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Amaç ve Kapsam

Haseki Tıp Bülteni, S.B.Ü. Haseki Eğitim ve Araştırma Hastanesi'nin süreli yayın organı olup, genel tıp içerikli, yayın dili Türkçe ve İngilizce olan, bağımsız ve önyargısız hakemlik ilkelerine dayanan uluslararası periyodik bir dergidir. Haseki Tıp Bülteni Mart, Haziran, Eylül, Aralık aylarında olmak üzere yılda dört sayı çıkmaktadır.

Haseki Tıp Bülteni'nin hedefi uluslararası düzeyde nitelikli, sürekli ve genel tıp konusunda özgün, periyodik olarak klinik ve bilimsel açıdan en üst düzeyde orijinal araştırmaları yayınlamaktır. Bununla birlikte eğitim ile ilgili temel yenilikleri kapsayan derlemeler, editöre mektuplar, olgu sunumları da yayınlar.

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Açık Erişim, "[hakem değerlendirmesinden geçmiş bilimsel literatürün], İnternet aracılığıyla; finansal, yasal ve teknik engeller olmaksızın, serbestçe erişilebilir, okunabilir, indirilebilir, kopyalanabilir, dağıtılabilir, basılabilir, taranabilir, tam metinlere bağlantı verilebilir, dizinlenebilir, yazılıma veri olarak aktarılabilir ve her türlü yasal amaç için kullanılabilir olması"dır. Çoğaltma ve dağıtım üzerindeki tek kısıtlama yetkisi ve bu alandaki tek telif hakkı rolü; kendi çalışmalarının bütünlüğü üzerinde kontrol sahibi olabilmeleri, gerektiği gibi tanınmalarının ve alıntılanmalarının sağlanması için, yazarlara verilmelidir.

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The Medical Bulletin of Haseki

Aims and Scope

The Medical Bulletin of Haseki is the official scientific journal of the University of Health Sciences Haseki Training and Research Hospital. It covers subjects on general medicine, published both in Turkish and English, and is independent, peer-reviewed, international periodical and is published quarterly (March, June, September and December).

The aim of The Medical Bulletin of Haseki is to publish original research papers of highest scientific and clinic value on general medicine. Additionally, educational material reviews on basic developments, editorial short notes and case reports are published.

The Medical Bulletin of Haseki is indexed in **Emerging Sources Citation Index (ESCI), Index Copernicus, EBSCO Database, Turkish Medline-National Citation Index, Excerpta Medica/EMBASE, SCOPUS, TÜBİTAK/ULAKBİM Türk Tıp Dizini, CINAHL, DOAJ, Hinari, GOALI, ARDI, OARE, AGORA, ProQuest, ROOT INDEXING, British Library, J-Gate, IdealOnline ve Türkiye Atıf Dizini** databases.

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Phone: +90 212 529 44 00/1874
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Yazarlara Bilgi

Haseki Tıp Bülteni, genel tıp alanlarını ilgilendiren tüm konulardaki yazıları yayınlar. Dergide orijinal makalelerin dışında derleme yazıları, orijinal olgu sunumları, editöre mektuplar, ve kongre/toplantı duyuruları da yayınlanır.

Dergide yayınlanacak yazıların seçimine temel teşkil eden hakem heyeti, dergide belirtilen danışmanlar ve gerekirse yurt içi/dışı otörler arasından seçilir.

Yazılarda Türk Dil Kurumu'nun Türkçe Sözlüğü ve Yazım Kılavuzu temel alınmalıdır. İngilizce yazılan yazılar özellikle desteklenmektedir.

Editör veya yardımcıları tarafından, etik kurul onayı alınması zorunluluğu olan klinik araştırmalarda onay belgesi talep edilecektir. Yazıların içeriğinden ve kaynakların doğruluğundan yazarlar sorumludur.

Yazarlar, gönderdikleri çalışmanın başka bir dergide yayınlanmadığı ve/veya yayınlanmak üzere incelemede olmadığı konusunda garanti vermelidir. Daha önceki bilimsel toplantılarda 200 kelimeyi geçmeyen özet sunumlarının yayınları, durumu belirtilmek koşulu ile kabul edilebilir. Tüm otörler bilimsel katkı ve sorumluluklarını bildiren formu doldurarak yayına katılmalıdırlar.

Tüm yazılar, editör ve ilgili editör yardımcıları ile en az üç danışman hakem tarafından incelenir. Yazarlar, yayına kabul edilen yazılarda, metinde temel değişiklik yapmamak kaydı ile editör ve yardımcılarının düzeltme yapmalarını kabul etmiş olmalıdırlar.

Makalelerin formatı 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication' (http://www.icmje.org) kurallarına göre düzenlenmelidir.

Anahtar kelimelerin Türkiye Bilim Terimleri (http://www.bilimterimleri.com)'nden seçilmelidir.

Dergi kaynaklarda kullanılırken Med Bull Haseki şeklinde kısaltılmalıdır.

Haseki Tıp Bülteni makale başvuru ücreti veya makale işlem ücreti uygulamamaktadır.

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Yazılar sadece online olarak kabul edilmektedir. Yazarların makale gönderebilmesi için web sayfasına (http://hasekitip.dergisi.org) kayıt olup şifre almaları gereklidir. Bu sistem on-line yazı gönderilmesine ve değerlendirilmesine olanak tanımaktadır.

Makale gönderimi yapılırken sorumlu yazarın ORCID (Open Researcher ve Contributor ID) numarası belirtilmelidir. http://orcid.org adresinden ücretsiz olarak kayıt oluşturabilir.

Bu sistem ile toplanan makaleler ICMJE-www.icmje.org, Index Medicus (Medline/PubMed) ve Ulakbim-Türk Tıp Dizini kurallarına uygun olarak sisteme alınmakta ve arşivlenmektedir. Yayına kabul edilmeyen yazılar, sanatsal resimler hariç geriye yollanmaz. Dergide yayınlanmak üzere editöre gönderilen yazılar A4 sayfasının bir yüzüne 12 punto, çift aralıkla, arial/times new roman karakteri ve kenarlarda 2,5 cm boşluk bırakılarak yazılmalıdır. Kullanılan kısaltmalar yazı içerisinde ilk geçtikleri yerde, parantez içinde, açık olarak yazılmalı, özel kısaltmalar yapılmamalıdır. Yazı içindeki 1-10 arası sayısal veriler yazıyla (Her iki tedavi grubunda, ikinci gün), 10 ve üstü rakamla belirtilmelidir. Ancak, yanında tanımlayıcı bir takısı olan 1-10 arası sayılar rakamla (1 yıl) cümle başındaki rakamlar da (Onbeş yaşında bir kız hasta) yazıyla yazılmalıdır. Yazının tümünün 5000 kelimeden az olması gerekmektedir. İlk sayfa hariç tüm yazıların sağ üst köşelerinde sayfa numaraları bulunmalıdır. Yazıda, konunun anlaşılmasında gerekli olan sayıda ve içerikte tablo ve şekil bulunmalıdır.

Başlık sayfası, kaynaklar, şekiller ve tablolar ile ilgili kurallar bu dergide basılan tüm yayın türleri için geçerlidir.

Hastalar mahremiyet hakkına sahiptirler. Belirleyici bilgiler, hasta isimleri ve fotoğraflar, bilimsel olarak gerekli olmayan durumlarda ve hasta (ebeveyn veya koruyucu) tarafından yayınlanmasına yazılı olarak bilgilendirilmiş bir onay verilmediği sürece yayınlanmamalıdır.

Bu amaçla, bilgilendirilmiş onay, hastanın yayınlanacak belirli bir taslağı görmesini gerektirir. Eğer gerekli değilse hastanın belirleyici detayları yayınlanmayabilir. Tam bir gizliliği yakalamak oldukça zordur ancak eğer bir şüphe varsa, bilgilendirilmiş onay alınmalıdır. Örneğin, hasta fotoğraflarında göz bölgesini maskelemek, yetersiz bir gizlilik sağlanmasıdır.

Haseki Tıp Bülteni'ne yayınlanmak amacıyla gönderilen ve etik kurul onayı alınması zorunluluğu olan deneysel, klinik ve ilaç araştırmaları için uluslararası anlaşmalara ve 2013'de gözden geçirilmiş Helsinki Bildirisi'ne uygun etik kurul onay raporu gereklidir (http://www.wma.net/en/30publications/10policies/b3/). Deneysel hayvan çalışmalarında ise "Guide for the care and use of laboratory animals (https://oacu.oir.nih.gov/regulations-standards) doğrultusunda hayvan haklarını koruduklarını belirtmeli ve kurumlarından etik kurul onay raporu almalıdırlar. Etik kurul onayı (onay numarası ile birlikte) ve "bilgilendirilmiş gönüllü olur formu" alındığı araştırmanın "Yöntemler" bölümünde belirtilmelidir. Yazarlar, makaleleriyle ilgili çıkar çatışması ve maddi destekleri bildirmelidirler.

Orijinal Makaleler

1) Başlık Sayfası (Sayfa 1): Yazı başlığının, yazar(lar)ın bilgilerinin, anahtar kelimelerin ve kısa başlıkların yer aldığı ilk sayfadır.

Türkçe yazılarda, yazının İngilizce başlığı da mutlaka yer almalıdır; yabancı dildeki yayınlarda ise yazının Türkçe başlığı da bulunmalıdır. Türkçe ve İngilizce anahtar sözcükler ve kısa başlık da başlık sayfasında yer almalıdır.

Yazarların isimleri, hangi kurumda çalıştıkları ve açık adresleri belirtilmelidir. Yazışmaların yapılacağı yazarın adresi de ayrıca açık olarak belirtilmelidir. Yazarlarla iletişimde öncelikle e-posta adresi ve mobil telefon kullanılacağından, yazışmaların yapılacağı yazara ait e-posta adresi ve mobil telefon mutlaka belirtilmelidir. Buna ek olarak sabit telefon ve faks numaraları da bildirilmelidir.

Çalışma herhangi bir bilimsel toplantıda önceden bildirilen koşullarda tebliğ edilmiş ya da özeti yayınlanmış ise bu sayfada konu ile ilgili açıklama yapılmalıdır.

Yine bu sayfada, dergiye gönderilen yazı ile ilgili herhangi bir kuruluşun desteği sağlanmışsa belirtilmelidir.

2) Özet (Sayfa 2): İkinci sayfada yazının Türkçe ve İngilizce özetleri (her biri için en fazla 200 sözcük) ile anahtar sözcükler belirtilmelidir.

Özet Bölümü: Amaç, Yöntemler, Bulgular, Sonuç şeklinde alt başlıklarla düzenlenir. Derleme, olgu sunumu ve eğitim yazılarında özet bölümü alt başlıklara ayrılmaz. Bunlarda özet bölümü, 200 kelimeyi geçmeyecek şekilde amaçlar, bulgular ve sonuç cümlelerini içermelidir.

Özet bölümünde kaynaklar gösterilmemelidir. Özet bölümünde kısaltmalardan mümkün olduğunca kaçınılmalıdır. Yapılacak kısaltmalar metindekilerden bağımsız olarak ele alınmalıdır.

3) Metin (Özetin uzunluğuna göre Sayfa 3 veya 4'den başlayarak)

Genel Kurallar bölümüne uyunuz.

Metinde Ana Başlıklar Şunlardır: Giriş, Yöntemler, Bulgular, Tartışma, Çalışmanın Kısıtlılıkları ve Sonuç.

Giriş bölümü çalışmanın mantığı ve konunun geçmişi ile ilgili bilgiler içermelidir. Çalışmanın sonuçları giriş bölümünde tartışılmamalıdır.

Yöntem bölümü çalışmanın tekrar edilebilmesi için yeterli ayrıntılar içermelidir. Kullanılan istatistik yöntemler açık olarak belirtilmelidir.

Bulgular bölümü de çalışmanın tekrar edilebilmesine yetecek ayrıntıları içermelidir.

Tartışma bölümünde, elde edilen bulguların doğru ve ayrıntılı bir yorumu verilmelidir. Bu bölümde kullanılacak literatürün, yazarların bulguları ile direkt ilişkili olmasına dikkat edilmelidir.

Çalışmanın Kısıtlılıkları bölümünde çalışma sürecinde yapılamayanlar ile sınırları ifade edilmelidir. Sonuç bölümünde çalışmadan elde edilen sonuç, gelecek çalışmalara ilişkin öneriler ile vurgulanmalıdır.

Teşekkür mümkün olduğunca kısa tutulmalıdır. Çalışma için bir destek verilmişse bu bölümde söz edilmelidir. (Teşekkür yalnızca "Başlık Sayfası" içerisinde gönderilmelidir.)

Metinde fazla kısaltma kullanmaktan kaçınılmalıdır. Tüm kısaltılacak terimler metinde ilk geçtiği yerde parantez içinde belirtilmelidir. Özette ve metinde yapılan kısaltmalar birbirinden bağımsız olarak ele alınmalıdır. Özet bölümünde kısaltması yapılan kelimeler, metinde ilk geçtiği yerde tekrar uzun şekilleri ile yazılıp kısaltılmalıdırlar.

4) Kaynaklar: Kaynakların gerçekliğinden yazarlar sorumludur.

Kaynaklar metinde geçiş sırasına göre numaralandırılmalıdır. Kullanılan kaynaklar metinde parantez içinde belirtilmelidir.

Kişisel görüşmeler, yayınlanmamış veriler ve henüz yayınlanmamış çalışmalar bu bölümde değil, metin içinde şu şekilde verilmelidir: (isim(ler), yayınlanmamış veri, 19..).

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a) Standart Makale: Intiso D, Santilli V, Grasso MG, Rossi R, Caruso I. Rehabilitation of walking with electromyographic biofeedback in foot-drop after stroke. Stroke 1994;25:1189-92.

b) Kitap: Getzen TE. Health economics: fundamentals of funds. New York: John Wiley & Sons; 1997.

c) Kitap Bölümü: Porter RJ, Meldrum BS. Antiepileptic drugs. In: Katzung BG, editor. Basic and clinical pharmacology. 6th ed. Norwalk, CN: Appleton and Lange; 1995. p. 361-80.

Birden fazla editör varsa: editors.

d) Toplantıda Sunulan Makale: Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Reinhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6-10; Geneva, Switzerland. North-Holland; 1992. p. 1561-5.

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Phone: +90 212 529 44 00/1874
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Original Article / *Özgün Araştırma*

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Stimulus Threshold for Providing Intraoperative Motor Evoked Potential

İntraoperatif Motor Uyarılmış Potansiyel Eldesi İçin Uyarı Eşiği

Emine Taşkıran, Dilek Akyüz*

İstanbul University-Cerrahpaşa, Department of Neurology, İstanbul, Turkey
**İzmir Katip Çelebi University, Department of Neurology, İzmir, Turkey*

Abstract

Aim: Intraoperative neurophysiological monitoring (IOM) has been increasingly used in surgeries associated with a risk of neurological impairment. Motor evoked potential (MEP), which is a part of intraoperative neurophysiological tests, evaluates motor function intraoperatively. Most anesthetic agents have negative effect on neurophysiologic recordings because of neuronal excitability changes. Our aim was to compare the effect of anesthetic methods consisting of volatile anesthetics and intravenous anesthetics (propofol + remifentanil) on eliciting of MEP during baseline recordings for spinal surgery which is a part of neurosurgical operations.

Methods: Fifty patients (29 males and 21 females; 21 to 85 years) who underwent spinal surgery with IOM in our department between 2016 and 2018 were randomly chosen for retrospective evaluation. A multipulse stimulation technique (6-9 stimuli) was used for electrical stimulation.

Results: There was a statistically significant difference in stimulus threshold in voltage stimulation between the two groups. In order to elicit muscle MEP, a higher voltage threshold had to be implemented for patients who had been given volatile anaesthesia compared to those who had been given total intravenous anesthesia (TIVA) (Mann-Whitney U test, p<0.005).

Conclusion: TIVA is considered better than volatile for eliciting muscle MEPs in lower stimulus threshold. In addition, TIVA provides easy recording in all proximal and distal muscles.

Keywords: Volatile, total intravenous anesthesia, motor evoked potential, voltage stimulus threshold

Öz

Amaç: İntraoperatif nörofizyolojik monitorizasyon (İOM) nörolojik kötüleşme riski olan cerrahilerde artan sıklıkta kullanılmaktadır. İntraoperatif nörofizyolojik testlerden biri olan motor uyarılmış potansiyeller (MUP) intraoperatif olarak motor fonksiyonları değerlendirir. Birçok anestetik ajan nöronal uyarılabilirliği değiştirdiği için nörofizyolojik kayıtlar üzerine negatif etki gösterir. Amacımız, spinal cerrahide başlangıç kayıtlarda MUP uyarım eşiğine volatil ve intravenöz (propofol + remifentanil) anestezikleri içeren anestezik metodların etkisini değerlendirmektir.

Yöntemler: 2016-2018 yılları arasında IOM eşliğinde spinal cerrahi için opere edilen rastgele 50 hasta (29 erkek ve 21 kadın; 21-85 yaş) retrospektif değerlendirme için çalışmaya alındı. Elektriksel uyarım için çoklu uyarım (6-9 uyarım) tekniği kullanıldı.

Bulgular: Sabit voltaj uyarımda uyarım eşiği için gruplar arasında istatiksel anlamlı farklılık saptandı. Volatil anestezik ile kas MUP eldesi için gereken uyarım eşiği TIVA grubundan daha yüksekti (Mann-Whitney U testi, p<0,005).

Sonuç: Total intravenöz anestezi daha düşük uyarım eşiğinde kas MUP eldesi için volatil anesteziğe daha üstün gözükmektedir. Ek olarak, TIVA proksimal ve distal kasların hepsinde daha kolay MUP eldesi sağlamaktadır.

Anahtar Sözcükler: Volatil, total intravenöz anestezi, motor uyarılmış potansiyel, voltaj uyarım eşiği

Address for Correspondence/Yazışma Adresi: Emine Taşkıran, İstanbul University-Cerrahpaşa, Department of Neurology, İstanbul, Turkey
E-mail: dreminetaskiran@gmail.com ORCID: orcid.org/0000-0002-4402-7606
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Introduction

Intraoperative neurophysiological monitoring (IOM), which aims the best quality of life after surgery, has been increasingly used in new advanced concept of neurosurgery, cardiovascular surgery and thyroid surgery (1-4). Primary intraoperative neurophysiological studies are motor- and sensory-evoked potentials (MEP and SEP), electromyography and, electroencephalography. Among these, MEP presents the functionality of motor pathway from the cortex to muscles during the surgery. Loss of MEP and decreased MEP amplitude indicate postoperative motor worsening. Thus, MEP evaluation during surgery is necessary for patients with lesions that may affect the motor pathways. Most of anesthetic agents used intraoperatively change excitability of the neuroaxis at the cortical, subcortical and spinal levels and have negative effect on neurophysiological recordings (5).

This study aims to compare the effect of anesthetic methods consisting of volatile anesthetics and intravenous anesthetics (propofol + remifentanil) on eliciting MEP during spinal surgery.

Methods

Subject and Data Collection

Fifty patients who underwent spinal surgery with the aid of IOM were included in this study. Sevoflurane, which is one of the most commonly used volatile anesthetics, was used in 20 patients and propofol plus remifentanil in 30 patients. Demographical characteristics, neurological examination findings, diagnosis, surgical level, anesthesia type, and intraoperative neurophysiological recordings of 50 patients were retrospectively evaluated. Those having a hemoglobin level of <10 gr/dL and hematocrit concentration of <35% in preoperative evaluations were excluded.

Transcranial Motor Evoked Potentials

MEPs were elicited by stimulating the motor cortex from the scalp and recording from muscles. Stainless steel needle electrodes (13-19 mm, Xi'an Friendship Medical Company) and corkscrew electrodes were used for MEP responses respectively. Stimulation was performed using a multipulse stimulation technique based on a train of 6 to 9 stimuli with 4 ms interstimulus interval (ISI), pulse width of 50 μs, and an intensity between 200 V and 600 V, delivered at C1-C2 for right (R) extremity and C2-C1 for left (L). C3-C4/C4-C3 montage was used when not being gained with C1-C2/C2-C1 montage. Out of these two groups, the one with the lowest voltage threshold to elicit mMEP was used. Abductor pollicis brevis (APB), tibialis anterior (TA) and abductor halluces (AH) were recorded in all patients. Trapezius, deltoid, biceps brachi, extensor digitorum communis and iliopsoas, quadriceps femoris (QF), vastus lateralis, gastrocnemius, and sphincter ani externus muscles were added depending on surgical level. Evaluation was made for muscles having the lowest muscle threshold (APB, TA and, AH).

The Cadwell elite IOM system was used for neurophysiological recordings.

