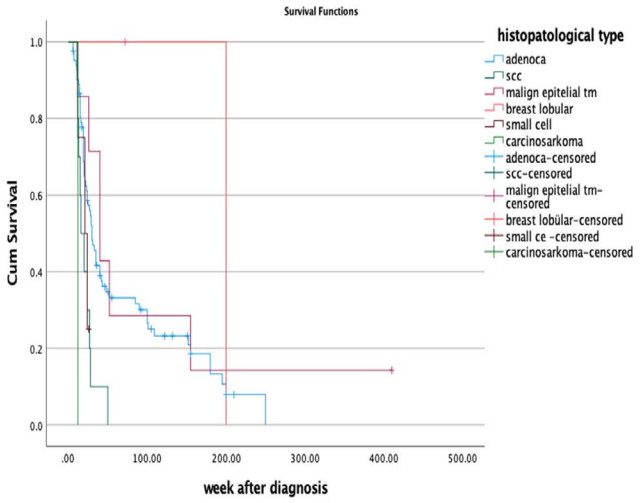

Figure 4. Carcinosarcomas have a much lower survival duration than other types

morphology had spread from the thyroid to the bone. It was discovered that in cases of positive or negative thyroglobulin, TTF-1, CK7, and CK20 slides, immunohistochemistry was used to make the diagnosis. 25% (n=1) of the cases of metastasis were found in the humerus and 75% (n=3) in the femur.

According to histopathology, three tumors with cutaneous-to-bone metastases were squamous cell carcinomas. It was discovered that immunohistochemistry was used to make diagnoses in cases where the findings of the PanCK, P63, P40, HMB45, and MelanA slides were either positive or negative. Nine tumors that spread from the kidney to the bone exhibited clear cell carcinoma of the kidney in terms of histopathology. It was discovered that CK7, CK20, AMACR, CD10, RCC, CA IX, vimentin, and PAX8 slides that were immunohistochemically positive or negative were used to make the diagnosis. Eight patients (88.9%) had metastases to their femur malignancy, whereas one patient (11.1%) had metastases to their forearm. Histopathologically, the tumor that spread from the bladder to the bone appeared to be papillary urothelial carcinoma.

It was discovered that CK7, CK20, GATA 3, and AMACR slides that were immunohistochemically positive or negative were used to make a diagnosis. The primary focus could not be determined with immunohistochemical results in 9 (8.4%) of the patients (Table 3). Of the patients, 69 (64.5%) had a primary diagnosis that was confirmed during follow-up, and 38 (35.5%) had metastases at the time of diagnosis (Table 3). Of the patients having metastases at admission, twenty-five (65.8%) and thirteen (34.2%) were female. Of the 69 patients with a recognized diagnosis, thirty-three (47.8%) were male and thirty-six (52.2%) were female. For 41% of male patients and 28.3% of female patients, the first diagnosis of metastasis was made.

The most common metastasis in men patients was to the femur bone (37, 60.7%); followed by humerus (6, 9.8%), pelvis (5, 8.2%), vertebrae (5, 8.2%), tibia (5, 8.2%), forearm (2, 3.3%) and hand bones (1, 1.6%). The femur (34, 73.9%) is the most often metastasized bone in female patients. Followed by humerus (7, 15.2%), vertebrae (3, 6.5%), tibia (1, 2.2%), and scapula (1, 2.2%). Male patients did not show signs of scapula metastases, whereas female patients did not show signs of metastases to the vertebrae, forearm, or hand bones. In our study, the median survival time for patients was 30 weeks for adenocarcinoma, 16 weeks for squamous cell carcinoma, 21 weeks for small cell carcinoma, 12 weeks for carcinosarcoma (Table 4). Log Rank analysis revealed a statistically significant difference ($p = 0.002$) in the survival duration between the tumors. Carcinosarcomas have a much lower survival duration than other types (Figure 4).

DISCUSSION

In our study, the relationship between the bones in which the metastatic tumor foci were localized in patients whose cancer spread to the bone, the histopathological characteristics of the tumors, the age, gender of the patients, and survival symptoms after diagnosis were investigated. Some patients may present to pathology with a preliminary diagnosis of a primary bone tumor because isolated metastases in long bones might resemble primary sarcomas (8). 40 (37.4%) of our patients had a preliminary diagnosis of bone cancer when they arrived at the clinic. Most patients with bone metastasis have been reported to be between 40 and 60 (9). In our study, the ages of the patients ranged between 36 and 84, and the median age was 62. Patients with bone metastases most often consult a doctor with bone pain. In evaluating these patients, accompanying pathological fractures can be detected (7, 10) (11). In our study, 78.5% of patients who complained of bone pain had pathological bone fractures. Pathological fractures, most commonly in the proximal femur, occur in 10–30% of patients (12). Our study noted that the primary tumor most frequently metastasized to the femur (60.5%) in the patients.

Bone metastases are primarily seen in the pelvis, sternum, femur, ribs, and vertebrae (7). In our study, the most frequently involved bone was the femur (n=71, 60.5%), followed by the humerus (n=13, 15.8%), vertebra (n=8, 7.4%), tibia (n=6, 5.6%) and pelvis (n=6, 5.6%), forearm (n=2, 1.8%), scapula (n=1, 0.9%), and distal phalanx of the hand (n=1, 0.9%). Metastasis of the male patient diagnosed with colon adenocarcinoma to the distal phalanx bone in the hand was noted. Research involving 712 individuals with bone metastases revealed metastases in various locations, including the elbow, knee and face bones (11). In our study metastasis was observed in different localizations such as hand bone, scapula and radius bone, and the distribution of these metastasis was varied according to gender. In male and female patients, the most common metastasis was observed in the femur bone (34, 73.9%) (37, 60.7%); metastases to the scapula bone were not observed in male patients and metastases to the vertebrae, forearm and hand bones were not observed in female patients.

Adenocarcinoma is the most common epithelial tumor among bone metastasis (13). In our study, the most common morphology was adenocarcinoma (77.6%), followed by squamous cell carcinoma (9.3%). The frequency of metastasis to bone is significantly affected depending on the primary focus. The rate of metastasis to bone in breast and prostate cancers is about 70%. Other primary tumors that metastasize to bone include thyroid carcinomas, malignant melanoma, and renal cell carcinoma of the kidney (6). Lung, breast and prostate was the most common metastatic malignancies according to our study. Bone metastasis has been reported at a rate of 3-15% in primary GI tract tumor malignancies (14). In our study, we observed upper GI metastases in 7 patients (6.5%) and GI metastases in 1 patient (0.9%) in 7.3% of the patients. Bone involvement can also be observed in hematological malignancies, such as lymphoma and plasma cell neoplasia (2). We did not include these patients in our study.

The primary focus cannot be detected in 3-15% of metastatic malignancies, and 5-20% of this is seen to be skeletal metastasis (9). Despite additional immunohistochemical methods in our study, the primary focus could not be detected in 9 (23.7%) patients. In a cohort study conducted in Denmark, the most common metastasizing primary cancers were prostate, breast, and lung, and 7.5% of patients showed metastasis to bone (15). In our study, the primary focus was most frequently detected in the lung, followed by breast and prostate. Metastasis to the humerus in 1 patient diagnosed with uterine carcinosarcoma was noted. A study conducted in the USA reported that patients with solid tumors had metastases to the bone more frequently in the first 2 years (9, 16). Our study detected metastases in 67 (62.6%) patients during follow-up after diagnosis. Bone is the third most common site of metastasis after lung and liver (8). There is limited data in the literature on which bone metastases occur depending on the location of the primary focus. In our study, we found that the primary tumors that metastasized to the femur were most commonly adenocarcinoma morphology (78.9%, n=56), and the primary focus was the lung (29.6%, n=21), followed by the breast (22.5%, n=16). We also observed that metastasis to the humerus bone occurred most frequently from lung and breast (30.8%, n=4). It was determined that 37.5% (n=3) of the primary tumors that metastasized to the vertebrae originated from the breast, and one patient (12.5%) originated from the prostate.

The tumor's primary focus and histopathological subtype and the time taken for bone metastasis vary (17). In our study, the time of patients with adenocarcinoma metastasis was 30 weeks. In comparison, it was 16 weeks for patients with squamous cell carcinoma metastasis and 40 weeks for patients with malignant epithelial tumor (Unable to determine specific subtypes) metastasis. It was observed that the duration was 21 weeks for patients diagnosed with small cell carcinoma, and 12 weeks for patients diagnosed with carcinosarcoma. According to Log Rank analysis regarding survival time among tumors, it was observed that the survival time of carcinosarcomas was significantly shorter than the others.

It has been shown that the primary cannot be found in 3-15% of all patients with cancer and that 5-20% of them have skeletal metastases (9). There is a study of CK7, CK20 panel, and organ-specific/organ-restricted markers in metastatic carcinoma to suggest or confirm the primary focus of metastasis (13). For skeletal metastases of unknown primary; the primary could be identified in 85% of cases by a series of methods such as anamnesis, physical examination, laboratory tests, imaging methods of abdomen and pelvis, and finally biopsy of the most accessible bone lesion (18). Errani et al. used the immunohistochemical method to determine the primary in 52% of metastasis cases of unknown origin (19). In our study, out of 107 patients with bone metastases, different immunohistochemical panels were applied to patients whose primary diagnosis was unknown or whose primary diagnosis was known but to confirm metastasis. CEA, CK19, EMA, NAPSIN A, PanCK, p63, synaptophysin, chromogranin, CDX-2, CK7, TTF-1, HMWCK, CD56 slides for lung adenocarcinoma, CK20, CK7 CDX-2, CEA, SATB2 slides for the GI system, CK20, CK7, CDX-2, CEA, CK19, SATB2 slides for hepatopancreatic biliary system, GCDFP15, GATA3, Mamaglobin, CK7, Estrogen, Progesterone, e-cadherin slides for breast ductal adenocarcinoma, PanCK, AMACR, CK7, CK20, NKX3.1 for prostate, PSA, PSAP, HMWCK, P63, PSAP slides for the prostate, thyroglobulin, TTF-1, CK7, CK20 slides for the thyroid, PanCK, P63, P40, HMB45, MelanA slides for the cutaneous, CK7, CK20, AMACR, CD10, RCC, CA IX, vimentin, PAX8 slides for the kidney, and CK7, CK20, GATA 3, AMACR for the bladder. Unfortunately, the primary focus could not be detected in a small number of patients (n: 9, 8.4%). A situation that may cause: This situation may be due to the decalcification process (waiting with 10% nitric acid until they become cuttable) before fixation.

Metastasis to hand bones is observed very rarely. In a study conducted in 1984 by reviewing all the literature, metastases to the hand bones were detected in 0.1% of all metastatic patients (163 patients). It has been observed that metastases from the lung, breast, and kidney are the most common (20). More recently, in a study conducted in 2014, GI metastases were noted in 25% of patients (21). Our study detected colon adenocarcinoma metastasis from the hand bones to the distal phalanx in 1 patient.

CONCLUSION

Among bone metastasis the most common side was femur and rarely others. Metastasis was most commonly in the morphology of adenocarcinoma, but determined also in different morphology, such as carcinosarcoma. It was observed that some of the patients were first diagnosed with metastasis. Rarely the primary focus could not be determined. The survival rate of metastatic tumors varies, with the lowest survival rate being in salivary gland tumor metastases. When evaluating tumor-containing bone materials, it should be remembered that metastases can present with various localizations and morphologies.

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OPEN ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Correlation Between BRAF^{V600E} Positivity and Recurrence and Poor Prognosis in Preoperative Fine Needle Aspiration Biopsy of Papillary Thyroid Carcinoma

Papiller Tiroid Kanserlerinde Preoperatif İnce İğne Aspirasyon Biyopsisinde Braf^{V600E} Pozitifliğinin Nüks ve Kötü Prognozla İlişkisi

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ÖZET

Amaç: Tiroid maligniteleri epitelyal ve non-epitelyal olmak üzere iki ana gruptan oluşmaktadır. Epitelyal olanlar; papiller, folliküler ve anaplastik karsinomlardır. Non-epitelyal olan ise medüller karsinomlardır. Papiller tiroid kanseri (PTK) tüm tiroid maligniteleri içerisinde %80'lik bir kısmı oluşturmaktadır ve bu maligniteler içerisinde tedaviye en iyi yanıt alınanlardan birisidir. Papiller tiroid kanseri gelişiminde tümörogenezisin moleküler genetiğinde A-Raf, B-Raf (BRAF^{V600E}) ve C-Raf olmak üzere 3 farklı Raf kinaz mevcuttur. Çalışmamızda papiller tiroid kanserlerinde cerrahi sonrası oluşan nükslerde BRAF^{V600E} pozitifliğinin nüks açısından prognostik faktörlerle ilişkisini ortaya koymayı amaçladık.

Gereç ve Yöntem: Papiller tiroid kanseri nedeni ile daha önce ameliyat edilmiş ve nüks gelişmiş olan hastaların ameliyat öncesi yapılan ince iğne aspirasyon biyopsi preparatları temin edildi. Bu preparatlar üzerinden kanserli bölge işaretlendi ve işaretli alanlardaki hücrelerden DNA izole edildi. İzole edilen DNA üzerinde pyrosequence dizi analizi yöntemi ile BRAF^{V600E} mutasyonu varlığı araştırıldı. Hastaların papiller tiroid kanserinde nüks açısından diğer prognostik kriterleri de kayıt altına alınarak BRAF^{V600E} mutasyonu ile ilişkileri istatistiksel olarak analiz edildi.

Bulgular: Papiller tiroid kanserlerinde nüks nedeni ile ameliyat edilen hastalarda tüm çalışma grubunda BRAF^{V600E} pozitifliği oranı %70,8 olarak bulunmuştur. Ayrıca kapsül invazyonu, yumuşak doku invazyonu, evresi, nüks zamanı ve lenf nodu metastazı ile BRAF^{V600E} mutasyonu arasında pozitif korelasyon gösterilmiştir.

Sonuç: BRAF^{V600E} mutasyonu ile papiller tiroid kanserlerindeki bazı kötü prognostik kriterler arasında pozitif ilişki gösterilmiştir.

Anahtar Kelimeler: Tiroid, İnce iğne biyopsi, Papiller, Kötü Prognoz, BRAF^{V600E}, Sitoloji

ABSTRACT

Aim: Papillary thyroid carcinoma (PTC) accounts for 80% of all thyroid malignancies and is one of the most responsive thyroid malignancies to treatment. There are three different Raf kinases implicated in the molecular genetics of tumorigenesis for the development of papillary thyroid cancer: ARAF, BRAF (BRAF^{V600E}), and CRAF. Among the BRAF^{V600E} mutations, T1799A (V600E amino acid translocation) is the most common and also observed in thyroid cancer. In our study, we aimed to establish the association between BRAF^{V600E} positivity in preoperative fine needle aspiration biopsy and prognostic factors in patients with recurrence.

Methods: Preoperative fine-needle aspiration biopsy slides were obtained from patients who had previously undergone surgery for PTC and had recurrence. The cancerous areas on the slides were marked and DNA was isolated from cells within the marked areas. The presence of the BRAF^{V600E} mutation was established from the DNA using the pyrosequencing method. Other patient prognostic criteria regarding the recurrence of PTC was also recorded and their correlation with the BRAF^{V600E} mutation was statistically analyzed.

Results: In patients who underwent surgery for recurrence of papillary thyroid cancer, the BRAF^{V600E} positivity rate was 70.8% of the study group. Furthermore, capsular invasion, soft tissue invasion, stage, recurrence time, and lymph node metastasis positively correlated with BRAF^{V600E} mutation.

Conclusion: A positive correlation between BRAF mutation and some poor prognostic criteria in PTC was observed.

Keywords: Thyroid, papillary, fine needle biopsy, poor prognosis, BRAF^{V600E}, cytology

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INTRODUCTION

Papillary thyroid carcinoma (PTC) accounts for 80% of all thyroid malignancies and is one of the most responsive cancers to treatment. The survival rate for PTC is 90–95% over 5–10 years. The diagnosis of PTC is made by radiological imaging and fine needle aspiration biopsy (FNAB) (1). There are three different Raf kinases implicated in the molecular genetics of tumorigenesis for the development of PTC: ARAF, BRAF ($BRAF^{V600E}$), and CRAF. Many variations of $BRAF^{V600E}$ mutations have been reported, with T1799A (V600E amino acid translocation) the most common and occurring in thyroid cancer (2,3).

There are published studies that show that papillary carcinomas with a $BRAF^{V600E}$ gene mutation are aggressive and have a poor prognosis. For this reason, it is becoming increasingly important for preoperative treatment protocol for PTCs and postoperative follow-up to determine the presence of the $BRAF^{V600E}$ mutation (4).

In our study, we aimed to investigate $BRAF^{V600E}$ mutation positivity using FNAB preparations from patients diagnosed with and surgically treated for PTC who were followed-up for at least 1 year and who experienced recurrence during the follow-up period. Therefore, by examining the FNAB specimens, we aimed establish the presence of the $BRAF^{V600E}$ mutation before patients underwent surgery and to compare the positive mutation rate in patients with recurrence with the positive mutation rate reported in the literature in patients diagnosed with PTC.

MATERIALS AND METHODS

Patients who underwent surgery with a diagnosis of recurrent PTC at Necmettin Erbakan University Faculty of Medicine, Department of General Surgery were included in this study. The Ethics Committee of Necmettin Erbakan University Faculty of Medicine granted approval for the research using non-drug and non-medical devices (decision number 2018/1396). 96 patients who had undergone surgery for PTC between 2007 and 2017 and who had recurrence during the follow-up period were included in the study. The follow-up period was at least 12 months.

As a criterion in patients diagnosed with recurrent disease, Thyroglobulin (Tg) levels were classified as: Tg >1 ng/ml with suppressed thyroid stimulating hormone (TSH), and Tg >2 ng/ml with stimulated TSH or evidence of recurrent disease seen on the ultrasonograph of the neck, recurrence on cross-sectional imaging (CT/MRI) and/or nuclear medicine imaging (if performed). It was accepted that a lesion was present. All patients included in the study were diagnosed with PTC, underwent total thyroidectomy or regional lymph node dissection with total thyroidectomy at the first operation, were older than 18 years. The inclusion criteria was that Tg was negative (Tg<1ng/ml) and there was no suspicion of residual tissue on the neck ultrasonography. None of the patients underwent prophylactic lymph node dissection, except for lymph node dissection for treatment purposes. Patients who had residual tissue after the first surgery, who did not receive radioactive iodine (RAI) treatment after surgical treatment, who underwent prophylactic lymph node dissection, and who were younger than 18 years were excluded from the study. Patients who were Tg and anti-Tg positive were considered inadequately treated and were excluded from the study. Each patient in the study group was screened for the presence of a BRAFV600E mutation by isolating DNA from the FNAB samples taken from the lesion before primary surgery. To establish the presence of the mutation, the areas with malignant cells on the FNAB preparations were first labeled under a light microscope (figure 1). After labeling, DNA was isolated and BRAFV600E positivity was determined by PCR (Figure 2a, 2b).

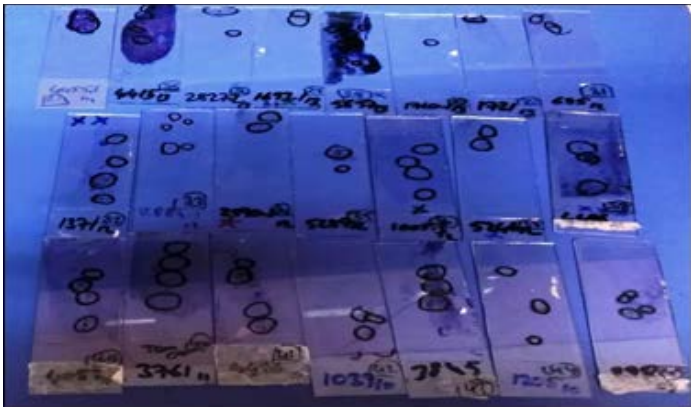

Figure 1. Labeled FNAB slides

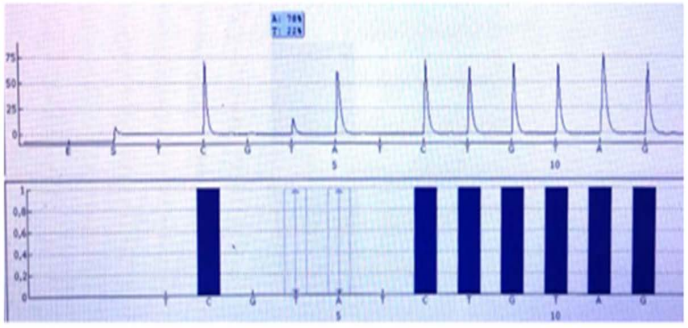

Figure 2a

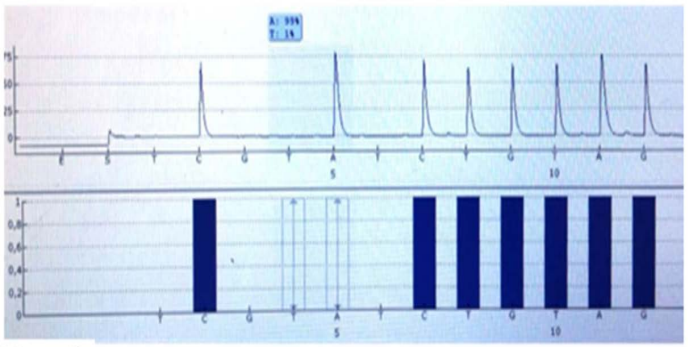

Figure 2b

Figure 2a. ABRAFV600E positive mutation
Figure 2b. BRAFV600E negative mutation

Table 1. Demographic and tumor characteristics of the study group

		Patients (n)
Total number of patients		96
Age (years)		45 (18–79)
Sex	Male	19 (20%)
	Female	77 (80%)
Tumor type	Classic	56 (58.3%)
	Follicular	30 (31.3%)
	Other	10 (10.4%)
Tumor diameter	1–2 cm	35 (36%)
	2,1–3 cm	31 (32%)
	3,1–4 cm	13 (13%)
	>4 cm	17 (15%)
Tumor location	Upper lobe	50 (52.1%)
	Lower lobe	46 (47.9%)
Multicentricity	Yes	64 (67.7%)
	No	32 (32.3%)
Capsular invasion	Yes	66 (77.6%)
	No	19 (22.4%)
Extracapsular invasion	Yes	43 (44%)
	No	53 (56%)
Lymph node metastasis at initial diagnosis	Yes	65 (67%)
	No	31 (33%)
BRAFV600E mutation	Yes	68 (70.8%)
	No	28 (29.2%)
Distant metastasis	Lung	3
	Bone and lung	1
AJCC stage	Stage 1	16 (16.6%)
	Stage 2	13 (13.5%)
	Stage 3	39 (40.6%)
	Stage 4	28 (29.3%)
Recurrence localization	Central area of neck	50 (52%)
	Lateral area of neck	18 (18.7%)
	Thyroidectomy loj	28 (29.3%)
Relapse time	<6 months	3 (3.1%)
	6–12 months	24 (25%)
	12–18 months	31 (32.2%)
	18–24 months	17 (17.7%)
	>24 months	19 (22%)

AJCC=American Joint Committee on Cancer. Other=oncocytic, columnar, and diffuse sclerosing variants.

Table 2. Relationship between continuous variables and $BRAF^{V600E}$ positivity

	BRAFV600E positive		BRAFV600E negative		
	Median	(Min–max)	Median	(Min–max)	P
Age	50	(18–79)	46.5	(19–79)	0.448
Tumor diameter (cm)	2.65	(1–6)	1.65	(1–3.5)	0.002
Multicentricity	2	(1–4)	2	(1–4)	0.709
Time of recurrence (months)	14	(7–65)	20	(4–66)	0.041
RAI dose (mCi)	99	(49–159)	101	(51–156)	0.744

RAI=radioactive iodine.

Statistical analysis

The data were grouped and analyzed using IBM SPSS 20.0 software. In the Kaplan–Meier (K–M) survival analysis, the time of PTC diagnosis and surgery was taken as the starting point and the time at which the study ended was the overall follow-up period.