Total intravenous anesthesia (TIVA) using propofol (1.5-2 mg/kg for anesthesia induction and 6-10 mg/kg/h for maintenance) plus remifentanil (0.15 μg/kg/min) was used in 30 patients while inhalation anesthesia (sevoflurane, minimum alveolar concentration (MAC) maximum 0.5, BIS 40-60) was used in 20. A short-acting muscle relaxant (rocuronium, 0.5 mg/kg) was used only for endotracheal intubation. Satisfactory recovery from neuromuscular block was monitored by the train-of-four technique before MEP recording. In addition, mean arterial pressure, end-tidal carbon dioxide and oxygen concentration were monitored by the anesthesia team before eliciting MEP responses in all cases.

All procedures were performed in accordance with the 1964 Helsinki Declaration and its later amendments. Due to the fact that the study was retrospective, ethical committee approval was not obtained. Written informed consent was obtained from the patients.

Statistical Analysis

The statistical package for the social sciences (SPSS) 22.0 was used for data analysis. For descriptive statistics, the measures used were percentage distributions for categorical variables, and means (medians) with standard deviation (ranges) and, ranges for continuous variables. Frequency distributions were compared using the chi-square test and means by independent samples t-test and one-way analysis of variance (ANOVA) for normal variables, The Mann-Whitney U test and Kruskal-Wallis H test were used for non normal variables.

Results

The mean age of the patients (29 males and 21 females) was 48.7 years (range: 21-85 years). Bilateral extremities of 50 patients were evaluated, yielding a data set of 100. All patients had a hemoglobin level of >10g/dL. The diagnosis and surgical levels in the two groups are shown in Table 1.

TIVA Group

Eight patients had an intradural extramedullary mass, five had an intramedullary mass, two had an extradural mass, nine had stenosis, and six patients had fracture. Operation levels were at the cervical spinal level in 15, thoracal in nine and, lumbar in six. Fourteen patients had motor deficits preoperatively, with three of them having

<3/5 motor power. Mean voltage value for TIVA group was 325 V, ranged from 170 to 450. In 60 extremities, APB-MEP was elicited in all except 2 which had severe motor paralysis. AH-MEP was not achieved in 6 (3 R, 3L) which had severe motor paralysis (<2/5) preoperatively and QF-MEP in 11 extremities, with eight out of 11 had motor paralysis at the preoperative neurological examination. MEP response variability was not detected in this group.

Volatile Group

Eight patients had an intradural extramedullary mass, 1- intradural intramedullary tumor, 1- extradural tumor, 7- stenosis, and 3- fracture. Operation levels in this group were cervical spinal level in 7, thoracal in 3, lumbar in 9 and, sacral in one. Seven patients had mild (>3/5) motor deficit preoperatively. Mean voltage value for volatile group was 423V, ranged from 330 to 600. MEP response variability was detected in two. R APB-MEP and bilateral proximal muscle MEPs (deltoid, biceps brachi) were elicited at the baseline recording in all except one having C1-C2 meningioma and no neurological deficit preoperatively. AH-MEP could not be elicited in one patient having severe motor deficit preoperatively and QF-MEP in 5 (3 R, 2 L) with three of those had motor deficit in preoperative neurological examination.

There was a statistically significant difference in stimulus threshold in voltage stimulation between the two groups (Mann-Whitney U test, p<0.005). Presence of motor deficit preoperatively did not have an impact on the increase of required voltage stimulation in the two groups.

Discussion

This study showed that voltage threshold to elicit mMEP with the multipulse stimulation technique–a train of 6 to 9 with an ISI of 4 ms, pulse width of 50 μs–in patients who had been given volatile anaesthesia was higher than that of TIVA. There are some studies evaluating suppressive effect of inhalation agents on MEPs (6). They generally pointed out reduction in MEP amplitude with volatile anesthetic usage in different MAC levels. In their study randomizing patients to three groups to receive halothane (HAL), isoflurane (ISO), or sevoflurane, Sekimoto et al. (6) reported that the amplitudes of MEPs were significantly reduced by all agents at 1.0 MAC, with the effect being less in HAL at 0.5 MAC. In this study, we focused on stimulus threshold for eliciting MEP response by the multipulse stimulation technique (6 to 9 stimuli with an ISI of 4 ms, pulse width of 50 μs) by sevoflurane agent at <0.5 MAC and TIVA. There was a statistically significant difference in stimulus threshold in voltage stimulation between the two groups (Mann-Whitney U test, p<0.005).

Table 1. Diagnosis and surgical levels for two groups

Diagnosis	Number of patient/TIVA	Number of patient/volatile
Intradural mass	9	7
Intramedullary mass	4	2
Extradural mass	2	1
Stenosis	9	7
Fracture	6	3
Surgical level	**Number of patient/TIVA**	**Number of patient/volatile**
Cervical	15	7
Thoracal	9	3
Lumbar	6	9
Sacral	0	1

TIVA: Total intravenous anesthesia

It is known that obtaining MEP response in lower extremities is difficult in patients with preoperative motor deficit. Chen et al. (7) reported that the success rate for obtaining reliable MEP response was 94.8% for upper extremities and 66.6% for lower extremities and it was only 39.1% for lower extremities in patients with preoperative motor deficit. This challenge could be demonstrated in patients who had been given volatile anaesthesia. In contrast, presence of preoperative motor deficit did not have an impact on the increase of required voltage stimulation in the two groups although provided MEP was much lower in patients with preoperative motor weakness in this study. This might be explained by very mild motor paralysis (>4/5) in our patients.

MEP response variability was detected in two patients of only volatile group in this study. Volatile agents decrease the possible motor neuron recruitment in anterior horn of the spinal cord and affect the propagation of a peripheral motor response negatively. Thus, volatile agents interfere with reliable MEP acquisition. Pelosi et al. (8) confirmed this situation in their study reporting that reliable recordings were present in 14 of 23 patients who received volatile agents and 28 of 29 who received propofol. In addition, bilateral MEP recruitment ratios were higher with propofol than with volatile anaesthesia. TIVA offers obvious advantages in obtaining MEP response and reliable acquisition during surgery.

Isofluran, enfluran, desfluran, sevoflurane and halotan are volatile anesthetics. In general, all have similar effects on evoked potentials. In this study, we evaluated the effect of sevoflurane on MEP acquisition and response variability. Volatile anaesthetics have an effect on pyramidal neurons, cortical interneurons, corticospinal axons and, alfa motor neuron (9-11). Inhibition via neocortical GABA-A receptors ensures cortical suppression (9,10). Suppressing effect on motor

pathway at the corticospinal and spinal level is based on blocking synaptic transmission over alpha motor neurons in the spinal cord and depressing Na transmission at the Ranvier nodes of corticospinal axons (11). Propofol's advantage over volatile anaesthesia is that it has a negligible effect on MEP at the spinal level. As seen in our study, MEP acquisition is achieved more easily and with lower voltage stimulation under TIVA.

Volatile anesthetics depress excitatory synaptic transmission by inhibiting presynaptic voltage-gated Na^+ channels at clinical concentrations. In contrast, the intravenous anesthetic propofol inhibits Na^+ channels only at supratherapeutic concentrations (12). Some new sedatives such as alpha2-agonist dexdemetomidine have been come up to decrease propofol dosage (13,14).

Study Limitations

All patients with and without motor weakness were included in this study. It would better to include patients with normal MEP findings without motor weakness for comparing the voltage stimulus thresholds for eliciting muscle MEPs between the two groups.

Conclusion

As a consequence, at a current time, TIVA is considered to be better than volatile anesthetic for monitoring muscle MEPs. Even in cases in which TIVA is used, however, it should be considered that higher levels of propofol may cause suppression on alpha motor neuron at the spinal level and thus misinterpretation of MEP loss or amplitude decreasing in this setting. With developing new anaesthetic agents, new studies aiming to evaluate their suppressing effect on MEPs and/or other evoked potentials would be required.

Authorship Contributions

Concept: E.T. Design: E.T. Data Collection or Processing: E.T., D.A. Analysis or Interpretation: E.T. Literature Search: E.T., D.A. Writing: E.T., D.A.

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

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Auralı Migren Hastalarında Non-spesifik Serebral Ak Madde Lezyonlarının Varlığı ve İlişkili Faktörlerin Değerlendirilmesi

Evaluation of Non-specific Cerebral White Matter Lesions and Related Factors in Patients with Migraine with Aura

Arife Çimen Atalar, Osman Özgür Yalın

İstanbul Eğitim ve Araştırma Hastanesi, Nöroloji Kliniği, İstanbul, Türkiye

Öz

Amaç: Migren hastalarında serebral ak madde lezyonlarına (AML) rastlanabildiği iyi bilinmekle birlikte, auralı migren (MWA) hastalarında AML'nin varlığı üzerine yoğunlaşmış sınırlı sayıda çalışma bulunmaktadır. Bu çalışmada amacımız MWA hastalarında non-spesifik AML'nin sıklığı ve ilişkili demografik ve çevresel faktörlerin araştırılmasıdır.

Yöntemler: Uluslararası Baş Ağrısı Topluluğu kriterlerine (ICHD-3) göre migren tanısı almış toplam 112 hasta çalışmaya dahil edildi. Detaylı sistemik ve nörolojik muayeneleri, kan basıncı, kilo, boy ölçümleri, hipertansiyon ve sigara kullanımları, klinik ve demografik bilgileri, görsel analog skala, allodini semptom kontrol anketi (ASK) ve migren dizabilite ölçeği skorları kaydedildi. Auralı ve aurasız migren grupları istatistiksel olarak karşılaştırıldı. AML oluşumuna etki edebilen bağımsız risk faktörlerinin analizi için ek olarak lojistik regresyon analizi uygulandı.

Bulgular: Süpratentoryal ve periventriküler non-spesifik AML, MWA grubunda aurasız migren grubuna kıyasla daha sıktı (p=0,008). Aura varlığı ve uzamış hastalık süresi AML oluşumunda bağımsız risk faktörleri olarak anlamlı bulundu (p=0,0020 ve p=0,019). Migren atak sıklığı, allodini varlığı ve ASK skorları MWA grubunda anlamlı düzeyde daha yüksekti (sırasıyla p=0,005, p=0,015 ve p<0,001).

Sonuç: Sonuçlarımız MWA hastalarında non-spesifik AML'nin aurasız gruba kıyasla daha sık olduğunu göstermektedir. Hastalık süresinin uzun olması ve aura varlığı bu lezyonların oluşumuna yatkınlık yaratmaktadır. Bulgularımızın ileride daha geniş hasta sayıları ile planlanacak çalışmalar ile desteklenmesi gerekmektedir.

Anahtar Sözcükler: Auralı migren, aurasız migren, manyetik rezonans, serebral ak madde, periventriküler

Abstract

Aim: Cerebral white matter hyperintensities (WMLs) are known to be observed in migraine patients but there only are a handful of studies focused on WMLs in migraine with aura (MWA). In this study, we aimed to investigate the frequency of WML and demographic and environmental factors associated with WML in patients with MWA.

Methods: A total of 112 patients diagnosed with migraine were enrolled. Detailed systemic and neurological examinations, blood pressure, weight and height measurements, presence of hypertension, smoking status, clinical and demographical data and visual analog scale, allodynia symptom checklist (ASC) and migraine disability assessment scale scores were recorded. The migraine groups with and without aura were compared statistically. A logistic regression analysis was performed to analyse the risk factors for the development of WMLs.

Results: Supratentorial and periventricular WMLs were more frequent in patients with MWA (p=0.008). Presence of aura and longer disease duration were independent risk factors for the development of WMLs (p=0.0020 and p=0.019, respectively). Migraine attack frequency, and ASC scores were higher in patients with MWA (p=0.005, p=0.015 and p<0.001, respectively).

Conclusion: Our results show a significant increase in non-specific WMLs in patients with MWA. A longer disease duration and presence of aura are significant risk factors for development of these lesions. Our findings warrant further research to validate our result.

Keywords: Migraine with aura, migraine without aura, magnetic resonance, cerebral white matter, periventricular

Yazışma Adresi/Address for Correspondence: Arife Çimen Atalar, İstanbul Eğitim ve Araştırma Hastanesi, Nöroloji Kliniği, İstanbul, Türkiye
Tel.: +90 212 560 65 59 E-posta: cimenatalar@yahoo.com.tr ORCID: orcid.org/0000-0003-0328-9607
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Giriş

Migren toplumun yaklaşık %15'ini etkileyen ve kadın popülasyonda erkeklere oranla 2-3 kat yüksek oranlarda rastlanan tekrarlayıcı baş ağrısı atakları ile karakterize kronik bir nörolojik hastalıktır (1-3).

Hastaların yaklaşık %25'inde aura adı verilen ve ağrı başlangıcından önce ortaya çıkan geçici özellikte görsel, duyusal veya konuşma ile ilgili nörolojik disfonksiyon bulguları eşlik edebilmektedir (4,5). Migrenli hastalarda beyin manyetik rezonans görüntüleme (MRG) incelemesinde vasküler lezyonların, özellikle de serebral ak madde lezyonlarının (AML) varlığı üzerine yapılmış pek çok çalışmanın varlığına karşın, özellikle AML saptanan auralı migren grubundaki olgularda klinik özelliklerin ele alındığı çalışma sayısı sınırlıdır (6). AML' nin patofizyolojisi halen net olmamakla birlikte önde gelen hipotezlerden biri migren atağı sırasında meydana gelen uzamış oligemi nedeniyle küçük çaplı penetran arterlerin sulama alanlarında hipoperfüzyon meydana geldiği ve bunun derin ak maddede mikrovasküler değişikliklere yol açtığı şeklindedir (7). Derin yerleşimli AML'nin özellikle süpratentoryal alanda sık görüldüğü, bunu daha az sıklıkta olmak üzere arka sisteme ait sulama alanlarındaki sessiz serebral iskemilerin ve infratentoryal alandaki lezyonların izlediği yakın zamanlı çalışmalarda bildirilmiştir (8,9). Bu lezyonların özellikle frontal ve parietal loblarda, ovoid ya da sirküler yapıda, net sınırlı, T2 ve FLAIR ağırlıklı görüntülemelerde hiperintens odaklar şeklinde görülebildiği bilinmektedir (8). Bu çalışmada amacımız 3. basamak bir baş ağrısı kliniğinde, auralı migren tanısı ile takip edilmekte olan hastalarda, beyin MRG'de non-spesifik serebral AML'nin varlığı ve sıklığını araştırmak ve bu lezyonların ortaya çıkmasında rol oynayabilecek demografik ve çevresel faktörleri belirlenmektir.

Yöntemler

Çalışmaya İstanbul Eğitim ve Araştırma Hastanesi Baş ağrısı polikliniğine, Şubat 2015-Aralık 2017 tarihleri arasında baş ağrısı yakınması ile başvuran hastalar arasından, bir nöroloji uzmanı tarafından, Uluslararası Başağrısı Derneği (10) kriterlerine uygun olarak tanı almış, sekonder baş ağrısı nedenlerinin dışlanması amacıyla kraniyal MRG incelemeleri yapılmış ve tekrar değerlendirmeye uygun olan, 62 auralı migren tanısı almış hasta ve kontrol grubu olarak 50 aurasız migrenli hasta dahil edilmiştir. Vasküler komplikasyon yaratabilecek sistemik komorbit hastalıkları bulunan hastalar (hipertansiyon, Diabetes Mellitus, hiperlipidemi vb.) çalışmaya dahil edilmemiştir. Yine Türkçe okuma ve yazması olmayan ve kraniyal MRG'de AML harici serebral lezyon saptanmış olan hastalar çalışma dışında bırakılmıştır.

Hastalık başlangıç yaşı, baş ağrısının süresi ve sıklığı, aura varlığı ve tipi, baş ağrısının klinik özellikleri ve lokalizasyonu, ağrının şiddeti, ağrıyı tetikleyici faktörler, varsa komorbid hastalıklar, ailede migren öyküsü varlığı, Görsel Analog skalası (VAS), Migren Dizabilite ölçeği (MIDAS), allodini varlığı gibi klinik ve demografik bilgiler bir baş ağrısı uzmanı tarafından yapılan yüz yüze görüşmelerde standart bir form uygulanarak kayıt altına alınmıştır.

Görüşmelerde kullanılan formlardan VAS skalası; yatay veya dikey planda 10 cm'lik düz bir çizgi üzerinde hastanın hissettiği ağrı şiddetini; 0=hiç ağrı yok ve 10=en şiddetli ağrı şeklinde işaretlemesi ve böylece hastanın ağrı yoğunluğunun subjektif olarak belirlenmesinde kullanılan bir ölçektir (11,12). Diğer bir anket olan Allodini Semptom Kontrol Listesi (ASK); 12 maddeden oluşan, kutanöz allodinin alt tiplerini ve allodini şiddetini belirlemeye yarayan, Türkçe geçerlik ve güvenilirlik çalışması yapılmış bir sorgulama formudur (13-15). Migrene bağlı dizabiliteyi değerlendirme amaçlı kullanılan MIDAS ise, beş sorudan oluşan, geçerliliği ve güvenirliği kanıtlanmış, Türkçe validasyonu yapılmış bir testtir. İş ve okul çalışması, ev işleri, aileyle geçirilen zaman ve sosyal durum üzerinde başağrısının son 3 ay içerisindeki yarattığı etkiyi inceleyerek dizabiliteyi belirlemede kullanılır (16). Her hastanın detaylı sistemik ve nörolojik muayeneleri bir nöroloji uzmanı tarafından yapılarak hastaların sistolik ve diyastolik kan basınçları, ağırlık ve boy ölçümleri standart cihazlar yardımıyla ölçülerek kaydedilmiştir (Riester/Seri Numarası: 4012835). Her hastada kardiyovasküler risk faktörlerinden hipertansiyon varlığı ve sigara kullanımı değerlendirilerek kaydedilmiştir. Hastalardan onam formu alınmış ve çalışma İstanbul Eğitim ve Araştırma Hastanesi Etik Kurulu tarafından onaylanmıştır (612/27.02.2015).

MRG Görüntüleme Protokolü

Çalışmaya dahil edilen her hastaya (Signa Hdxt, GE Medical Systems, Milwaukee, WI, USA) ile 1,5 Tesla MRG ile serebral görüntüleme yapılmıştır. Hastalara difüzyon sekansı, aksiyel FSE T2, aksiyel FLAIR, aksiyel SE T1, koronal FSE T2, T1 aksiyel ve sagittal DFOV sekanslarını içeren standart bir inceleme protokolü uygulanmıştır. T2 ağırlıklı ve FLAIR sekanslarda hiperintens ve T1 ağırlıklı sekanslarda hipointens olarak izlenen, boyut olarak ≥3 mm olan lezyonlar serebral AML olarak tanımlanmıştır (17). Elde edilen görüntüler AML'nin varlığı ve yerleşim yerlerine göre sınıflandırılmıştır.

İstatistiksel Analiz

İstatistiksel analizler için NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) programı kullanıldı. Çalışma verileri değerlendirilirken tanımlayıcı istatistiksel metotlar (ortalama, standart sapma, medyan, birinci çeyreklik, üçüncü çeyreklik, frekans, yüzde, minimum,

maksimum) kullanıldı. Nicel verilerin normal dağılıma olan uygunlukları Shapiro-Wilk testi ve grafiksel incelemeler ile sınandı. Normal dağılım gösteren nicel değişkenlerin iki grup arası karşılaştırmalarında bağımsız gruplar t-testi, normal dağılım göstermeyen nicel değişkenlerin iki grup arası karşılaştırmalarında Mann-Whitney U testi kullanıldı. Nitel verilerin karşılaştırılmasında Pearson ki-kare test ve Fisher'ın exact testi kullanıldı. Süpratentoryal lezyon oluşumu üzerine etki eden bağımsız risk değişkenlerinin değerlendirilmesinde multivaryant lojistik regresyon analizi uygulandı. İstatistiksel anlamlılık p<0,05 olarak kabul edildi.

Bulgular

Demografik Özellikler ve Baş Ağrısı Özellikleri

Çalışmaya dahil edilen toplam 112 olgunun 62'si (%44,6) auralı migren ve 50'si (%55,4) aurasız migren grubundaydı. Hastaların yaş ortalaması 34,08±11,96 yıldı. Olguların %81,3'ü (n=91) kadın, %18,8'i (n=21) erkekti. Hastaların eğitim süreleri ortalama 8,66±4,64 yıl, boy ortalamaları 163,83±8,20 cm ve kilo ortalamaları 71,96±16,11 kilogram olarak hesaplandı. Hastaların sistolik kan basıncı ve diyastolik kan basıncı ortalamaları sırasıyla 118,63±16,82 mmHg ve 76,06±1,47 mmHg olarak bulundu. Auralı migren grubunda 26 hasta ve aurasız grupta 18 hasta sigara kullanmakta olup, sigara kullanımı ortalama 4,46±8,18 paket/yıl olarak hesaplandı. Tüm olguların 19'u (%17) kronik migren tanısı almıştı. Hastaların 76'sının (%67,9) ailesinde migren öyküsü mevcuttu. İlaç aşırı kullanım öyküsü olan 17 hasta (%15,2) bulunuyordu. Hasta grubunun baş ağrısı karakteristikleri Tablo 1'de özetlenmiştir.

Hasta grubunun kraniyal MRG'leri değerlendirildiğinde, 88 (%78,6) hastanın görüntülemesi tamamen normal iken, 24 (%21,4) hastanın süpratentoryal ve periventriküler alanda AML'i olduğu görüldü. Saptanan lezyonlar ağırlıklı olarak frontal ve parietal lob alanlarında yer almaktaydı (%91,6). Hastaların hiçbirinde arka sistem sulama alanında ak madde lezyonu mevcut değildi. AML olan olgular migren alt gruplarına göre sınıflandırıldığında, sadece beş hasta (%20,8) aurasız migren grubuna dahil iken, 19 hasta (%79,1) auralı migren grubunda yer almaktaydı. AML bulunan olgular tüm auralı migren grubunun %30,6'sını oluşturmaktaydı. Auralı ve aurasız gruplar arasında AML varlığı bakımından istatistiksel olarak anlamlı farklılık mevcuttu (p=0,008) (Tablo 2).

Kraniyal MRG lezyonu olmayan ve süpratentoryal AML'si olan gruplar kendi aralarında kıyaslandığında, AML saptanan olgularda migren atak sürelerinin anlamlı olarak daha uzun olduğu görüldü (p=0,049). Hastalık süresi de AML olan grupta istatiksel anlamlılık sınırındaydı (p=0,054). Diğer özellikler bakımından iki grup arası fark bulunmadı (p>0,05). Tablo 3'te kraniyal MRG incelemeleri normal ve süpratentoryal AML'si olan olguların verileri karşılaştırılmıştır.

AML oluşumu ile ilişkili bağımsız faktörleri belirlemek amacıyla lojistik regresyon analizi yapıldığında, aura varlığı olan olgularda AML gelişme ihtimalinin, aurası bulunmayan olgulara oranla 3,641 kat artmış olduğu saptandı [Odds oranı (OR): 3,641, güven aralığı (%95 GA): 1,221,10,862, p=0,029].

Ayrıca, baş ağrısı hastalık süresindeki her bir birim artışın AML gelişme ihtimalini 1,055 katı arttırdığı tespit

Tablo 1. Hasta grubunun baş ağrısı karakteristikleri

Hastalık süresi (yıl)	Min-maks	0,25-45
	Ort ± SS	10,13±9,82
Atak sıklığı (ay)	Min-maks	0,2-30
	Ort ± SS	9,73±9,17
Atak süresi (saat)	Min-maks	3-72
	Ort ± SS	29,08±2,73
Ağrının karakteri	Künt	4 (3,6)
	Sıkıştırıcı	6 (5,4)
	Zonklayıcı	100 (89,3)
	Zonklayıcı + sıkıştırıcı	2 (1,8)
Allodini varlığı	Yok	40 (35,7)
	Var	72 (64,3)
ASK skoru	Min-maks	0-17
	Ort ± SS	5,15±4,53
MIDAS skoru	Ort ± SS	27,18±3,48
Ağrı şiddeti/VAS skoru	Min-maks	0-10
	Ort ± SS	8,30±1,52
Fiziksel aktivite ile tetiklenme	Hayır	6 (5,4)
	Evet	106 (9,6)
Bulantı varlığı	Hayır	20 (17,9)
	Evet	92 (82,1)
Kusma varlığı	Hayır	69 (61,6)
	Evet	43 (38,4)
Fotofobi	Hayır	22 (19,6)
	Evet	90 (80,4)
Fonofobi	Hayır	18 (16,1)
	Evet	94 (83,9)
Mens ile ilişki	Hayır	69 (61,6)
	Evet	43 (38,4)
Emosyonel stres ile ilişki	Hayır	25 (22,3)
	Evet	87 (77,7)
Açlık ile ilişkisi	Hayır	37 (33)
	Evet	75 (67)

ASK: Allodini Semptom Kontrol anketi, MIDAS: Migren Dizabilite ölçeği, VAS: Görsel Analog skalası, Ort: Ortalama, min: Minimum, maks: Maksimum, SS: Standart sapma

edildi [OR: 1,055, (%95 GA): 1,009,1,103, p=0,019]. Atak süresinin univaryant analiz sonuçlarında istatistiksel olarak anlamlı bulunmasına karşın, multivaryant analizde anlamlılık düzeyine ulaşmadığı görüldü (Tablo 4).

Tartışma

Çalışmamız sonucunda, auralı migren hastalarında kraniyal MRG'de süpratentoryal ve periventriküler alanda AML'nın, aurasız gruba kıyasla daha yüksek oranlarda bulunduğunu ve aura varlığı ile hastalık süresinin uzun olmasının, auralı migrenli olgularda AML oluşumu üzerinde bağımsız risk faktörleri oluşturabileceğini gördük. Çalışmamızın bir diğer anlamlı sonucu ise, auralı migren grubundaki olgularda aylık atak sıklığı, allodini varlığı ve allodini şiddetini yansıtan ASK skorlarının aurasız gruba kıyasla daha yüksek bulunmasıydı.

Migren hastalarında serebral AML'ye %29'lara varan oranlarda rastlanabildiği yapılan çalışmalarda bildirilmiştir (18). Bu hastalarda özellikle arka sistem sulama alanında sessiz iskemiler, infratentoryal alanda T2 hiperintens

Tablo 2. Auralı ve aurasız migrenler arasında demografik, klinik ve MRG özelliklerinin karşılaştırılması

		Aurasız	Auralı	p
Hastalık süresi (yıl)	Medyan (Q1, Q3)	6 (3,12)	7 (3,15)	[c]0.369
Atak sıklığı (ay)	Medyan (Q1, Q3)	4.5 (3,8)	9 (4,20)	[c]0.005**
Atak süresi (saat)	Medyan (Q1, Q3)	24 (7,48)	24 (10,48)	[c]0.055
Allodini varlığı	Yok	24 (48)	16 (25.8)	[b]0.015*
	Var	26 (52)	46 (74.2)	
SKB	Ort ± SS	119.00±19.49	118.32±14.47	[d]0.833
DKB	Ort ± SS	76.71±12.12	75.53±10.99	[d]0.593
ASK skoru	Medyan (Q1, Q3)	2 (0,6)	6.5 (4,11)	[c]<0.001**
VAS skoru	Medyan (Q1, Q3)	8 (7,9)	9 (8,10)	[c]0.106
MIDAS skoru	Ort ± SS	27,12±2,48	28,19±3,52	0.07[d]
MRG	Normal	45 (90)	43 (69.4)	[b]0.008**
	AML	5 (10)	19 (30.6)	

MRG: Manyetik rezonans görüntüleme, SKB: Sistolik kan basıncı, DKB: Diyastolik kan basıncı, ASK: Allodini semptom kontrol anketi, VAS: Görsel analog skalası, MIDAS: Migren Dizabilite ölçeği, AML: Serebral ak madde lezyonları, Ort: Ortalama, SS: Standart sapma, Q1: Birinci çeyreklik, Q3: Üçüncü çeyreklik
[a]Fisher's exact testi, [b]Pearson ki-kare testi, [c]Mann-Whitney U testi, dBağımsız gruplar t testi
*p<0.05, **p<0.01

Tablo 3. Kraniyal MR incelemeleri normal olan/süpratentoryal ak madde lezyonu olan olguların karşılaştırılması

		Normal	AML	p
Aura	Hayır	45 (90)	5 (10)	[b]0,008**
	Evet	43 (69,4)	19 (30,6)	
İlaç aşırı kullanım öyküsü	Hayır	82 (79,6)	21 (20,4)	[a]0,400
	Evet	6 (66,7)	3 (33,3)	
Kronik migren	Hayır	73 (78,5)	20 (21,5)	[a]0,999
	Evet	15 (78,9)	4 (21,1)	
Hastalık süresi (yıl)	Medyan (Q1, Q3)	5 (3, 13)	10 (4.5, 25)	[c]0,054
Atak sıklığı (ay)	Medyan (Q1, Q3)	6 (3, 12)	6 (4, 16)	[c]0,708
Atak süresi (saat)	Medyan (Q1, Q3)	24 (8, 48)	36 (18, 48)	[c]0,049*
Allodini varlığı	Yok	35 (87,5)	5 (12,5)	[b]0,086
	Var	53 (73,6)	19 (26,4)	
ASK skoru	Medyan (Q1, Q3)	4 (1, 8)	7 (3, 9)	[c]0,135
VAS skoru	Medyan (Q1, Q3)	8 (7, 9)	9 (8, 10)	[c]0,162
MIDAS skoru	Ort ± SS	28,22±3,11	29,21±2,19	0,146[d]

ASK: Allodini Semptom Kontrol anketi, VAS: Görsel Analog skalası, MIDAS: Migren Dizabilite ölçeği, AML: Serebral ak madde lezyonları, MR: Manyetik rezonans, Q1: Birinci çeyreklik, Q3: Üçüncü çeyreklik, Ort: Ortalama, SS: Standart sapma
[a]Fisher's exact testi, [b]Pearson ki-kare testi, [c]Mann-Whitney U testi, dBağımsız gruplar t-testi
*p<0,05, **p<0,01

Tablo 4. Lojistik regresyon analizi sonuçları				
	Beta	**Wald**	**p**	**OR (%95 Güven aralığı)**
Sabit	-2,109	28,294	<0,001**	0,121
‡Aura (Auralı)	1,292	5,370	0,020*	3,641 (1.221, 10,862)
Hastalık süresi	0,054	5,490	0,019*	1,055 (1,009, 1,103)

OR: Odds oranı
İlgili değişken için aurasız migren kategorisi referans kategori olarak alınmıştır
*p<0,05, **p<0,01

lezyonlar ve süpratentoryal derin AML'nin varlığına dair birtakım çalışmalar bulunmakla birlikte, auralı migren hastalarında AML'nin varlığı ve sıklığı üzerine yapılmış çalışma sayısı oldukça kısıtlıdır (19,20).

AML patofizyolojisi tam olarak aydınlatılamamakla birlikte, multifaktöriyel olduğu düşünülmekte ve özellikle migren atağı esnasında oluşabilen lokal kan akımındaki azalmaya bağlı hipoperfüzyon suçlanmaktadır (21). Ayrıca migren atağı esnasında meydana gelebilen lokal vazojenik ödem, vazokonstrüksiyon, oluşan nörojenik enflamasyon ve koagülasyon eğilimindeki artış da yapılan çalışmalarda olası mekanizmalar arasında bildirilmektedir (21-26). Yine uzamış aura esnasında kortikal yayılan depresyon dalgası ve buna bağlı meydana gelen perfüzyon artışının beyin omurilik sıvısının ekstravazasyonuna yol açarak, bu lezyonların oluşumunda rol oynayabileceği iddia edilmiştir (27,28). Çalışmamız sonucunda, migrenli hastaların genelinde %21,4 oranında, auralı migren alt grubunda ise %30,6 oranında AML görülebildiğini ve bu lezyonlara öncelikli olarak süpratentoryal alanda frontal ve parietal loblarda ve periventriküler derin ak maddede rastlanabildiğini gördük. Bu konuya dair yapılmış olan çalışmalarda, migrenli hastalar alt grup ayrımı yapılmadan analiz edildiğinde, serebral AML varlığı daha yüksek oranlarda (%14-39) bildirilmiş olmasına karşın (29), auralı migren grubu için çalışmamızın sonuçları literatürde bildirilmiş toplum temelli çalışmaların sonuçları ile benzer bulunmuştur (%30,6 ya karşılık %34,3) (20,30).

Kruit ve ark. (31) 2009 yılında yapmış oldukları CAMERA (Cerebral Abnormalities in Migraine: An Epidemiological Risk Analysis) çalışmasında, 295 migrenli hasta (161 auralı migren ve 134 aurasız migrenli olgu) ile 140 sağlıklı kontrol hastası karşılaştırılmış ve migren atak frekansı yüksek olan ve hastalık süresi uzun olan migrenli olgularda AML oluşma riskinin daha yüksek olduğu bildirilmiştir. Bizim çalışmamızda da benzer biçimde hastalık süresi, auralı migrenli olgularda AML oluşumu üzerinde etkili bulunmasına karşın, bahsi geçen çalışmadan farklı olarak atak sıklığı bakımından istatiksel açıdan anlamlı bir fark bulunamamıştır (sırasıyla p=0,049 ve p=0,708). Galli ve ark. (30) auralı migreni olan 90 hasta üzerinde yapmış oldukları bir başka çalışmada ise, AML oranı %30 olarak bulunmuş ve atak süresi, atak frekansı, başlangıç yaşı ve aura tipi ile AML oluşum riski arasında herhangi bir ilişki saptanmamıştır. Yazarlar sadece kadın popülasyonda, ileri yaşın ak madde lezyonu oluşumu açısından bir risk oluşturabileceğini bildirmişlerdir. Bizim çalışmamızda ise kadın ve erkek popülasyon arasında AML oluşumu bakımından herhangi bir farklılık bulunmamaktaydı.

Migren hastalarında AML'nin en sık yerleştiği lokalizasyonlara dair, yapılmış olan çalışmalarda literatürde farklı sonuçlar bildirilmektedir. Bazı çalışmalarda bu lezyonların en sık serebellum ve beyin sapında yerleşebildiği bildirilmekteyken (31), kimi çalışmalar ise süpratentoryal alanda bu lezyonlara daha yüksek oranda rastlanabildiğini iddia etmektedir (8,20). Bizim çalışmamızda, en sık süpratentoryal sahada, frontal ve parietal derin ak madde alanlarında ve bunu takiben periventriküler derin ak madde alanında AML'leri mevcuttu. Çalışmamıza dahil olan olguların hiçbirinde arka sistem sulama alanına ait lezyon saptanmadı. Bu durumun çalışma grubumuzda hipertansiyon, diyabet gibi risk faktörleri olan hastaların dışlanmış olması ve ileri yaştaki olguların alınmamış olması ile ilgili olabileceğini düşünüyoruz.

Çalışmamızın diğer bir sonucu olan auralı migrenli grupta allodini oranlarının yüksek bulunması beklenen bir sonuç olup, yapılan çalışmalar allodininin migrenin kronikleşmesine ve migrenle ilişkili dizabiliteye yol açabilen önemli bir risk faktörü olduğu bildirmektedir (32-36). Auralı migren hastalarında %70,5'lere varan oranlarda allodini görülebildiği ve allodini varlığı ile allodini şiddetinin, kronik migrenli olgularda daha yüksek olduğu da göz önüne alındığında, çalışmamızın sonuçları bu bulguları destekler niteliktedir (37-39).

Migren hastalarında normal bireylere oranla iskemik inme riskinin iki kat ve kraniyal MRG'de AML gelişme riskinin yaklaşık 4 kat artmış olduğunu bildiren meta-analiz çalışmaları bulunmaktadır (31,40,41). Fakat bu konuya dair yakın zamanda yapılmış prospektif ve uzun takip süresi içeren çalışmaların sonucunda, migren ve migrene dair özelliklerden çok ilerleyen yaş ile ilişkili birtakım vasküler risk faktörlerinin ön plana çıkarak bu hastalarda iskemik inme insidansını artırabileceği öne sürülmektedir (18). Bu konu halen netleşmemiş olmakla beraber, migren hastalarında AML varlığının önemli bazı klinik ve prognoza dair yansımaları olabileceği öngörülebilir. Bunlar arasında, bahsedildiği gibi diğer eşlik eden risk faktörleri, genetik ve çevresel faktörlerin de katkısıyla iskemik inme riskini artırabilmesinin yanı sıra kognitif etkilenmeye de yol açabileceği halen tartışılmalı konular arasındadır (42,43). Bu nedenle çalışmamız, özellikle auralı migren alt grubunda bu lezyonların varlığına dikkat çekmesi ve uzamış hastalık

süresinin bu lezyonların oluşumu üzerinde bağımsız bir risk faktörü oluşturabileceğini göstermesi bakımından dikkat çekicidir.

Çalışmamızın bazı kısıtlılıkları mevcuttur. Öncelikle gösterilen tüm dikkate rağmen çalışmamız sınırlı sayıda hasta ile yapılmış bir çalışmadır bu nedenle genel auralı migren popülasyonunu temsil etmede yetersiz kalabilir. Çalışmamıza normal kontrol alınmamış olması ve kontrol grubu olarak aurasız migrenli grubun alınmış olması da sonuçlarımıza etki etmiş olabilir fakat vasküler risk faktörleri taşıyan hastaların dışlanmış olmasının bu kısıtlılığı kısmen giderebileceği kanaatindeyiz. Son olarak üçüncü basamak bir baş ağrısı merkezinde ve genel olarak daha ağır migren yakınmaları ile başvuran hastalarda yapılmış olması nedeniyle sonuçlarımızın bu durumdan etkilenmiş olması mümkündür.

Sonuç

Çalışmamızın sonuçları, migren hastalarında aura varlığı ve uzamış hastalık süresinin, kraniyal MRG'de non-spesifik AML gelişme riskini artırabildiğini destekler niteliktedir. Bu lezyonların oluşum mekanizmalarının araştırılmasını temel alarak planlanacak geniş sayıda hasta içeren, prospektif nitelikteki çalışmaların patofizyolojinin aydınlatılmasına ışık tutarak gelecekte etkin tedavilerin ortaya çıkmasına zemin hazırlayabileceği kanaatindeyiz.

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Inflammation, Left Ventricular Mass Index and Chronic Renal Failure in Diabetic Patients

Diyabetik Hastalarda Enflamasyon, Sol Ventrikül Kitle İndeksi ve Kronik Böbrek Yetmezliği

Egemen Cebeci, Nilay Şengül Samancı*, Sami Uzun, Serhat Karadağ, Meltem Gürsu, Tuba Elif Şenel Özler, Mustafa Velet*, Savaş Öztürk

University of Health Sciences Turkey, Haseki Training and Research Hospital, Clinic of Nephrology, İstanbul, Turkey

**University of Health Sciences Turkey, Haseki Training and Research Hospital, Clinic of Internal Medicine, İstanbul, Turkey*

Abstract

Aim: The aim of this study was to determine the relationship between left ventricular hypertrophy (LVH) and inflammatory markers in patients with type 2 Diabetes Mellitus (T2DM) with diabetic nephropathy at different stages.

Methods: Our study was a cross-sectional study involving patients with various stage of T2DM. Patients with LVH were identified by 2D echocardiography. Plasma human tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, vaspin, vispatin and midkine were measured.

Results: A total of 59 T2DM patients (56% women) with a mean age of 56.1±8.8 years were included in the study. The mean left ventricular mass index was 129±30. LVH was detected in 62.7% of the patients. Patients with an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2 had a higher incidence of LVH than patients with an eGFR ≥60 mL/min/1.73 m^2 (p=0.03). The TNF-α levels in patients with LVH with low eGFR was found to be statistically significantly higher than in patients without LVH (p=0.047). The level of vaspin was statistically significantly higher in patients with LVH (p=0.01).

Conclusion: LVH was found to be more frequent in patients with low eGFR, and from inflammatory markers, it was found to be associated only with TNF-α and vaspin.

Keywords: Left ventricular mass index, inflammatory markers, diabetic nephropathy

Öz

Amaç: Çalışmamızın amacı farklı evrelerdeki tip 2 Diabetes Mellitus (T2DM) hastalarında sol ventrikül hipertrofisi (SVH) ve enflamatuvar biyobelirteçlerin arasındaki ilişkiyi ortaya koymaktır.

Yöntemler: Kesitsel çalışmamıza çeşitli evrelerdeki T2DM hastaları dahil edildi. SVH 2D ekokardiyografi cihazı ile değerlendirildi. Tümor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, vaspin, vispatin ve midkine serumda ölçüldü.

Bulgular: Ortalama yaşı 56,1±8,8 yıl olan 59 T2DM hastası (%56'sı kadın) çalışmaya dahil edildi. Sol ventrikül kitle indeksi 129±30 idi. SVH'si hastaların %62,7'sinde saptandı. Tahmini glomerüler filtrasyon hızı (tGFH) <60 mL/dk/1,73 m^2 olan hastalarda tGFH'ı ≥60 mL/dk/1.73 m^2 olan hastalara göre SVH daha yüksek oranda saptandı (p=0.03). Düşük tGFH olan hastalarda SVH'ı olan hastalar SVH'ı olmayan hastalara göre TNF-α seviyesi istatistiksel olarak daha yüksek saptandı (p=0.047). Vaspin SVH'si olan hastalarda daha yüksekti (p=0.01).

Sonuç: SVH düşük tGFH'li hastalarda daha sıktı ve enflamatuvar biyobelirteçlerden sadece TNF-α ve vaspin ile ilişkili bulundu.