RESULTS

The demographic and clinical characteristics of the 96 cases included in our study are shown in table 1. The most common PTC variant in our study group was the classical type. In the BRAFV600E-positive group, 85% of the PTCs were the classic type. The tumor diameter was significantly higher in

Table 3. Relationship between AJCC clinical stage and BRAF^{V600E} mutation

			BRAFV600E positive	BRAFV600E negative	Total
ACCJ stage	Stage 1	n	6	10	16
		%	37.50	62.50	100.00
	Stage 2	n	9	4	13
		%	69.20	30.80	100.00
	Stage 3	n	28	11	39
		%	71.80	28.20	100.00
	Stage 4	n	25	3	28
		%	89.30	10.70	100.00
Total		n	68	28	96
		%	70.80	29.20	100.00

Pearson's chi–squared P value=0.004.

the group with BRAF mutation (table 2). The time to disease relapse was significantly shorter in the BRAF^{V600E}-positive group than in the negative group. There was no statistically significant difference between the BRAF^{V600E} positive and negative groups in terms of RAI dose given for treatment, age, and multicentricity. When tumor, node, and metastasis staging is performed according to the American Joint Committee on Cancer (AJCC) system, most patients were in stages 3 and 4. As the disease progresses towards stage 4, the rate of BRAF^{V600E} positivity increased. While the BRAF^{V600E} positivity rate in stage 1 was 37.50%, at stage 4 it was 89.30% (table 3). There was a statistically significant association between the high stage of patients at diagnosis and BRAF^{V600E} positivity (P=0.004). 18.9% of patients relapsed with lymph node metastasis in the cervical area, 29.50% in the thyroidectomy area, and 51.60% in the central area and underwent reoperation. Lymph node metastases were mostly seen in the central area as the localization of recurrence. BRAF^{V600E} mutation was present in 73.50% of patients with lymph node metastases in the central area. Although the number of recurrences in the cervical area was low in the overall distribution (18.90%), the highest rate of BRAF^{V600E} positivity was seen in recurrences detected in the cervical area (77.30%). As for BRAF^{V600E} mutation positivity, the distribution between groups was similar when analyzed by site of recurrence and there was no statistically significant difference (P=0.376). However, in BRAF^{V600E}-positive patients who had recurrence, the recurrence rate was highest in the central region at 53.70%. Kaplan–Meier analysis was performed to investigate the relationship between BRAF^{V600E} mutation and disease-free survival (figure 3).

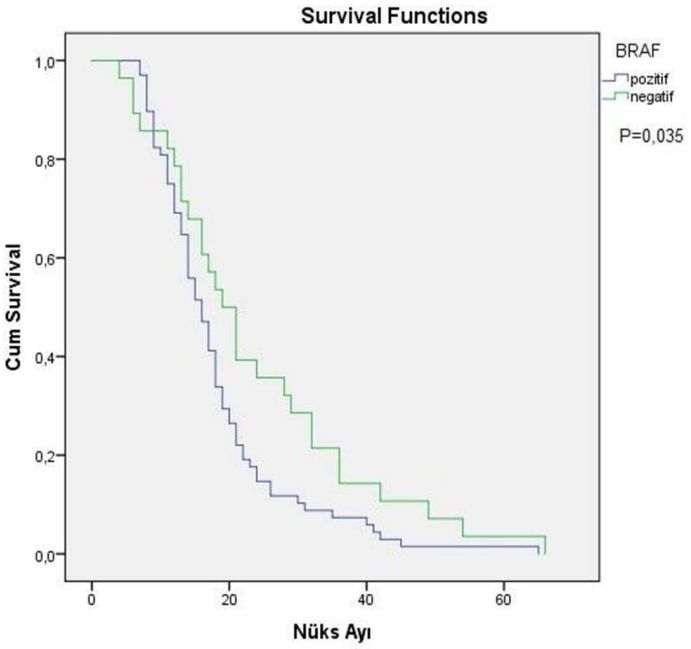

Figure 3. Relationship between disease-free survival and BRAF^{V600E} mutation

Mean and median values of disease-free survival time (months) were calculated. Since the recurrence time data were not normally distributed, Kaplan–Meier analysis was calculated with the median values. With a 95% confidence interval, the expected disease-free survival time in the BRAF^{V600E}-negative group was 19 months, while it was 16 months in the BRAF^{V600E}-positive group and this difference was statistically significant (P=0.035).

DISCUSSION

The BRAF^{V600E} gene mutation is known to be associated with PTC. In this study, patients who had previously undergone surgery with a diagnosis of PTC were in remission at evaluation after primary treatment and patients who developed recurrence during follow-up were evaluated. In this group of patients with recurrence, the association between prognosis-related parameters, such as tumor diameter, capsular invasion, soft tissue invasion, lymph node metastasis, and pathological subtype with BRAF^{V600E} mutation was investigated. A positive correlation between capsular invasion, soft tissue invasion, stage, recurrence time, and lymph node metastasis were seen with BRAF^{V600E} mutation.

The mutation rate of the BRAFV600E gene in our study group was 70.8%. In the literature, BRAF^{V600E} mutation rates in patients with PTC were between 25–81% in various studies. Kurtulmuş et al. (5) found a BRAF^{V600E} mutation rate of 31.7% in their study, Khan et al. (6) reported a rate of 25%, and Chunping Liu et al. (7) in a meta-analysis of 34 studies, the range of

$BRAF^{V600E}$ mutation positivity was 14–81%. Although there are varying rates of $BRAF^{V600E}$ positivity associated with PTC in the literature, the average prevalence is generally reported to be around 45%. Since our study group consisted of patients with complete recurrence, a significantly higher $BRAF^{V600E}$ mutation positivity was found compared with the literature, with a rate of 70.8% compared to a study group in which $BRAF^{V600E}$ was investigated with a diagnosis of PTC. A reason for this increase might be because we studied a group with poor prognosis and possible recurrence rather than all patients diagnosed with PTC.

In our study, patients were categorized into four groups at preoperative assessment according to the AJCC staging system. Chunping Liu et al. (7) in a meta-analysis, reported the rate of patients with advanced stage and $BRAF^{V600E}$ positive gene as 37.5%. A correlation was found between mutation positivity and advanced stage. Tufano et al. (8) showed a strong association between AJCC stage 3 and stage 4 and the $BRAF^{V600E}$ mutation. In our study, a significantly higher $BRAF^{V600E}$ mutation was found in advanced stage patients (stage 3–4). Caroli Li et al. (9) showed a positive correlation with the positivity of BRAF mutation in patients with a tumor diameter of more than 1 cm. In our study, the correlation between the increase in tumor diameter and $BRAF^{V600E}$ positivity was statistically significant ($P<0.05$). While the median tumor diameter in the $BRAF^{V600E}$-positive group was 2.65 cm, it was 1.65 cm in the $BRAF^{V600E}$-negative group. A close correlation was found between $BRAF^{V600E}$ positivity and capsular and soft tissue invasion. The risk of capsular invasion was 5.61 times higher in the $BRAF^{V600E}$-positive group than in the negative group.

The expected disease-free survival time in the $BRAF^{V600E}$-negative group was 19 months, while it was 16 months in the $BRAF^{V600E}$-positive group, which was statistically significant ($P=0.035$) and it was observed that the mutation positive patients relapsed earlier than the $BRAF^{V600E}$-negative patients. However, it is possible that relapse occurs earlier in the $BRAF^{V600E}$-positive group and that other prognostic factors not directly related to the mutation are also associated with $BRAF^{V600E}$. It is known in the literature from studies conducted independently of BRAFV600 that the duration of disease-free survival is shortened for reasons such as an increase in tumor diameter, capsular invasion, multicentricity, and extra-thyroidal spread (10). Since $BRAF^{V600E}$ mutation is associated with parameters, such as capsular invasion, soft tissue invasion, and tumor diameter in our study, the results are indirectly associated with disease-free survival.

Patients were grouped by site of recurrence as central, cervical, or locus. There was no statistically significant difference between the areas of recurrence and the presence of $BRAF^{V600E}$ mutation status. However, in the $BRAF^{V600E}$-positive group, relapse occurred mostly in the central area. There are no studies in the literature on the relationship between the location of the primary tumor and $BRAF^{V600E}$; however, a study by T. Zhang et al. (11) found a higher rate of lymph node metastasis in the central area for tumors in the lower lobe when comparing tumor location and lymph node metastasis location. In our study, no statistically significant differences were found with respect to $BRAF^{V600E}$ mutation and tumor localization in the upper and lower lobe, which has a high rate of tumor localization. However, tumors were proportionally more frequent in the upper lobe. Assuming that the $BRAF^{V600E}$ mutation, which is a somatic mutation, is influenced by environmental factors, a panoramic X-ray of the jaw in the neck area often used by dentists, is performed. It is thought that PTC is more common in the upper lobes, partly due to exposure to radioactivity. However, a larger series of studies with controlled patient groups should be conducted to clarify this opinion.

Although many studies have found a significant association between the $BRAF^{V600E}$ mutation and poor prognostic criteria, the $BRAF^{V600E}$ mutation has not yet been definitively included in the globally accepted PTC management algorithms. However, if PTC can be detected preoperatively by FNAB, which is a minimally invasive method, and if it is accepted as a prognostic criterion by new and large-scale multicenter studies, the role of $BRAF^{V600E}$ mutation in the management of PTC will increase further.

CONCLUSION

In our study, the rate of $BRAF^{V600E}$ positivity was high in patients who underwent surgery for recurrence of PTC. Moreover, there was a positive correlation between $BRAF^{V600E}$ mutation and poor prognostic criteria, such as capsular invasion, soft tissue invasion, stage, time to recurrence, and lymph node metastasis. It is a fact that new studies from larger comparative series are needed to reveal the prevalence of $BRAF^{V600E}$ mutation and its relationship with poor prognostic criteria in PTC. As such, it is likely that the $BRAF^{V600E}$ mutation will be included in future guidelines with the contribution of new studies that will provide more definitive evidence.

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OPEN ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Evaluation of The Relationship Between Metabolic Parameters and Vitamin D Levels in Children with Insulin-Dependent Diabetes Mellitus

İnsülin Bağımlı Diabetes Mellituslu Çocuklarda Metabolik Parametreler ile Vitamin D Seviyesi Arasındaki İlişkinin Değerlendirilmesi

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ÖZET

Amaç: D vitamini hiperlipidemi, kardiyovasküler hastalık riski ve glikoz/insülin metabolizmasında önemli bir rol oynar. Bu çalışmanın amacı; tip 1 diyabetes mellituslu çocukların metabolik profilleri, aterojenik indeks ve kardiyovasküler hastalık riski ile serum vitamin D düzeyleri arasındaki ilişkiyi araştırmaktır.

Gereçler ve Yöntem: Çalışmaya 307 tip 1 diyabetes mellituslu hasta dahil edildi. Antropometrik ve klinik ölçümler, biyokimyasal parametreler, aterojenik plazma indeksi (AIP) değerleri ve serum vitamin D düzeyleri değerlendirildi.

Bulgular: Çalışmaya yaş ortalaması 11.5 ±3.87 olan, 152'si (%49.5) kız, 155'i (%50.5) erkek olmak üzere 307 diyabet hastası dahil edildi. Hastalarımızın büyük çoğunluğunda Vitamin D eksikliği (%77.5; (n=193)) tespit edildi. Vitamin D düzeyi düşük hastaların günlük ortalama insülin dozu 0.91±0.31 U/kg/gün; ortalama hemoglobin A1C (HbA1C) seviyesi %11.77; vitamin D düzeyi normal olanlarda ise ortalama insülin dozu 0.94±0.28 U/kg/gün; HbA1C seviyesi %12.19 olarak bulundu (p> 0.05). Kızlarda ortalama aterojenik plazma indeksi düzeyi 0.28±0.33 iken; erkeklerde 0.25±0.31 idi (p > 0.05). Vitamin D eksikliği olan vakaların aterojenik plazma indeksi ortalaması 0.29±0.31 iken, vitamin D eksikliği olmayanlarda aterojenik plazma indeksi ortalaması ise 0.13±0.28 olarak bulundu (p< 0.001).

Sonuç: Aterojenik plazma indeksi; vitamin D eksikliği olan hastalarda daha yüksek bulunmuştur. Çalışma sonucunda elde edilen verilere göre; Vitamin D düşüklüğü ve aterojenik plazma indeksi yüksekliği kardiyovasküler komplikasyonları öngörmede önemli belirteçlerdir. Tip 1 diyabetes mellituslu çocuklarda bu parametrelerin yakın takibinin uzun dönem morbiditeyi önlemede yol gösterici olacağı düşünülmektedir.

Anahtar Kelimeler: Tip 1 Diabetes Mellitus, Aterojenik İndeks, D Vitamini

ABSTRACT

Objective: Vitamin D play an important role in hyperlipidemia, cardiovascular disease risk, and glucose/insulin metabolism. The aim of this study is; To investigate the relationship between serum vitamin D levels and metabolic profiles of children with type 1 diabetes mellitus (T1DM), atherogenic index, and cardiovascular disease risk.

Materials and Methods: Anthropometric and clinical measurements, biochemical parameters, atherogenic plasma index (AIP) values and serum vitamin D levels were evaluated.

Results: The study included 307 diabetic patients, 152 (49.5%) females and 155 (50.5%) males, with a mean age of 11.5±3.87. Vitamin D deficiency (77.5%; (n=193)) was detected in the majority of our patients. The mean daily insulin dose of patients with low vitamin D levels was 0.91±0.31 U/kg/day; mean hemoglobin A1C (HbA1C) level 11.77%; in those with normal vitamin D levels, the mean insulin dose is 0.94±0.28 U/kg/day; the HbA1C level was found to be 12.19% (p>0.05). While the mean atherogenic plasma index level in females was 0.28±0.33; it was 0.25±0.31 in males (p>0.05). While the mean atherogenic plasma index of cases with vitamin D deficiency was 0.29±0.31, the mean of atherogenic plasma index in those without vitamin D deficiency was found to be 0.13±0.28 (p<0.001).

Conclusion: Atherogenic plasma index was higher in patients with vitamin D deficiency. According to the data obtained as a result of the study; low vitamin D and high atherogenic plasma index levels are important predictors of cardiovascular complications. It is thought that close monitoring of these parameters will be a guide in preventing long-term morbidity in children with type 1 diabetes mellitus.

Keywords: Type 1 Diabetes Mellitus, Atherogenic Index, Vitamin D

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INTRODUCTION

Type 1 Diabetes Mellitus (T1DM) is one of the most common chronic diseases of childhood and is caused by partial or absolute insulin deficiency due to the destruction of the beta cells of the pancreas (1). It has been reported in epidemiological studies that there is a high prevalence of vitamin D deficiency in children and adolescents with T1DM and a relationship between both (2). Studies have shown that 25(OH)D levels of T1DM patients are lower than the control groups, and vitamin D supplementation in early infancy reduces the risk of T1DM by approximately 30% (3). Vitamin D has anti-inflammatory and immunomodulatory effects that may affect the autoimmune pathology of T1DM (4). Epidemiological studies suggest an inverse relationship between circulating 25(OH)D levels and coronary vascular disease risk biomarkers, including the atherogenic lipid profile (5). It has been suggested that Vitamin D has both direct and indirect effects in changing lipid profile by increasing lipoprotein lipase activity in adipose tissue and decreasing serum levels of triglycerides (TG) (6).

MATERIALS AND METHODS

A total of 307 T1DM patients who were followed up in the pediatric endocrinology outpatient clinic, 249 and 58 who were not checked for vitamin D levels were retrospectively included in the study. While 193 of the patients had vitamin D deficiency, this level was found to be normal in 56 patients. Vitamin D levels were considered deficient in those below 20 ng/mL (7). Inclusion criteria were determined as being in the 0-18 age range and having a diagnosis of insulin-dependent diabetes mellitus. Non-insulin dependent diabetes mellitus patients, history of drug use that impairs vitamin D metabolism and those with disease were determined as exclusion criteria.

The body weights of the children and adolescents included in the study were measured in kilograms (kg) by using a NAN brand mechanical weighing device and their height was measured with a standard type stable Holtain Limited height measuring device (sensitive to 1 millimeter) and recorded in centimeters (cm). Measurements were made in-room clothes, on an empty stomach, and standing. Body mass index (BMI) was calculated using the formula (Bodyweight (kg)/height (m2)). Body weights, heights, and body mass indices were evaluated with the Standard Deviation Score (SDS). SDSs were calculated using an appropriate computer program prepared according to age and gender (8). Patients with BMI-SDS≥+2 are obese, those between +1.5, +1.99 are overweight; those between -2, +1.5 are normal weight; those with <-2 were considered to be underweight (9).

Tanner staging was used for puberty classification (10,11). Patients with Tanner stage 2 and above were evaluated as pubertal, and those with stage 1 were evaluated as prepubertal (12). After resting all patients for 10 minutes, blood pressure was measured with a mercury sphygmomanometer in a sitting position using a cuff suitable for their age. The value at which the first Korotkoff sound was heard was recorded as systolic blood pressure, and the value at which the sound disappeared was recorded as diastolic blood pressure. Systolic and diastolic blood pressure limits were analyzed from tables determined according to height percentile and age. Those with a blood pressure level above the 95th percentile were considered hypertensive (13).

HbA1C, lipid profile (Total cholesterol, TG, low-density lipoprotein (LDL), high-density lipoprotein (HDL)), and vitamin D levels were recorded at the first admission and follow-up of patients with type 1 diabetes mellitus. Each patient gave examinations in the morning, after 8-12 hours of fasting, in the form of venous blood samples. Biochemical parameters were determined by routine methods using the Abbot Architect c 8000 brand device. A Siemens Centaur device was used to measure the 25OHD level.

The Atherogenic Index (AIP) was calculated as log (TG/HDL-C). Those with AIP ratios of 0.11 and below were considered at low risk; those >0.11 and ≤0.21 at medium risk and AIP >0.21 at high risk (14). The study was conducted retrospectively. So there was no need for patient informed consent form. The study was conducted as a medical specialization thesis. Ethics committee approval was received.

Statistical Analysis

The data obtained in this study were evaluated with the SPPS 25 program. The Chi-square test was used for comparing the differences between categorical variables. In cases where independent numerical variables were not normally distributed, the Mann-Whitney U test was used. In statistical analysis, the significance level was taken into account as <0.05 (p-value).

Approval of research protocols by institutional committee was taken with the number 2020/2549.

RESULTS

In our study, there were 307 diabetic patients, 152 (49.5%) females and 155 (50.5%) males, whose ages ranged from 2.1 to 18 (Mean = 11.52±3.87). Examining the distribution of diabetic patients by puberty, 114 (37.1%) patients were prepubertal and 193 (62.9%) patients were pubertal. The mean doses of insulin usage of our patients were calculated as 0.90±0.30 U/kg/g. HbA1C levels in the first year of diagnosis (11.9%) were found to be significantly higher than the third (10%) and fifth years (10.2%) (respectively; $t(225)=9.56$, $p<0.001$;$150)=5.74$, $p<0.001$). There was no significant difference between the HbA1C levels in the third and fifth years ($p>0.05$).

When the BMI of the cases with and without vitamin D deficiency was examined, the mean BMI of the cases with vitamin D deficiency was 19.50±5.74 kg/m^2, while the cases without vitamin D deficiency were calculated as 18.57±8.12 kg/m^2. It was observed that there was no significant difference between the means of the groups ($p>0.05$). In our study, while 77.5% (n=193) of the patients whose vitamin D level was checked had vitamin D deficiency, this level was normal in 22.5% (n=56). In patients with low vitamin D levels, the mean insulin dose was 0.91±0.31 U/kg/day, and the mean HbA1C level was 11.7%; In normal cases, it was 0.94±0.28 U/kg/day, and the mean HbA1C level was 12.1%. When the insulin use dose and the mean HbA1C value are compared in cases with

low and normal vitamin D levels, it is seen that there is no significant difference (p>0.05).

When the AIP levels of the patients were classified according to puberty and gender, a significant difference was found in AIP levels in the prepubertal and pubertal groups ($\chi^2(2)=10.34$, p<0.01). While the mean AIP value of prepubertal patients is 0.18±0.33, it is 0.31±0.30 in pubertal patients. On the contrary, there is no significant difference in the AIP levels of males and females (p>0.05). While the mean AIP value in females is 0.28±0.33, the mean AIP value in males is 0.25±0.31. Table 1can be examined for detailed information. The results obtained when comparing the HbA1C value of the patients with high atherogenic index in the first year and those of the patients with normal and low AIP show that there is a significant difference ($F(2,250)=3.87$, p<0.05). It is observed that the mean HbA1C value of the patients with low AIP levels in the first year is significantly lower than that of the patients with high AIP levels. Table 2 can be examined for detailed information. When the mean AIP values of the cases with and without vitamin D deficiency were examined, the mean AIP of the cases with vitamin D deficiency was 0.29±0.31, while this rate was calculated as 0.13±0.28 in those with normal vitamin D deficiency. The results obtained show that there is a significant difference between the two groups (p<0.001).

When the mean systolic and diastolic blood pressure of the cases with and without Vitamin D deficiency were examined, the mean systolic-diastolic blood pressure of the vitamin D deficient cases was 103.94±11.24/65.31±8.91 mmHg; in cases without vitamin D deficiency, mean systolic-diastolic blood pressure was calculated as 104.11±10.62/64.64±9.53 mmHg. The results show that there is no significant difference in the mean systolic and diastolic blood pressure of the cases with and without vitamin D deficiency (p>0.05).

When the mean of TG, cholesterol, HDL, and LDL values of the cases with and without vitamin D deficiency was examined, it was observed that the TG levels of the cases with vitamin D deficiency were significantly higher ($t(236)=-4.09$, p<0.001). However, there was no significant difference between the groups in terms of cholesterol, HDL, and LDL means (p>0.05). Table 3 can be examined for detailed information.

Table 1. Classification of Atherogenic Index Levels of Patients According to Puberty and Gender

		Atherogenic Index Level			
		Low	**Medium**	**High**	**Total**
Prepubertal	n	43	15	38	96
	%	44.8	15.6	39.6	
Pubertal	n	47	37	98	182
	%	25.8	20.3	53.8	
Total	n	90	52	136	278
	%	32.4	18.7	48.9	
Female	n	42	27	64	133
	%	31.6	20.3	48.1	
Male	n	48	25	72	145
	%	33.1	17.2	49.7	
Total	n	90	52	136	278
	%	32.4	18.7	48.9	

Table 2. First-Year HbA1C Values of Cases with High Atherogenic Index, Medium and Low Risk

				95% Confidence Interval	
Level	**Mean**	**S**	**n**	**Lowest**	**Highest**
Low	11.34	2.81	82	10.72	11.95
Medium	11.94	3.18	47	11.13	12.76
High	12.46	2.73	124	11.96	12.96
Total	12.00	2.88	253	-	-

Table 3. TG, Cholesterol HDL and LDL Values of Cases with and without Vitamin D Deficiency

Vitamin D Level	**Variables**	**n**	**Lowest Value**	**Highest Value**	**Mean**	**S**
Low	TG(mmol/L)	185	1.27	54	6.66	5.18
	Cholesterol(mmol/L)	185	4.94	20.2	9.41	2.37
	HDL(mmol/L)	183	0.85	5.98	2.95	0.75
	LDL(mmol/L)	183	0.99	11.7	5	1.82
Normal	TG(mmol/L)	53	1.55	12.4	4.55	2.27
	Cholesterol(mmol/L)	53	5.66	14.11	9.1	1.95
	HDL(mmol/L)	52	1.41	7.33	3.16	0.91
	LDL(mmol/L)	52	1.78	8.25	5	1.5

DISCUSSION

It has been suggested that vitamin D has both direct and indirect effects in changing lipid profile by increasing lipoprotein lipase activity in adipose tissue and decreasing serum levels of TG (6). There is no study in the literature on the evaluation of vitamin D levels of pediatric patients with T1DM diagnosis by atherogenic index. In our study, the mean AIP of cases with vitamin D deficiency was 0.29±0.31, while the mean AIP of those without vitamin D deficiency was found to be 0.13±0.28 ($p<0.001$). The fact that the mean value of AIP is above 0.21 in T1DM patients with vitamin D deficiency is an indication that vitamin D deficiency poses a high risk for cardiovascular diseases in these patients

The age at which T1DM occurs in childhood has a bimodal distribution, with a peak at four to six years of age and a second in early adolescence (ages 10 to 14) (15). In the study conducted by Al-Shaikh et al in Saudi Arabia in 2016, the mean age of the patients was 13.9±3.8 years (13.86±3.88 for males and 14.06±3.86 for females) (16). In our study, the mean age of the patients was found to be 11.52±3.87 (11.74 for males and 11.3 for females). In a study conducted in Egypt by Hafez et al in 2019, 48% of the patients were found prepubertal and 52% pubertal (17). In our study, it is seen that 37.1% patients were prepubertal and 62.9% were pubertal, and it contains similar findings with the studies in the literature.