Anahtar Sözcükler: Sol ventrikül kitle indeksi, enflamatuvar belirteçler, diyabetik nefropati

Address for Correspondence/Yazışma Adresi: Egemen Cebeci, University of Health Sciences, Haseki Training and Research Hospital, Clinic of Nephrology, İstanbul, Turkey
E-mail: egemencebeci@hotmail.com ORCID: orcid.org/0000-0002-7393-5144

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Introduction

Diabetes Mellitus (DM) affects many people around the world, and has been one of the most important health problems with its microvascular and macrovascular complications and increasing prevalence. In addition, it is a chronic disease associated with atherosclerosis and increased cardiovascular events (1). Left ventricular hypertrophy (LVH) is physiological adaptation to chronic afterload pressure, which leads to pathological changes in the structure and function of the cardiovascular system. DM is related with LVH and lower myocardial function independent of age, sex and hypertension (2). The Framingham study showed that the presence of LVH was associated with increased mortality (3).

Diabetic nephropathy (DNP) is the most common reason for end stage renal disease (ESRD) in the developed countries. LVH is a risk factor for mortality in patients with ESRD. Inflammation is one of the earliest events in cardiac stress situations such as pressure and volume overload, and it involves elevated levels of inflammatory cytokines. Inflammatory markers also affect cardiovascular functions either by paracrine effects or by directly affecting the vascular wall (4). In this study, we studied the frequency of LVH and the relationship of inflammatory markers with LVH in diabetic patients with low estimated glomerular filtration rate (eGFR).

Meanwhile, the inflammatory factors, such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, vaspin, midkine and visfatin, are studied in this paper. We aimed to determine the relationship of LVH with DNP, drugs, inflammatory markers and laboratory markers in a population of patients with type 2 DM (T2DM).

Methods

Our study was a cross-sectional study involving patients with DM at different stages of follow-up in the nephrology clinic of our hospital. The American Diabetes Association *criteria for the diagnosis of diabetes* were used for the diagnosis of T2DM (5). Informed consent was obtained from all patients eligible for inclusion.

Patients younger than 18 years and older than 70 years, type 1 diabetic patients, patients with acute renal dysfunction or albuminuria, non-diabetic renal disease, advanced chronic liver disease, positive hepatitis serology, high transaminase level, autoimmune disease, malignant disease, advanced cardiac or respiratory disease, history of systemic infectious or inflammatory disease or acute ischemic vascular disease within the past three months, and those without written informed consent were excluded from the study.

Age, gender, height, weight, and waist and hip circumference were recorded for all patients. Body mass index (BMI) was calculated using the formula [BMI=weight (kg)/(height)^2 (m)]. Duration of DM, presence of kidney failure, and time from diagnosis of kidney failure were recorded. GFR values were estimated using the Chronic Kidney Disease Epidemiology Collaboration equation.

All the drugs the patients were using were recorded. Patients with LVH underwent 2-dimensional echocardiography (HDI 5000 Sono CT machine with a transducer 2.5 mHz). The echocardiographic technique, calculation of dimensions, and different cardiac volumes were realized according to recommendations of the American Society and European Association of Echocardiography. The echocardiographic evaluation included endocavitary dimensions of the left ventricle and other cardiac chambers. Left ventricular mass was calculated according to the Devereux formula: (6) $0.8\times1.04\times[(LVIDd + PWTd + VSTd)^3 - (LVIDd)^3]+0.6$

LVH was determined as left ventricular mass index (LVMI) greater than 115 g/m^2 for men and greater than 95 g/m^2 for women.

Venous blood samples were taken from all patients after 12 hours of fasting and placed in gel-free dry tubes and EDTA tubes. The samples were centrifuged at 1000 G for 10 minutes and serum and plasma samples were stored at -80 °C until analysis. After the sample collection was completed, serum and plasma were melted and biochemical studies were performed. Glucose, Hemoglobin A1c (HbA1c), urea, creatinine, uric acid, sodium (Na), potassium (K), calcium (Ca), phosphorus (P), total protein, albumin, parathyroid hormone, total cholesterol, high-sensitive C-reactive protein, IL-1, IL-6 and TNF-α, LDL cholesterol, VLDL cholesterol, triglycerides, aspartate transaminase, and alanine transaminase levels were measured in all patients. Among hematological parameters, hemoglobin (Hb), total leukocyte count, mean corpuscular volume, platelet count, transferrin saturation and ferritin level were measured. The analysis of the blood samples was made in our hospital's biochemistry laboratory. Biochemical assays were performed using an Architect c16000 (Abbott Diagnostics, Chicago, Ill., USA) instrument as recommended by the manufacturer. HbA1c levels were studied by high pressure liquid chromatography (HPLC) with a TOSOH G7 (Tosoh Bioscience, South San Francisco, Calif.) analyzer. HORIBO ABX pentra dx 120 (Horiba Medical, Montpellier, France) was used for the measurement of hematological parameters.

Measurement of human TNF-α, IL-1 and IL-6 levels was performed by an enzyme-linked immunosorbent *assay* (ELISA) using a BIOTEK ELX50 Microplate Strip Washer and BIOTEK EL 800 Absorbance Microplate Reader (BioTec Inc., Winooski, VT, USA). ELISA

kit from Adipo Bioscience (Santa Clara, CA) was used to measure serum vaspin, visfatin, midkine with the sandwich ELISA method.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors. Ethics committee approval was not obtained due to the cancellation of the regulations about ethics committees by the state council while our non-experimental study had been conducted. Informed consent was obtained from all individual participants included in the study.

Statistical Analysis

The SPSS 15.0 for Windows was used for statistical analysis. Categorical variables were given in numbers and percentages and numerical variables were given in mean ± standard deviation and minimum and maximum. The Mann-Whitney U test was used when independent numerical comparisons between two groups did not satisfy the normal distribution condition. The ratios of categorical variables were tested by a chi-square test. Determinant factors were analyzed by logistic regression analysis using the forward method. A p value of less than 0.05 was considered statistically significant.

Results

A total of 59 T2DM patients (33 women and 26 men) with a mean age of 56.1±8.8 years were included in the study. The mean DM duration was 12.3±8.3 years, left ventricular mass was 240.8±60.6 cm^3, and LVMI was 129±30. LVH was detected in 62.7% of the patients. 83.1% of the patients had retinopathy. The mean BMI was 30.3±4.9, average waist circumference, hip circumference and waist-to-hip ratio was 102.9±8.6 cm, 107.0±10.2 cm and 0.97±0.10, respectively. Characteristics of patients are given in Table 1. The mean systolic blood pressure (BP) and diastolic BP was 157±26 (118-240) mmHg and 81±11 (58-100) mmHg, respectively. A total of 27 (45.8%) patients were hypertensive. There was no significant difference in the number of LVH patients between hypertensive and normotensive groups [17 (63.0%) patients vs 20 (62.5%) patients, respectively, p=0.97].

Sixty-four point four percent of the patients were using insulin. Oral anti-diabetic drug (OAD) usage rate was 61%. Fifty-two point were using metformin, 20.3% - acarbose, 15.3% - sulphonylurea, 6.8% - other secretegogs (repaglinid, nateglinid), 3.4% - glitazone and 5.1% - other OAD. Antihypertensives used by the patients were as follows: ACEi (35.6%), ARB (32.2%), diltiazem (11.9%), and other antihypertensives (42.4%). The rate of statin use was 45.8% and the rate of aspirin use was 59.3%. Insulin usage rate was significantly higher in patients with LVH (p=0.019). There was no difference in ACE inhibitors usage between patients with diabetic hypertension with and without LVH (p=0.92).

According to CKD EPI value, 59.3% of patients had an eGFR of ≥60 mL/min/1.73 m^2 and 40.7% of them had and eGFR of <60 mL/min/1.73 m^2. The incidence of LVH was statistically significantly higher in patients with an eGFR of <60 mL/min/1.73 m^2 than in those with an eGFR of >60 mL/min/1.73 m^2 (p=0.03). The TNF-α levels in patients with LVH with low eGFR was found to be statistically significantly higher than in patients without LVH (p=0.047) (Table 2).

The average values of the evaluated markers were as follows: visfatin: 3.3±3.4, midkine: 365±404, vaspin: 1.5±0.9, IL-1: 35.5±51.3, IL-6: 8.7±9.2, TNF-α: 22.3±46.5.

Among the evaluated markers, vaspin was statistically significantly higher in patients with LVH (p=0.01). There was no statistically significant difference in other markers between patients with and without LVH (Table 3). The mean values of urea, creatinine, phosphorus and leukocyte were significantly higher in patients with LVH and Hb,

Table 1. Baseline characteristics of the patients

Age, mean ± SD (min-max)	56.1±8.8 (29-70)
Gender, n (%)	
Women	33 (55.9)
Men	26 (44.1)
Postmenopausal, n (%)	26 (78.8)
DM duration (year), mean ± SD (min-max)	12.2±8.5 (0-30)
LV mass (cm^3), mean ± SD (min-max)	240.8±60.6 (116-402)
Left ventricular mass index, mean ± SD (min-max)	129.3±30.3 (65.2-213.3)
Left ventricular hypertrophy, n (%)	
No	22 (37.3)
Yes	37 (62.7)
Diabetic retinopathy, n (%)	49 (83.1)
Weight, mean ± SD (min-max)	80.6±12.8 (55-118)
Height, mean ± SD (min-max)	163.4±8.0 (150-180)
Body mass index, mean ± SD (min-max)	30.3±4.9 (22.0-42.6)
Waist circumference, mean ± SD (min-max)	102.9±8.6 (85-126)
Hip circumference, mean ± SD (min-max)	1070±10.2 (56-127)
Waist hip ratio, mean ± SD (min-max)	0.97±0.10 (0.85-1.64)

DM: Diabetes Mellitus, LV: Left ventricule, SD: Standard deviation, min: Minimum, max: Maximum, n: Number

hematocrit, and mean CKD EPI values were statistically significantly lower than in those without LVH (Table 4).

Discussion

Increased left ventricular mass has been associated with cardiovascular morbidity and mortality (7). Diabetic patients have an additional cardiovascular risk. The association between LVH and cardiac morbidity is well established, especially in the presence of myocardial ischemia, fibrosis and scar tissue, and atrial fibrillation. Inflammation, fibrosis and oxidative stress, as well as ischemia play a significant role and are the leading pathways. And so, we studied some inflammatory markers associated with LVH (8). Vaspin is a visceral adipose tissue-derived serine protease inhibitor. It was first studied in visceral white adipose tissues of Otsuka Long-Evans Tokushima fatty (OLETF) rats with abdominal obesity, T2DM, insulin resistance, hypertension, and dyslipidemia. (9). There is a possibility that vaspin has an effect in insulin resistance. Our study showed that there was a potential correlation between vaspin and chronic inflammation in diabetic patients. With these hypotheses, vaspin may have a role in LVH in diabetic patients. We showed that vaspin was statistically significantly higher in diabetic patients with LVH.

Table 2. Markers in the patients with low eGFR

	Left ventricular hypertrophy		
eGFR (<60 mL/min/1.73 m²)	**No**	**Yes**	**p**
Visfatin	3.2±1.3	2.7±1.1	0.499
Midkine	303±160	467±447	0.644
Vaspin	1.0±0.5	1.7±0.9	0.081
IL-1	41.1±26.2	38.8±72.4	0.110
IL-6	8.6±4.8	11.0±12.7	0.803
TNF alfa	8.9±9.7	15.6±9.3	0.047

eGFR: Estimated glomerular filtration rate, TNF: Tumor necrosis factor, IL: Interleukin

Tablo 3. Markers in patients with and without left ventricular hypertrophy

	Left ventricular hypertrophy		
	No	**Yes**	**p**
Visfatin	2.7±1.2	3.6±4.1	0.419
Midkine	278±202	416±481	0.605
Vaspin	1.2±1.0	1.6±0.8	0.01
IL-1	34.6±45.9	36.1±54.9	0.894
IL-6	6.7±5.2	9.7±10.7	0.268
TNF alfa	29.4±74.8	18.2±13.0	0.188

TNF: Tumor necrosis factor, IL: Interleukin

Tablo 4. Laboratory values in patients with and without left ventricular hypertrophy

	Left ventricular hypertrophy		
	No	**Yes**	**p**
Glucose	213.4±83.7	205.7±68.4	0.531
Urea	41.5±25.2	64.2±41.1	0.014
Creatinine	1.0±0.6	1.5±1.1	0.022
Uric acid	5.2±1.5	5.8±1.6	0.246
Sodium	138.3±2.2	139.2±3.2	0.083
Potasium	4.6±0.4	4.8±0.4	0.080
Calcium	9.8±0.5	9.7±0.6	0.295
Phosphorus	3.5±0.5	4.2±0.9	0.002
Total protein	7.5±0.4	7.4±0.5	0.642
Albumin	4.2±0.5	4.1±0.4	0.070
Parathyroid hormone	73.1±38.2	77.3±67.1	0.666
Total cholesterol	217.5±75.0	236.7±68.4	0.132
High density lipoprotein	44.0±9.7	44.2±9.3	0.718
Low density lipoprotein	131.8±52.4	150.4±56.0	0.079
Very low density lipoprotein	50.0±58.4	43.5±21.1	0.605
Trigliseride	252.2±291.6	217.6±105.4	0.684
Aspartate aminotransferase	19.3±6.2	21.3±8.9	0.551
Alanine aminotransferase	24.6±11.3	22.0±11.5	0.286
HbA1C	9.0±2.4	8.7±1.5	0.969
Hemoglobin	13.4±1.5	12.1±2.0	0.005
Hematocrit	41.2±4.3	36.7±5.6	0.004
Leukocyte	7.3±1.4	8.3±1.9	0.046
Mean corpusbuler volume	85.8±4.7	87.0±3.8	0.252
Platelets	279.6±64.0	269.7±68.0	0.466
Ferrous	63.6±21.2	66.7±28.5	0.919
Total Iron Binding Capacity	350.3±65.5	313.8±63.0	0.042
Transferrin saturate	0.19±0.07	0.22±0.09	0.347
C-reactive protein	0.6±0.5	0.7±0.7	0.753
Vitamin D	21.7±10.9	36.0±34.1	0.095
Insulin	12.4±9.8	13.5±9.0	0.512
C peptid	2.8±1.5	3.5±2.3	0.426
Ferritin	65.5±45.7	111.5±128.9	0.513
Proteinuria	1415.3±1773.0	2848.3±2552.6	0.057
Microalbuminuria	66.1±93.7	287.0±436.1	0.081
eGlomeruler filtration rate	85.8±30.6	60.0±31.3	0.004

HbA1C: Hemoglobin A1c

Midkine has protective effects against ischaemia, reperfusion injury, cardioprotection, angiogenesis, vascular stenosis, and cardiac remodeling. Midkine protects the heart and brain from acute ischemia, reperfusion injury and infarction via its anti-apoptotic effect (10). Based on this information, we looked at the midkine values in diabetic LVH patients. No statistically significant difference was found between diabetic patients with and without LVH.

Visfatin, an adipocytokine, is produced by visceral adipose tissue and has insulin-mimetic action. Visfatin acts as an insulin analog on the insulin receptor (11). Adipocytokines participate in different stages of atherosclerosis, from endothelial dysfunction to plaque destabilization. Visfatin is a proinflammatory cytokine and is secreted in response to inflammation and upregulates cytokines such as IL-1, TNF-α, and IL-6, and probably has a potential role in the pathogenesis of inflammatory disorders (12). In their study, Dahl et al. (13) claimed that visfatin was an inflammatory mediator, synthesized by foam cell macrophages within unstable atherosclerotic lesions and played a role in plaque destabilization. And so according to this knowledge, we studied visfatin, IL-1, TNF-α, and IL-6 levels in diabetic patients who have LVH. There was no statistically significant differance between diabetic patients with and without LVH.

Among the markers, only vaspin was statistically significantly higher in patients with LVH.

There was no correlation between LVH and waist circumference, BMI, duration of DM and duration of chronic kidney disease, but there was a significant relationship between GFR and LVH. Patients with LVH were more likely to have low GFR. It is known that LVH correlates with DNP (14).

The rate of insulin use was significantly higher in patients with LVH (p=0.019). Hyperglycemia is associated with LVH. In their study including 16 insulin-dependent patients, Weinrauch et al. (15), noted that left ventricular mass improved with glycemic control over time and they have also described improvement in autonomic function by metabolic control. In this study, they showed that left ventricular remodeling and parasympathetic improvement could both be attained by aggressive treatment of hyperglycemia.

Patients with an eGFR of <60 mL/min/1.73 m^2 had a statistically significantly higher incidence of LVH than patients with an eGFR of ≥60 mL/min/1.73 m^2 (p=0.03). Also, the laboratory parameters of urea, creatinine and phosphorus were significantly higher (p=0.014, p=0.022 and p=0.002, respectively); Hb, Htc, and GFR mean were statistically significantly low (p=0.005, p=0.004 and p=0.004, respectively) in patients with LVH. It means that there is a significant relationship between DNP and LVH. In diabetic patients with renal insufficiency, coronary artery disease, overload, uremia, and hypertension are the most common explanations for cardiac dysfunction.

Additionally, hyperglycemia, increases catecholamine levels and downregulates cardiac adrenoreceptors, leading to ventricular diastolic dysfunction and ventricular hypertrophy (16). Mechanisms for cardiac dysfunction in uncontrolled DM have been studied in animal models. The ventricular dimension grows with collagen accumulation due to advanced glycated end products, a pathological process that can be prevented by blood sugar regulation. In addition, ACE inhibitors prevent accumulation of advanced glycated end products in the ventricular tissue (17). However, in our study, there was no difference in patients with diabetic hypertension using ACE inhibitors with or without LVH (p=0.92).

In a study, Weinrauch et al. (15) declared that diabetic patients with nephropathy and severe cardiac autonomic dysfunction may have parallel improvement in left ventricular mass by restoring glycemic control through intensive therapy.

When we looked at the relationship between LVH and inflammatory markers in patients with low GFR, the TNF-α levels in patients with LVH with low GFR was found to be statistically significantly higher than in patients without LVH (p=0.047). TNF-α contributes to the development and progression of DNP and is correlated with increased levels of albuminuria and nephropathy (18). It is well established that TNF-α plays an important role in cardiac contractile dysfunction and cardiac hypertrophy (19). Takei et al. (20) have shown that increased TNF-α receptors might be responsible for increased left ventricular mass.

Conclusion

LVH was found to be more frequent in patients with low eGFR and TNF-α levels in patients with LVH with low GFR was found to be statistically significantly higher than in patients without LVH and, from inflammatory markers, LVH was found to be associated only with vaspin. It was also found that the rate of insulin use was significantly higher in patients with LVH; there was no difference in patients with diabetic hypertension using ACE inhibitors with or without LVH.

Authorship Contributions

Concept: E.C., M.V., S.Ö. Design: E.C., M.V., S.Ö. Data Collection or Processing: N.Ş.S. Analysis or Interpretation: E.C., M.G. Literature Search: S.U., S.K., T.E.Ş.Ö. Writing: E.C., S.Ö.

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Programmed Death-ligand 1 Expression Analysis for Non-small Cell Lung Cancer in Tissues Sampled Using Different Methods

Farklı Yöntemlerle Örneklenen Akciğer Küçük Hücreli Dışı Karsinom Dokularında Programlı Ölüm Ligandı 1 Ekspresyon Analizi

Halide Nur Ürer, Mehmet Zeki Günlüoğlu*, Levent Cansever**, Mehmet Ali Bedirhan**

University of Health Sciences Turkey, Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, Clinic of Medical Pathology, İstanbul, Turkey

**Medipol Mega University Hospital, Clinic of Thoracic Surgery, İstanbul, Turkey*

***University of Health Sciences Turkey, Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, Clinic of Thoracic Surgery, İstanbul, Turkey*

Abstract

Aim: This study aims at investigating the concordance of programmed death-ligand 1 (PD-L1) expression in non-small cell cancer tissues that have been sampled using different methods and its relationship with the pathologic parameters of tumors.

Methods: PD-L1 expression assays were made on the cell blocks taken using fine needle aspiration, the small tissue samples representing endoscopic biopsy and the large tissue samples representing the resected tumor taken from the tumors of 100 subjects diagnosed with non-small cell lung cancer; their percentage values were evaluated and their groups were determined based on these values.

Results: The average difference in expression rates found through different sampling methods was close to 0, and the values of such differences changed mostly in a narrow range. In paired comparisons, a good level of concordance was observed between all the sampling methods. The values were 0.8865 (SE: 0.0306, CI: 0.8264-0.9466) in the comparison of tumor tissue (TT) and small biopsy (SB): 0.8637, (SE: 0.033, CI: 0.7989-0.9285) TT to cell block (CB): 0.8916, (SE: 0.0272, CI: 0.8383-0.9449) SB tissue to the CB.

Conclusion: Therefore, it can be concluded that the PD-L1 expression does not differ between the tissues sampled through various methods in non-small cell lung cancers.

Keywords: Non-small cell carcinoma, PD-L-1, cell block, different expression

Öz

Amaç: Çalışmada amacımız farklı yöntemlerle örneklenmiş küçük hücreli dışı karsinomda programmed death-ligand 1 (PD-L1) ekspresyon uyumunu ve tümör patolojik parametrelerle ilişkisini araştırmaktır.

Yöntemler: Küçük hücreli dışı akciğer karsinom tanısı alan 100 olgunun tümöründen ince iğne aspirasyonu hücre bloğu, endoskopik biyopsiyi temsil eden küçük doku ve rezeksiyon tümör temsil eden büyük doku örneğinde PD-L1 ekspresyon tayini yapılıp yüzde değerleri ve buna göre grupları değerlendirildi.

Bulgular: Örnekleme yöntemleri tarafından saptanmış ekspresyon oranlarının ortalama farkının 0'a yakın olduğu gibi, fark değerlerinin, büyük oranda, dar uyum içinde olduğu bulundu. İkişerli karşılaştırmalarda, tüm örnekleme yöntemleri arasında, PD-L1 ekspresyon oranı açısından iyi düzeyde uyum olduğu izlendi. Tümör dokusu-biyopsi dokusu karşılaştırmasında 0,8865 (SE: 0,0306, CI: 0,8264-0,9466), tümör dokusu-hücre bloğunda 0,8637 (SE: 0,033, CI: 0,7989-0,9285); biyopsi dokusu-hücre bloğunda 0,8916 (SE: 0,0272, CI: 0,8383-0,9449) idi.

Sonuç: Sonuçta akciğerin küçük hücreli dışı karsinomlarında PD-L1 ekspresyonu farklı yöntemlerle örneklenmiş dokular arasında fark göstermemektedir.

Anahtar Sözcükler: Küçük hücreli dışı karsinom, PD-L1, hücre bloğu, farklı yöntemler

Address for Correspondence/Yazışma Adresi: Halide Nur Ürer, University of Health Sciences Turkey, Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, Clinic of Medical Pathology, İstanbul, Turkey
Phone: +90 505 265 93 65 E-mail: nururer@gmail.com ORCID: orcid.org/0000-0002-0549-9969

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Introduction

The treatments of non-small cell lung cancer have shown fast improvements in recent years, the most outstanding being cancer immunotherapy (1,2). In carcinogenesis, suppression of the host immune system by tumor cells facilitates tumor proliferation. Blocking the programmed death-ligand 1 (PD-L1) receptors on the cell surface that create immune tolerance is an activating cancer immunotherapy method (3). Removal of the blockage triggers T lymphocyte immunization against cancer cells.

The decision for immunotherapy is made upon evidencing the presence of a PD-L1 expression in the tissue. The samples obtained using various methods can be used to identify the ligand. Moreover, the distribution of PD-L1 expression in tissues is variable in non-small cell cancers (4). This raises the question whether the expression values of different intervention methods used in the diagnosis of cancer have the same proliferation value.

This study aims at investigating the concordance of PD-L1 expressions in non-small cell cancer tissues sampled using different methods and its relationship with the pathologic parameters of tumors.

Methods

This study was conducted in the Department of Pathology in Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital. One hundred subjects diagnosed with non-small cell lung cancer based on the preoperative or intraoperative frozen sections were enrolled in the study.

The tissues whose routine pathological macroscopic assessments and samplings in their pulmonary tumor resection material had been completed were included in this study. Tumors larger than 2.9 cm and diagnosed with non-small cell lung cancer were the inclusion criteria. Patients with a tumor ≤2.9 cm, those with undetectable tumor tissue (TT) after neoadjuvant therapy and those diagnosed with a disease other than non-small cell cancer were excluded.

First, cell block (CB) were sampled using fine needle aspiration from the residual tumor that remained on the resection and was not yet involved in buffered formalin fixation. Secondly, 2-5 pieces of tissue 2-3 mm in diameter, representing endoscopic small biopsy (SB) were sampled from the residual tissue. Finally, after the completion of the macroscopic TT sampling, a piece of TT at least 1-2 cm^2 in diameter and 2-3 mm in thickness was taken.

The demographic characteristics of the subjects, anatomic resection types, tumor diameters, histopathological diagnoses, tumor diameters, lymphovascular involvements, peritumoral lymphocytic inflammation and lymph node metastasis statuses (no metastasis=N0, inter-and intra-lobular lymph node metastasis=N1, mediastinal unilateral lymph node metastasis=N2) were explored.

Study designing has been approved by Local Ethical Committe of University of Health Sciences, İstanbul Training and Research Hospital Ethics Committee. The study protocol number and date are 1058/04.08.2017.

Immunohistochemical Analysis

A commercial product approved for lung cancer was chosen for the immunohistochemical analysis of PD-L1 (5). After deparaffinization, the slides were subjected to immunohistochemical analysis using the PD-L1 antibody (SP263 Roche/Ventana) and an Optiview DAB IHC detection kit (Roche/Ventana) was used as a universal kit.

While assessing the PD-L1 expression, complete or incomplete membranous staining was accepted as a positive value. The intensity of staining was not considered in this assessment. The tumor cells in the entire area of each sample were counted. Further, the tumor cells exhibiting PD-L1 expression were counted; and PD-L1 percentage value was found based on the percentage value, they were grouped as none (0), less than 1, equal to or greater than 1, less than 5, equal to or greater than 5, less than 10, equal to or greater than 10, less than 50 and equal to or greater than 50.

Statistical Analysis

The analyses were conducted using the R software and interactive software. The one-sample t-test was used to compare the mean values. To demonstrate the concordance, Lin's concordance correlation coefficient and Cronbach's alpha coefficient were used for measured variables and weighted kappa and Fleiss' kappa values for ordinal variables. To show correlations, Pearson's correlation coefficient was used for measurable variables and Spearman's correlation coefficient for ordinal variables. Additionally, any probability level less than 0.05 was considered significant.

Results

A total of 100 patients had a preoperative or intraoperative diagnosis of non-small cell cancer. The demographic characteristics and surgical data of the subjects and histopathological findings are shown in Table 1.

The surgical procedures included 70 lobectomies, four bilobectomies, 26 pneumonectomy, four chest wall resections and one carinal sleeve resection.

The PD-L1 expression was found to be less than 1 in 35 subjects (35%) and 1 or more in 65 (65%) (Figure 1). The PD-L1 expression value groups and histopathological tumor distribution are shown in Table 2.

The general distribution of PD-L1 expression (Figure 2), and its distribution in squamous cell carcinoma (Figure 3) and in adenocarcinoma (Figure 4), were shown. Moreover, its highest value among TT, SB and CB were considered.

The least PD-L1 expression was found in the other carcinomas group. No expression was found in mucinous adenocarcinoma, large-cell neuroendocrine carcinoma and typical and atypical carcinoid tumors. The expression was found to be 5-10% in adenosquamous cell carcinoma and over 50% in one of the pleomorphic carcinomas. It was limited only to the carcinoma component in that tumor. Its value was 0 in the other pleomorphic carcinoma.

The concordance of the PD-L1 expression percentage values was explored in the samples of TT, SB and CB.

Assessment of the expression values showed that 39% of the tumors expressed PD-L1 at a low level and 46% of them at a high level in the TT; a moderate level of expression was hardly encountered (Table 3).

Table 1. The demographic and histopathological findings

Total cases	**n=100**
Female/Male	15/85
Age	60.2 (39-78)
Histopathological diagnosis	
Squamous cell carcinoma	58
Keratinizing squamous cell carcinoma	40
Nonkeratinizing squamous cell carcinoma	17
Basaloid squamous cell carcinoma	1
Adenocarcinoma	31
Mucinous adenocarcinoma	3
Adenosquamous cell carcinoma	1
Carcinoid tumor	4
Typical carcinoid tumor	3
Atypical carcinoid tumor	1
Large-cell neuroendocrine carcinoma	1
Pleomorphic carcinoma	2
Tumor diameter (cm)	2.9-11
Lymphovascular invasion	
Positive/Negative	71/29
Peritumoral lymphocytic inflammations	
No/minimal/medium/intense	43/30/20/7
Lymph nodal metastasis	
N0/N1/N2	56/33/11

N0=No metastasis, N1=Inter-and intra-lobular lymph node metastasis, N2=Mediastinal unilateral lymph node metastasis, n: Number

Discussion

The concordances of the PD-L1 expression values obtained with the three sampling methods were compared. A graphical evaluation of the concordance (Figure 5), showed that the expression values found by the sampling methods were very close to each other in each paired comparison as seen in the scatter plots, and for this reason, the best fit line determined for the distribution

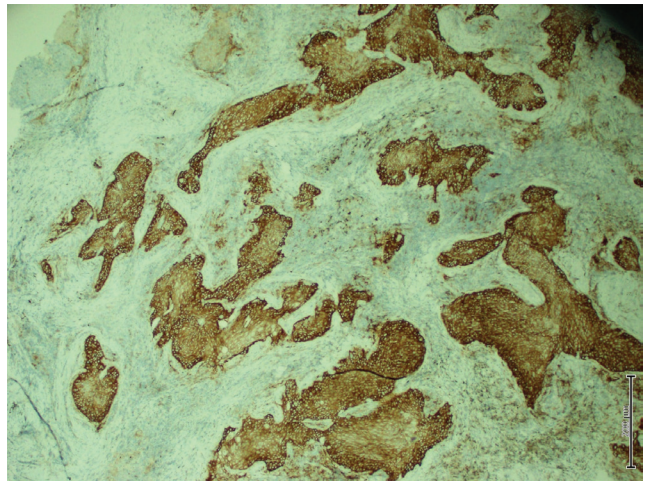

Figure 1. Programmed death-ligand 1 expression in small biopsy

Table 2. Distribution of programmed death-ligand 1 expression

Histologic type	**0 (%)**	**<1 (%)**	**≥1<5 (%)**	**≥5<10 (%)**	**≥10<50 (%)**	**>50 (%)**	**Total (%)**	**Discordant case number**
Squamous cell carcinoma	8	6	1	2	10	15	58	16
Adenocarcinoma	7	5	1	0	6	6	31	6
Mucinous adenocarcinoma	3	-	-	-	-	-	3	-
Adenosquamous cell carsinoma	-	-	-	-	-	-	1	1
Typical carcinoid tumor	3	-	-	-	-	-	3	-
Atypical carcinoid tumor	1	-	-	-	-	-	1	-
Large-cell neuroendocrine carcinoma	1	-	-	-	-	-	1	-
Pleomorphic carcinoma	1	-	-	-	-	1	2	-
Total	24	11	2	2	16	22	77	23

was close to 45 degrees. The Bland-Altman plots revealed that in each paired comparison, the mean difference of the expression values found in the sampling methods was close to 0 in each paired comparison and the difference values were within the narrow concordance limits (limits of agreement-LOA); there was no trend showing that the difference changed as the values changed; the difference remained consistently low even when the values changed.

The levels of concordance between the sampling methods were calculated with reference to the PD-L1 expression values that were found. A good degree of concordance in terms of PD-L1 expression values was observed between all sampling methods in paired comparisons [Lin's correlation coefficient: pc was 0.9371 (CI: 0.9081-0.9572)] for the concordance between TT-SB, 0.9522 (CI: 0.9301-0.9675) for the concordance between TT-CB and 0.9689 [(CI: 0.9542-0.9789) for the concordance SB-CB)]. The Cronbach's alpha correlation coefficient for the concordance between all the three methods was calculated to be 0.9837 (CI: 0.977-0.989), which indicates a good level of concordance.

The concordance was also analyzed based on the results obtained through classification of the expression values. All methods revealed the same expression values in 77 samples. In the other 23 samples, the expression values were different from the others in at least one sampling method.

The concordance of the sampling methods with each other was analyzed with respect to their expression values in pairs. A very good concordance between the sampling methods was observed in this analysis [(Weighted Kappa level was 0.8865 (SE: 0.0306, CI: 0.8264-0.9466)] for the TT-SB comparison, 0.8637 (SE: 0.033, CI: 0.7989-0.9285) for the TT-CB comparison and 0.8916 (SE: 0.0272, CI: 0.8383-0.9449) for the SB-CB comparison). Even when the three sampling methods were assessed together, there was a good concordance among them (Fleiss' Kappa: 0.7823 (SE: 0.0326, CI: 0.7184-0.8463).

The factors that may affect the concordance of the sampling methods were analyzed. For this, the correlation of the differences in the expression values found in each pair of sampling methods with the variables was explored. Further, the same exploration was repeated by making the expression groups ordinal and finding the difference between the rates obtained from the sampling methods (Table 4).

Through this analysis, it was observed that although significant, a weak difference occurred only between the expression rates of TT and CB and a stronger difference between the expression rates found in the two methods

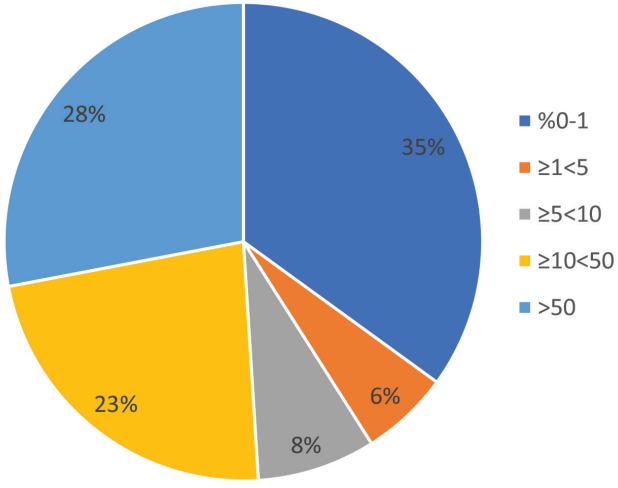

Figure 2. General programmed death-ligand 1 expression

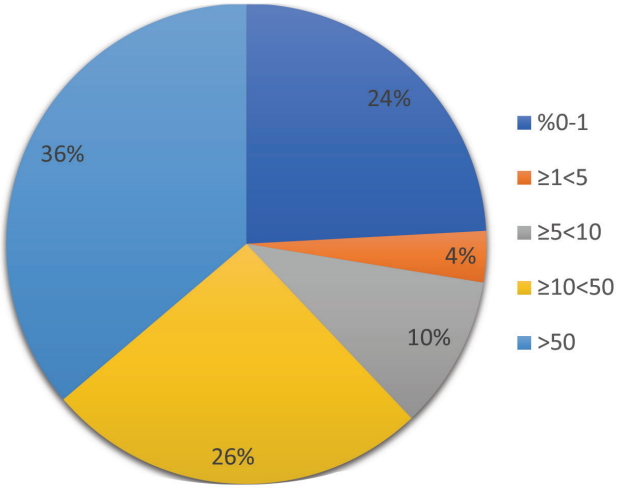

Figure 3. Programmed death-ligand 1 expression in squamous cell carcinoma

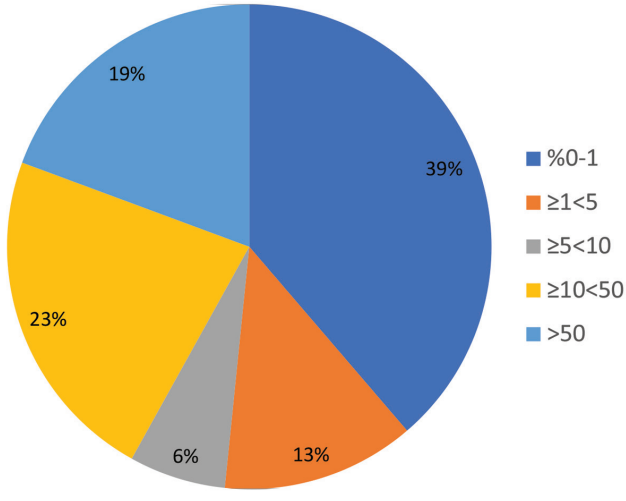

Figure 4. Programmed death-ligand 1 expression in adenocarcinoma

in squamous cell carcinomas than in adenocarcinomas (r=0.2, p=0.04); this difference found between the expression rates in the two methods increased as the tumor diameter increased (r=0.2, p=0.045). Similarly, the difference between the expression rates in the two methods decreased as the N stage advanced (r=0.22, p=0.03).

Conclusion

The PD-L1 expression is critically important in lung cancer checkpoint inhibitory therapy. The identification of PD-L1 in tumor cells is standardized using immunohistochemical methods (6). The coding of the selected tissue at a preanalytical stage, fixation, processing, section thickness, determination of priorities in molecular and immunohistochemical analyses, selection of PD-L1 clone at the analytic stage, and the correct methods of conducting assessments and quality control have been specified in practice (7,8). The use of different PD-L1 clones has also been shown in non-small cell lung cancer (9,10). In fact, the administration of immunotherapy according to the threshold values of the expression has created a new vision in the treatment of non-small cell lung cancer.

The aim in determining the treatment option is the presence and rating of an in vitro PD-L1 expression in the TT. In this study, the presence of expression was explored in tissues that were sampled using different methods from the same tumor. Instead of taking samples from the tumor resection tissue through the microarray method, three different tissues that were taken simultaneously but separately from the same tumor were examined. Although studies comparing the expression in tumor anatomic resection tissue in the samples taken by way of endoscopic biopsy, transthoracic fine needle aspiration and mediastinoscopic lymph node biopsy are available, in this study, the concordance of expression was found to be very high in the tissue samples (11). As a result, it was found that CB representing the tumor and limited tissue samples give correct and reliable results in the assessment of PD-L1 expression. Moreover, there are also studies comparing the expression value in tumor cells in biopsy and resection samples (12,13). In fact, the presence of immune reaction varying across regions in tumors is a known fact (14). A study conducted by Munari et al. (15) has shown that the minimum amount of tissue should be 3-4 pieces of core biopsies for a maximum yield. The concordance was found to be 92.4% at an expression score of 1% and over, but there was a marked difference in stratified score groups (16). Further, SB samples were argued not to be sufficient for assessment due to strikingly non-concordant results. The reason for these different results obtained from the methods may be the combination of the intensity of membranous staining and the proportion of tumor cells showing positivity. On the other hand, the presence of a weak concordance can be associated with the chosen PD-L1 clone (11). Similarly, the presence of expression in the same cell can vary in the pre-and post-chemotherapeutic stages (17). In fact, mucinous adenocarcinoma and signet-ring cell carcinoma also involve difficulties in the assessment of expression.