Treatment of type 1 diabetes mellitus requires lifelong administration of exogenous insulin (18). According to ISPAD; At the onset of T1DM, typically 0.5-0.75 U/kg/day total insulin doses are selected, and then these doses are adjusted daily to reach the target glycemia (19). In our study, the mean daily insulin dose of the patients was found to be 0.90±0.30 U/kg/day. Hemoglobin A1C reflects the mean blood sugar level of 2-3 months and is used to predict the risk of developing diabetes complications. Although there are different approaches regarding the HbA1C level of T1DM patients in the pediatric population in the literature, the mean value is expected to be below 7.5% (20). In our study, it was found that the HbA1C level in the first year of diagnosis was 11.90%.

In a study conducted in Egypt by Hafez et al in 2017, the insulin dose of T1DM patients with vitamin D deficiency was 1.2±0.38 U/kg/day; In those with normal levels of vitamin D; 0.99±0.16 U/kg/day was found. HbA1C levels were 9.38±1.99% for patients with vitamin D deficiency; For those with normal vitamin D levels, it was found to be 8.56±0.49% (21). In our study, the mean daily insulin dose of patients with low vitamin D levels was 0.91±0.31 U/kg/day; The HbA1C level was found to be 11.77%. In patients with normal vitamin D levels, the mean insulin dose was 0.94±0.28 U/kg/day; The HbA1C level was found to be 12.19%. In the studies in the literature, no significant difference was found in the insulin use dose and HbA1C level in patients with vitamin D deficiency and normal. We think that this is since the vitamin D level of our patients was not regularly checked at the time of diagnosis and during the period when the HbA1C level was checked.

In a study conducted in Saudi Arabia in 2016 by Al-Shaikh et al. the mean systolic-diastolic blood pressure measurement was 113.2±11.2/67.8±9.0 mmHg in those with insufficient vitamin D, and 111.9±12.8/67.9±8.5 mmHg in those with normal levels. Again in this study, the BMI of those with vitamin D deficiency was 21.6±4.5 kg/m^2; the BMI of normal ones was found to be 20.1±3.9 kg/m^2 (16). In our study, the mean systolic blood pressure of cases with vitamin D deficiency was 103.94±11.24 mmHg; the mean diastolic blood pressure was calculated as 65.31±8.91 mmHg. Besides, the mean systolic blood pressure of cases without vitamin D deficiency was 104.11±10.62 mmHg; Mean diastolic blood pressure was found to be 64.64±9.53 mmHg, and no significant difference was found in those with deficient and normal vitamin D levels ($p>0.05$). While the mean BMI of our patients with vitamin D deficiency was 19.50±5.74 kg/m^2, the mean BMI of cases without vitamin D deficiency was calculated as 18.57±8.12 kg/m^2. It was observed that there was no significant difference between the means of both groups ($p>0.05$). Our study contains findings similar to the literature.

In a study conducted by Sapunar et al in 2018 with 208 children in Chile, the AIP value in males was 0.25±0.31; 0.26±0.23 in females; it was found to be 0.25±0.30 in prepubertal children and 0.26±0.21 in pubertal children (22). In a study conducted by Nogay on 400 children in our country in 2017, the AIP value in females in pubertal patients was −0.15±0.22; It was found to be −0.15±0.25 in males. AIP rate in females in prepubertal patients was −0.04±0.20; It was found to be −0.24±0.31 in males (23). In the results obtained from our study, while the mean AIP value in females was 0.28±0.33; the mean AIP value in males is 0.25±0.31. However, the mean AIP value in prepubertal cases was found to be 0.18±0.33, while it was 0.31±0.30 in pubertal patients. In the studies in the literature, we think that there is a difference in the mean of AIP since the cases are not diagnosed with T1DM. Again, we believe that this difference between pubertal and prepubertal cases is due to the malnutrition in patients and the increase in blood glucose regulation during adolescence.

In a study conducted by Zabeen et al (2018) in Bangladesh, 65% of 576 T1DM patients have dyslipidemia. It was found that 50% of the patients had a high TG level, 66% had a high LDL level, and 48% had a low HDL level. A higher mean HbA1C (9.8%[8.4-11.8] versus 7.9%[9.3-10.5]) was found in patients with dyslipidemia compared to those without (24). In our study, in the comparison of the relationship between AIP and HbA1C levels, the mean HbA1C level in the first year of patients with high AIP values was 12.4%; those with low risk were found to be 11.34%. No study was found in the literature on the comparison of the relationship between AIP and HbA1C in T1DM patients; It was also found in our study that dyslipidemia affects the increase of HbA1C.

In conclusion Blood sugar regulation must be ensured in patients with type 1 diabetes mellitus and optimal vitamin D support should be given importance in patients with diabetes mellitus.

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OPEN ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Efficacy of Bevacizumab-Based Terapy in Patient With Metastatic or Recurrent Cervical Cancer: Real Life Data

Metastatik veya Nüks Serviks Kanserli Hastalarda Bevacizumab Bazlı Tedavinin Etkinliği: Gerçek yaşam Verisi

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ÖZET

Amaç: Bu çalışmanın amacı metastatik serviks kanseri (SK) tanısı konulan hastalarda bevacizumab (BEV) bazlı tedavilerin güvenliğini ve etkinliğini, gerçek yaşam verileri baz alınarak değerlendirmektir.

Gereçler ve Yöntem: Bu çalışma, retrospektif gözlemsel bir analiz içermektedir. Çalışmaya Ocak 2012-Aralık 2022 tarihleri arasında Tıbbi Onkoloji bölümünde BEV tedavisi alan metastatik SK tanılı hastalar dahil edilmiştir.

Bulgular: Bu çalışmaya ortalama yaşı 51 (medyan: 21-78) ve tedavi sonrası ortalama takip süresi 16,6 ay olan 40 hasta dahil edildi. Yaygın metastatik bölgeler arasında %72,5 (n=29) lenf nodu, %55 (n=22) periton, %35 (n=14) akciğer, %22,5 (n=9) karaciğer ve %15 (n=6) kemik yer almaktadır. Tedavi yanıtlarına ilişkin olarak hastaların %12,5'inde (n = 5) tam yanıt , %45'inde (n = 18) kısmi yanıt , %17,5'inde (n = 7) stabil yanıt, %25'inde ise (n = 10) progresyon saptandı. Medyan progresyonsuz sağkalım 8,5 ay (%(95% CI: 6.838 – 10.295) ve genel sağkalım ise 16,3 ay (95% CI: 11.305 – 21.362) olarak bulundu. Kemik metastazı varlığı (p=0,024) ve obezite (p=0,020) sağkalım sonuçlarını etkileyen istatistiksel olarak anlamlı faktörlerdir. Yaş, patoloji alt grupları, metastatik bölge sayısı, tümör gradı, başlangıç evresi, tedavi öncesi cerrahi ve radyoterapi, ve BEV ile eşzamanlı uygulanan sitotoksik ajan türü gibi çeşitli faktörlere bağlı olarak genel sağkalım sonuçlarında istatistiksel olarak anlamlı bir fark bulunmadı (p > 0.05).

Sonuç: Metastatik SK tanılı hastaların prognozu kötüdür. BEV'in kemoterapi ajanlarıyla kombinasyonları bu hasta grubunun tedavisinde etkili ve güvenlidir.

Anahtar Kelimeler: Serviks kanseri, bevacizumab, kemoterapi

ABSTRACT

Aim: This study's goal is to evaluate the safety and efficacy of bevacizumab (BEV)-based therapies in patients with metastatic cervical cancer (CC) using real-life data.

Materials and Methods: This study constitutes a retrospective observational analysis. Patients diagnosed with metastatic CC who received BEV treatment in the Medical Oncology department between January 2012 and December 2022 were included in the study.

Results: This study encompassed 40 patients, with a median age of 51 years (range: 21-78), and a median follow-up duration post-treatment of 16.6 months. Predominant metastatic sites included the lymph nodes 72.5% (n=29), peritoneum 55% (n=22), lungs 35% (n=14), liver 22.5% (n=9) and bones 15%(n=6). Regarding treatment responses, 12.5% (n = 5) of patients achieved complete response , 45% (n = 18) achieved partial response, 17.5% (n = 7) had stable disaese, and 25% (n = 10) experienced disease progression. The median progression-free survival was found 8.5 months (95% CI: 6.838 – 10.295), and the median overall survival was 16.3 months (95% CI: 11.305 – 21.362). The presence of bone metastasis (p=0.024) and obesity (p=0.020) are statistically significant factors affecting survival outcomes. There were no statistically significant differences in survival outcomes due to several factors, including age, pathology classification, number of metastatic sites, tumor grade, initial staging, previous surgeries and radiotherapy before starting therapy, and the type of cytotoxic agents administered with BEV (p > 0.05).

Conclusions: Metastatic CC has a challenging prognosis. Combinations of BEV with chemotherapy agents are effective and safe in the treatment of this patient group.

Keywords: Cervical cancer , bevacizumab, chemotherapy

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INTRODUCTION

Cervical cancer (CC) is the fourth most common malignancy among women worldwide after breast, colorectal and lung cancers, with 600,000 new cases and 340,000 deaths annually (1). While the incidence of CC has considerably diminished in developed countries due to cytologic screening and HPV vaccination campaigns, it still remains the predominant gynecological malignancy (2). In the management of primary CC, therapeutic approaches encompass surgical interventions for early-stage disease and the utilization of concurrent cisplatin chemotherapy (ChT) combined with pelvic radiation therapy for locally advanced lesions (3). Additionally, even the progress in the prevention and detection of CC is huge, individuals identified with advanced or recurrent stages experience unfavorable prognoses. In the United States, the 5-year survival rate for CC diagnosed at the locally advanced stage is 57%. Yet, for those categorized as stage IV, the rate diminishes to 16% or lower, and for recurrent cases, it dips below 5% (4). In the past, the standard treatment involved cisplatin as a monotherapy, followed by the adoption of a platin and paclitaxel combination (5).

Bevacizumab (BEV), a synthetic antibody against vascular endothelial growth factor, impedes tumor development by suppressing angiogenesis (6). Due to randomized trials, incorporating BEV into ChT regimens has demonstrated favorable results in terms of response rates and overall survival (OS) outcomes (7). Based on these outcomes, current recommendations endorse this regimen as the standard therapeutic approach for metastatic CC (8). Despite these accomplishments, there remains a necessity for innovative therapies to address metastatic CC in both initial and subsequent treatment lines (9).

Randomized prospective trials are essential to ascertain a drug's efficacy and safety. However, since these studies frequently encompass selected patient groups, variations from real-world results are possible. In our research, we aimed to retrospectively assess the safety and efficacy of combining BEV with ChT in patients with metastatic CC, mirroring real-life clinical practices.

MATERIALS AND METHODS

The study was carried out with the permission of the Istanbul University, Istanbul Faculty of Medicine Scientific Research Evaluation and Ethics Committee (Date:13.10.2022, Decision No: 2022/1651). It was conducted in strict adherence to the principles outlined in the Declaration of Helsinki and in accordance with the recommended guidelines for good clinical practice. Retrospective analysis was performed on patients who were hospitalized between January 2012 and December 2022. Individuals diagnosed with metastatic CC and subjected to ChT protocols incorporating BEV were included for this study. All patients received BEV at a dose of 15 mg/kg every three weeks until disease progression, severe toxicity and adequate treatment duration. Patients with insufficient data for statistical evaluation were omitted from the study. Comprehensive demographic and clinical information, encompassing age at diagnosis, familial history, stage, histological findings, perioperative interventions, the count of BEV cycles administered, specific ChT protocols, radiotherapy regimens, surgical procedures, and associated toxicities, were conducted from the medical database. This information was meticulously documented and organized for subsequent analysis.

Clinical and radiological evaluations were performed at approximately two-three month intervals to determine the effectiveness of the treatment. Utilizing the Response Evaluation Criteria in Solid Tumors guidelines as a reference, treatment outcomes were segmented into four categories: complete response (CR), partial response (PR), stable disease (SD), and progressive disease. Using this classification, we determined the optimal response exhibited by patients according to the set criteria. The overall response rate (ORR) was derived from the combined instances of CR and PR. Concurrently, the disease control rate (DCR) was ascertained by encompassing cases categorized as CR, PR, and SD. Progression-free survival (PFS) was determined as the time from initiation of BEV treatment to progression.The time from beginning BEV to death from any cause was defined as OS. An univariate analysis was executed to examine the influence of clinicopathological factors on OS. A multivariate analysis was conducted, incorporating both the notable factors identified in the univariate analysis of this study and those recognized as significant in the current literature. To ensure precise and trustworthy data, patient statuses were verified by cross-referencing with the Ministry of Health's death registration system.

Survival curves were generated employing the Kaplan-Meier methodology. The log-rank test was utilized to conduct univariate analysis. The Cox regression model was employed for multivariate analysis to determine the independent impacts of different variables on the desired outcomes. Statistical evaluations were performed utilizing SPSS version 25 (IBM Corp., Armonk, NY, USA).

RESULTS

The current study included 40 patients diagnosed with metastatic CC. The median age of the patients was 51 years (range, 21–78 years). Based on pathological features, 82.5% (n = 33) of the patients were diagnosed with squamous carcinoma, 10% (n = 4) exhibited adenocarcinoma, 5% (n = 2) clear cell carcinoma, and 2.5% (n = 1) presented with the adenosquamous subtype. The predominant sites of metastatic spread included the lymph nodes at 72.5% (n=29), peritoneum at 55% (n=22), lungs at 35% (n=14), liver at 22.5% (n=9), bones at 15% (n=6) and brain at 2.5% (Table 1). Prior to receiving BEV treatment, 75% (n = 30) underwent definitive radiotherapy, while 45% (n=18) had surgical interventions. Among the participants, 17.5% (n=7) underwent perioperative ChT. Additionally, approximately 32.5% of the patients, equivalent to 13 individuals, had received palliative ChT before initiating BEV. After BEV treatment, 22.5% (n = 9) of patients received palliative ChT.

Table 1. Clinical and pathological features of the patients.

Characteristics		n (%)
Age at diagnosis	<50 years	17 (42.5)
	≥50 years	23 (57.5)
Pathologic subtypes	Squamous	33 (82.5)
	Adenocancer	4(10)
	Clear cell	2 (5)
	Adenosquamous	1 (2.5)
Grade status	Grade 1-2	30 (75)
	Grade 3	10 (25)
BMI (kg/m^2)	<19	1 (2.5)
	19-25	11 (27.5)
	25-30	17 (42.5)
	>30	11 (27.5)
Stage at diagnosis	Stage 1	6 (15.0)
	Stage 2	14 (35.0)
	Stage 3	10 (25.0)
	Stage 4	10 (25.0)
Sites of metastasis	Liver	9 (22.5)
	Periton	22 (55.0)
	Lungs	14 (35.0)
	Bone	6 (15.0)
	Brain	1 (2.5)
	Lymphadenopathy	29 (72.5)
	Others	9 (22.5)
The number of metastatic sites	≤ 2 sites	19 (47.5)
	> 2 sites	21 (52.5)
Surgeries prior to bevacizumab	Yes	18 (45.0)
	No	22(55)
Radioterapy before bevacizumab based terapy	No	8 (20)
	Definitive	30 (75)
	Palliative	2 (5)
Perioperative chemoterapy before bevacizumab based terapy	No	33 (82.5)
	Yes	7 (17.5)
Chemotherapy regimens used in combination with bevacizumab	Paclitaxel + Carboplatin	17 (42.5)
	Cisplatin + Paclitaxel	15 (37.5)
	Gemcitabine +Carboplatin	2 (5.0)
	Gemcitabine + Cisplatin	1 (2.5)
	Weekly Paclitaxel	3 (7.5)
	Others	2 (5.0)
Palliative chemotherapy before Bevacizumab	No	27 (67.5)
	Yes	13 (32.5)
After bevacizumab treatment	Chemotherapy	9 (22.5)
	Other (HT, Surgery, RT)	3 (7.5)

Table 2. Responses to bevacizumab-based treatment in metastatic or recurrent cervical cancer.

	Total n=40 n (%)
Response ratios	
Complete response	5 (12.5)
Partial response	18 (45)
Stable disease	7 (17.5)
Progression	10 (25.0)
Objective response ratio	23 (57.5)
Disease control ratio	30 (75)

Table 3. Grade >2 side effects of bevacizumab-based therapy

Variables	n = 40 n (%)
Hypertension	7(17.5)
Proteinuria	1(2.5)
Fistula	2(5)
Thromboembolic events/hemorrhage	4(10)
Febrile neutropenia	2 (5)
Congestive heart failure	0

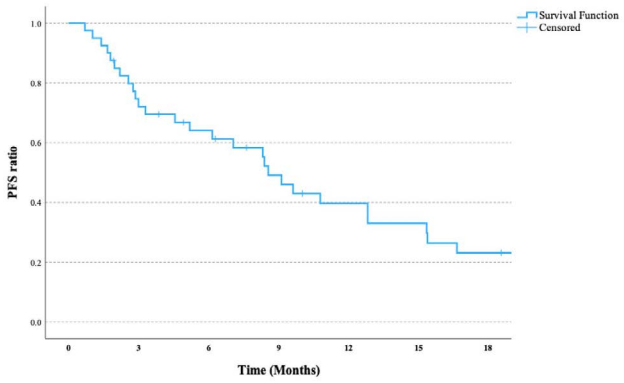

Figure 1. Kaplan–Meier curve of progression-free survival in patients with metastatic cervical cancer treated with chemotherapy plus bevacizumab

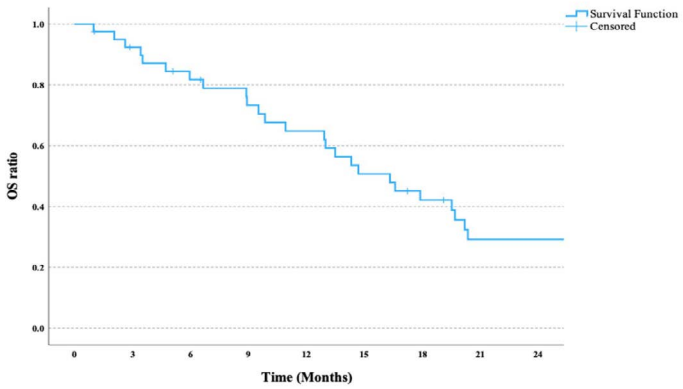

Figure 2. Kaplan–Meier curve of overall survival in patients with metastatic cervical cancer treated with chemotherapy plus bevacizumab

Table 4. Univariate analysis for survival analysis

		Total N	Ex N	Survivors N	Survival rate (%) (24 months)	P-value
Age at diagnosis	<50	17	12	5	29	0.409
	≥50	23	18	5	22	
Obesite	Non-obese	12	9	3	25	0,009**
	Obese	28	21	7	25	
Radioterapy before bevacizumab based terapy	No	8	6	2	25	0,156
	Definitive	30	22	8	27	
	Palliative	2	2	0	0	
Pathologic subtype	Squamose	33	24	9	27	0.080
	Others	7	6	1	14	
Grade status	Grade 1-2	33	24	9	27	0.724
	Grade 3	7	6	1	14	
Primary surgery before bevacizumab	No	22	18	4	18	0.910
	Yes	18	12	6	33	
Palliative chemotherapy before bevacizumab	No	27	19	8	30	0.139
	Yes	13	11	2	15	
Liver	No	31	23	8	26	0,908
	Yes	9	7	2	22	
Peritoneum metastasis	No	18	11	7	39	0.375
	Yes	22	19	3	14	
Lung metastasis	No	26	18	8	31	0.427
	Yes	14	12	2	14	
Bone metastasis	No	34	25	9	27	0,009**
	Yes	6	5	1	17	
Brain metastasis	No	39	29	10	25	0,006**
	Yes	1	1	0	0	
Number of metastatic sites	≤ 2 Sites	13	11	2	15	0.076
	> 2 Sites	18	12	6	33	
Chemotherapy regimens used in combination with bevacizumab	Paclitaxel + Carboplatin	17	13	4	24	0,732
	Paclitaxel + Cisplatin	15	11	4	27	
	Others	8	6	2	25	

Table 5. Multivariate Cox Regression Analysis for Overall Survival

	P-value	HR	95% CI	
			Lower	Upper
Age (<50years vs. ≥ 50)	0,611	1,224	0,561	2,672
Obesite (non-obese vs. obes)	0,020*	2,758	1,175	6,475
Grade (1-2 vs.3)	0,879	0,934	0,387	2,254
Number of metastatic sites (≤ 2 sites vs. > 2 sites)	0,451	1,392	0,589	3,289
Bone metastasis (yes vs. no)	0,024*	3,494	1,179	10,354
Brain metastasis (yes vs. no)	0,401	2,815	0,252	31,446

Multivariate analysis model p-value *p<0,05

The median number of ChT cycles administered in conjunction with BEV was 6 (range, 1–30 cycles). Patients, on average, received 8 cycles of BEV, with the range spanning from 1 to 30 cycles. Regarding the ChT regimens used with BEV, the most prevalent ones included paclitaxel plus carboplatin, used in 42.5% (n = 17) of patients, and cisplatin plus paclitaxel, employed in 37.5% (n = 15). In terms of treatment outcomes, 12.5% (n = 5) of patients accomplished a CR, 45% (n = 18) achieved a PR, 17.5% (n = 7) maintained SD, while 25% (n = 10) faced disease progression. The ORR stood at 57.5% (n = 23), and the DCR was 75% (n = 30), as depicted in Table 2. When it comes to main side effects, roughly 17.5% (n = 7) of patients exhibited hypertension categorized as grade >2. Additionally, proteinuria of grade ≥3 was observed in 2.5% (n = 1) of patients, while gastrointestinal fistula occurred in 5% (n = 2) (Table 3). The cessation of BEV treatment occurred due to disease progression in 72.5% (n = 29) of patients, adverse effects in 7.5% (n = 3), and completion of an adequate treatment duration in 7.5% (n = 3).

Following treatment with BEV, the median follow-up duration was 16.6 months. The median PFS accounted for 8.5 months (95% CI: 6.838 – 10.295), as illustrated in Figure 1, whereas the median OS was 16.3 months (95% CI: 11.305 – 21.362) (Figure 2). The existence of bone metastases emerged as a statistically significant factor associated with lower survival rates (95% CI: 1,179 – 10,354) (p=0.024; p<0.05). Moreover, there were no statistically noteworthy differences in survival outcomes related to various factors, including age, pathology classification, metastatic region count, tumor grade, initial staging, prior chemotherapy surgeries and radiotherapy before starting BEV, and the type of ChT administered alongside BEV (p > 0.05) (Tables 4 and 5).

DISCUSSION

Despite the progress made in screening and diagnostic techniques for CC, a considerable number of cases are diagnosed each year, and a significant proportion of these cases progress to advanced stages (10). For individuals diagnosed with stage IVB disease or those experiencing recurrent disease characterized by metastases across multiple sites particularly those that cannot be encompassed within a single radiation field or metastatic disease that is not responsive to localized treatments, the primary aim of treatment is palliative care. In these advanced scenarios, long-term survival remains a challenge. The introduction of novel targeted therapies, particularly the incorporation of BEV with platinum-based ChT, has led to enhanced OS rates as evidenced by randomized studies involving patients with metastatic CC (11).