Table 3. Frequency of programmed death-ligand 1 expression values by sample types and their distribution

Sample type	Expression values						
	<1 (%)	≥1<5 (%)	≥5<10 (%)	≥10<50 (%)	>50 (%)	Median	IQR
Tumor tissue	39	7	8	21	25	3	1-4.75
Small biopsy	39	10	6	19	26	3	1-5
Cell block	36	5	12	22	25	3	1-4.75

IQR: Interquartile range

Table 4. The difference between the expression values and histpathologic parameters

Histologic type			Grade		Diameter		N status	
PD-L1 expression values	CC	p	CC	p	CC	p	CC	p
TT-SB	-0.03	**0.74**	0.03	**0.79**	0.16	**0.10**	-0.09	**0.35**
TT-CB	-0.2	**0.04**	-0.06	**0.62**	0.2	**0.045**	-0.22	**0.03**
SB-CB	-0.11	**0.29**	-011	**0.29**	0.014	**0.89**	-0.14	**0.17**
PD-L1 expression group								
TT-SB	-0.019	**0.85**	-0.02	**0.82**	0.11	**0.26**	-0.009	**0.93**
TT-CB	-0.09	**0.38**	-0.02	**0.87**	0.16	**0.11**	-0.09	**0.37**
SB-CB	-0.10	**0.31**	0.006	**0.95**	0.07	**0.48**	-0.10	**0.31**

PD-L1: Programmed death-ligand 1 TT: Tumor tissue SB: Small biopsy CB: Cell block
*CC: Correlation coefficient, N: Nodal status

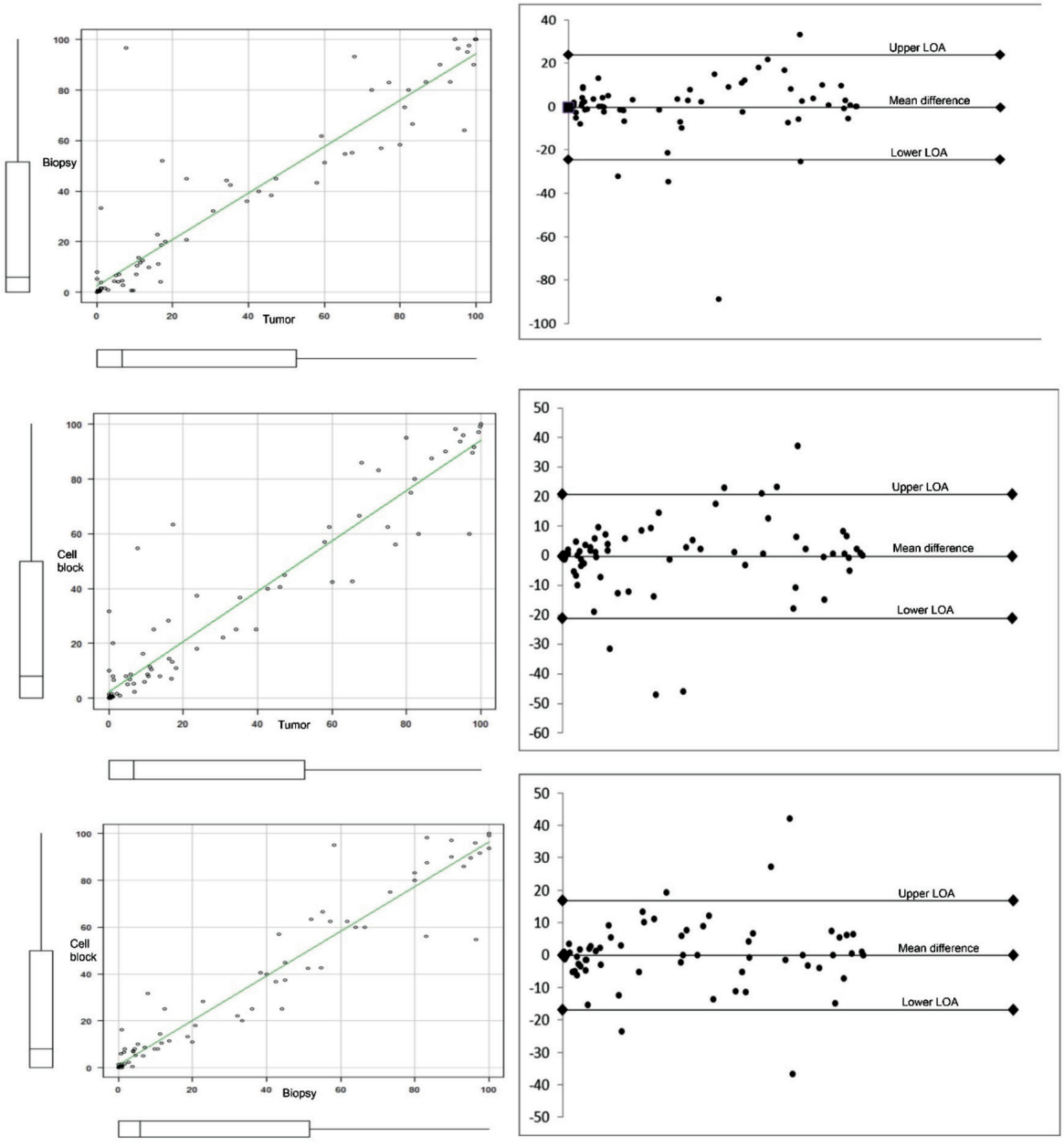

Figure 5. Concordance between samples
LOA: Limits of agreement

PD-L1 expression has been explored in poorly-differentiated lung cancers. The expression rate was found to be over 90% in pleomorphic carcinoma (18). The reaction is present more evidently in the sarcomatoid component of the tumor than in its carcinoma component. Along with adenocarcinoma, squamous cell carcinoma and neuroendocrine tumors, this study also considered pleomorphic carcinoma and adenosquamous cell carcinoma. The expression appeared at a rate of 2/3 in these tumors. Although the result of this study would require further support by further studies, it helps in creating an immunotherapy option in poorly-differentiated non-small cell lung cancer with a high probability.

The diameter, as one of the tumor parameters, was included in this study to create a difference, although insignificantly, in the expression between TT and CB. Accordingly, cytological samples may represent the PD-L1 expression lesser than expected because of the diameter

in T3 and T4 tumors. In a study conducted by Sakata et al. (19), the EBUS needle aspiration was compared to tumor resection. This is supported by the fact that cytological samples are less sensitive than large tissues. However, this difference can be eliminated by combining needle aspiration with tru-cut biopsy.

The PD-L1 expression does not show any difference among tissues sampled using different methods in non-small cell lung cancer. It can also be concluded that small biopsies and cytological samples with completed CB are able to represent the tumor itself. However, it should be noted that the tumor parameters may have an effect on expression.

Authorship Contributions

Concept: H.N.Ü. Design: H.N.Ü. Data Collection or Processing: H.N.Ü, L.C., M.A.B. M.Z.G. Analysis or Interpretation: H.N.Ü. Literature Search: H.N.Ü. Writing: H.N.Ü.

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

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Is Complement Factor H Tyr402His Variant a Potential Cause of Ankylosing Spondylitis?

Kompleman Faktör H Tyr402His Varyant Ankilozan Spondilitin Olası Bir Nedeni Olabilir mi?

Sacide Pehlivan, Mazlum Serdar Akaltun*, Mustafa Pehlivan**, Savaş Gürsoy*, Ayşe Feyda Nursal***

İstanbul University Faculty of Medicine, Department of Medical Biology, İstanbul, Turkey

**Gaziantep University Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Gaziantep, Turkey*

***Gaziantep University Faculty of Medicine, Department of Haematology, Gaziantep, Turkey*

****Hitit University Faculty of Medicine, Department of Medical Genetics, Çorum, Turkey*

Abstract

Aim: Ankylosing spondylitis (AS) is an autoimmune disease caused by chronic inflammatory response. Complement system is the major component of the innate immune defence. In this study, we investigated the potential association between complement factor H *(CFH)* gene Tyr402His variant (rs1061170) with AS in a Turkish population.

Methods: Seventy-eight AS patients and 80 healthy individuals were enrolled in the present study as case and control subjects, respectively. The Tyr402His variant of *CFH* gene was analysed by PCR-RFLP method.

Results: There was no statistically significant difference between AS patients and healthy controls in terms of *CFH* Tyr402His genotype and allele frequencies. However, the visual analogue scale (VAS) daytime and the AS Quality of Life (ASQoL) were significantly different according to *CFH* Tyr402His genotype distribution (p=0.032 and p=0.036, respectively). VAS of daytime and ASQoL were higher in subjects carrying Tyr402His variant Tyr/Tyr + Tyr/His genotypes compared to those carrying His/His genotype.

Conclusion: This is the first study evaluating the association between *CFH* Tyr402His and susceptibility to AS in a Turkish population. Although *CFH* Tyr402His variant was not considered a candidate gene for AS susceptibility in our samples, some clinical findings seem to be associated with genotype distribution of *CFH* Tyr402His variant.

Keywords: Ankylosing spondylitis, complement factor H, variant

Öz

Amaç: Ankilozan spondilit (AS) kronik enflamatuar cevabın neden olduğu otoimmün bir hastalıktır. Kompleman sistemi doğal bağışıklığın esas savunma sistemidir. Bu çalışmada, Türk toplumunda kompleman faktör H *(CFH)* geni Tyr402His varyantı (rs1061170) ve AS arasındaki olası ilişkiyi araştırdık.

Yöntemler: Bu çalışmaya 78 AS hastası ve 80 sağlıklı birey, olgu ve kontrol bireyleri olarak alındı. *CFH* Tyr402His varyantı PPCR-RFLP yöntemi ile analiz edildi.

Bulgular: AS hastaları ve sağlıklı kontroller arasında *CFH* Tyr402His genotip ve alel sıklıkları açısından istatistiksel olarak önemli fark yoktu. Ancak, gündüz vizüel analog skala (VAS) ve AS Yaşam Kalitesi Ölçeği (ASQoL) *CFH* Tyr402His genotip dağılımına göre önemli derecede farklıydı (p=0.032, p=0.036, sırasıyla). Gündüz VAS ve ASQol Tyr402His varyantı Tyr/Tyr + Tyr/His genotiplerini taşıyan kişilerde His/His genotipi taşıyanlara göre daha yüksekti.

Sonuç: Bu, Türk toplumunda *CFH* Tyr402His varyantı ve AS yatkınlığı arasındaki ilişkiyi değerlendiren ilk çalışmadır. *CFH* Tyr402His varyantı bizim örneklerde AS yatkınlığı için aday bir gen olmasa da, bazı klinik bulgular *CFH* Tyr402His varyantın genotip dağılımı ile ilişkili görünmektedir.

Anahtar Sözcükler: Ankilozan spondilit, kompleman faktör H, varyant

Address for Correspondence/Yazışma Adresi: Ayşe Feyda Nursal, Hitit University Faculty of Medicine, Department of Medical Genetics, Çorum, Turkey
E-mail: feyda.nursal@gmail.com ORCID: orcid.org/0000-0001-7639-1122
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Introduction

Ankylosing spondylitis (AS) is a chronic, progressive autoimmune illness that involves the axial and sacroiliac joints. The majority of patients with AS eventually manifest spine malformations, resulting in functional impairment (1). The prevalence of AS ranges between 0.1% and 1.4% worldwide, and it is seen more frequently in Eurasia (2). AS occurs more frequently in males, with a male/female ratio of 2:1 (3). Similar to other autoimmune diseases, the pathogenesis of AS remains unclear. Genetic and environmental factors may play a role in the etiology. Previous studies reported that major histocompatibility alleles, particularly HLA-B27, may account for upto one-third of the genetic effect. (4), hence suggesting that there could be other susceptible genes that play significant roles in the onset of this disease. Dysregulation or overactivation of the immune system appears to be crucial since several studies demonstrated several immune cells, secreted-mediators, and markers that are involved in the pathogenesis of AS (5).

The complement system plays a key role in innate immunity which has functions varying from eliminating foreign pathogens to modulating immune responses and playing a part in the homeostasis chiefly through its cleaved products, such as pro-inflammatory C3a and C5a, opsono-cytophagic C3b/iC3b, and cytolytic membrane attack complex (MAC, with C5b-9n components) (6). The complement regulator, complement factor H (*CFH*, OMIM 134370) regulates the alternative pathway of the complement system; it has anti-inflammatory effect and protects the host tissue from damage. It has been reported that genetic variation in the *CFH* gene, which is found on 1q31.3 region of chromosome 1, is related with a higher risk of inflammatory diseases (7).

A single nucleotide polymorphism (SNP), Tyr402His, found in exon 9 of the *CFH* gene manifests a tyrosine to histidine change at amino acid position 402 in the CFH protein that modifies the complement activity (8). Complement dysfunctions, such as unregulated activation and inadequate regulation, exerts its harmful potential against host cells, implying that the complement system plays a crucial role in several human disorders, including autoimmune, inflammatory, and infectious diseases (9).

With this background, we postulated that the *CFH* gene might be a risk factor for AS. In the present study, we aimed to examine the association of CFH Tyr402His variant in patients with AS and control subjects in Turkey.

Methods

Study Population

Seventy-eight AS patients as cases were recruited from the Department of Physical Medicine and Rehabilitation at the Medical Faculty, Gaziantep University (Gaziantep, Turkey) The patients were diagnosed with AS after routine examinations, X-ray, computed tomography and nuclear magnetic resonance imaging according to the Modified New York Criteria for AS (10). Exclusion criteria were diabetes mellitus, cancer, severe liver and kidney failure, and being on therapy for any chronic inflammatory disease. Meanwhile, the control group was composed of 80 healthy individuals. The patients and control groups were matched in terms of age and ethnic background. All subjects provided written informed consent after being informed about the details of the study. The ethics committee of the Gaziantep University Ethics Committee approved the project in accordance with the tenets of the Helsinki Declaration and the National Ethical Guideline for Medical Research (no: 2016/308).

Assessment Criteria

Visual Analog scale (VAS) was applied to assess the level of pain. Pain during night time and daytime were evaluated. Disease activity was assessed by the Bath AS Disease Activity index (BASDAI) from 0 (no symptoms) to 10 maximal symptoms) on a numeric scale. Functional impairment was evaluated by the Bath AS Functional index (BASFI) from 0 to 10. Higher values of BASFI indicate poorer functional ability. To assess the quality of life, the AS Quality of Life (ASQoL) questionnaire was used.

Genotyping

DNA samples were extracted from the peripheral blood in all subjects by the salting out method (11). Then the DNA samples were stored at -20 °C. The *CFH* Tyr402His genotype was determined by using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (12). Briefly, a 244-bp DNA fragment containing the variant site was amplified with the primer pairs of *CFH*- F (5'- ACT GTG GTC TGC GCT TTT G3') and CFR-R (5'-TTT TTG GAT GTT TAT GCA ATC TT-3'). PCR was performed in a 10-µL reaction mixture containing 25 ng DNA, 0.1 mM each primer, and 1 µ Maxima® HotStart Green PCR MasterMix (Thermo Scientific). The thermal profile consisted of an initial denaturation step 2 minutes at 94 °C, followed by 34 cycles of 30 seconds at 94 °C, 40 seconds at 60 °C, 55 seconds at 72 °C, and a final elongation step of 5 minutes at 72 °C. PCR product was digested by FastDigest *NlaIII* restriction enzyme (Thermo Scientific) at 37 °C for 5 minutes. The restriction products were separated in 2% agorose gel and visualised by ultraviolet illumination. The CFH Tyr/Tyr genotype consisted of a single 244-bp band; the 402 His/His genotype had two bands, 161-bp and 83-bp, whereas the Tyr/His heterozygous genotype had three bands: 244-bp, 161-bp, and 83-bp. Random samples were selected,

50% of experiments were repeated, and the concordance rate was 100%.

Statistical Analysis

Analysis of the data was performed using the computer software SPSS 15.0 (SPSS, Chicago, IL) and Open Epi Info software package program. Continuous data were given as mean ± SD (standard deviation) and (min-max). Differences in CFH Tyr402His genotype distribution between the patients and controls were compared with the chi-square test and, Fisher's exact test was used when needed. The Hardy-Weinberg equilibrium (HWE) was calculated using the de-finetti program (Online HWE and Association Testing-Institut für Human genetik, Munich, Germany). The association between the CFH Tyr402His variant with clinical manifestations, namely VAS (night time and daytime), BASDAI, BASFI, and ASQoL was investigated through the Mann-Whitney U test. A p value of less than 0.05 (two-tailed) was regarded as statistically significant.

Results

Genotype and allele frequencies of *CFH* Tyr402His variant are listed in Table 1. Among 78 patients and 80 healthy controls with *CFH* Tyr402His variant, Tyr/Tyr homozygote accounted for 39.7%, 40.0%, Tyr/His heterozygote accounted for 44.9%, 41.3%, and His/His genotype accounted for 15.4%, 18.7 in patients and controls, respectively. The frequency of Tyr allele was 62.18%, 60.63% and His allele was 37.82%, 39.37% in patients and controls, respectively. There was no significantly difference in distribution of either genotypes or alleles between AS patients and healthy subjects (Table 1).

Next, the association between the CFH Tyr402His variant and clinical manifestations, namely VAS (night time and daytime), BASDAI, BASFI, and ASQoL was investigated (Table 2). VAS of daytime and ASQoL were significantly different among AS patients with Tyr/Tyr + Tyr/His and His/His genotype of *CFH* Tyr402His variant (p=0.032). VAS of daytime and ASQoL were significantly increased in patients carrying Tyr/Tyr + Tyr/His genotypes in comparison with those who had His/His genotype (Table 2).

Discussion

AS belongs to the spondyloarthritis family of diseases in which certain clinical, genetic, and immunologic characteristics are common. Chronic inflammation in the joints of the vertebrae results in serious chronic pain and stiffness. This in turn leads to ankylosis, immobility and consolidation of a joint due to the disease (13). On the contrary, dysregulation or excessive activation of the immune system appears to be crucial since some researchers reported that various immune cells, secreted-

Table 1. Genotype and allele frequency of *CFH* gene Tyr402His variant between groups

CFH Tyr402His	Patients	Controls	OR	95% CI	p
Genotypes	**n=78 (%)**	**n=80 (%)**			
Tyr/Tyr	31 (39.7)	32 (40)	1.043&	0.553-1.970&	1.000&
Tyr/His	35 (44.9)	33 (41.3)	0.942*	0.471-1.884*	0.866*
His/His	12 (15.4)	15 (18.7)	1.313*	0.522-1.884*	0.562*
Alleles					
Tyr	97 (62.18)	97 (60.63)	-	-	-
His	59 (37.82)	63 (39.37)	0.937&	0.595-1.473&	0.818&

OR: Odds ratio, CI: Confidence interval, n: Number, CFH: Complement factor H
*OR (95%CI) was adjusted by age and sex, &: Fisher's Exact Test.

Table 2. Association of *CFH* Tyr402His genotypes with various clinical features of the patients

Characteristics	Tyr/Tyr + Tyr/His	His/His	p*
	n=66 (SD)	**n=12 (SD)**	
VAS of night time	5.02 (2.33)	3.92 (1.97)	0.079
VAS of day time	4.08 (2.51)	2.50 (1.73)	0.032
BASDAI	4.35 (1.85)	3.40 (1.86)	0.082
BASFI	4.05 (1.71)	2.95 (1.71)	0.053
ASQol	8.89 (4.60)	5.92 (3.45)	0.036

SD: Standard deviation, VAS: Visual Anolog scale, BASDAI: Bath Ankylosing Spondylitis Disease Activity index, BASFI: Bath Ankylosing Spondylitis Functional index, ASQol: Ankylosing Spondylitis Quality of Life, CFH: Complement factor H
*Mann-Whitney U test

mediators, and markers played an important role in the pathogenesis of AS.

The complement system is a part of the innate immunological mechanism that contains effector molecules and receptors which help in both fighting against the invasion of pathogens and regulation of the immune system. The complement cascade can be triggered by variety of molecules, such as bacterial cell-wall components and antigen-antibody complexes, leading to the activation of one of the three major complement pathways. These include classical, alternative or lectin pathways (14). The complement system contains membrane-bound regulators and receptors along with many plasma proteins that interact with several cells and mediators of the immune system (15). These interactions differ with regard to the pathophysiologic setting, and they take place at various stages of an immune reaction. Impairment in the balance of complement activation and regulation will lead to detrimental results and can contribute to several inflammatory diseases, such as age-related macular degeneration, rheumatoid arthritis (RA), systemic lupus erythematosus, and Alzheimer's disease (16-18). There are a growing number of proofs implying that the complement system influences the skeletal system (19). Herewith, the complement system modulates bone metabolism and turnover both under physiological and pathophysiological conditions. Actually, it was seen that the state of complement activation affects and modulates the development and progression of some bone-related acute and chronic inflammatory disorders (19).

The complement system has a usually well-defined effect especially in chronic inflammatory disorders, all of which are associated with extreme bone loss. Numerous complement proteins and their cleaved products have been found in synovial fluids of patients with RA, including the early complement components C1q and C4 (20), as well as pro-inflammatory cleavage products C3a (21), and C5a (22). This accumulation of complement components occurring in arthritis patients implies a mechanism of local complement generation and activation in the demarcated area of inflamed joints. For more investigation of complement involvement in arthritis, animal model trials have been conducted with a wide variety of complement-deficient or complement-co strains, most of which being based on the collagen-induced arthritis models of RA. These models virtually imitate the autoimmune and progressive features of human RA, with cartilage and bone destruction (23).

The CFH protein is a critical regulator of the alternative pathway of the complement cascade that involves the elimination of pathogens and immune complexes, and modulates adaptive immunity (24). CFH hinders complement activation by preventing the development and facilitating the decay of C3 convertase and acting as a cofactor for factor I-mediated degradation of C3b, both in plasma and on cell surfaces. Many studies showed complement activation in AS by the importantly increased complement components or activation products such as C3, C4 and C3d, and by the complement activation triggers including IgA, IgG, C-reactive protein (CRP), serum amyloid A, apolipoprotein A (25). Besides, the complement activation products including C3a and C5a may alter the expression of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α in blood cells (26).

The CFH gene contains 23 exons and spans more than 94 kb of genomic DNA (27). The CFH Tyr402His variant is found in an area of CFH which binds to both heparin and C-reactive protein, and this binding could be modified by a tyrosine (Y) to histidine (H) substitution in CFH protein, leading to dysregulation of CFH (28). The adequate formation of C-reactive protein-CFH complex on cell surfaces is critical in order to reduce complement activation and diminish the secretion of the proinflammatory cytokine TNF-α (29). Previous studies reported that the *CFH* Tyr402His variant might be related to an enhanced activation of complement cascade both systemically and locally (30).

These observations led us to hypothesize that *CFH* Tyr402His variant may be involved in the pathogenesis of AS through inflammation. However, there has been no study investigating the association of the *CFH* Tyr402His variant with AS to date. We first considered the possibility that *CFH* Tyr402His variant is related to the inflammatory process in AS in a Turkish cohort. We found no evidence for the association of *CFH* Tyr402His variant with AS risk. Previous studies have demonstrated that the CFH variants were not associated with RA (31,32). Then, we compared the clinical characteristics of AS patients and genotypes of *CFH* Tyr402His. We detected a slightly significantly higher VAS of daytime and ASQoL score in subjects carrying Tyr/Tyr + Tyr/His genotypes compared to that in those with His/His genotype.