In the GOG 240 study, 452 women with metastatic or recurrent cervical carcinoma were randomly assigned to either receive ChT alone or in combination with BEV. The trial demonstrated an enhancement in OS by 3.7 months, with figures of 17 months for those on BEV versus 13.3 months without, regardless of the specific ChT regimen they were receiving concurrently. Additionally, patients administered BEV exhibited superior ORR at 48%, compared to 36% (12). Considering all the above-mentioned findings, this trial advocates for the utilization of ChT in conjunction with BEV as an initial treatment approach for metastatic CC.

In a retrospective investigation by Lee et al., utilizing real-world data, the effectiveness of pairing BEV with platinum-based doublet ChT in managing metastatic CC was examined. The study encompassed 52 patients. Ultimately, the PFS and OS stood at 9.8 months and 15.3 months, respectively. Regarding response rates, the study revealed a CR rate of 15.4%, a PR rate of 34.6%, and a stable response rate of 19.2%. The ORR among these patients was 69% (13). In this real-world analysis, we aimed to explore the effectiveness and safety of approach mainly focused on using BEV for treating recurrent and metastatic CC. According to our findings, PFS was determined to be 8.5 months, OS was 16.3 months, and the ORR was 57.5%. These results are similar to the results of the studies referenced above.

In the research led by Matsumiya et al., the identification of bone metastasis among patients with CC was consistently linked with a diminished OS prognosis (14). Likewise, our study identified bone metastases as a statistically notable factor correlating with decreased OS rates.

In the study of Gross et al., it was observed that survival rates increased in patients diagnosed with CC with a BMI ≥30.

Similarly, in our study, we determined that obese patients had better OS. In our research, the administration of BEV was generally well-received, with predominant grade 3–4 adverse effects encompassing hypertension, proteinuria, thromboembolic incidents, and febrile neutropenia. Only a minimal 7.5% (n = 3) of patients discontinued treatment due to toxicity reasons. These observations align with findings reported in earlier studies (15,16).

This study is subject to certain constraints. The retrospective nature of the design introduced heterogeneity within the patient group, leading to some missing data. Additionally, being a single-center study poses a potential risk of selection bias.

In summary, in real-world clinical settings, BEV-based therapy for recurrent or metastatic CC demonstrates feasibility and tolerability. Additionally, the presence of bone metastases and obesity were found to be statistically significant factors for a survival outcomes in this patient cohort.

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OPEN ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Prospective Follow-Up and Results of Neutralizing Antibody Levels of Patients During The Pandemic Period

Pandemi Döneminde Hastaların Nötralizan Antikor Düzeylerinin Prospektif İzlemi ve Sonuçları

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ÖZET

Amaç: COVID-19 küresel çapta salgına neden olmuştur. İnfeksiyon kontrolü, tekrarlayan infeksiyonların önlenmesi ve aşı çalışmalarında salgısal bağışık yanıtın aydınlatılması yol göstericidir.

Gereç ve Yöntemler: Çalışmaya 18 yaşından büyük, gebelik ve antikor cevabını etkileyen bağışıklık sistemi baskılanmış ek hastalığı olmayan, SARS-CoV-2 PCR testi pozitif 100 hasta dahil edildi. Hastalardan 7, 15 ve 30.günlerle, 3. ve 6. aylarda kan numunesi alınarak nötralizan IgM ve IgG antikor düzeyleri hem pozitif veya negatif olarak hem de kantitatif olarak kaydedildi.

Bulgular: Olguların nötralizan IgM ve IgG antikorları sırasıyla 3. ayda % 65 ve % 94; 6. ayda % 35 ve %100 pozitif olarak bulundu. Halsizlik, öksürük, nefes darlığı, ishal semptomları olan hastalarda, göğüs tomografisinde akciğerde tutulumu olanlarda antikor düzeyleri daha yüksek oranlarda bulundu. Lenfosit sayısı, C-Reaktif Protein, prokalsitonin düzeyleri ile antikor düzeyleri arasında pozitif yönde korelasyon görüldü

Sonuç: COVID-19 geçiren hastalarda büyük oranda nötralizan antikorların oluştuğu ve 6 ay boyunca devamlılık gösterdiği tespit edildi. Bu bulgular, SARS-CoV-2 infeksiyonuna karşı oluşan immunolojik yanıtın anlaşılmasına katkıda bulunmakta ve uzun vadeli bağışıklık ile aşı stratejileri üzerinde etkileri olabileceğini göstermektedir.

Anahtar Kelimeler: Antikor düzeyi, COVID-19, Nötralizan antikor

ABSTRACT

Objective: The COVID-19 pandemic has affected the entire world. Understanding the humoral immune response is crucial for protection against the disease, prevention of reinfections and guiding vaccine development.

Materials and Methods: A hundred patients who were over 18 years of age, did not have pregnancy or additional immunosuppressive diseases and had a positive SARS-CoV-2 PCR test were included in the study. Blood samples were taken from the patients on the 7th day, 15th day, in the 1st month, 3rd month and 6th month and COVID-19 IgM and IgG antibody levels were recorded both as positive or negative and quantitatively.

Results: The COVID-19 IgM and IgG antibodies of the patients were found positive at the following rates: 65% and 94% in the 3rd month, and 35% and 100% in the 6th month, respectively. Higher antibody levels were observed in patients with symptoms such as fatigue, cough and shortness of breath, those with lung involvement in chest tomography. Positive correlations were found between lymphocyte count, C-reactive protein, procalcitonin levels and antibody levels.

Conclusion: Our findings indicated the presence of a significant level of neutralizing antibodies which persisted for 6 months in patients who recovered from COVID-19. These results contribute to understanding the immunological response to COVID-19, and may have implications for long-term immunity and vaccine strategies.

Keywords: Antibody level, COVID-19, Neutralizing antibody

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INTRODUCTION

The virus, which was first identified in December 2019 in Wuhan, China, and could not be controlled and spread around the world in a short time causing a pandemic and led to severe acute respiratory syndrome, was called coronavirus-2 (SARS-CoV-2) and the disease was called coronavirus disease 2019 (COVID-19) (1). SARS-CoV-2 infection can progress in a wide spectrum, ranging from asymptomatic or upper respiratory tract disease to severe pneumonia (2). The humoral immune response is necessary and beneficial for the clearance of cytopathic viruses and the establishment of the immune memory required to prevent recurrent infections. After infection, virus-specific IgM, IgG, IgA and neutralized IgG antibodies are detected (3). Although detection times in circulation vary, approximately 5 days (3-6 days) for IgM and IgA, and an average of 14 days (10-18 days) for IgG (4). It has been found that IgM levels increase before IgG and decrease within a month, while IgG levels increase later and persist for a long time (5).

SARS-CoV-2 Spike (S) protein is the most basic immunological target as it is responsible for the entry of the virus into the host cell through the angiotensin-converting enzyme 2 (ACE-2) receptor, its neutralizing antibody induction capacity and its species-specific antigenic specificity. S protein consists of two subunits called S1 and S2 respectively, which are responsible for binding to the host cell receptor and fusion of host cell membranes. It contains two important domains: S1 N-terminal domain (S1-NTD) and S1 C-terminal domain (S1-CTD). One or both of the S1 domains potentially bind the receptor and function as the receptor-binding domain (RBD). Studies show that SARS-CoV-2-specific antibody-related neutralization is predominantly associated with epitopes within the S protein RBD of the virus (6).

It is essential to explain the secretory immune response well in protecting against COVID-19 infection, preventing recurrent infections and in vaccine applications. In our study, in addition to the dynamics of the humoral immune response against infection; It was aimed to investigate whether there is a significant relationship between these dynamics and the epidemiological characteristics of the patients and clinical and laboratory findings.

MATERIALS AND METHODS

The study was carried out with the approval of Necmettin Erbakan University Non-drug and medical device research ethics committee, meeting number 120 dated December 18, 2020 and decision number 2020/2937. One hundred patients, who were followed up in the COVID-19 clinic or admitted to the outpatient clinic at Necmettin Erbakan University, and volunteered, ≥18 years of age, did not have pregnancy or severe immunocompromised comorbidities that would prevent antibody formation, and whose SARS-CoV-2 Polymerase Chain Reaction (SARS-CoV-2 PCR) test was positive, were included in the study. Informed consent was obtained from the patients before the study. When blood samples were taken from the patients on the 7th, 15th day and in the 1st, 3rd and 6th months after PCR positivity, the plasma part was separated by centrifugation and the samples were stored at (-80) degrees. After the sample collection was completed in 9 months, SARS-CoV-2 IgM and IgG antibodies were investigated in the samples. For the qualitative and quantitative determination of IgM and IgG antibodies in serum and plasma, the Chemiluminescence Microparticle Immunoassay (CMIA) method was used using SARS-CoV-2 IgM and IgG II Quant kits (ARCHITECT SYSTEM). This method quantitatively detects IgG and IgM antibodies against the RBD region of SARS-CoV-2. IgG >50 AU/mL is positive, <50 AU/mL is negative. IgM response is evaluated with the threshold value of ≥1.0 S/C according to the index (S/C) calculated with the help of the reaction relative light unit (RLU) measured by the CMIA method. IgM >1 Index unit was reported as positive and <1 Index unit was reported as negative. It was determined and reported that the positive percent agreement (PPA) of the test was 95% and the confidence interval (CI) was 95%, the negative percent agreement (NPA) was 99.55% and the confidence interval (CI) was 95%. The neutralizing IgG and IgM results of the patients, measured on the 7th and 15th days, and in the 1st, 3rd and 6th months, were recorded both as positive or negative and quantitatively.

The patients age, gender, inpatient or outpatient follow-up, and hospitalization days were recorded; their symptoms were questioned. Ways of transmission were defined as domestic transmission, workplace transmission, healthcare setting and unknown groups. Physicians, technicians, nurses, caregivers and medical secretaries who had close contact with the patient were included in the healthcare worker group. Hospital staff and non-hospital-related professional groups such as kitchen staff and technical staff were included in the non-healthcare group. Diabetes Mellitus, hypertension, asthma, Chronic Obstructive Pulmonary Disease (COPD), chronic renal failure and other comorbidities were questioned. Influenza, conjugated pneumococcal and polysaccharide pneumococcal vaccines of the cases were recorded. PCR positivity days and COVID-19 vaccinations of the patients were questioned and recorded in detail. According to the thorax computed tomography (CT) results, the cases were recorded in 3 groups: those for whom CT was not performed, those whose CT result was reported as normal, and those whose CT result was reported as highly suspicious for COVID-19 pneumonia. The patients' leukocyte, lymphocyte, neutrophil and platelet counts and CRP, procalcitonin, D-dimer, fibrinogen, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and ferritin levels at the time of admission were recorded.

Statistical analysis

The collected data were analyzed in computer environment. Data entry and statistical analysis were performed using the SPSS for Windows version 18.0 (SPSS Inc. Chicago, IL, USA) package program. The suitability of the data for normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogrorov-Smirnov/Shapiro-Wilk tests). In evaluating numerical data, arithmetic mean (Mean), standard deviation (SD), median, minimum and maximum (min-max) values were employed, and frequency distributions

and percentages were used to summarize categorical data. Chi-square ($\chi 2$) test and Fisher Exact test were used to compare categorical data. The relationship between non-normally distributed numerical data and categorical data was evaluated with the Man-Whitney U test. The Kruskal Wallis test was used to evaluate three or more non-normally distributed groups with numerical data. Posthoc Man-Whitney U test and Bonferroni correction were performed for pairwise comparisons between groups with significant Kruskal Wallis test results. Correlations of non-normally distributed numerical variables were analyzed with the Spearman correlation coefficient (r). In the evaluation of Spearman Correlation Coefficients, between 0.05-0.30 was considered a low or insignificant relationship, between 0.30-0.40 a low-moderate relationship, between 0.40-0.60 a moderate relationship, between 0.60-0.70 a good relationship, between 0.70-0.75 a very good relationship, and between 0.75-1.00. was considered a perfect relationship. Positive correlation coefficients indicate that the variables increase or decrease together, and negative correlation coefficients indicate that as one variable increases, the other decreases, or vice versa (7). $p<0.05$ were considered statistically significant.

RESULTS

The average age of 100 patients included in the study was 37.4±11.7 (19-68) and 51% were male. The average length of stay for patients who were hospitalized and monitored was 8.69±3.8 (2-15) days. The rates of patients receiving influenza and conjugated pneumococcal vaccines in the same year were found as 11% and 1%, respectively. None of the patients had received polysaccharide pneumococcal vaccine. 10 of those vaccinated were healthcare workers. Thorax CT imaging was performed in 29% of the patients. While normal findings were detected in 41.4% of these patients, findings compatible with highly suspicious COVID-19 were detected in 58.6%. 98 of the patients were symptomatic; while the most common symptoms were muscle-joint pain, fever and fatigue; hypertension, Diabetes Mellitus and COPD-asthma were the most common comorbidities (Table 1). Laboratory findings of patients included in the study with a diagnosis of COVID-19 revealed high levels of leukopenia, lymphopenia, AST and ALT elevations, as well as other parameters. When the IgM and IgG dynamics of the cases were investigated on the 7th, 15th, 30th days and in the 3rd and 6th months, respectively, IgM was 96% positive and IgG was 98% positive on the 15th day (Table 2).
In our study, in which antibody dynamics were followed for 6 months, IgM levels were close to the normal index unit value (1.5 index unit) in the 6th month while IgG continued to remain at very high values (average 1053 AU/mL) (Figure 1 and Figure 2).

It was investigated whether there was a statistical relationship between the epidemiological and clinical characteristics of the patients and the dynamics of COVID-19 IgM and IgG antibodies. When antibody levels were evaluated according to the age of the patients, a low-medium level positive correlation was observed. When antibody levels were evaluated between inpatients and outpatients, there was a significant difference, being higher in inpatients ($p<0.05$). Of all patients, 61% were healthcare workers. When antibody levels were compared between groups of healthcare workers and non-healthcare workers, the 1st and 3rd month IgG levels were observed high in favor of the non-healthcare worker group ($p<0.05$), while no significant difference was detected

Table 1. Epidemiological and Clinical Characteristics of Patients

Epidemiological and Clinical Characteristics	Number (%)
Female Gender	49 (49)
Healthcare Professional	61 (61)
Comorbidities (exist)	26 (26)
Hypertension	11 (42.3)
Diabetes Mellitus	6 (23.1)
COPD-Asthma	5 (19.2)
Immunosuppressive State	3 (11.5)
Coronary Artery Disease	2 (7.7)
Chronic Renal Failure	1 (3.8)
Others (hypothyroidism, spondylitis etc.)	6 (23.1)
Those who have symptoms	98 (98)
Muscle and Joint Pain	61 (62.2)
Fever	48 (49)
Fatigue	46 (46.9)
Cough	34 (34.6)
Anosmia	31 (31.7)
Ageusia	23 (23.5)
Headache	20 (20.4)
Dyspnea	14 (14.3)
Diarrhea	5 (5.1)
Others (vomiting, rheum, sore throat)	26 (26.5)

COPD: Chronic Obstructive Pulmonary Disease

Table 2. Laboratory Findings and Antibody Dynamics of Patients

Laboratory Findings	**Mean ± SD**	**Minimum- Maximum**	**Normal Value**		
C-Reactive Protein (CRP)	13.33±31.53 mg/L	0.2-209 mg/L	0-5 mg/L		
Procalcitonin	0.8±1.64 ng/mL	0.03-6 ng/mL	0-0.046 ng/mL		
Leukocyte Count	5.662±2.003 u/L	1.000-12.000 u/L	4.000-10.000 u/L		
Lymphocyte Count	1.490±634 u/L	370- 3.800 u/L	800-5.500u/L		
Neutrophil Count	3.693±1.830 u/L	1.200-9.600u/L	1.500-7.300u/L		
Platelet Count	219.000± 64.400 u/L	108.000-401.000 u/L	150.000-400.000 u/L		
D-dimer	132±132.5 mg/L	18-1005 mg/L	0-0.55 mg/L		
Ferritin	129±143 ug/L	7-892 ug/L	30-400 ug/L		
Fibrinogen	317±81 mg/dL	202-609 mg/dL	200-400 mg/dL		
AST	32±109 U/L	13-1076 U/L	0-33 U/L		
ALT	36±82 U/L	13-768 U/L	0-32 U/L		
Antibody Dynamics	**7th Day**	**15th Day**	**30th Day**	**3th Day**	**6th Day**
IgM Level	6.1±12.2	11.8±16.3	9.15±13	4.32±8	1.5±2.5
Detection rate (%)	69	96	76	65	35
IgG Level	344±1120	1949±6755	1738±2730	1142±1707	1053±1895
Detection rate (%)	49	98	98	94	100

SD: Standard Deviation

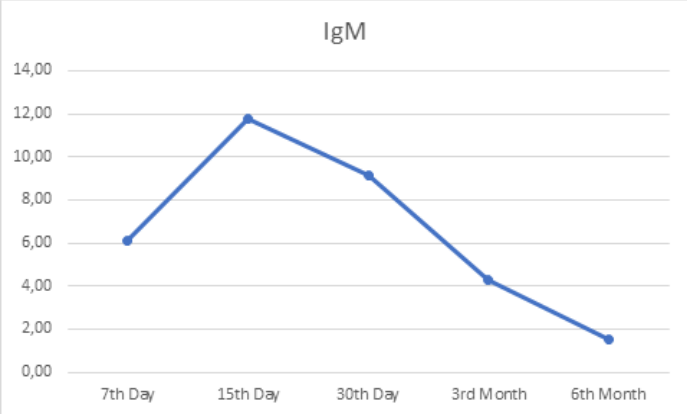

Figure 1. Average IgM Antibody Dynamic Changes (>1 Index unit, positive)

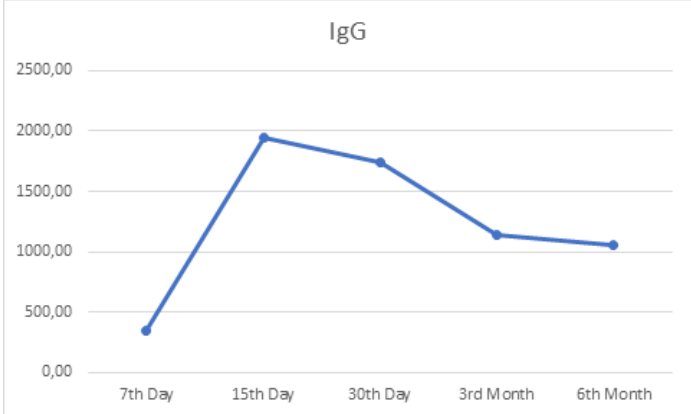

Figure 2. Average IgG Antibody Dynamic Changes (>50 AU/mL, positive)

in other times and antibody types. When the relationship between the route of transmission and the antibody response was investigated, a significant relationship was found only in the 6th month IgG levels due to the lower antibody levels in the family transmission group ($p<0.05$) while there was no significant difference between the other groups. There was no significant difference for 6 months between COVID-19 neutralizing antibody levels and the gender of the patients and whether they had received influenza or pneumococcal vaccination ($p>0.05$).

When the relationship between the complaints and symptoms of the patients and their antibody levels was evaluated, IgM and IgG values were reported higher on the 7th day in symptomatic patients ($p<0.05$). While antibody levels were higher in those with complaints and symptoms of fatigue, cough, dyspnea, and diarrhea than in those without, Levels were lower in those with headache than in those without ($p<0.05$). When thorax CT results and antibody levels were compared, IgM and IgG levels were significantly higher in patients with high-risk involvement on CT compared to patients who did not undergo CT. IgG levels on the 15th day and 1st month were higher in patients with high-risk CT findings than in patients without CT and with normal CT findings ($p<0.05$). There was no relationship between the presence or absence of fever, muscle-joint pain, loss of taste, loss of smell and other symptoms and antibody levels ($p>0.05$).

It was explored whether there was a statistical relationship between the laboratory indicators of the patients and the dynamics of COVID-19 IgM and IgG antibodies. There was a low-moderate positive correlation between CRP and IgG level on the 15th day, and a positive moderate correlation between the IgG level on the 30th day. Again, there was a low positive

Table 3. Correlation Coefficients (r) Relationship Between Laboratory Findings and Antibody Levels of Patients

Laboratory Findings	7th Day IgM	7th Day IgG	15th Day IgM	15th Day IgG	30th Day IgM	30th Day IgG	3rd Month IgM	3rd Month IgG	6th Month IgM	6th Month IgG
CRP	0.45**	0.092	0.263	0.399*	0.243	0.416**	0.107	0.287	0.079	0.236
D-dimer	0.162	0.180	0.273	0.363*	0.251	0.297	0.139	0.240	0.132	0.230
Fibrinogen	0.098	0.140	0.257	0.335*	0.277	0.324*	0.122	0.298	0.083	0.209
ALT	0.057	0.060	0.307*	0.338*	0.322*	0.381*	0.289	0.396*	0.222	0.157
AST	0.093	0.115	0.310*	0.373*	0.341*	0.412**	0.282	0.334*	0.236	0.124
Ferritin	0.133	0.117	0.167	0.200	0.206	0.187	0.204	0.253	0.059	0.084
Leucocyte	-0.040	0.114	-0.035	0.054	0.035	0.098	-0.033	-0.077	0.013	-0.003
Neutrophile	-0.126	0.043	-0.038	0.123	0.041	0.140	0.000	-0.72	0.037	0.041
Lymphocyte	0.158	0.227	-0.037	-0.067	-0.061	-0.090	-0.064	-0.162	-0.007	-0.231
Thrombocyte	-0.007	0.087	0.013	0.043	-0.080	0.041	-0.095	-0.161	-0.022	-0.071
Procalcitonin	0.542**	0.548**	0.250	0.295	0.224	0.361*	0.347*	0.390*	0.152	0.139
Sedimentation	-0.086	0.049	0.151	0.245	0.092	0.246	0.004	0.168	0.152	-0.017

r: Spearman Correlation Coefficient *: Positively low-moderate relationship **: Moderate, good, very good relationship in positive direction.

correlation or insignificant correlation between 7th day IgG, 15th day IgM, 30th day IgM, 3rd month IgG and 6th month IgG levels. A low to moderate positive correlation was reported between D-dimer level and 15th day IgG, while a low positive correlation or insignificant correlation was observed between 15th day IgM, 30th day IgM, 30th day IgG, 3rd month IgG and 6th month IgG. A positive low to moderate correlation was detected between fibrinogen level and day 15 IgG and day 30 IgG levels. There was a positive low-medium level correlation between ALT and AST levels and 15th day IgM, 15th day IgG, 30th day IgM, 30th day IgG, 3rd month IgG levels. A low or insignificant positive correlation was reported between ferritin level and IgG only in the 3rd month. While a positive, moderate correlation was found between procalcitonin level and 7th day IgM and 7th day IgG, a low and moderate positive correlation was detected between 30th day IgG, 3rd month IgM and IgG levels. While there was a low positive correlation or insignificant correlation between the lymphocyte count and 7th day IgG and 6th month IgG, no correlation was observed between leukocyte, neutrophil, platelet count and erythrocyte sedimentation rate values and antibody levels (Table 3).

DISCUSSION

It is unclear which components of the immune system are important for SARS-CoV-2 infection and the antibody levels required to maintain immunity. Most patients develop a humoral immune response in the early period, which leads to the emergence of neutralizing antibodies in a majority of cases with SARS-CoV-2 infection. However, the duration of the developing immune response and its protective capacity have not been fully elucidated. In some of the studies, it has been shown that neutralizing and protective anti-SARS-CoV-2 antibodies that appear after infection reduce the possibility of re-infection in the 13 months following the infection (8). Despite the difficulty of measuring neutralizing antibodies outside the laboratory environment, recent studies have shown that IgG levels are associated with neutralizing antibody levels (9). Antibodies are detected 6 days after the symptoms occur and increase during the first 3-4 weeks (4). In our research, we studied how these antibodies changed over 6 months and whether there was a significant relationship with clinical, laboratory and epidemiological features.

Figueiredo-Campos et al. (10) in their 6-month COVID-19 antibody seroprevalence studies; the female and male ratios in COVID-19 patients were reported as 52% and 48%, respectively. In the study of Cervia et al. (11) in which the systemic and mucosal specific antibody response was evaluated in mild and severe COVID-19 cases, it was reported that 54.7% of the cases were male and 45.3% female. In the COVID-19 antibody study conducted by Simanek et al. (12), it was reported that 51% of 110 patients were male and 49% were female. We have seen that antibody studies are generally carried out with numbers of patients between 100-200. In this study, 51% of 100 patients were male and 49% were female. In most studies evaluating the antibody response in COVID-19 patients, the comorbidities of the patients were most frequently reported as hypertension, diabetes mellitus and coronary artery disease (10, 11, 13, 14). In our study, the most common comorbidities were hypertension and diabetes mellitus.

In Sandri et al.'s (15) study, when the patients were asked about the possible way of transmission; while 27% did not report any means of transmission, 40.2% had contact with a diagnosed patient, 21.5% had contact with a co-worker, 3.5% had family contact and 7.8% had other contact. In our study, the most common possible route of transmission was reported as healthcare environment with 59%. Since the majority of the patients were healthcare workers, similar to the other study, healthcare-related contact was the most frequently detected route of transmission, while family contact was the second most common route of transmission in our study. In the study of Simanek et al. (12), it was stated that 6.1% of the cases had received influenza vaccination. In the study of Liu et al. (13), it was reported that COVID-19 IgG levels of those vaccinated with pneumococcal vaccine were low and there was no difference in COVID-19 antibody levels after influenza vaccination. In a different study conducted in Italy,

it was stated that there was an inverse relationship between COVID-19 cases and influenza vaccine, that the influenza vaccine played a protective role, and that it might be possible for the prognosis of COVID-19 infection to be better in these people by inhibiting the accompanying infection (16). In our study, 11% of the patients declared that they had influenza and 2% had conjugated pneumococcal vaccination; no significant difference in antibody response was detected in both patient groups. Although the majority of cases are healthcare workers, it is noteworthy that the vaccination rate is very low. This issue should also be studied separately in our country.

During the course of COVID-19, the reliability of antibody testing increases with the time passed after the onset of symptoms, and at least 14 days after the onset of symptoms is the most appropriate period for antibody testing (17). In our study, COVID-19 IgM seroconversion rates were highest on the 15th day and gradually decreased to the lowest rate in the 6th month; the percentage of IgG seroconversion was highest in the 6th month. In our study, while IgM and IgG were positive in 69 % and 49 % of the patients, respectively, in the first week, these rates increased on the 15th day. Although it decreased in the 3rd month, it was determined that IgM positivity continued in 35% of the patients and IgG positivity continued in all patients in the 6th month. Zhao et al. (18) in their study evaluating 173 inpatients, seroconversion rates were reported as 93.1%, 82.7% and 64.7% for total antibody (Ab), IgM and IgG, respectively, and the median seroconversion time was the 11th day, the 12th day and 14th days. While antibody positivity was < 40% in the first week, it rapidly increased to 100 % (total Ab), 94.3% (IgM) and 79.8% (IgG) from the 15th day. Also, in a meta-analysis evaluation, the IgM seroconversion rate was found as approximately 75.3%; the mean IgM seroconversion rates on day 7, day 14, day 21, day 28 and >28 days were 37.5%, 73.3%, 81.3%, 72.3% and 73.3%, respectively. Mean IgG seroconversion rate was 85.8%; on the 7th, 14th, 21st, 28th day and >28th days, it was found as 35.4%, 80.6%, 93.3%, 84.4% and 98.9%, respectively. In this meta-analysis, IgM and IgG seroconversion rates were low in the first week of infection, at 37.5% and 35.4%, respectively; While the IgM detection rate decreased to 81.3% on the 21st day and to 73.3% after the 28th day, the IgG detection rate increased to 93.3% on the 21st day and to 98.9% after the 28th day (19). In the study by Wajnberg et al. (20) in which they evaluated more than 30,000 COVID-19 PCR positive cases, approximately 93% of the cases had medium-high titer anti-spike antibodies, and more than 90% of them had a detectable neutralizing antibody response. It was determined that these antibody titers were stable for 3 months and modest decreases were observed in the 5th month. In a different study, it was observed that the neutralizing antibody levels of cases diagnosed with COVID-19 started to decrease after approximately 6-8 months (21). In another different cohort study evaluating symptomatic COVID-19 cases in North America, it was stated that IgM and IgA responses to SARS-CoV-2 RBD were transient and seroreversion occurred within 2.5 months after the onset of symptoms in the majority of patients, However, the IgG response remained positive for more than 90 days and seroreversion was minimal (22). In a population-based serosurveillance study in Iceland, the seropositivity rate in COVID-19 patients with PCR positivity was found over 90%, and it was reported that the patients remained seropositive even after 120 days and no decrease in antibody levels was detected (23). In a different study by Zhang et al. (24), it was shown that although there may be a variable decrease in the antibody titer against SARS-COV-2 in most patients, antibody positivity may remain even after 194 days. In our study, the fact that the IgG detection rate decreased from 98% on the 15th day to 94% in the 3rd month and then increased to 100% in the 6th month can be related to the re-increase in antibody levels due to the COVID-19 vaccine administration that coincided with this period. As found in other studies and in our research, similar results are achieved in intermittent monitoring of antibody dynamics; while IgM levels, which are initially high, decrease over time, IgG levels increase and remain constant at high rates. COVID-19 vaccine applications and difficulties in obtaining study kits during the pandemic period have not made it possible to monitor antibody dynamics for a longer period of time.

In the study of Sandri et al. (15), it was stated that the IgG level was higher in individuals between the ages of 31-50, and an age-related decreasing trend was observed in the analysis of the frequency of IgG positivity in different age ranges. Additionally, it was determined that this relation was particularly prevalent in patients between the ages of 20 and 40 or older than 60, and in women rather than men. In the same study, it was also found that there was no significant difference in plasma IgG levels between men and women. When patients over the age of 60 were evaluated, the antibody level was higher in men than in women, but it was emphasized that the prognosis was worse in men, which was interesting. No significant difference was found in other age groups. In a study investigating the IgG and IgA response, there was no significant relationship between the formation of antibodies and age and gender (11). In our study, no significant relationship was observed between gender and antibody formation and level, but there was a low positive correlation between the 15th day IgG, 30th day IgG and 3rd month IgM level and age, and a low-medium level positive correlation between the 30th day IgM level and age.

Sandri et al. (15) and Cervia et al. (11) did not find a relationship between IgG positivity and comorbid diseases. In our study, 1st and 6th month IgG levels were approximately 2 times higher in patients with comorbid diseases than in those without, and there was a significant relationship ($p < 0.05$). Also, in our study, there was a significant relationship due to lower 6th month IgG levels in the family transmission group. This relationship may be due to the fact that healthcare workers have been given priority for COVID-19 vaccination, so non-healthcare workers have not yet been vaccinated.

Studies have reported that there is a delayed and weaker immune response in asymptomatic COVID-19 infection and that the IgG titer decreases more and faster (24, 25). IgM and IgG titers are higher in severe cases compared to mild

COVID-19 cases (25-28). In the study of Liu et al. (29) in which they evaluated COVID-19 antibody dynamics, there was no difference in IgG levels in mild and severe cases in the first 2 weeks, but after the 2nd week, a stronger IgG response was observed in severe cases compared to mild cases. Similar to these findings, in our study, 15th day IgM and IgG, 30th day IgM and IgG, 3rd and 6th month IgG levels were significantly higher in inpatients than in outpatients ($p<0.05$). In this context, it can be concluded that those who have more severe COVID-19 infections have a stronger immune response to maintain immunity both in the early and long term.

In a study evaluating symptoms, researchers determined that all patients with myalgia, cough, fever, asthenia, dyspnea, angina pectoris, anosmia/dysgeusia, tachycardia or pneumonia had higher IgG levels than asymptomatic patients, and that, in addition to pneumonia, fever, anosmia/dysgeusia and chest pain were the symptoms that best characterized the IgG positive population (15). In our study, day 7 IgG and IgM levels were found higher in symptomatic patients, but contrary to expectations, no correlation was observed between fever, muscle-joint pain, anosmia/dysgeusia and antibody levels. There was a positive correlation between symptoms of fatigue, dyspnea, cough, diarrhea and antibody levels, while a negative correlation was observed between headache and antibody levels.

In a study conducted on 243 healthcare workers in which IgM and IgG antibodies were tested, the positive IgM and IgG rates in cases with findings on thorax CT were 1.6 and 1.3 times higher, respectively, than in those without CT findings (30). Similarly, in our study, antibody levels were higher in patients with involvement on thorax CT, especially those with high-risk involvement. This result suggests that the antibody response is stronger due to the more severe clinical course.

When the literature is reviewed, it is seen that the relationship between laboratory parameters and the severity of COVID-19 infection has been frequently investigated, but there are not enough studies on the relationship between COVID-19 antibodies and laboratory parameters. In a study conducted with 28 intensive care unit patients, researchers reported that they could not detect a significant relationship when leukocyte, neutrophil and lymphocyte counts, ferritin, CRP and procalcitonin levels and antibody levels were evaluated separately (31). In our study, no correlation was observed between the leukocyte, neutrophil and platelet levels of the cases and the antibody level, but there was a positive correlation between the lymphocyte count, ferritin, D-dimer, fibrinogen, CRP, procalcitonin, AST, ALT levels and antibody levels. We believe that this situation is due to the relationship between the immune response to the disease and the antibody response.

In addition, in a 25-year-old female patient in our study population, two SARS-CoV-2 PCR tests taken 24 hours intervals were positive because she complained of fever and cough again 3 months after the first infection, and reinfection was diagnosed (this patient's SARS-CoV-2 PCR test taken on days 10 and 11 after initial infection was negative). While the 7th 15th day and 1st month IgM and IgG results of this patient, who had no known comorbidities or immunosuppression, were negative, the 3rd month IgM level 61 index units; IgG 112 and 6th month IgM 1, IgG 190 index units were positive. It was observed that the patient had an antibody response after reinfection. Although statistical evaluation cannot be made because it is a single case, this case suggests that IgG antibodies against the SARS-CoV-2 spike antigen are protective.

While the limitations of this study are that the sample size was small and the fact that some of the patients participating in the study were vaccinated during the very intense period of the pandemic, the study was conducted in a period when there was no previous COVID-19 infection and most of the time the COVID-19 vaccine was not available; monitoring the neutralizing antibody dynamics on the 7th, 15th and 30th days and in the 3rd and 6th months, and investigating the correlation and duration of the patients' epidemiological and clinical characteristics, laboratory parameters and serum protective antibody levels reveal the very strengths of the study.

In conclusion; It has been determined that neutralizing antibodies are formed to a large extent in those who have had COVID-19 infection and usually persist for at least 6 months. Antibody levels were found higher in symptomatic patients, those who were hospitalized, and those with moderate-high suspicious infiltration in the lungs. Also, a significant relationship was detected between laboratory indicators such as CRP, AST, ALT, procalcitonin, ferritin, D-dimer and antibody dynamics. These findings contribute to the understanding of the immunological response to COVID-19 and suggest that they may have implications for long-term immunity and vaccine strategies.

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OPEN ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Evaluation of PD-1 / PD-L1 Expressions in Patients with Diffuse Large B Cell Lymphoma and Chronic Lymphocytic Leukemia

Diffüz Büyük B Hücreli Lenfoma ve Kronik Lenfositer Lösemili Hastalarda PD-1 / PD-L1 Ekspresyonlarının Değerlendirilmesi

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ÖZET

Amaç: Programlanmış ölüm 1 (PD-1) / PD-ligandı (PDL) yolu, T hücresi aracılı immün yanıtlarının düzenlenmesi için önemli bir kontrol noktasıdır. Kanserler ne yazık ki PD-1/ PD-L1 yolunun immünsüpresif etkilerinden de yararlanmaktadır. Yaygın Büyük B Hücreli Lenfoma (YBBHL) ve Kronik Lenfositer Lösemi (KLL)'de PD-1/ PD-L1'in önemi ile ilgili yeterli kanıt yoktur. Çalışmamızda YBBHL ve KLL'de PD-1/ PD-L1 ekspresyonun varlığını ve prognostik önemini değerlendirmeyi amaçladık.

Gereçler ve Yöntem: Hematoloji Kliniğinde Ocak 2010 ile Eylül 2018 tarihleri arasında takip edilen 18-80 yaş arası 26 YBBHL hastası ve 27 KLL hastası çalışmaya dahil edildi. İmmunhistokimyasal boyama için Tıbbi Patoloji Anabilim Dalı arşivinde bulunan hastalara ait parafin blokları kullanıldı. YBBHL hastalarının lenf bezi biyopsi materyalleri ve KLL hastalarının kemik iliği biyopsi materyalleri değerlendirildi. PD-1 neoplastik olmayan dokularda boyandı ve PD-L1 neoplastik hücrelerde boyandı. Boyanma yüzdesi %5'in üzerinde olanlar pozitif kabul edildi.

Bulgular: PD-1; YBBHL olgularının %65,4'ünde (n=17) pozitif, KLL olgularının %14,8'inde (n=4) pozitif saptandı. PD-L1; YBBHL olgularının %69,2'sinde (n=18) pozitif, KLL olgularının PD-L1 %3,7'sinde (n=1) pozitif saptandı. PD-1 ve PD-L1 ekspresyon ve boyanma sıklığı istatistiksel olarak anlamlı şekilde YBBHL'da daha yüksek bulundu (p=<0.001). PD-L1'de YBBHL tanılı olgularda evreler arasında ekspresyon açısında anlamlı fark bulundu (P=0,004).

Sonuç: Çalışmamızda YBBHL'de literatürden daha yüksek oranda PD-1/ PD-L1 ekspresyonu tespit edildi. KLL'de diğer çalışmalara göre daha düşük oranda PD-1/PD-L1ekspresyonu saptandı. PD-1/ PD-L1 ekspresyonun YBBHL'de KLL'den daha fazla olduğu gösterildi.

Anahtar Kelimeler: Yaygın Büyük B Hücreli Lenfoma, Kronik Lenfositer Lösemi, PD-1, PD-L1.

ABSTRACT

Aim: The programmed death 1 (PD-1) / PD-ligand (PDL) pathway is an important checkpoint for regulation of T cell mediated immune responses. Malignancies unfortunately also benefit from the immunosuppressive effects of the PD-1/PD-L1 pathway. There is not enough evidence regarding the importance of PD-1/ PD-L1 in Diffuse large B cell lymphoma (DLBCL) and Chronic lymphocytic leukemia (CLL). We aimed to evaluate the presence and prognostic significance of PD-1 / PD-L1 expression in DLBCL and CLL.

Materials and Methods: 26 DLBCL patients and 27 CLL patients aged between 18-80 years, who were followed up between January 2010 and September 2018 in Hematology Clinic, were included. For immunohistochemical staining, paraffin blocks belonging to the patients in the archive of Medical Pathology Department were used. Lymph node biopsy materials of DLBCL patients and bone marrow biopsy materials of CLL patients were evaluated. PD-1 was stained in non-neoplastic tissues and PD-L1 was stained in neoplastic cells. Those whose staining percentage was above 5% were considered positive.

Results: PD-1; It was detected positive in 65.4% (n=17) of DLBCL cases and 14.8% (n=4) of CLL cases. PD-L1 was detected positive in 69.2% (n=18) of DLBCL cases, and PD-L1 was positive in 3.7% (n=1) of CLL cases. The frequency of PD-1 and PD-L1 expression and staining was found to be statistically significantly higher in DLBCL (p=<0.001). In PD-L1, a significant difference in expression was found between stages only in cases diagnosed with DLBCL (P=0,004).

Conclusion: In our study, a higher rate of PD-1/ PD-L1 expression was detected in DLBCL than in the literature. A lower rate of PD-1/ PD-L1 expression was detected in CLL compared to other studies. It has been shown that PD-1/ PD-L1 expression is higher in DLBCL than in CLL.

Keywords: Diffuse large B cell lymphoma, Chronic lymphocytic leukemia, PD-1, PD-L1.

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INTRODUCTION

The programmed death 1 (PD-1)/PD-ligand (PDL) pathway is important for the regulation of T cell-mediated immune responses (1). The complex consists of the transmembrane protein PD-1/CD279 and its two ligands PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273). PD-1 is expressed on activated T cells, B cells, natural killer cells, macrophages, as well as a large proportion of tumor-infiltrating lymphocytes (TIL) (2). PD-1 functions as an important immune checkpoint in the regulation of T cell-mediated responses. PD-L1 is expressed mainly by antigen-presenting cells (APCs) as well as various non-hematopoietic cells and tumor cells (3).

PD-L1 is activated by PD-1 and causes reversible inhibition of T cell activity and proliferation (4–6). Malignant cells can also exploit the immunosuppressive effects of the PD-1-PD-L1 pathway (7). Many tumors are known to express PD-L1 as a line of defense against TILs (8). The importance of PD-1/PD-L1 expression in some solid tumors and in Hodgkin's lymphoma (HL), a hematological neoplasm, is well known. Anti-PD-1/PD-L1-directed treatments have begun to be used successfully in the clinics (9). However, there is insufficient evidence regarding the importance of PD-1/PD-L1 in Diffuse large B cell lymphoma (DLBCL) and Chronic lymphocytic leukemia (CLL). In the current study, we aimed to evaluate the presence and prognostic significance of PD-1/PD-L1 expression in DLBCL and CLL.

MATERIALS AND METHODS

Patients

The current study included 26 DLBCL and 27 CLL cases who received chemotherapy and were followed up at the Hematology Clinic between January 2010 and September 2018, and whose post-chemotherapy response data was available. Lymph node biopsy materials of DLBCL patients and bone marrow biopsy materials of CLL patients were evaluated. Patients between the ages of 18 and 80 years were included in the study. DLBCL disease stage at diagnosis was determined according to the Ann Arbor staging system, and risk scoring was calculated according to the International Prognostic Index (IPI) score. CLL staging was carried out according to the RAI classification. Stage at diagnosis and risk scores were determined from patient files. The study was approved by Non-Interventional Clinical Research Ethics Committee (Decision no: 08, Date: 09.11.2018). Financial support for the study was obtained from the Scientific Research Project unit (project number TTU-2019-7741).

Immunohistochemical Analysis

Paraffin blocks of specimens collected from patients diagnosed with DLBCL and CLL were used for immunohistochemical staining. The appropriate paraffin blocks were selected from Hematoxylin - Eosin sections. The presence of sufficient tissue from the paraffin blocks for immunohistochemical examination was first evaluated. Next, 4 micrometer thick sections were obtained from the selected paraffin blocks with a Leica RM® 2135 (Leica MICROSYSTEMS, GERMANY) brand rotary microtome device and placed on poly-L-lysine coated slides. Primary antibodies against PD-1 (NAT105) and PDL-1 (SP263) were used. The sections were stained with these antibodies using the Ventana Benchmark XT immunohistochemistry automatic staining system and Ventanaultra View Universal DAB Detection Kit (REF 760-500, Ventana Medical Systems, Inc., Arizona, USA) accompanied by appropriate positive controls. The stainings were evaluated at different magnifications on an Olympus BX53F (Olympus, Tokyo, Japan) light microscope. The staining intensity was evaluated in non-neoplastic tissues for PD-1 and in neoplastic cells for PD-L1 as follows:

Table 1. Laboratory Data

	DLBCL Mean±SD	CLL Mean±SD
Hb (g/dl)	12.5 ± 2.1	12.1 ± 2.98
WBC (x109/L)	7.68 ± 3.16	7.53 ± 8.81
PLT(x109/L)	220.23 ± 103.27	152.51 ± 85.73
Neutrophil count (x109/L)	5.37 ± 2.84	8.05 ± 7.86
Lymphocyte count (x109/L)	1.59 ± 1.85	59.99 ± 74.61
Eosinophil count (x109/L)	0.90 ± 0.87	0.33 ± 0.60
Monocyte count (x109/L)	0.51 ± 0.43	5.99 ± 11.84
MCV(fL)	86.23 ± 5.36	91.59 ± 7.99
Glucose (mg/dl)	112.07 ± 61.23	102 ± 24.11
ALT (U/L)	28.65 ± 27.09	20.55 ± 14.10
AST (U/L)	35.03 ± 6.68	23.44 ± 7.94
Uric acid (mg/dl)	5.15 ± 2.20	5.35 ± 1.55
Creatine (mg/dl)	1.06 ± 0.50	0.83 ± 0.265
Urea (mg/dl)	1,03 ± 2,05	3,31 ± 6,06
Ferritin(ng/ml)	318.39 ± 410.92	151.19 ± 265.69
Sedimentation (mm/h)	33.26 ± 26.16	27.33 ± 29.20
CRP (mg/L)	49.19 ± 5.,69	19.13 ± 29.64
Albumin (g/dl)	3.56 ± 0.67	4.13 ± 0.762
Ki-67(%)	67.11 ± 22.27	12.03 ± 10.917

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CRP: C-reactive protein, Hb: hemoglobin, PLT: platelet count, MCV: Mean corpuscular volume, WBC: white blood cell, DLBCL: Diffuse large B cell lymphoma, CLL:Chronic lymphocytic leukemia

(-) or (0): No staining
(+) or (1): Weak staining
(++) or (2): Moderately strong staining
(+++) or (3): No strong staining

The sections with a staining percentage above 5% were considered positive, and those below 5% were considered negative.

Table 2. PD-1 / PD-L1 expression frequency and staining intensity in DLBCL-CLL.

	DLBCL (n:26)		CLL (n:27)		P value
	N	%	N	%	
PD-1 expression					
Positive	17	65.4	4	14,8	
Negative	9	34.6	23	85,2	<0.001
PD-1 staining intensity					
-(0)	7	26.9	19	70,4	
+ (1)	3	11.5	5	18,5	0,001
++ (2)	13	50	3	11,1	
+++ (3)	3	11.5	0	0	
PD-L1 expression					
Positive	18	69.2	1	3,7	
Negative	8	30.8	26	96,3	<0.001
PD-L1 staining intensity					
-(0)	7	26.9	26	96,3	
+ (1)	0	0	0	0	
++ (2)	1	3,8	0	0	
+++ (3)	18	69.2	1	3,7	<0.001

Those with a staining percentage above 5% were considered positive, and those below were considered negative. DLBCL: Diffuse large B cell lymphoma, CLL:Chronic lymphocytic leukemia

Table 3. Prognostic significance of PD-1 / PD-L1 in DLBCL.

		PD-1			PD-L1		
	n	Positive (%)	Negative (%)	P value	Positive (%)	Negative (%)	P value
Stage							
1	6	4 (66,6)	2 (33,3)		4 (66,7)	2 (33,3)	
2	5	2 (40)	3 (60)	0,058	2 (40)	3 (60)	0,004
3	11	10(90,9)	1 (9,1)		11 (100)	0 (0)	
4	4	1 (25)	3 (75)		1 (25)	3 (75)	
ECOG Performance Status							
1	11	8 (72,7)	3 (27,3)		8 (72,7)	3 (27,3)	
2	3	2 (66,7)	1 (33,3)	0,615	2 (66,7)	1 (33,3)	0,962
3	5	2 (40)	3 (60)		3 (60)	2 (40)	
4	7	5 (71,4)	2 (28,6)		5 (71,4)	2 (28,6)	
IPI score							
Low risk	9	7 (77,8)	2 (22,2)		7 (77,8)	2 (22,2)	
Low-intermediate risk	2	0 (0)	2 (100)	0,131	0 (0)	2 (100)	0,145
High-intermediate risk	9	5 (55,6)	4 (44,4)		6 (66,7)	3 (33,3)	
High risk	6	5 (3,3)	1 (16,7)		5 (83,3)	1 (16,7)	
Disease subtype							
Germinal Center	6	5 (83,3)	1 (16,7)		4 (66,7)	2 (33,3)	
Non-Germinal	20	12 (60)	8 (40)	0,380	14 (70)	6 (30)	1,00
Ki 67 index							
<30	1	1 (100)	0 (0)		1 (100)	0 (0)	
>30	25	16 (64)	9 (36)	1,00	17 (68)	8 (32)	1,00
Treatment response							
Complete response	5	4 (80)	1 (20)		4 (80)	1 (20)	
Partial response	8	4 (50)	4 (50)	0,505	4 (50)	4 (50)	0,263
Unresponsive	9	7 (77,8)	2 (22,2)		8 (88,9)	1 (11,1)	
Stable disease	4	2 (50)	2 (50)		2 (50)	2 (50)	

ECOG: Eastern Cooperative Oncology Group, IPI: International Prognostic Index, PD-1: Programmed death 1, PD-L1: Programmed death ligand-1, DLBCL: Diffuse large B cell lymphoma,

Table 4. Prognostic significance of PD-1/PD-L1 in CLL.