Study Limitations

There are some limitations of the present study that should be considered. Initially, we centred on only a variant involved in the pathway of CFH, other regulatory genes in the signalling pathway may also play a role in the pathogenesis of AS. Secondly, due to the relatively small sample size, the number of some homozygous variants was low in groups and thus decreased the statistical power. Finally, absence of assessment of expression levels of CFH is also a limitation of this study. The strengths of our study are its prospective nature.

Conclusion

In conclusion, this is the first research investigating the relationship between *CFH* Tyr402His genotype distribution and AS and also their association with clinical findings. Although we found no significant association between *CFH* Tyr402His variant and AS risk, our results suggest a possible association of *CFH* Tyr402His variant with clinical features including VAS and ASQoL. Genetic variants are important in AS pathogenesis, and further studies with larger populations may help control the clinical findings of patients with AS and facilitate the development of the new therapeutic agents.

Authorship Contributions

Concept: S.P., M.P., A.F.N. Design: S.P., S.G. Data Collection or Processing: M.S.A., S.G. Analysis or Interpretation: S.P., A.F.N. Literature Search: M.P., A.F.N. Writing: A.F.N.

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Comparison of Retrograde Intrarenal Surgery and Micro-percutaneous Nephrolithotomy for Kidney Stones 5-10 mm in Diameter

5-10 mm Böbrek Taşlarında Retrograde Intrarenal Cerrahi ile Mikroperkütan Nefrolitotomi Sonuçlarının Kıyaslanması

Bayram Güner, Bilal Günaydın*

Edremit Körfez Hospital, Clinic of Urology, Balıkesir, Turkey

**Ömer Halisdemir University Faculty of Medicine, Department of Urology, Niğde, Turkey*

Abstract

Aim: To compare the results of micro-percutaneous nephrolithotomy (micro-PNL) and retrograde intrarenal surgery (RIRS) for symptomatic renal stones 5-10 mm in diameter.

Methods: A total of 86 patients, who underwent RIRS (n=53) and micro-PNL (n=23), were evaluated retrospectively. Urine analysis, serum creatinine level, urine culture and non-contrast computed tomography scan were performed in all patients preoperatively. Kidney stones were opaque in all cases. Unresolved coagulopathy, active urinary infection, morbid obesity, missing data and pregnancy were considered the criteria for exclusion. The groups were compared in terms of operative time, Visual analogue scale score, analgesic requirement, retreatment, transition to other treatment, complication and stone-free rates and length of hospital stay.

Results: Both surgical techniques were similar for all parameters except need for analgesics, which was higher in the micro-PNL group (p=0.026). The stone-free rate was 85.7% in the RIRS group and 78.2% in the micro-PNL group (p=0.43).

Conclusion: Both methods can be administered as alternative modalities with high success and low complication rates. However, analgesics requirement was higher in micro-PNL group.

Keywords: Micro-percutaneous nephrolithotomy, retrograde intrarenal surgery, kidney stone, ureteroscopy

Öz

Amaç: 5-10 mm böbrek taşına sahip hastalarda retrograd intrarenal cerrahi (RIRC) ve mikroperkütan nefrolitotomi (mikro-PNL) operasyonlarının sonuçlarını karşılaştırmak amaçlandı.

Yöntemler: RIRC ve mikro-PNL yapılan hastaların kayıtları hastane dijital veri tabanı ve servis dosyalarından tarandı ve bunlar içerisinden opak taşa sahip 53'ü RIRC olmak üzere toplam 86 hasta çalışmaya dahil edildi. Düzeltilemeyen koagülopatiye, aktif üriner enfeksiyona, morbid obeziteye, eksik veriye ve opak olmayan taşa sahip hastalar çalışmadan dışlandı. Operasyon öncesi tüm hastalara tam idrar tetkiki, kreatinin, idrar kültürü, kontrastsız bilgisayarlı tomografi yapıldı. Gruplar demografik veriler, taş karakteristikleri, operasyon süresi, Görsel Ağrı skoru, ağrı kesici ihtiyacı, yeniden tedavi ve diğer tedaviye geçiş oranları, komplikasyon oranları, hastanede kalış süresi ve başarı açılarından karşılaştırıldı.

Bulgular: Ağrı kesici ihtiyacı mikro-PNL grubunda daha yüksek bulundu (p=0,026). Bakılan diğer parametreler her iki grupta benzer idi. Taşsızlık oranı RIRC grubunda %85,7; mikro-PNL grubunda %78,2 idi (p=0,43).

Sonuç: RIRC ve Mikro-PNL, 5-10 mm arası böbrek taşlarında benzer başarı oranlarına sahip olmasına karşın analjezik ihtiyacı açısından RIRC daha avantajlıdır.

Anahtar Sözcükler: Mikroperkütan nefrolitotomi, retrograd intrarenal cerrahi, böbrek taşı, üreteroskopi

Address for Correspondence/Yazışma Adresi: Bayram Güner, Edremit Körfez Hospital, Clinic of Urology, Balıkesir, Turkey
E-mail: gunerbayram@yahoo.com ORCID: orcid.org/0000-0001-7602-2255
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Introduction

Minimally-invasive treatment options are one of the most important determinants of current practice in patients with urinary stone disease. Owing to technological improvements, there are extremely rapid developments especially in the treatment of kidney stones. In addition to advances in extracorporeal shock wave lithotripsy (SWL), advances in retrograde intrarenal surgery (RIRS) and miniaturized percutaneous nephrolithotomy (mini-PNL) have reduced the use of open surgery.

The European Association of Urology urolithiasis guideline recommends standard PNL as the first choice in the treatment of kidney stones larger than 2 cm (1). Although PNL is accepted as a safe method, it can lead to life-threatening hemorrhages. Considering that hemorrhage in standard PNL is directly related to the instruments used, the diameters of the instruments have been reduced over the years. In the following years, developments have continued with defining smaller diameter systems such as ultra-m-PNL, super mini-PNL and micro-PNL techniques (2-5). Flexible ureteroscopy has superiorities such as the use of natural orifices, short-stay hospitalization, the advantage of concurrent access to all locations of the renal calyceal system, avoidance of complications resulting from dilatation and adjacent organ injuries in percutaneous interventions, low morbidity, and stone-free rates similar to that with percutaneous interventions. Micro-PNL does not need dilatation and especially, the risk of hemorrhage is lower than in standard PNL. Although there is no consensus on the optimal treatment for 5-20 mm kidney stones. SWL, RIRS and PNL (ultra mini-PNL, super mini-PNL and micro-PNL) alternatives can be used. In this study, we aimed to compare the results of RIRS and micro-PNL performed in symptomatic kidney stones 5 to 10 mm in size.

Methods

A total of 86 patients (14 females, 72 males), who were admitted to our center with renal stones smaller than 1 cm between June 2013 and November 2018 were included in the study. Patients who underwent micro-PNL were defined as group 1 and patients who underwent RIRS were defined as group 2. A complete urinalysis, serum creatinine measurement, urine culture and non-contrast computed tomography scan was performed in each patient. All patients had opaque stone. The procedure was performed by a single surgeon. Unresolved coagulopathy, active urinary infection, morbid obesity, having missing data and pregnancy were considered as the exclusion criteria.

Micro-percutaneous Nephrolithotomy

Following insertion of a 3-5F open-ended ureteral catheter in the lithotomy position under spinal anesthesia, micro-PNL procedure was performed via direct access to the stone or, in necessary cases, through retrograde pyelography with the help of a 4.85F all-seeing needle in the prone position. The procedure was performed by a single surgeon.

Retrograde Intrarenal Surgery

Flexible ureteroscopy (Karl Storz Flex X2, Stuttgart) was performed routinely under spinal anesthesia in all cases except for two patients. A guidewire was placed in the renal pelvis through a semirigid ureteroscope. The procedure was performed without using a ureteral access sheath in most cases. In only three patients, a 9.5/11F ureteral access sheath was used because of cost effectiveness and risk of ureteral trauma. A 270-micron laser fiber was used for fragmentation (1-1.5 Joule, 5-10Hz) (Dornier Medilas H Solvo 30W). At the end of fragmentation, a 4.8F 26 cm J stent was inserted in all patients via a semirigid ureteroscope and was removed on the 14th day postoperatively. The procedure was performed by a single surgeon.

Extracorporeal shock wave therapy was not performed before the procedures. All the patients, who underwent micro-PNL, were discharged on the postoperative day 1 and ureteral and Foley catheters were removed. All patients were followed up at 10th day and every 3 months postoperatively. None of the patients received medical therapy other than analgesic and antispasmodic treatment. Oral quinolone was given for three days postoperatively. Stone free rates were demonstrated by the absence of residual fragment on the combination of X-ray and ultrasonography two weeks postoperatively. Complications were evaluated according to the Modified Clavien Classification System (1).

Statistical Analysis

Data were analyzed by using the Statistical Package for the Social Sciences version 20 (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as mean ± standard deviation on tables and categorical data were expressed with frequency (n) and percentages (%). The distribution of the variables was measured by the Kolmogorov-Smirnov test. The independent samples t-test was used to compare independent groups. Pearson's correlation coefficient was used to examine the relationship between variables. Pearson's chi-squared and Fisher's exact tests were used to compare the categorical data. The data were analyzed at 95% confidence level and the threshold for statistical significance was accepted as $p<0.05$ for all analysis.

Results

One patient in micro-PNL group needed J stent placement on the 3rd postoperative day because of

pain and hydronephrosis. Fever occurred in only one patient in micro-PNL group. In RIRS group, three patients required ureteral access sheath and one patient required retreatment for the stone. In RIRS group, complete fragmentation could not be achieved due to bleeding in two patients and stone free status was achieved with SWL. Flexible ureteroscopy was not performed in these patients because the visualization would be poor due to bleeding. In micro-PNL group, two patients underwent flexible ureteroscopy due to problems in stone fragmentation. In three patients in RIRS group, the operation was converted to micro-PNL because the kidney could not be accessed due to ureteral stenosis. No hemoglobin decrease occurred in any of the patients. The demographic data and stone characteristics are given in Table 1. There was no statistically significant difference between the groups in terms of intra- and postoperative parameters except for analgesic requirement (Table 2).

Discussion

The main treatment modalities used in the surgical treatment of kidney stones consist of retrograde endoscopic procedures performed using natural orifices and the procedures that provide percutaneous access in various diameters and sizes (1). In both techniques, advances in miniaturization, high image quality and irrigation advantage have enriched the treatment alternatives. Currently, urologists are more familiar with retrograde procedures. Rapidly expanding ureteroscopy procedures have led us to gain an alternative procedure in kidney stones especially smaller than 2 cm with a high success rate versus percutaneous interventions. There is no doubt that failure of SWL treatment to achieve desired success in lower pole stones has led to use of alternative procedures (5-8).

Flexible ureteroscopy has superiorities such as use of natural orifices, short-stay hospitalization, advantage of concurrent access to all locations of the renal calyceal system, and avoidance of complications resulting from dilatation and adjacent organ injuries in percutaneous interventions. Also, flexible ureteroscopy has low morbidity and the stone free rates similar to that of percutaneous interventions. Meta-analysis studies showed that the stone-free rates of macro-percutaneous interventions were still higher than other alternatives, but these procedures have higher complication rate, risk of bleeding and longer hospital stay than, especially, RIRS. Although there are contradictory publications, it has been shown that RIRS, rather than miniaturized percutaneous interventions, may be recommended as a standard treatment in kidney stones smaller than 2 cm especially in obese patients with high morbidity risk (9).

Extracorporeal shock wave therapy, standard PNL, micro-PNL and RIRS have been performed in our center since 2011. One of the main indications for RIRS is renal and upper ureteral stones of 10-20 mm. Use of ureteral access sheath is not a routine in our center. However, we used flexible ureteroscopy in about 80-90 cases. With careful use, we were able to perform an acceptable number of flexible ureteroscopies without the use of a ureteral access sheath.

In the literature, there are several studies comparing mini-PNL with RIRS, but the stone size was limited to 10-

Table 1. Patient characteristics according to groups

Parameters		Micro-PNL (n=23)	RIRS (n=63)	p
Age		53.04±11.25	51.94±10.41	0.67
Stone diameter		8.91±1.12	8.06±1.51	0.018*
Laterality	Right Left	17 (73.9%) 6 (26.1%)	26 (41.3%) 37 (58.7%)	0.007*
Number of stone	Single Multiple	21 (91.3%) 2 (8.7%)	59 (93.7%) 4 (6.3%)	0.70
Localization	Upper pole Middle pole Lower pole Pelvis	0 7 (30.4%) 11 (47.8%) 5 (21.7%)	3 (4.8%) 15 (23.8%) 34 (54%) 11 (17.5%)	0.64
Comorbidity	+ - DM Ht CVD CAD	6 (26.1%) 17 (73.9%) 4 (17.4%) 1 (4.3%) 0 1 (4.3%)	23 (36.5%) 40 (63.5%) 10 (15.9%) 6 (9.5%) 3 (4.8%) 4 (6.3%)	0.36 - 0.92 0.39 0.26 0.64

PNL: Percutaneous nephrolithotomy, RIRS: Retrograde intrarenal surgery, DM: Diabetes Mellitus, Ht: Hypertension, CVD: Cardiovascular disease, CAD: Coronary artery disease, n: Number

Table 2. Operative and postoperative data

Parameters	Micro-PNL (n=23)	RIRS (n=63)	p
Operation time (min)	54.87±12.08	55.87±12.06	0.700
VAS score	4.48±1.41	5.02±6.4	0.752
Need for analgesics	6 (26%)	5 (7.9%)	0.026*
Switching to other treatment or ESWL	2 (8.6%)	5 (9.4%)	0.164
Hospitalization time (day)	1 23 (100%)	61 (96.8%)	0.387
	2 0	2 (3.2%)	
Complication	4 (17.4 %)	8 (12.7%)	0.578
Grade 1	2	3	-
Grade 2	1	2	-
Grade 3a	-	2	-
Grade 3b	1	1	-
Need for retreatment	0 (0)	1 (1.8)	0.164
Stone free rate	18 (78.2%)	54 (85.7%)	0.434

PNL: Percutaneous nephrolithotomy, VAS: Visual analogue scale, RIRS: retrograde intrarenal surgery, ESWL: Extracorporeal shock wave litotripsi, min: Minute

20 mm in general. In our study, we aimed to compare two procedures in a more specific group of patients. The reasons were lack of comparative studies with micro-PNL and the assumption of lower success rates due to increase in stone diameter and decrease in tract size secondary to deterioration in irrigation and image quality in the micro-PNL technique. In one of the few meta-analysis on this subject, it was emphasized that as stone diameter decreased, stone-free rates increased and micro-PNL and RIRS had comparable stone-free rates. It was also suggested that micro-PNL using a 4.85F "all-seeing" needle was a good alternative to RIRS with favorable stone-free rates and decreased complications related to dilatation. It has been reported that micro-PNL was an alternative to RIRS in patients with a narrow infundibulopelvic angle, stenosis of the calyceal neck and long calyceal neck and in cases where the stone cannot be reached due to deflection angle of the ureteroscope (10). Several factors should be considered when choosing the method in percutaneous procedures: small diameter tools are used for removal of the stone in small fragments which may lead to increased intrapelvic pressure, prolonged fragmentation time, increased metabolic acidosis risk, migration and difficult intrarenal navigation. With regard to flexible ureteroscopy however, problems with the insertion of the accessory sheath, two-stage procedure requirement, ureteral injuries, and problems secondary to J stent placement are presented as the most important factors affecting the method to be chosen. Undoubtedly, the experience of the surgeon is also important (11). Conversion from flexible ureteroscopy to micro-PNL was performed in order to avoid additional anesthesia and procedure requirement and cost effectiveness for the patients. The success rates were reported to be similar in four studies comparing micro-PNL and RIRS methods and it was stated that these two methods were alternative to each other as in our study (9-12). In a prospective randomized study, success rate, analgesic requirement, hemoglobin decrease and pain were similar between patients undergoing RIRS and micro-PNL, however, prolonged fluoroscopy time and hospital stay as well as increased radiation exposure due to "all-seeing needle" were found to be the main disadvantages of micro-PNL (12). In our study, although the stone size decreased partially, we found that there was no significant difference between the two methods in terms of both complication and success rates except the need for analgesic use. Complications rates, hemoglobin drop and length of hospital stay decrease as the diameter of the instrument decreases in percutaneous procedures. We believe that apart from the anatomical factors, the most important parameters in the choice of technique are the technical competence and surgical experience. We believe that the results of our study are meaningful because the operations were performed by the same surgeon with the same tools.

Study Limitations

The disadvantages of this study include the relatively small number of patients and obtaining the results from a single center and surgeon.

Conclusion

With the development of minimally invasive therapies, surgical intervention alternatives for kidney stone are increasing. RIRS and micro-PNL may be an alternative treatment for small kidney stones, but it should be noted that there may be perioperative conversions between these two treatment modalities. Although it seems favorable that percutaneous interventions can be performed with a small needle, we should state that it still needs improvement due to image quality and irrigation problems. The availability of RIRS equipment due to the possibility of conversion to RIRS in micro-PNL cases, who do not want SWL, may prevent repeated interventions. In our study, both surgical techniques were similar for all parameters except analgesic requirement, which was higher in the micro-PNL group.

Authorship Contributions

Concept: Ba.G., B.G. Design: Ba.G., B.G. Data Collection or Processing: Ba.G., B.G. Analysis or Interpretation: Ba.G., B.G. Literature Search: Ba.G., B.G. Writing: Ba.G., B.G.

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Comparison of Nutritional Screening Tools in Patients Undergoing Surgery for Gastric Cancer

Mide Kanseri Nedeniyle Cerrahi Uygulanacak Hastalarda Beslenme Tarama Araçlarının Karşılaştırılması

Reyyan Yıldırım, Bahar Candaş*, M. Arif Usta, Oğuz Erkul, Serdar Türkyılmaz, Ali Güner

Karadeniz Technical University Faculty of Medicine, Department of General Surgery, Trabzon, Turkey

**Karadeniz Technical University Faculty of Health Sciences, Department of Surgical Disease Nursing, Trabzon, Turkey*

Abstract

Aim: Nutritional screening tools are mainly used to identify patients at risk of malnutrition. We aimed to compare commonly used nutritional tools in assessing the nutritional status of patients undergoing surgery for gastric cancer

Methods: Consecutive patients undergoing surgery for gastric cancer between January 2017 and May 2019 were retrospectively evaluated from the comprehensive database. Nutritional Risk Screening-2002 (NRS), Malnutrition Universal Screening Tool, Subjective Global Assessment, Mini Nutritional Assessment-Short Form (MNA-SF), Malnutrition Screening Tool, and Short Nutritional Assessment Questionnaire scores were calculated for all patients. The assessment capabilities of these tools were compared using the European Society for Clinical Nutrition and Metabolism (ESPEN) diagnostic criteria for malnutrition as the reference standard. The distinctive abilities of the tool risk groups were also evaluated using parameters reflecting nutritional status, including albumin, lymphocyte count, and fat-free mass index.

Results: One hundred forty patients with the mean age of 64.2±11.8 years were analyzed, and 29 (20.71%) of whom were diagnosed as malnourished based on the ESPEN criteria. The strongest association (phi=0.62, large effect) and the highest agreement (kappa=0.59, moderate agreement) between tools and malnutrition were found for MNA-SF. This exhibited the highest specificity (0.84, 95% CI: 0.76 to 0.90), positive predictive value (0.58, 95% CI: 0.42 to 0.73), accuracy (0.84, 95% CI: 0.77 to 0.90), area under curve (0.850, 95% CI: 0.777 to 0.923), and diagnostic odds ratio (32.29, 95% CI: 10.02 to 104.04). Statistically significant decreases in all three parameters were observed only for the NRS risk groups. Additionally, MNA-SF exhibited a statistically significant decrease in the fat-free

Öz

Amaç: Beslenme tarama araçları çoğunlukla malnutrisyon riski olan hastaları belirlemek için kullanılır. Bu çalışmada mide kanseri nedeniyle ameliyat planlanan hastaların beslenme durumlarını değerlendirmede sıklıkla kullanılan beslenme araçlarını karşılaştırmayı amaçladık.

Yöntemler: Ocak 2017-Mayıs 2019 tarihleri arasında mide kanseri nedeniyle ameliyat olan hastalar, kapsamlı veri tabanından elde edilen bilgiler ile retrospektif olarak değerlendirildi. Tüm hastalar için Nutritional Risk Screening -2002 (NRS), Malnutrition Universal Screening Tool, Subjective Global Assessment, Mini Nutritional Assessment - Kısa Form (MNA-SF), Malnutrition Screening Tool, ve Short Nutritional Assessment Questionnaire araçlarının skorları hesaplandı. Bu araçların beslenme değerlendirme becerilerinin karşılaştırılmasında, referans standart olarak Avrupa Klinik Beslenme ve Metabolizma Derneği'nin (ESPEN) malnutrisyon tanı kriteri kullanıldı. Araçların risk gruplarını ayırt edici özellikleri ise albümin, lenfosit sayısı ve yağsız kitle indeksi gibi beslenme durumunu yansıtan parametreler kullanılarak değerlendirildi.