		PD-1			PD-L1		
	n	**Positive (%)**	**Negative (%)**	**P value**	**Positive (%)**	**Negative (%)**	**P value**
Stage							
0	1	0 (0)	1 (100)	0,710	0 (0)	1 (100)	0,842
1	6	1 (16,7)	5 (83,3)		0 (0)	6 (100)	
2	5	0 (0)	5 (100)		0 (0)	5 (100)	
3	15	3 (20)	12 (80)		1 (6,7)	14 (93,3)	
4	0	0 (0)	0 (0)		0 (0)	0 (0)	
ECOG Performance Status							
1	8	2 (25)	6 (75)	0,708	0 (0)	8 (100)	0,397
2	7	1 (14,3)	6 (85,7)		1 (14,3)	6 (85,7)	
3	4	0 (0)	4 (100)		0 (0)	4 (100)	
4	8	1 (87,5)	7 (12,5)		0 (0)	8 (100)	
Ki67 index							
<30	23	2 (8,7)	21 (91,3)	0,92	1 (4,3)	22 (95,7)	1,00
>30	4	2 (50)	2 (50)		0 (0)	4 (100)	
Treatment response							
Complete response	11	2 (18,2)	9 (81,8)	0,358	0 (0)	11 (100)	0,206
Unresponsive	5	0 (0)	5 (100)		1 (20)	4 (80)	
Stable disease	3	0 (0)	3 (100)		0 (0)	3 (100)	
Progressive disease	8	2 (25)	6 (75)		0 (0)	8 (100)	

ECOG: Eastern Cooperative Oncology Group, IPI: International Prognostic Index, PD-1: Programmed death 1, PD-L1: Programmed death ligand-1, CLL:Chronic lymphocytic leukemia

Statistical Analysis

The data were evaluated with the SPSS 22.0 statistical program. Frequency distributions were examined. Descriptive statistics are given as percentages and averages. Chi-square and Fisher's exact test were used for categorical variables. The suitability of numerical variables for normal distribution was evaluated with the Kolmogorov-Smirnov test. When parametric test conditions were met for numerical variables, Student-t test was used for binary variables, analysis of variance (ANOVA) was used in groups with more than 2 variables, Mann-Whitney U test was used when parametric conditions were not met, and Kruskal Wallis test was used in groups with more than two variables. The study was conducted with a 95% confidence interval, and $p<0.05$ was considered to be statistically significant.

RESULTS

The average age of the DLBCL cases at diagnosis was 56.4 ± 17.26 years; 46.2% (n=12) were female and 53.8% (n=14) were male. The average age of CLL cases at diagnosis was 61.40 ± 10.95 years, 44.4% (n=12) female and 55.6% (n=15) male. The distribution of the age at diagnosis of DLBCL and CLL was evaluated with the Mann-Whitney U test; no statistically significant difference was detected (p= 0.130). The mean leukocyte count was found to be higher in CLL patients (75326/µL) than in DLBCL patients (7683/µL). The Ki-67 index was found to be higher in DLBCL (67.11%) compared to CLL (12.03%). All other laboratory data are shown in Table 1.

The expression of PD-1/PD-L1 and their staining intensities in DLBCL and CLL (Table 2) were evaluated with the Pearson Chi-Square test; the proteins were found to be expressed statistically significantly more in DLBCL than in CLL (p=0.00) (Table 2). We observed that PD-1 was expressed in 65.4% and PD-L1 in 69.2% of the patients with DLBCL, while PD-1 was expressed in 14.8% and PD-L1 in 3.7% of the patients with CLL. No statistically significant difference in Ann Arbor stage, Eastern Cooperative Oncology Group (ECOG) score, IPI score, disease subtype, Ki67 index, treatment response and PD-1 expression could be identified in the cases diagnosed with DLBCL (P>0.05). A significant difference in PD-L1 expression was identified; additionally, a significant difference could be identified in the expression of PDL-1 between Ann Arbor stages (P=0.004) (Table 3).

We also evaluated the relationship between the expression of PD-1 and PD-L1 and RAI stage, ECOG score, IPI, Ki67 index, and treatment response in the cases diagnosed with CLL using the Chi-Square test. No statistically significant difference could be identified for either PD-1 or PD-L1 (Table 4).

DISCUSSION

The current study was designed to evaluate the expression of PD-1 and PD-L1 in patients with DLBCL and CLL. Yang et al. evaluated the plasma levels of PD-L1 in patients with B-cell lymphoma. The highest PD-L1 expression was found in DLBCL, followed by small lymphocytic lymphoma, mucosa-associated lymphoid tissue lymphoma, mantle cell lymphoma; the lowest expression of PD-L1 was detected in follicular lymphoma (10). Corroborating these data, we also observed stronger PD-L1 expression in DLBCL compared to CLL.

In one of the largest studies reported to date, PD-L1 and PAX5 were analyzed together in biopsy samples from 1253 DLBCL patients. PD-L1 was reported to be positively expressed in 11% of the tumor cells while PD-L1 (mPD-L1) was expressed in the tumor microenvironment in 15.3% of the cases. Both

tumor PD-L1(+) and mPD-L1(+) DLBCL were associated with non-germinal center B-cell type and Epstein-Barr virus (EBV) positivity. Patients with PD-L1(+) DLBCL had lower overall survival (OS) compared to patients with PD-L1(-) DLBCL. Contrary to our findings, a previous study has reported no significant difference in OS between mPD-L1(+) and mPD-L1(-) DLBCL (11). Another study investigated the relationship between the prognosis of DLBCL patients and PD-1 expression on the surface of CD4+ T cells. Patients with ≥30.25% PD-1 expression on CD4+ T cells had significantly lower event free survival (EFS) and OS compared to patients with <30.25% PD-1 expression on CD4+ T cells (10, 12). Kwon et al. reported that 61.1% of tumor cells in patients diagnosed with DLBCL showed expression of PD-L1. Strong PD-L1 expression on tumor cells was significantly associated with the presence of B symptoms and EBV infection and tended to be higher in activated B cell-like cells (16.7%) compared to the germinal center B cell (GCB)-like immunophenotype (2.5%). Increased infiltration of PD-1(+) cells was associated with prolonged progression-free survival (PFS) (P = 0.005) and OS (P = 0.026) in DLBCL patients treated with rituximab-cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP). The prognostic significance of PD-L1 expression, however, was not reported in the study (13).

DLBCL is divided into two subtypes: GCB type, which generally has a good prognosis, and the non-GCB (activated B cell-like (ABC)) type, which shows poor prognosis. Compared with classical HL, the expression of PD-L1 is known to be much lower in DLBCL (except for some specific subtypes). Only 10-24% of DLBCL cases are known to be positive for PD-L1; of these, PD-L1 expression is observed more frequently in non-GCB than in the GCB subtype. Moreover, PD-L1 expression is detected more frequently in de-novo DLBCL than in transformed DLBCL. PD-L1 expression is seen in approximately two-thirds of EBV+ DLBCLs, while it is seen in 5–10% of EBV-negative DLBCLs (11, 14–16). In the current study, we observed more PD-L1 expression in the GCB arm (83.3%) compared to the non-GCB arm (60%), although the difference was not statistically significant.

PD-L1 was reported to be expressed in 70% of classical HL, 54% of nodular lymphocyte-predominant HL, 35% of primary mediastinal B-cell lymphomas, and 31% of primary DLBCL (17). Gassner et.al reported that T cells express high levels of the inhibitory exhaustion markers PD-1 and lymphocyte-activation gene 3 (LAG3), whereas CLL cells express high levels of PDL-1. The fraction of exhausted T cells was shown to increase with the progression of CLL. The same study also demonstrate that exhausted T cells could be reinvigorated and show CLL cytotoxicity by the inhibition of PD-1/PD-L1 interaction in vivo (18).

Grzywnowicz et.al demonstrated the expression of PD-1 and PD-L1 on the surface of CLL cells and reported that the expression of PD-1 was higher in CLL cells compared to healthy donors. However, the relationship between PD-1 and PD-L1 with time to progression and OS could not be demonstrated (19). Similarly, another study also reported higher expression of PD-1/PD-L1 in CLL patients compared to the control group; this high expression was associated with RAI stage, CD38, ZAP-70, chromosome karyotype (20). Another study that evaluated the effect of EBV status on CLL prognosis also reported a higher expression of PD-1/PD-L1 in the patient group compared to the control group. High expression of PD-1/PD-L1 was associated with poor prognostic markers (RAI stages of CLL, del 17p13, ZAP70 and CD38 expression), failure of complete remission, shorter PFS and OS (21). In the current study, we detected PD-1/PD-L1 expression in CLL cells; however, contrary to the literature, we could not identify any significant relationship between PD-1/PD-L1 expression and stage, ECOG, IPI, disease type, Ki67 index, and treatment response.

A Phase Ib study using Nivolumab in patients with relapsed/refractory hematological malignancies reported an objective response of 40% in follicular lymphoma, 36% in DLBCL, 15% in Mycosis Fungoides, 40% in peripheral T-cell lymphoma and 4% in Multiple myeloma. Genetic alterations of PD-L1 and PD-L2 were rare among the Non-Hodgkin's lymphoma patients evaluated in the referred study (22).

Study Limitations

One of the limitations of the current study is the short follow-up period after treatment. CLL and DLBCL are diseases that are known to have high treatment response and longer survival compared to other malignancies. Another limitation of our study is that the number of patients in both DLBCL and CLL was too small to show statistically significant differences in subgroup analyses.

CONCLUSION

Higher PD-1 and PDL-1 expression and staining intensity were observed in DLBCL patient samples compared to the cases diagnosed with CLL. The current study suggests that PD-1/PD-L1 expression in B-cell lymphomas can be used diagnostically and may be a target for immunotherapy, especially in DLBCL.

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OPEN DERLEME / REVIEW

HLA-Related Drug Hypsensitivity Reactions

HLA İlişkili İlaç Aşırı Duyarlılık Reaksiyonları

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ÖZET

İlaç reaksiyonları (İR) iki temel grupta sınıflandırılır. İlki, immünolojik mekanizma aracılı alerjik reaksiyonları; ikincisi, spesifik immünolojik mekanizmanın katılımı olmadan gelişen ilaç aşırı duyarlılık reaksiyonlarıdır (İADR). Morbidite ve mortalite prevalansı İADR'de yüksektir. Bu şiddetli reaksiyonların bazıları spesifik insan lokosit antijen (HLA) alelleri ile bağlantılıdır. Çünkü, HLA genleri genomun en yüksek oranda polimorfik lokuslarını barındırır. Dolayısıyla, İADR etyolojisinde farmakolojik doz etkisinden ziyade genetik yatkınlık farmokogenetik araştırmaların son dönem merak uyandıran alanlarından birini oluşturmaktadır. İADR ile spesifik HLA ilişkileri coğrafi bölgeler ve etnik gruplar arasında farklılık gösterebilir. Aynı populasyonun bireyleri arasında dahi HLA allellerine göre ilaç yanıtı ve duyarlılığı farklılık teşkil edebilir. Çok sayıda çalışma, spesifik HLA alellerinin İADR geliştirme riskini artırdığına dair kanıtlar sağlamıştır. Çünkü, ilaca yanıt veren T hücresi aktivasyonunda HLA, ilaçlar ve T hücresi reseptörleri arasındaki etkileşim HLA allellerine göre değişebilmektedir. Allel türüne göre, T hücresinin aktivasyonu, antijen ile etkileşimi doğurduğu immün cevap farklılığı doğuran en önemli moleküler sistemdir. Bu özellikler HLA allerinin İADR üzerinden farmakogenetik ilişkisini ortaya koyarken, farmakokinetik etki içinde HLA allel çeşitlerine göre ilacı hızlı metabolize eden veya hiç metabolize etmeden toksik yanıt geliştiren bireyler de olabilir. Bu doğrultuda, toplumlarda farmakogenomik ilişkilerin incelenmesi, HLA alleleri ile ilaç duyarlılığı için yeni verilerin keşfedilmesi, kanıt oranıyla bulunanların doğrulanması hem ilaç yararlanımı için sağlık kalitesi üzerinden hem de maliyet etkinliği üzerinden büyük fayda sağlayacaktır. İlaç kullanımından önce HLA kimliklendirmesi ile kişiye özel farmakolojik tedavi, gelişen toplumlarda güncel tedavi hedeflerinin başında gelmektedir. Bu derlemede, ilaç aşırı duyarlılık reaksiyonlarına yol açan modeller, en sık reaksiyon veren ilaçlar, ilişkili HLA alleleri ve izlenen klinik tablolar araştırılmıştır.

Anahtar Kelimeler: HLA, ilaç, aşırı duyarlılık reaksiyonları

ABSTRACT

Drug reactions (DR) are grouped into two basic classes. Allergic reactions through by immunological mechanisms is one of them; the second is the drug hypersensitivity reactions (DHR) that occur without the engagment of a specific immunological response. Prevelance of DHR is a extensive cause of morbidity and mortality. These severe reactions are originated from specific human leukocyte antigen (HLA) alleles. Since, HLA genes include the high rate of polymorphic loci in the genome significantly. Thus, genetic predisposition rather than the pharmacological dose effect in the etiology of DHR account for one of the most intriguing sites of pharmacogenetic research. Specific HLA associations with DHR may be varry between geographic regions and ethnic groups. Even among individuals of the same population, drug response and sensitivity may differ according as HLA alleles. Numerous investigations have provided evidence that some of HLA's increase the risk of growing DHR. In view of the fact that the interconnection between HLA, drugs and T cell receptors in drug-responsive T cell reaction may vary widely depending on HLA alleles. According to, the allele type, the activation of the T cell and its interaction with the antigen is the most significant molecular system that causes variations in the immune response. While these features reveal the didtinctive genetic relationship of HLA alleles through DHR, there may be individuals who metabolize the drug rapidly or develop a toxic response without metabolizing it at according to HLA allele types within the pharmacokinetic effect. In this line, determining pharmacogenomic datas in societies, discovering new evidence for HLA alleles and drug sensitivity, and verifying the findings with the evidence rate will provide great benefits both in terms of health quality for drug use and cost effectiveness. Personalized pharmacological treatment with HLA identification before drug use is one of the current treatment targets in developing countries. In this review, the models that cause drug hypersensitivity reactions, the most frequently reacting drugs, the associated HLA alleles and the clinical presentations were investigated.

Keywords: HLA, drug, hypersensitivity reactions

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INTRODUCTION

OIn the region of the Major Histocompatibility Complex (MHC) of the human genome, HLA class I and class II loci are located. HLA genes are highly polymorphic about 8000 functional HLA-A/B/C alleles and 3000 HLA-DR/DQ/DP alleles (1). Drug hypersensitivity reaction (DHR) is explained by the World Health Organization (WHO) as "a harmful and undesirable answer to normal doses of a drug used for the prophylaxis, diagnosis or treatment of disease". While most DHRs are considered predictable (type A) based on the known pharmacologic effect of the drug, immune responses caused by a smaller number of drugs are generally considered unpredictable (type B) and dose most polymorphisms are located in the antigen-binding cleft peptide binding region. The purpose of HLA genes are to provide the antigens produced by HLA class I or to present peptides stem from on the cell surface HLA class II for immune system. These peptides are recognized by circulating T cells via T cell receptors (TCRs). Since antigen-MHC association is responsible for the formation of pharmacologic adverse reactions, HLA genotype is an important risk factor in drug reactions (2).

A drug hypersensitivity reaction is defined as "a harmful and undesired response to normal doses of a drug used for the prophylaxis, diagnosis or treatment of a disease" by the WHO. Most DHRs are accepted as predictable (type A) sourced from pharmacological interconnection of the drug, while immune responses caused by a smaller number of drugs are generally considered unpredictable (type B) and independent of dose (3). Type B reactions constitute only about 13% of DHRs and are more severe than the type A reactions. A subtype B of DHR is T cell-mediated drug hypersensitivity. T cell-mediated reactions are characterized by systemic reactions (drug-induced hypersensitivity sNGSrome) and skin reactions. These reactions occur after 3-4 days of drug remedy and sometimes longer >30 days. Consistent with removal of antigen, symptoms decrease with discontinuation of drug treatment; but, represent of the drug may be fatal, owing to rapid activation of a memory T cell population (4). Studies considering the role of HLA alleles triggiered T cell have revealed corporations between DHRs and polymorphic HLA alleles. The association between DHRs and HLA class I-II alleles in some ethnic communities has been clearly established. There are also strong associations between DHR and genetic polymorphisms of some enzymes that metabolize drugs. In this compilation, the models leading to drug hypersensitivity reactions, the associated HLA alleles and the clinical pictures observed are reviewed.

1.Models Leading to Drug Hypersensitivity Reactions

Reactions developed with HLA alleles are T cell-mediated Type IV delayed-type reactions. It is not defined drug-mediated T cell activation yet so, different models have been proposed. According to these models, HLA genes give out drugs to TCRs in three different ways (figure 1).

Hapten model has been demostrated that the drug has an irreversible covalent bond with the peptide. Drug-modified peptides are generated. These drug-modified peptides are then presented to TCRs by HLA molecules (5). The second mechanism is hold on the pharmacologic interconnection with immunity (P-I). The drug links directly with HLA complexes in a non-covalent bond here to trigger activation. Since the drug interconnets with peptides on the surface of cell presented by HLA in the P-I model, it does not depend on intracellular protein processing mechanism. Theoretically, the drug first triggers the T cell by interacting with HLA or TCRs. However, both scenarios involve the formation of a drug-peptide HLA-TCR complex. In both models, TCRs receive binding signals from HLA, peptide and drug, the only difference is in the nature of the binding of the drug molecule with HLA (6). The third mechanism is the modified peptide repertoire model. There is a non-covalent interaction between the drug and the HLA molecule. The drug interacts with the antigen binding cleft of the HLA molecule instead of interacting at the -TCR interface. It

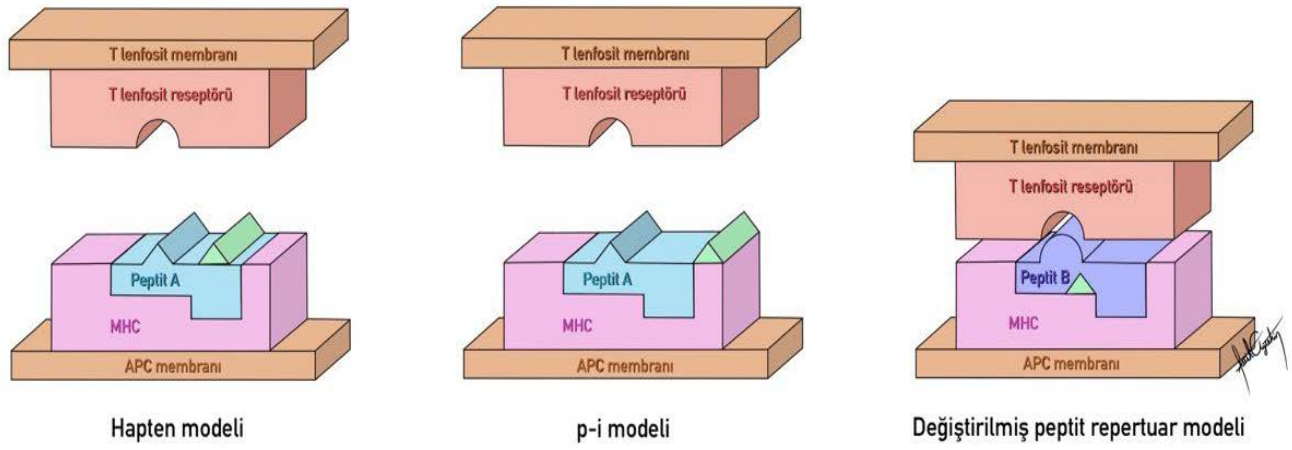

Figure 1. Drug presentation patterns of HLA molecules to the TCR (Figure is created by Mehmet Fatih Aytekin)

occupies some part of the space available for peptide binding. It changes the peptide binding preferences and peptit binding motif of the HLA molecule while doing this. The emerged HLA-drug-peptide complexes are conformationally different from the constitutive HLA-peptide complexes and are recognized as unfamiliar by circulating T cells (7).

Figures show drug presentation of HLA alleles in the MHC region to the TCR. *The green pyramid symbolizes the drug.

2.Clinical Consequences of Drug Hypersensitivity Reactions

HLA antigens vary according to individual, race, community and disease. The relationship between HLA class I and II alleles and drug allergy is specific to drug, phenotype (clinical picture) and ethnicity. The most important HLA-ADR associations found in studies conducted in the last 10 years are as follows (table 1).

2.1. Stevens Johnson Syndrome (SJS)/Toxic Epidermal Necrolysis (Ten)

Stevens Johnson Syndrome characterized by acute, rare and potentially fatal skin reactions involving loss of skin and by systemic symptoms. In more than 80 percent of cases, drugs are the triggering factor. SJS is less common but unpredictable reaction for DHR involving drug-specific CD8+ cytotoxic lymphocytes, the Fas-Fas ligand (FasL) pathway, and the tumor necrosis factor-alpha (TNF) pathway. Clinically, these two manifestations are distinguished from each other by examining body surface area: it is defined as SJS if there is less than 10% skin damage, TEN if there is more than 30% epidermal detachment, and SJS-TEN if there are skin problems between 10% and 30% (8).

2.2. Drug-induced Hypersensitivity Syndrome (DIHS)/Drug Reaction Syndrome with Eosinopophilia and Systemic Symptoms (DRESS)

DRESS is a rare T cell-mediated delayed-type drug hypersensitivity reaction that is serious and potentially fatal. It is more common in adults. The syndrome affects not only the skin but also other organs such as the liver, kidneys and heart. The "R" in the acronym DRESS, which previously stood for "rash", was later changed to "reaction". The mortality rate is approximately 10%, depending on the age of the patient, underlying comorbidities and the drug involved. Typically occurs in two weeks or two months after starting a medicine. It characteristically occurs with fever, widespread rash, lymphadenopathy, hematologic abnormalities and involvement of one or more internal organs (9).

2.3. Maculopapular Exanthema (MPE)

It is a frequently observed drug-induced cutaneous hypersensitivity with a favorable outcome. It is a T cell-mediated reaction. It occurs within 1 week after the initiation of the causative drug use and regresses in 7-14 days. It usually has a mild course and is observed in generalized form less frequently (10).

2.4. Drug-Induced Liver Injury (DILI)

Drug-Induced Lıver Injury (DILI) has been the most significant source of acute liver failure. It is an important but rare side effect that can range from asymptomatic elevation in liver tests to acute liver failure, transplantation or death. DILI has classically been categorized as direct dose-dependent or idiosyncratic, but indirect injury has emerged as the third type of drug-induced liver injury. Idiosyncratic DILI cannot be predicted (11).

2.5. Agranulocytosis

It is a rarely seen, potentially life-threatening state. It can be attributed to drugs in >70% of cases. In agranulocytosis, the peripheral neutrophil count is < 0.5 x 10 9. It often presents with a severe sore throat, but isolated fever, pneumonia or septicemia are not uncommon

3. HLA Alleles Associated with Drug Hypersensitivity Reactions

Well-known drugs associated with HLA alleles causing drug hypersensitivity reactions are given in table 1. Data of pharmacogenomics including HLA alleles are regularly updated and available on the 'Pharmacogenomics Knowledge Base' with significant evidence (13). Prominent drug-HLA- DHR associations are described below.

3.1. Abacavir

Abacavir-induced hypersensitivity reaction develops against HIV-1 remedy. Fever, common rash, fatigue, gastrointestinal symptoms and shortness of breath are observed. It has been reported that HLA-B*57:01 carrying was a significant risk abacavir-induced hypersensitivity syndrome (14). According to predict 1 and shape studies that patients with HLA-B*57:01 carriage should not use abacavir. (15,16). The FDA has issued a package insert warning for abacavir stating that "HLA-B*57:01 detecting should be performed in all patients before abacavir is started for the first time or given again, with or without prior allelic detecting" (17). HLA-B*57:01 related with abacavir-induced hypersensitivity at about %3 in Middle Eastern. The prevalence of the allele in Caucasian race, African-Americans and Thai populations is approximately 6%. It is 2% and 4%, respectively (18) In Chinese and Koreans.

3.2. Carbamazepine

Carbamazepıne is used for epilepsy treatment, bipolar disorder and the other neurological disorders. SJS/TEN, maculopapular exanthema and DRESS reactions have been reported following carbamazepine treatment (19). People carriying HLA-B*15:02 have been risk with SJS/TEN (20). It is important to note that not all patients with carbamazepine-associated SJS/TEN reactions carried the HLA-B*15:02 allele. In recent studies carbamazepine-SJS/TEN have been assosiated with HLA-B*15:21, HLA-B*15:11 and HLA-B*15:08 genotypes (20,21). DRESS/DIHS observed with carbamazepine usage is not related with HLA-B*15:02. Carbamazepine-induced immunity related drug reactions and HLA-A*31:01 allele has been associated in Chinese, Northern European, Japanese and Korean origin (22). The US Food and Drug Administration (FDA) states on its website that HLA B*15:02 allele scanning should be performed before starting carbamazepine in high-risk groups (people of Southeast Asian origin) and if positive, the drug should not be started. It is also recommended to avoid all aromatic antiepileptic drugs in HLA B*15:02 positive individuals (23).

The prevalence of HLA-A*31:01 in Caucasians and Asians was found to be high as another allele associated with carbamazepine cutaneous adverse reactions (24). In another study, it was found evidince between HLA-A*31:01 and carbamazepine. Whereas the HLA-A*02:01 allele is associated with an significant risk triggired by carbamazepine and lamotrigine with maculopapular exanthema (25,26).

3.3. Phenytoin

Phenytoin is used for epileptic remedy has been related with severe cutaneous effect including SJS/TEN, DRESS and maculopapular eruption as well. HLA-B*13:01, HLA-B*15:02 and HLA-B*51:01 carriage have been risk factor for the treatment of phenytoin. In an investigation further, phenytoin-SJS/TEN was reported to be associated with HLA-B*15:02 carriers (27-30). It was identified that severe skin reactions caused by phenytoin were significantly related with CYP2C9*3 carried. In a study, in which combined displaying was done for detecting CYP2C9 variants and HLA-B*15:02 carriage, it was shown that the sensitivity of phenytoin-SJS/TEN was 62.5% in combination (20).

3.4. Allopurinol

Allopurinol is used against hyperuricemia and gout. The variable in approximately 2% of patients starting treatment, is responsible for a drug sensivity reaction primarily of cutaneous phenotype. In 2005, the HLA-B*58:01 genotype was shown to be related with allopurinol-trigered SJS/TEN and DRESS in Han Chinese individual descent. It has been similar association in Thai, Korean and Japanese populations (29).

HLA-B*58:01 is estimated to be responsible for almost 50% of allopurinol-induced adverse effect in European and Japanese people. It is thought to be a dose-dependent reaction (19). A very strong HLA -DHR association between HLA B*58:01 and allopurinol-associated SJS/TEN has been demonstrated in different populations. Besides, HLA-A*33:03, which is significantly found in Europeans for allopurinol. HLA-C*03:02 is another allele that has been shown to be associated (9).

3.5. Nevirapine

In recent years, HLA-C*04:01 was significantly associated with an increased risk of cutaneous reactions and hepatotoxicity hypersensitivity to nevirapine is an antiretroviral. Black Africans, Caucasians and Thais populations can be ranged from 8% to 14% or even higher in the Iranian-Baluch population, where it can be 28%. HLA-B*35:01 and HLA-DRB1*01:01 are associated with nevirapine hypersensitivity as well (31).

3.6. Lapatinib

Lapatinib is a therapeutic drug used for breast cancer and carrier of -DRB1*07:01 have been found increased risk in patients treated with lapatinib. These HLA alleles are present by 15-25% of Caucasian, Asian, African and Hispanic indivudials and the risk have been found more less in Japanese. Prevalence can range from 10% to 25% in GME. Accoring to FDA lapatinib drug label is that liver can be adviced by monitoring for lapatinib treatment, irrespective of HLA allelles (32).

3.7. Aspirin

Using of aspirin and carried of HLA-DPB1*03:01 have been found significant risk by 15% in Sudanese and Tunisians with asthma. It was found an associations in the White Polish inviduals and Koreans. However, studies will be needed to validate the findings of the HLA-DPB1*03:01 allele in different populations (32,33).

4. Methods Used for Detection of HLA Alleles

Since the association between HLA alleles and developing DHR has been shown in many studies, genetic testing for determining HLA alleles is very important for new users of the above drugs. Haplotype results are either "positive" HLA-B*57:01 is detect in one or both copies of the HLA-B gene or "negative" no copies of HLA-B*57:01 are detect. Since HLA genes are expressed in a codominant manner, there are no intermediate phenotypes. Although most of the technologies have been developed to detect the HLA-B*57:01 allele, one of the first prominent alleles identified, tests can also be applied to test for other alleles (34). Several techniques are available for HLA genotyping methods.

5. DNA-Related Molecular Genetic Tests

DNA sequence-specific primers (SSP), sequence-specific oligonucleotides (SSO), real-time polymerase chain reaction (real-time PCR), Sanger and next generation sequencing (NGS/Next generation sequencing-NGS) are all DNA-related molecular genetic methods. DNA based HLA types amplified by PCR are common laboratory procedures. PCR amplification of DNA is used as a means of enriching a selected DNA region. Different methods are used for HLA typing after this stage; SSP (sequence specific primer), SSO (sequence specific oligonucleotide), RFLP (restriction fragment length polymorphism) and reverse SSOP dot blot technologies (33).

Fortyping,HLA-SSOusessequence-specificoligonucleotides (SSOs) to determine which HLA alleles are present in a sample that has been amplified by using biotin-labeled primers in PCR. It is the sequence-specific oligonucleotide-hybridization probes used following amplification that provide specificity. Microbead and fluorescence detection technology have been combined with an automated software technique in SSO which is made with Luminex. For each locus, an array of microspheres is used, which can be recognized by their specific color resulting from two internal fluorescent dyes. Each microbead is combined with a single probe capable of hybridization with a biotin-labeled complementary amplicon. When hybridization occurs, fluorescence signal ,stemming from the fluorescent (streptavidin-PE)-labeled amplicons captured by the beads, can be quantified by measuring. That is, all SSOs are analyzed at the same time. Luminex and Auto-Lipa devices can also be studied on a gel basis. It is a low resolution method.

HLA-SSP is a method in which only the desired alleles are amplified with specific primers. Exactly matched primers are used. The amplified PCR product is processed by agarose gel electrophoresis. The image obtained following the electrophoresis process is evaluated as 'Score' by means of various software. HLA-SSOP dot blot; DNA amplified with PCR is passed on the membrane by dot technique. The probes on the membrane are subjected to DNA for hybridization, then

the membranes are washed very well. Hybridization of the probe indicates the presence of target HLA antigens (35).

Restriction fragment length polymorphism (RFLP) is a method that is no longer used today. Alleles can also be detected by sequencing methods after PCR. These tests are high resolution. Sanger sequencing and new generation sequencing methods are used. Due to its accuracy, high throughput and speed, NGS is increasingly becoming the preferred method for HLA typing. The most important benefit of NGS is that it eliminates ambiguities at a cost comparable to Sanger sequencing and without the need for additional screens. The international ImMunoGeneTics project/human leukocyte antigen (IMGT/HLA) database is actively used in the variant calling process of NGS. The use of flow cytometry-monoclonal antibody and patch testing are tests that are not performed with PCR and DNA.

CONCLUSION

HLA-ADR pharmacogenomic relationships are not a well-studied area in our country. The daily use of next-generation sequencing and bioinformatic algorithms developed for HLA genotyping will soon provide an opportunity to reduce the information gap in this field in countries around the world. In the near future, new HLA-related pharmacogenomic markers will be identified, especially in populations where genomic projects are ongoing. Revealing the pharmacogenomic HLA profiles of countries will play an important role in reducing drug-related side effects. Polimorphism of HLA allelles especially due to ethnic origin should motivate HLA-related investigations in different populations. As a result of the data obtained, scanning the relevant HLA allele before the drug usage will be important for cost-effectiveness evaluation.

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OLGU SUNUMU / CASE REPORT

Radiation-Induced Angiosarcoma of The Breast: A Case Report with FDG PET/CT Imaging Findings

Radyasyona Bağlı Meme Anjiyosarkomu: FDG PET/BT Görüntüleme Bulgularıyla Bir Olgu Sunumu

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ÖZET

Giriş: Meme anjiyosarkomu, vasküler endotelden köken alan, tüm yumuşak doku meme tümörlerinin yaklaşık %1'ini oluşturan ve kötü prognoz taşıyan nadir görülen bir tümördür. İki farklı tipte ortaya çıkar: Primer meme anjiyosarkomu (PMAS) ve sekonder meme anjiyosarkomu (SMAS). PMAS tipik olarak meme kanseri veya radyoterapi öyküsü olmayan genç kadınları etkiler; sıklıkla meme parankiminden kaynaklanır ve ara sıra cilt tutulumuyla birlikte hızla büyüyen, genellikle ağrısız, ele gelen bir kitle olarak ortaya çıkar. Buna karşın SMAS yaşlı kadınlarda görülür, meme dermisinden kaynaklanır, bazen parankimi tutar, multifokalite gösterir. Ciltte renk değişikliği ve şişlik gibi karakteristik özellikler sunar. Mevcut literatürde, memenin radyasyonla ilişkili anjiyosarkomunu Flor-18 florodeoksiglukoz pozitron emisyon tomografisi/bilgisayarlı tomografi (FDG PET-BT) kullanılarak yapılan görüntüleme bulguları ile rapor eden vakaların eksikliği bulunmaktadır. Bu vaka raporu, sol meme kanseri nedeniyle meme koruyucu cerrahi ve ardından radyoterapi uygulanan 55 yaşındaki kadın hastayı ayrıntılarıyla anlatmaktadır.

Olgu: Spesifik olmayan mamografik ve ultrason özellikleri olan hastada radyoterapiden 48 ay sonra radyasyona bağlı anjiyosarkom gelişti. FDG PET-BT'de meme derisinde FDG tutulumunun arttığı nodüler lezyonlar görüldü. Artmış FDG tutulumu gösteren bu nodüllerin yapılan patolojik inceleme sonucunda radyasyona bağlı meme anjosarkomu olduğu doğrulandı. Hastaya tedavi amacıyla total mastektomi operasyonu yapıldı.

Sonuç: Meme koruyucu cerrahi ve radyoterapi öyküsü olan hastalarda, takip sırasında semptomların ortaya çıkması, radyasyona bağlı anjiyosarkomun gelişimini akla getirmelidir. Erken tanı çok önemlidir ve FDG PET-BT lokal görüntüleme ve uzak organ metastazı taraması açısından faydalı olabilir.

Anahtar Kelimeler: Radyasyona bağlı anjiosarkom, Meme Koruyucu Cerrahi, Radyoterapi, FDG PET-BT

ABSTRACT

Introduction: Breast angiosarcoma is a rare tumor arising from the vascular endothelium, accounting for approximately 1% of all soft tissue breast tumors and carrying poor prognosis. It manifests in two distinct types: Primary breast angiosarcoma (PBAS) and secondary breast angiosarcoma (SBAS). PBAS typically affects young women without a history of breast cancer or radiotherapy, often originating from the breast parenchyma with occasionally skin involvement presenting as a rapidly growing, usually painless, palpable mass. In contrast, SBAS occurs in older women, originates from the breast dermis, occasionally involves the parenchyma, displays multifocality, and presents characteristic features such as skin discoloration and swelling. There is a lack of cases in the current literature reporting radiation-associated angiosarcoma of the breast with imaging findings using Fluorine-18 fluorodeoxyglucose positron emission tomography/computed tomography (FDG PET-CT). This case report details the development of radiation-associated angiosarcoma in a 55-year-old woman who underwent breast-conserving surgery and subsequent radiotherapy for left breast cancer.

Case: The patient developed radiation-associated angiosarcoma 48 months after radiotherapy, with non-specific mammographic and ultrasound features. FDG PET-CT revealed increased FDG uptake in the breast skin and nodular lesions. Pathological examination of the nodules with increased FDG uptake confirmed radiation-induced breast angiosarcoma. The patient underwent a total mastectomy for treatment.

Conclusion: In patients with a history of breast-conserving surgery and radiotherapy, presenting symptoms during follow-up should prompt consideration of radiation-associated angiosarcoma. Early diagnosis is crucial, and FDG PET-CT can be beneficial for local visualization and distant organ metastasis screening.

Keywords: Radiation-associated angiosarcoma, breast-conserving surgery, radiotherapy, FDG PET-CT

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INTRODUCTION

Breast angiosarcoma (AS) is a rare tumor arising from the vascular endothelium, comprising approximately 1% of all soft tissue breast tumors and carrying poor prognosis. It manifests in two distinct types: primary breast angiosarcoma (PBAS) and secondary breast angiosarcoma (SBAS). PBAS typically affects young women without a history of breast cancer or radiotherapy, often originating from the breast parenchyma with occasionally skin involvement presenting as a rapidly growing, usually painless, palpable mass (1,2). In contrast, SBAS occurs in older women, originates from the breast dermis, occasionally involves the parenchyma, displays multifocality, and presents characteristic features such as skin discoloration and swelling (2,3).

SBAS can occur in the ipsilateral extremity with chronic lymphedema outside the radiation field after radical mastectomy and axillary dissection (known as Stewart-Treves syndrome). It can also present as radiation-associated breast angiosarcoma (RABAS), whose frequency is increasing due to the rising prevalence of breast-conserving surgery followed by adjuvant radiotherapy. RABAS develops in the ipsilateral chest wall or breast within the "twilight zone," where radiation is not homogeneously distributed (2,3). The incidence of RABAS ranges between 0.9-1.1 per 1000 cases (4). In the literature, the latent period for AS development post-radiotherapy is reported to range from 3 to 20 years, with an average duration of 6-8 years (4,5).

In the existing literature, there is a dearth of reported cases documenting radiation-associated angiosarcoma of the breast along with imaging findings utilizing Flour-18 fluorodeoxyglucose positron emission tomography (FDG PET-CT) (6). The primary objective of this study is to present a case involving a patient who underwent breast-conserving surgery and received radiotherapy on the same breast for the treatment of breast cancer. Subsequently, during the follow-up period post-treatment, the patient developed radiation-induced secondary AS. Furthermore, our aim encompasses the presentation of the FDG PET-CT imaging findings associated with this particular patient.

CASE

A 55-year-old female patient with no family history of cancer underwent breast-conserving surgery in August 2015 due to left breast cancer. Concurrently, axillary lymph node sampling on the same side was performed. The postoperative pathology report revealed the tumor stage as pT1 pN0. The tumor exhibited positive estrogen receptor (ER) at 90%, positive progesterone receptor (PR) at 40% with a +3 intensity, negative Cerb-2, Ki-67 proliferative index at 20%, and positive E-cadherin, indicating invasive ductal carcinoma.

Following the surgery, the patient received a total radiation dose of 60 Gy over 30 days: 50 Gy to the left breast (2 Gy per day, 5 days a week) and 10 Gy to the tumor bed (2 Gy per day for 5 days in one week). Also hormonal therapy consisting of two years of tamoxifen and three years of letrozole was administered for five years following the radiation therapy. Until August 2019, the patient underwent regular follow-ups, including blood tests, tumor markers, breast ultrasounds, mammograms, and computed tomography (CT) scans, revealing no pathological findings. However, in August 2019, mammography showed non-specific features such as volume reduction in the operated area of the left breast, skin retraction in the outer quadrant, and mild thickening of the breast skin. The same day, ultrasound revealed a slightly heterogeneous area in the outer-middle quadrant of the left breast, corresponding to the operation site, with vertical irregularly bordered hypoechoic nodular lesions measuring 5x4 mm. An incisional biopsy from this area resulted in the diagnosis of AS. Magnetic resonance imaging (MRI) was not conducted; instead, FDG PET-CT imaging was performed in October 2019 for possible metastasis screening. The FDG PET-CT imaging indicated increased FDG uptake in the thickening of the breast skin in the outer-upper segment, along with millimetric nodular soft tissue lesions with elevated FDG uptake (Figure 1). The maximum standard up-take value (SUV max) of the tumor was measured as 3.2.

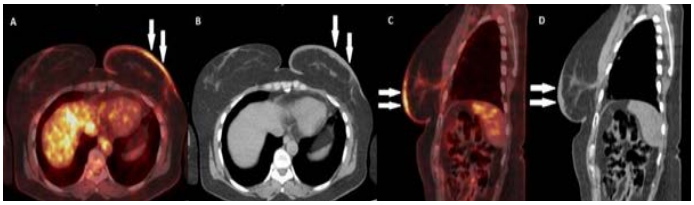

Figure 1. In the axial (A: fusion, B: CT) and sagittal sections (C: fusion, D: CT) of the left breast skin, a thickening indicating FDG uptake is observed in the area indicated by the arrows.

In February 2020, the patient underwent a left breast mastectomy and lymph node sampling on the same side. In

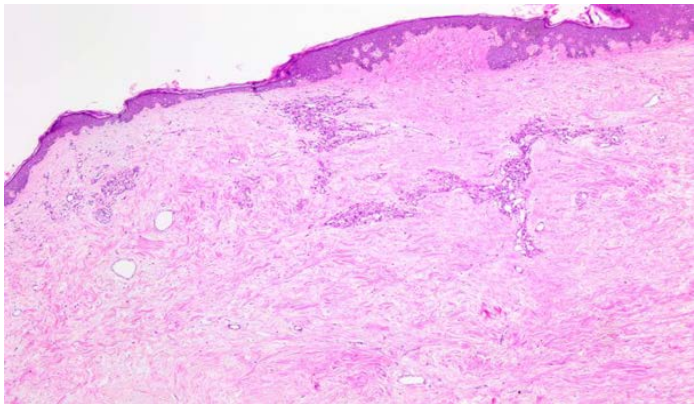

Figure 2. Microscopic appearance of angiosarcoma: A tumoral lesion consisting of irregularly shaped anastomosing vascular channels under the skin, lined with atypical endothelial cells. HE,x50

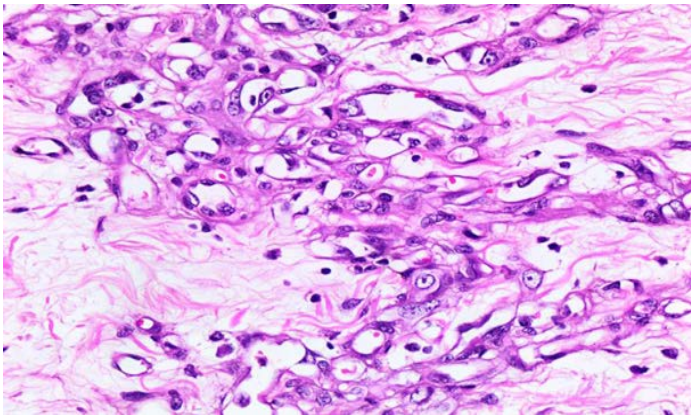

Figure 3. Microscopic appearance of angiosarcoma: Tumor cells are polygonal shaped, plump and pleomorphic cells. HE,x400

the macroscopic examination of the mastectomy material, a 3x3x1.5 cm sized lesion with a white fibrotic appearance in most areas and a dirty yellow calcified appearance in some areas was observed. In addition, the presence of nodules, the largest of which was 5 mm in diameter, in the white fibrotic area under the skin in the old incision area was noted. Microscopic examination of nodular lesions reveald a tumoral lesion consisting of irregularly shaped anastomosing vascular channels under the skin, lined with polygonal shaped, plump, atypical endothelial cells (Figures 2,3). Immunohistochemically, the tumor cells were diffusely strongly positive for CD34, CD31, and FLI-1, focally weakly positive for CD117 and negative for HHV-8, CK7 (Figure 4). Eighteen mitoses were counted in ten highpower fields using PHH3, and the Ki67 proliferation index was 40%. The surgical margins were tumor-negative. In the frozen material of the sentinel lymph node in the left axilla, one reactive lymph node was

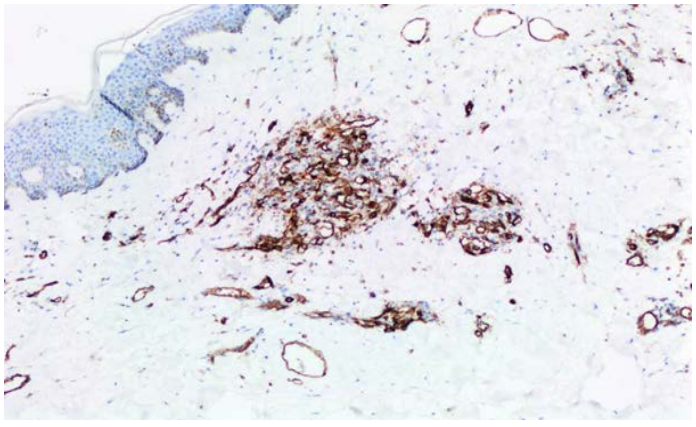

Figure 4. Immunohistochemical CD31 positivity of tumor cells in angiosarcoma. CD31x100

observed and no metastasis was detected. Based on the pathology results, the tumor in the left breast, attributed to the prior radiation therapy, was considered RABAS.

Our patient received six cycles of adjuvant chemotherapy following the surgery.

DISCUSSION

SBAS developing as a result of radiation can manifest in the early period, approximately 3-4 years after radiotherapy, or even emerge in the late period, up to 25 years later (4,5,7). In our case, the onset of the disease after radiotherapy was found to be consistent with the literature, occurring at 48 months. "Despite the 5-year overall survival rate being over 80% in breast cancer, the prognosis of RABAS is notably poor, with a 5-year overall survival rate generally ranging between 20% and 54% (1,7,8). Factors such as tumor size exceeding 5 cm, advanced age of the patient, multiple skin lesions, and high tumor grade have been reported to be associated with a poor prognosis (5,7,9). After a postoperative period of 48 months, no evidence of recurrence or metastasis has been identified in our patient. We attribute the favorable prognosis in our case to the small size of the tumor and the patient's young age. In RABAS, mammography typically shows non-specific findings such as skin retraction, skin thickening, and distortion of breast parenchyma. Despite these findings, approximately one-third of patients may not exhibit any specific symptoms (10,11). In our case, non-specific features were observed, including skin retraction and mild thickening of the breast skin in the outer quadrant. Similarly, ultrasound revealed a slightly heterogeneous area in the outer-middle quadrant of the left breast, corresponding to the operation site, with irregularly bordered hypoechoic nodular lesions measuring 5x4 mm, a pattern frequently reported in the literature (12). Although magnetic resonance imaging (MRI) was not performed in our study, MRI has been shown to be the most sensitive technique for detecting the primary tumor, recurrence, and residual lesions in RABAS (13). FDG PET-CT is commonly used for staging malignant tumors and post-treatment follow-ups. While its routine use for staging early-stage breast cancers is not recommended, FDG PET-CT is recommended in advanced-stage breast cancer and in cases where distant metastasis is suspected (14). Breast AS is not considered a commonly encountered neoplasm; therefore, there is limited research examining the use of FDG PET-CT in the imaging of this tumor (6). In our case, FDG PET-CT imaging revealed heterogeneous FDG uptake in the breast skin and increased FDG uptake in small millimetric nodular lesions on the skin. When a suspicious lesion arises in a previously irradiated area, the most suitable method for diagnosing RABAS is recommended to be the application of a core biopsy or diagnostic excision (13). Treatment decisions should be made promptly. Due to the rarity of the disease, there is no consensus on treatment, and it varies from simple wide excision to radical mastectomy. There is no clear consensus on chemotherapy (9,15).

CONCLUSION

In patients who have undergone breast-conserving surgery and received radiotherapy on the same breast for breast cancer, presenting symptoms such as thickening of the skin, bruising, discoloration, and rash on the same side during follow-up should prompt consideration of RABAS in the differential diagnosis. Early diagnosis is crucial, and for treatment planning, FDG PET-CT imaging can be beneficial for local visualization and screening for distant organ metastasis.

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OLGU SUNUMU / CASE REPORT

A Case of Lung Cancer Presenting with Paraneoplastic Pruritus

Paraneoplastik Kaşıntı İle Prezente Olan Akciğer Kanseri Olgusu

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ÖZET

Kaşıntı, dermatolojik rahatsızlıklar arasında sıkça görülen ve kişiyi kaşımaya zorlayan hoş olmayan bir duygu olarak tanımlanır. Kronik kaşıntı altı haftadan daha uzun süren kaşıntı olarak tanımlanır. Liken simpleks kronikus (LSC), kronik kaşıntı sonrasında ortaya çıkan kuru, pullu ve kalın cilt lekeleri olarak tanımlanır. Tipik olarak cildin belirli bir bölgesinin alışkanlık olarak kaşınmasının sonucudur. Ayrıca, paraneoplastik kaşıntı, solid tümörlerin erken bir belirtisi olarak ortaya çıkabilir. Bu vaka sunumu, bir cilt biyopsisinin sonucunda doğrulanmış olan LSC tanısı konan 73 yaşındaki bir erkek hastanın tetkik ve tedavi sürecini sunmaktadır. Hastada cilt biyopsi sonrası LSC tanısı almasının ardından yapılan malignite taramasında, akciğerde solid nodül ve karaciğerde multipl metastatik kitleler tespit edildi. Hastaya hem akciğer kitleden hem de karaciğerdeki metastatik kitleden biyopsi yapıldı. Biyopsilerin patolojik değerlendirilmesi sonucunda TTF-1 ve sinaptofizin pozitifti. Hem TTF-1 hem de sinoptofizin pozitif olan hasta Mikst tümör olarak değerlendirildi. Tetkikler sonucunda hastaya, mikst bileşenli akciğer adenokarsinomu ve küçük hücreli akciğer kanseri tanısı konmuştur. Kronik kaşıntı etyolojisini değerlendirmek için, hematolojik hastalıklar, endokrin hastalıklar, karaciğer hastalıkları, enfeksiyonlar ve ilaçlara bağlı etkenler, nörolojik ve psikiyatrik nedenler gibi olasılıklar araştırıldı. Ancak, kronik kaşıntının etyolojisi tam olarak belirlenememiştir. Akciğer kanseri için karboplatin ve etoposid tedavisi başlandıktan sonra, hastanın kaşıntı şikayetinde belirgin azalma olması, kronik kaşıntı ile malignite arasında bir ilişki olabileceğini düşündürmektedir. Ne yazık ki, hasta akciğer kanseri tanısı konulduktan iki ay sonra hipoksi, pnömoni ve multi-organ yetmezliği nedeniyle hayatını kaybetmiştir. Bu vaka, kronik kaşıntının kanser belirtisi olma potansiyelini vurgulamaktadır. Paraneoplastik kaşıntı genellikle hematolojik malignitelerle ilişkilendirilse de, bu durumun solid tümörlerde de görülebileceği unutulmamalıdır.

Anahtar Kelimeler: Kaşıntı, lichen simplex chronicus, paraneoplastik, karsinom

ABSTRACT

Pruritus, defined as an unpleasant sensation that compels scratching, is a common occurrence in dermatological disorders. Chronic pruritus is defined as itching that persists for a period exceeding six weeks. Lichen simplex chronicus (LSC) is a chronic dermatological condition that presents with dry, scaly, and thickened skin lesions, which typically arise from habitual scratching. Additionally, paraneoplastic pruritus may serve as an early indicator of the presence of solid tumours. This case report outlines the diagnostic and therapeutic process of a 73-year-old male diagnosed with lichen simplex chronicus (LSC), confirmed by skin biopsy. Subsequent malignancy screening revealed the presence of solid nodules in the lungs and multiple metastatic masses in the liver. Biopsies of the lung nodule and liver mass demonstrated positivity for TTF-1 and synaptophysin, leading to a diagnosis of mixed component lung adenocarcinoma and small cell lung cancer. In order to ascertain the underlying cause of the patient's chronic itching, a comprehensive investigation was conducted, which considered a range of potential factors, including haematological diseases, endocrine disorders, liver diseases, infections, drug-related factors, and neurological and psychiatric causes. Despite a comprehensive evaluation, the precise etiology of the chronic itching remained elusive. The initiation of carboplatin and etoposide therapy for lung cancer resulted in a significant reduction in the patient's itching, which may suggest a possible link between chronic itching and malignancy. Unfortunately, the patient succumbed to hypoxia, pneumonia, and multiple organ failure two months post-diagnosis. This case serves to illustrate the potential of chronic pruritus as a symptom of cancer. While paraneoplastic pruritus is often associated with haematological malignancies, this report highlights that it can also occur with solid tumours. It is important for clinicians to be aware of this potential association, as it allows for timely diagnosis and treatment.

Keywords: Pruritus, lichen simplex chronicus, paraneoplastic, carcinoma

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INTRODUCTION

Pruritus, defined as an uncomfortable sensation, is one of the most common clinical findings known for centuries in dermatology (1). Pruritus can be acute or chronic depending on the duration of clinical findings; Chronic pruritus is defined as pruritus lasting longer than 6 weeks (2). Causes of systemic chronic pruritus include hematologic diseases, malignancies, endocrine diseases, liver diseases, postmenopausal pruritus, HIV infection, pregnancy, senile pruritus, brachioradial pruritus, notalgia paresthetica, neuropathic pruritus, and drugs (3). Less than 10% of patients with chronic pruritus can have a malignant disease (4). Lichen simplex chronicus (LSC) is defined as patches of dry, scaly, and thick skin that appear after chronic pruritus. It is typically the result of habitual scratching or rubbing of a particular area of the skin (5). We diagnosed lung small cell carcinoma and lung adenocarcinoma in a patient who presented with LSC after chronic pruritus. In this report, we present this case of paraneoplastic pruritus due to malignancy.

CASE

A 73-year-old male patient was admitted to the hospital in September 2022 with complaints of pruritus all over his body. He had no comorbidities. The patient reported experiencing pruritus for 8-10 months. An investigation into the etiology of chronic pruritus was initiated. Physical examination revealed dry, scaly, and thickened skin patches (Figure 1). A skin biopsy was performed, resulting in a diagnosis of lichen simplex chronicus (LSC) (Figure 2A). These lesions are associated with chronic pruritus. Following pathology results, the investigation into the etiology of chronic pruritus continued. Due to the onset of chronic pruritus at an advanced age, malignancy screening was conducted. Abdominal computed tomography (CT) revealed multiple hypodense lesions, with the largest lesion measuring 3 cm in the liver. Thoracic CT identified a 17 mm solid nodule with irregular borders in the anterior aspect of the right lung upper lobe and an 8 mm nodule in the anteromedial aspect of the right lung upper lobe. Biopsies were performed from both liver and lung masses, and pathological examination of both biopsy materials revealed the presence of areas stained with TTF-1 and synaptophysin, suggesting a dual tumor. Lung adenocarcinoma and small cell lung cancer metastases were considered (Figures 2B,C,D,E,F). The patient underwent screening for hematological diseases, endocrine diseases, liver disease, infection, and drug-related causes of chronic pruritus. No other underlying cause for the chronic pruritus was identified. Chronic pruritus was attributed to malignancy. Treatment with carboplatin and etoposide was initiated for lung cancer. Additionally, the patient received topical antihistamines, topical anesthetics, topical steroids, and oral antihistamines for pruritus. Following chemotherapy, the patient's pruritus and skin lesions improved. However, two months after the lung cancer diagnosis, the patient passed away due to hypoxia, pneumonia, and multiorgan failure.

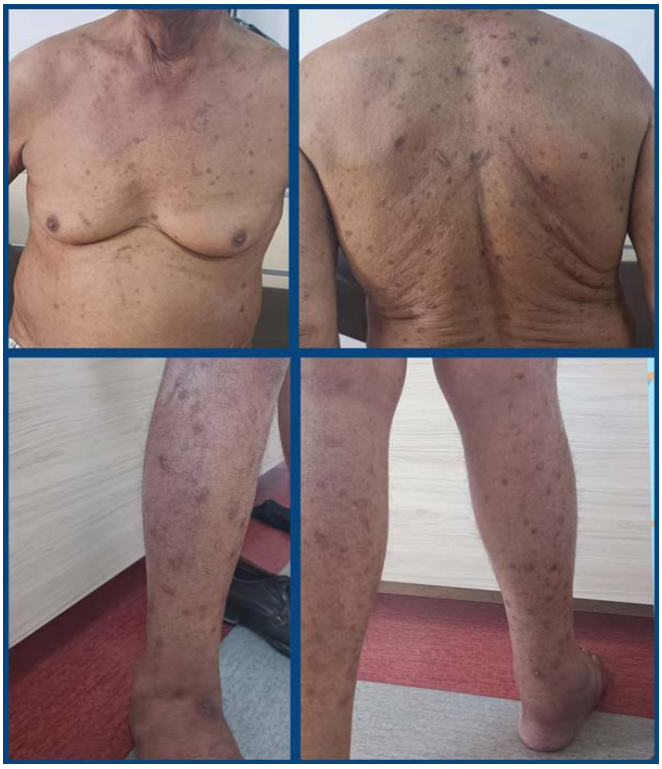

Figure 1. Dry, scaly, and thickened skin patches of the patient

DISCUSSION

Paraneoplastic syndromes are clinical conditions that arise as a result of the influence of cytokines, antibodies, or the immune system's reaction against the tumor, irrespective of the tumor's local or metastatic effects. There is a correlation between the stage of the paraneoplastic syndrome and the size of the tumor. Additionally, it can manifest in early-stage disease (6). Small cell lung cancer (SCLC) is the most common cause of paraneoplastic syndromes (7). Inappropriate Antidiuretic Hormone Syndrome (SIADH) is a common finding in 10% of cases of SCLC (8). Cushing syndrome, which develops due to ectopic corticotropin release in SCLC, is also frequently observed (9). Hypercalcemia in lung cancer may be caused by the secretion of parathyroid hormone-related protein (PTHrP) from the tumor or, less commonly, by bone metastases or primary hyperparathyroidism (10).

Pruritus that lasts for 6 weeks or longer is referred to as chronic pruritus (11). Lichen simplex chronicus (LSC) is defined as patches of dry, scaly, and thickened skin that develop following chronic pruritus. It may manifest as paraneoplastic pruritus. Hematological malignancies are more commonly associated with pruritus than solid tumors (3). The most common hematologic malignancies associated with pruritus include Hodgkin lymphoma, non-Hodgkin lymphoma, polycythemia vera, leukemias, mycosis fungoides, plasma cell dyscrasias, and cutaneous lymphoma (12). In some studies, pruritus was detected in 48-70% of polycythemia vera patients, approximately 30% in Hodgkin's disease, and

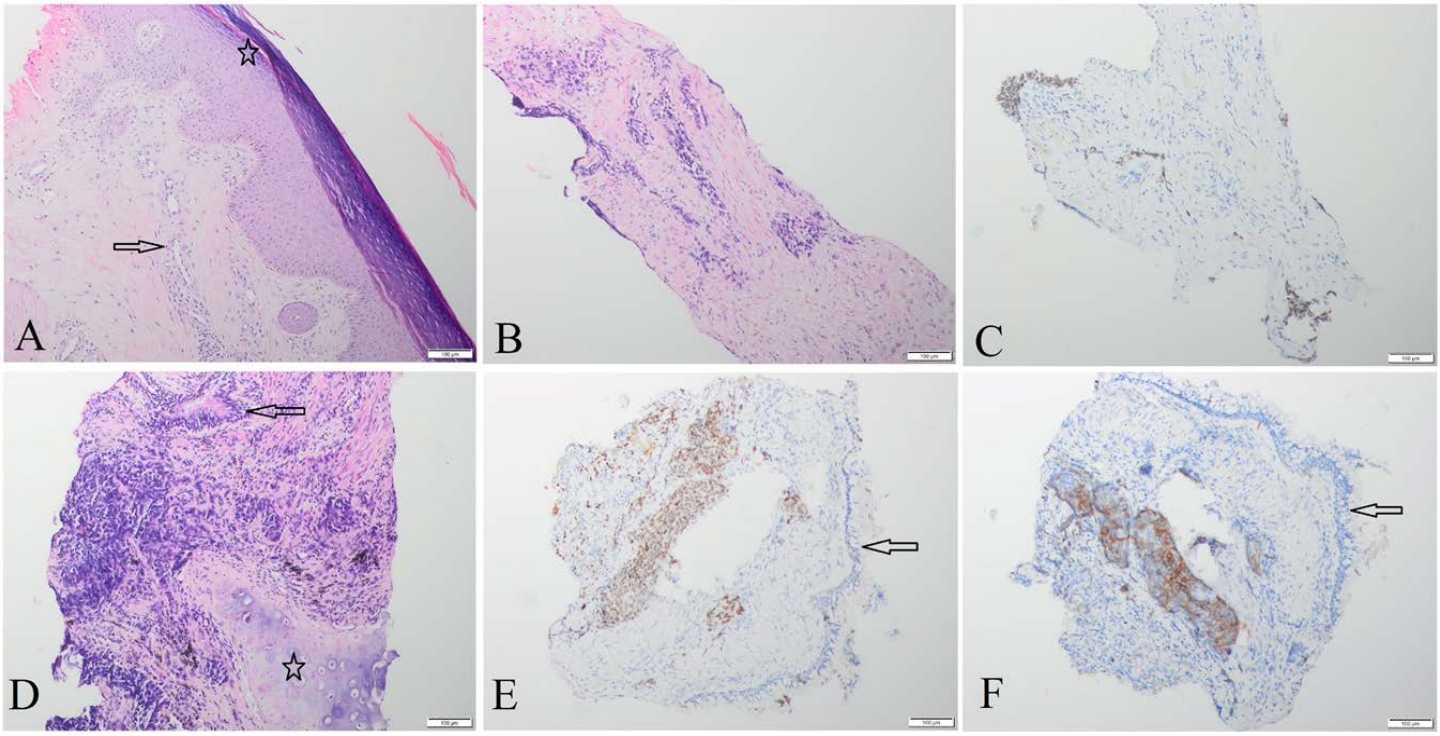

Figure 2. A) In the skin tissue, hypergranulosis (asterisk), irregular acanthosis in the epidermis, hyperkeratosis on the surface, perivascular lymphocytes and rare eosinophils (arrow) in the dermis are observed. B) In the biopsy sample taken from the liver, infiltrative tumor cells forming groups within the fibrotic stroma are observed. C) Nuclear TTF-1 positivity is observed in tumor cells observed in the liver. D) In the lung biopsy sample, tumoral infiltration is observed within the fibroinflammatory stroma (chondroid tissue of the bronchial wall is marked with an asterisk). E) Nuclear TTF-1 positivity is observed in tumor cells observed in the lung. F) Cytoplasmic Synaptophysin positivity is observed in some of the tumor cells (Arrows in D, E and F point to the bronchial surface epithelium) (A, B, D: Hematoxylin&Eosin, 100x magnification; C, E: Immunohistochemical TTF-1, 100x magnification; F: Immunohistochemical Synaptophysin, 100x magnification).

approximately 3% in other hematological malignancies. Chronic pruritus has also been reported in breast cancer, non-melanoma skin cancer, biliary tract cancer, and gastric carcinoid tumors. (13,14). Treatment of LSC aims to reduce pruritus and minimize existing lesions. Rubbing and scratching increase LSC. Pruritus and inflammation can be treated with a lotion or steroid cream applied to the affected area of the skin (15). Jaxon et al. investigated pruritus in more than 25,000 patients with solid cancer during follow-up and treatment. A total of 203 patients were included in the study. The most common tumor associated with pruritus was breast cancer at 36.5%, followed by lung cancer at 23.2%. In this study, pruritus was evaluated during the cancer treatment process, and no data were provided on the rate of pruritus etiology at the time of diagnosis. Our patient had chronic pruritus and had no additional disease other than the underlying cancer, and her pruritus was relieved after chemotherapy. In this study, pruritus was assessed during the treatment process, but patients diagnosed due to pruritus were not reported (16). In paraneoplastic pruritus, pruritus decreases or disappears with the treatment of the underlying malignancy. If the malignant disease recurs, the pruritus may also recur (4).

In conclusion, chronic pruritus can be a symptom of paraneoplastic syndrome. It is most commonly associated with hematological malignancies. However, it should be kept in mind that, although rare, it may be due to underlying solid malignancies. Therefore, patients with chronic pruritus should be screened for malignancy.

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OPEN ORJINAL GÖRÜNTÜ RAPORU / ORIGINAL IMAGE REPORT

Reactive Lymphocytosis Mimicking Acute Lymphoblastic Leukemia in A Patient with DRESS Syndrome

DRESS Sendromlu Hastada Akut Lenfoblastik Lösemiyi Taklit Eden Reaktif Lenfositoz

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A 30-year-old female patient, who had been taking salazopyrine for reactive arthritis for three months, presented with fever and icterus for the last 2 weeks, accompanied with erythematous maculopapular rash located on the proximal areas of the trunk and upper extremities for the last two days. The patient also had facial edema on admission. Oral and genital mucosa appeared normal on inspection. On physical examination, the patient had peripheral cervical, axillary and inguinal lymph nodes not exceeding 2 cm in diameter. There were intrabdominal and mediastinal lymph nodes of 1 to 2 cm in diameter with no signs of hepatosplenomegaly or mass lesion observed in computed tomography evaluation. Hemogram analyses revealed leukocytosis with a white blood cell count of 20300/µL, lymphocyte count of 7920/µL, absolute neutrophil count of 10960/µL and a monocyte count of 790/µL with no eosinophilia. Hemoglobin level was 12.1 g/dl and platelet count was 233000/µL. Antinuclear antibody (ANA) was positive with a titer of 1/100. Epstein-Barr virus (EBV) DNA was 486 copies/ml. EBV EBNA Ig G and EBV-VCA Ig G were positive, while EBV IgM was negative. Other viral tests (HIV, HBV, HCV, HSV) and Rose Bengal test were negative. CRP was 23 mg/dL and ferritin was 1304 ng/ml. Sedimentation rate was 41 mm/h. In biochemical analyses, transaminases were elevated to 5 times the upper limit of normal (ULN). Lactate dehydrogenase was elevated to 4 times the ULN. Total bilirubin was elevated as 5.6 mg/dL with direct bilirubin level of 4.4 mg/dL. There were slight elevations observed in alkaline phosphatase and gamma glutamyl transferase (1.5 times the ULN). Other biochemical parameters were within the normal limits. In the peripheral smear, mature lymphocytes, neutrophils and large granular lymphocytes were observed, along with large lymphomonocytoid cells (Figure-1A and 1B), which were approximately 4 erythrocytes in size, resembling acute lymphoblastic leukemia blasts with no granules in their basophilic cytoplasm, and having large nuclei, thin chromatin and nucleoli in some of the nuclei. Flow cytometry analysis of peripheral blood revealed no immunophenotypically compatible cells with acute leukemia blasts. Ninety percent of the lymphocytes were identified as T cells immunophenotypically. In the bone marrow biopsy, the cellularity was between 80-85% and the myeloid/erythroid ratio was 7 and increased which favors myeloid predominance. No infiltrative disease or maturation arrest was observed. A diagnosis of DRESS (Drug Reaction with Eosinophilia and Systemic Symptoms) associated with salazopyrine was suspected and Registry of Severe Cutaneous Adverse Reactions (RegiSCAR) scoring system was applied (1). The patient had a score of 6 and was categorized as “definite” according to the RegiSCAR scoring system. With corticosteroid and cyclosporine A treatment, the patient's clinical course hasimproved and hemogram parameters has returned to normal values within 3 weeks. Her lymphadenopathieshave also disappeared.

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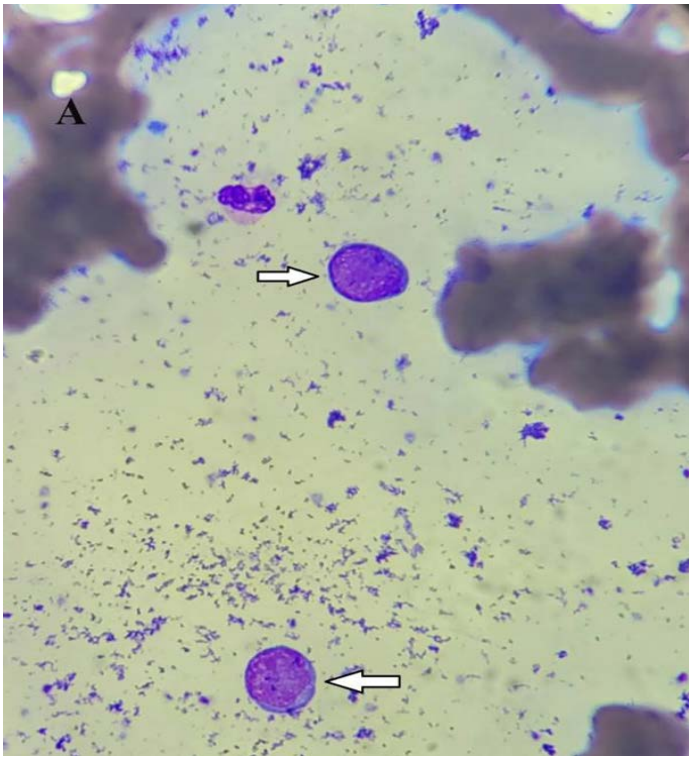

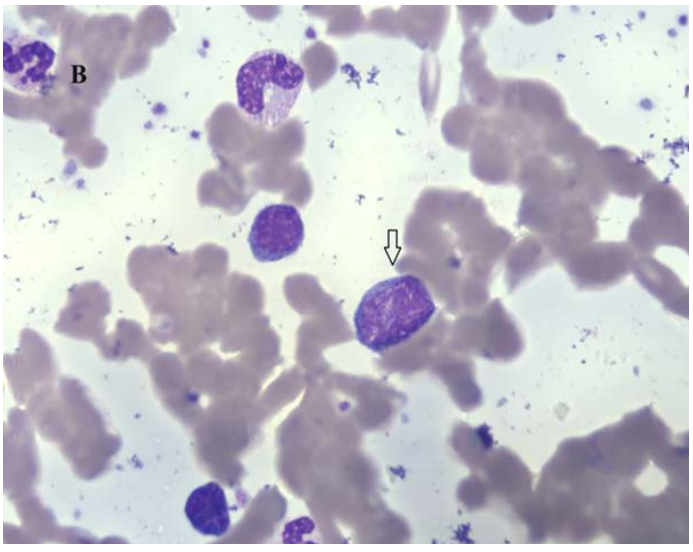

Figure 1A and 1B. Lymphomonocytoid cells mimicking acute lymphoblastic leukemia, (White arrows), Wright-Giemsa stain; x100 objective, original magnification x1000

Keywords: Lymphocytosis, Leukemia, DRESS Syndrome

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