Bulgular: Bu çalışmada yaş ortalaması 64.2±11.8 olan toplam 140 hasta analiz edildi ve bu hastaların 29'u (%20.71) ESPEN kriterlerine göre malnutre olarak saptandı. Tarama araçları ile malnutrisyon arasındaki en güçlü ilişki (phi=0.62, yüksek etki) ve en yüksek anlaşma (kappa=0.59, orta düzeyde anlaşma) gösteren araç olarak MNA-SF bulundu. Mini Nutritional Assessment - Kısa Form en yüksek özgüllüğe (0.84, %95 CI: 0.76-0.90), pozitif prediktif değere (0.58, %95 CI: 0.42-0.73), doğruluğa (0.84, %95 CI: 0.77-0.90), AUC değerine (0.850, %95 CI: 0.777-0.923) ve tanısal odds oranına (32.29, %95 CI: 10.02-104.04) sahipti. Her üç parametrede de istatistiksel olarak anlamlı düşüşler sadece NRS'nin risk grupları için gözlendi. Ek olarak, MNA-SF, düşük ve

Address for Correspondence/Yazışma Adresi: Ali Güner, Karadeniz Technical University Faculty of Medicine, Department of General Surgery, Trabzon, Turkey
E-mail: draliguner@yahoo.com ORCID: orcid.org/0000-0001-7801-8081
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Abstract

mass index (-1.60, 95% CI: -2.49 to -0.71) between low- and high-risk groups.

Conclusion: Although all the tools analyzed were effective to a certain extent, MNA-SF, designed as a screening and assessment tool, was the most effective tool for assessing nutritional status based on the ESPEN malnutrition criteria in patients undergoing surgery for gastric cancer.

Keywords: Nutritional assessment, nutritional screening tools, malnutrition, stomach neoplasms, surgical procedure

Öz

yüksek riskli gruplar arasında yağsız kitle indeksinde (-1.60, %95 CI: -2.49 ila -0.71) istatistiksel olarak anlamlı bir düşüş gösterdi.

Sonuç: Analiz edilen tüm araçlar belli bir dereceye kadar etkili olmasına rağmen, MNA-SF, ESPEN malnutrisyon kriterlerine göre, mide kanseri nedeniyle ameliyat planlanan hastalarda beslenme durumunu değerlendirmede en etkili araç olarak saptanmıştır.

Anahtar Sözcükler: Beslenmenin değerlendirmesi, beslenme tarama araçları, malnutrisyon, mide neoplazileri, cerrahi prosedür

Introduction

The main guidelines published by the European Society for Clinical Nutrition and Metabolism (ESPEN), the American Society for Parenteral and Enteral Nutrition and Enhanced Recovery After Surgery strongly recommend perioperative nutritional therapy, particularly for patients with malnutrition, as well as those at nutritional risk (1-3). Since the benefit of nutritional therapy has been demonstrated in patients under severe nutritional risk, risk stratification before surgery and identifying patients who are malnourished or at risk of malnutrition have become essential elements of the preoperative period (4-6).

Several screening and assessment tools are available, including the Subjective Global Assessment (SGA), Nutritional Risk Screening-2002 (NRS), Malnutrition Universal Screening Tool (MUST), Mini Nutritional Assessment (MNA), Malnutrition Screening Tool (MST), and the Short Nutritional Assessment Questionnaire (SNAQ) (2,7-9). All these tools have been validated in distinct patient populations. However, no consensus on which is the optimal tool has been reached in studies comparing their accuracy (10-14). A systematic review of 32 screening tools for hospitalized patients demonstrated that no single tool by itself was capable of performing nutritional screening or assessment. The authors therefore recommended applying different tools in the same patient population, rather than development of a new tool (14).

Gastric cancer is one of the most common cancer types worldwide and is frequently accompanied by malnutrition. Preoperative malnutrition has been shown to cause poor short- and long-term outcomes in patients undergoing surgery for gastric cancer (15). Among the different screening tools, the NRS has largely been employed to assess nutritional status in this patient population, and has been identified as a predictor of postoperative complications, length of hospital-stay, and overall survival (16,17). The only study to compare the NRS, MUST, and the MNA-Short Form (MNA-SF) in gastrointestinal cancer patients identified the MUST as the best tool for identifying malnourished gastric cancer patients (18). However, there were a number of limitations to that study; other valuable tools, including SGA, were not evaluated, only geriatric patients (over 70 years) were included, and the fat-free mass index (FFMI), a key item of the ESPEN malnutrition criteria, was not been used. There is therefore still no consensus on the optimal tool for assessing nutritional status in gastric cancer patients in the preoperative period.

The aim of the present study was to compare and evaluate commonly used nutritional tools in assessing the nutritional status of patients with gastric cancer during preparation for surgery.

Methods

Patients

Consecutive patients undergoing preparation for gastric cancer surgery at the Karadeniz Technical University Department of Surgery, Turkey, between January 2017 and May 2019 were retrospectively evaluated for this study. Exclusion criteria were: (1) emergency surgery, (2) presence of malignancy other than adenocarcinoma, (3) receipt of neoadjuvant chemotherapy, (4) impossibility of assessment using screening tools due to disability or incompetence, and (5) insufficient data. Written informed consent was routinely obtained from all participants at the time of admission. Approval for the study protocol was granted by the Institutional Ethics Committee of Karadeniz Technical University (2019/193).

Data collection

A prospectively maintained comprehensive database was used for this study. All data were collected and recorded by medical doctors within two days before surgery. Parameters including demographics, patient comorbidities, smoking status, aim of surgery (curative vs palliative), disease stage, laboratory data, anthropometric data (current weight, actual weight, amount of weight loss, time elapsed during this weight loss, body mass index (BMI), fat-free mass (FFM)), changes in food intake, Eastern

Cooperative Oncology Group scores, symptoms (such as loss of appetite, functional capacity, and neurological symptoms), and physical examination findings (such as ascites, edema, and skin elasticity) were routinely recorded onto the electronic database.

Validated screening tools, including NRS, MUST, SGA, MNA-SF, MST, and SNAQ, were selected for analysis. NRS is routinely performed for all hospitalized patients in our institution. Tools other than NRS are applied using the data in the database by an experienced medical doctor. All screening tools were assessed by a single team member. In case of uncertainty concerning scores, the existing nursing documentation in electronic patient records or files was checked, and the case was consulted with the study coordinator.

Height was measured using a Charder™ MS4900 device. Weight and FFM were measured using a Tanita™ SC-330 portable calibrated digital scale. BMI (current weight/height^2) and FFMI (FFM/height^2) were also calculated.

Nutritional Screening Tools

Nutritional Risk Screening 2002 (NRS): The NRS score is obtained by evaluating the two main components, impaired nutritional status and disease severity, and the age criterion is also added. For impaired nutritional status, weight loss ratio, decrease in dietary intake, and BMI are assessed on a scale of 0 to 3. Severity of disease based on disease-related nutritional requirements is also assessed on a scale of 0 to 3. These two scores were then summed, and another point was added to the total score in case of patients older than 70 (possible maximum score is 7). Patients with an NRS score ≥3 are considered to be nutritionally at-risk.

Malnutrition Universal Screening Tool (MUST): Three components are used to calculate the MUST score. BMI (on a scale of 0 to 2), weight loss ratio (on a scale of 0 to 2), and acute disease effect score (0 or 2) are assessed, and all scores are added to calculate the overall risk of malnutrition. Scores of 0 and 1 are regarded as low risk and medium risk, respectively, while scores ≥ 2 are considered high risk.

Subjective Global Assessment (SGA): Medical history (food intake, weight loss ratio, symptoms capable of affecting oral intake, and functional capacity) and physical examination (loss of body fat, loss of muscle mass, edema, and ascites) are assessed for the SGA. Patients are classified as grade A (well-nourished), grade B (mildly/moderately malnourished), or grade C (severely malnourished).

Mini Nutritional Assessment-Short Form (MNA-SF): MNA-SF is a short version (6 items) of the original MNA form (18 items). Food intake, weight loss, mobility, psychological stress or acute disease, neuropsychological problems, and BMI are assessed (for a maximum score of 14). Scores of 12-14 represent normal nutritional status, while scores of 8-11 indicate risk of malnutrition, and scores of 0-7 indicate malnourishment.

Malnutrition Screening Tool (MST): Weight loss and decreased food intake are assessed, and scores are summed. Patients scoring 0 or 1 are considered not at risk, and those scoring 2 or more are regarded as at risk.

Short Nutritional Assessment Questionnaire (SNAQ): Weight loss, decreased appetite, and use of supplemental drinks are used for SNAQ. A score of 2 indicates moderate malnourishment, and 3 indicates severe malnourishment.

Parameters for Comparison and Evaluation

Although nutritional tools are designed for different purposes, we categorized our patients into two groups (low risk vs high risk) in order to permit comparison and evaluation of the tools. NRS and MST, which contain two categories, were used as they were. NRS scores <3 were defined as low risk, and scores ≥3 as high risk (19). A MST score of 0 or 1 was defined as low risk, and a MST score of ≥2 as high risk (20). Screening tools containing three categories were grouped into two categories based on the current evidence (21,22). A MUST score of ≥2, SGA grade C, a MNA-SF score of ≤7, and a SNAQ score of ≥3 were defined as representing high-risk groups, while the remaining scores were defined as low risk.

The ESPEN diagnostic criteria for malnutrition were used as a reference standard to compare the validity of the tools in assessing nutritional status, and relationship between the screening tool risk groups and diagnosis of malnutrition were analyzed. Based on the ESPEN diagnostic criteria, any of two alternative sets of criteria confirm the diagnosis (7).

Option 1: BMI <18.5

Option 2: >10% weight loss (indefinite length of time) or >5% weight loss over 3 months,

and

• Low BMI (BMI<20 if under 70 years or BMI<22 if over 70) or

• Low FFMI (<15 for females and <17 for males)

Validated parameters reflecting nutritional status, including albumin, lymphocyte count, and FFMI, were used to evaluate the screening tools (23-25). Commonly used parameters, such as weight, weight loss, and BMI, were not used for this evaluation, since all these represent items in the tools described above.

Statistical Analysis

Continuous variables are presented as mean ± standard deviation or as median (1st-3rd quartiles). Student's t-test or the Mann-Whitney U test was used to compare categorical variables. The chi-square test (or

Fisher's exact test) was used to compare the proportion of malnourished patients according to the different screening tools. The Phi coefficient, a measure of association between two binary variables, and the Kappa coefficient, a measure of agreement between categorical variables, were used to explore the relationships between screening tools and malnutrition. Phi was interpreted as adapted by Cohen; 0.1-0.3, small effect size; 0.3-0.5, medium effect size; ≥0.5, large effect size. Kappa scores were interpreted as 0-0.19, poor concordance; 0.20-0.39, fair agreement; 0.40-0.59, moderate agreement; 0.60-0.79, substantial agreement; and ≥0.80, almost perfect agreement.

Since there is no single perfect indicator for a screening test, various aspects of screening tools were evaluated using multiple indicators including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, area under the receiver operating curve (AUC), and diagnostic odds ratio (OR) including their 95% confidence intervals (CI). All p values were two-sided, and statistical significance was defined as $p<0.05$. R software (R Foundation for Statistical Computing, Vienna, Austria) with required packages was used for statistical analyses and graphical representation.

Results

Patients

One hundred forty patients meeting the inclusion criteria were included in the analyses. Patient demographics and clinical characteristics are shown in Table 1.

Comparison of Screening Tools Based on ESPEN Malnutrition Criteria

Twenty-nine (20.71%) patients were diagnosed as malnourished based on the ESPEN malnutrition criteria. Distributions of numbers of non-malnourished vs malnourished patients according to the different screening tools are presented in Figure 1.

The chi-square test revealed statistically significant associations between all screening tools and malnutrition. The strongest association was observed between the MNA-SF (phi=0.62, large effect) and malnutrition, while NRS, SGA, and MST exhibited small effect size associations (phi <0.3). Cohen's kappa was also run to determine if there was any agreement between the screening tool and malnutrition, the highest agreement being observed for MNA-SF (kappa=0.59, moderate agreement). NRS and MST both exhibited poor agreement (kappa ≤0.20).

Various measures for test validity are presented in Figure 2. Four screening tools exhibited higher sensitivity (MUST, MNA-SF, MST, and SNAQ). The MNA-SF also exhibited the highest specificity. PPV and NPV for MNA-SF were 0.58 and 0.96, respectively. Overall accuracy for MNA-SF was 0.84.

Lower limits of confidence intervals for AUC values exceeded 0.5 for all screening tools. The highest AUC

Table 1. Patient demographics and clinical characteristics of the patients

Characteristics		Data† (n=140)
Age		64.2 ±11.8
Gender	Female	40 (28.6%)
	Male	100 (71.4%)
Comorbidity‡	Yes	84 (60%)
	Diabetes Mellitus	29 (20.1%)
	Hypertension	60 (42.9%)
	Ischemic heart disease	16 (11.4%)
	Heart failure	6 (4.3%)
	Liver disease	2 (1.4%)
	Chronic respiratory disease	16 (11.4%)
	Chronic renal disease	6 (4.3%)
	Cerebrovascular disease	6 (4.3%)
ASA score	ASA-I	13 (9.3%)
	ASA-II	83 (59.3%)
	ASA-III	43 (30.7%)
	ASA-IV	1 (0.7%)
Smoking status	Current or ex-smoker	78 (55.7%)
Pathological stage	Stage-I	21 (15%)
	Stage-II	40 (28.6%)
	Stage-III	60 (42.9%)
	Stage-IV	19 (13.5%)
Intent for surgery	Curative	114 (81.4%)
	Palliative	26 (18.6%)
Hemoglobin	-	11.92±2.06
Albumin	-	3.8 (3.4-4.1)
Total protein	-	6.7 (6.1-7.1)
Lymphocyte count	-	1790 (1217-2232)
Weight	-	67.75 (60-76.5)
Weight loss ratio	-	7.96 (3.59-13.85)
Body mass index	-	24.67 (22.37-28.18)
Fat free mass index	-	18.88 ± 2.57

ASA: The American Society of Anesthesiologists
†: Data were presented as n (percentage), mean ± standard deviation or median (1st-3rd quartile)
‡: Some patients have more than one comorbidity

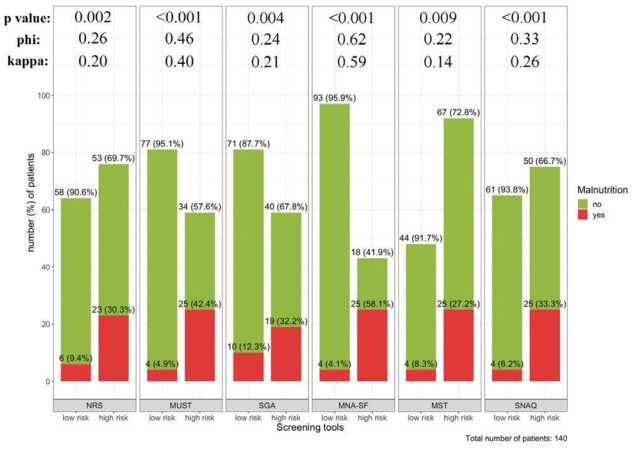

Figure 1. Distribution of the numbers (percentages) of non-malnourished vs malnourished patients according to the screening tools (based on ESPEN malnutrition criteria)
P value (the chi-square test) and phi coefficient for association, and Cohen's kappa coefficient for agreement between screening tools and malnutrition

NRS: Nutritional Risk Screening-2002, MUST: Malnutrition Universal Screening Tool, SGA: Subjective Global Assessment, MNA-SF: Mini Nutritional Assessment - Short Form, MST: Malnutrition Screening Tool, SNAQ: Short Nutritional Assessment Questionnaire

value was observed for the MNA-SF. Diagnostic OR was highest for the MNA-SF (32.29, 95% CI: 10.02 to 104.04) and lowest for the SGA (3.37, 95% CI: 1.43 to 7.96). Diagnostic OR values for other tools were NRS: (4.19, 95% CI: 1.59 to 11.09), MUST: (14.15, 95% CI: 4.57 to 43.82); MST: (4.10, 95% CI: 1.34 to 12.60), and SNAQ: (7.63, 95% CI: 2.49 to 23.36).

Evaluation of Albumin, Lymphocyte Counts, and FFMI for the Screening Tool Risk Groups

Albumin, lymphocyte, and FFMI values of the low and high-risk groups were evaluated for each screening tool. Changes between low and high risks are also shown (Table 2 and Figure 3). Statistically significant decreases for all three parameters were observed only for the NRS risk groups.

All tools exhibited a statistically significant decrease in albumin values between low- and high-risk groups. A marked difference was observed for SGA (-0.40, 95% CI: -0.59 to -0.21). The lowest albumin value for the high-risk group was obtained from the MUST and SGA tools.

Only the NRS demonstrated a statistically significant decrease in lymphocyte values between low and high-risk groups (p=0.013). The lowest lymphocyte values for the

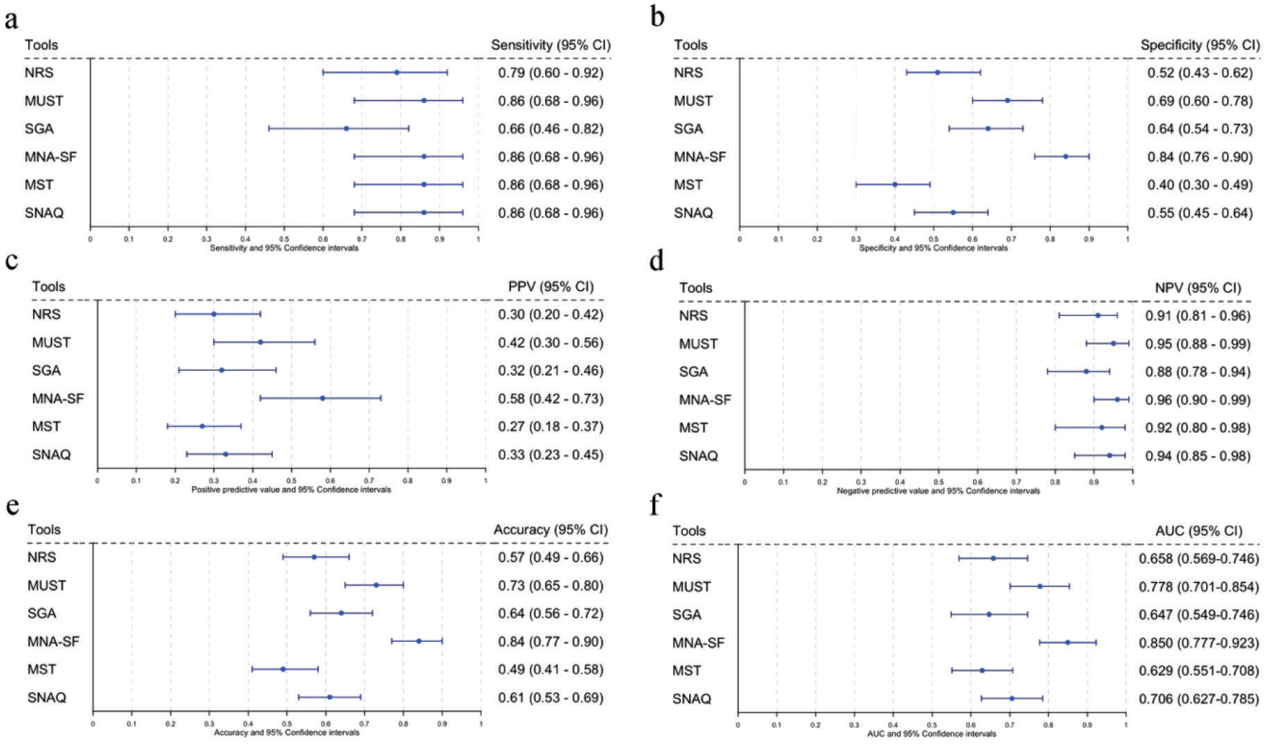

Figure 2. Sensitivity **(a)**, specificity **(b)**, PPV **(c)**, NPV **(d)**, accuracy **(e)**, AUC (f) values with 95% confidence intervals of the screening tools for assessing nutritional status based on the ESPEN malnutrition criteria

PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the receiver operating curve, CI: Confidence intervals, NRS: Nutritional Risk Screening-2002, MUST: Malnutrition Universal Screening Tool, SGA: Subjective Global Assessment, MNA-SF: Mini Nutritional Assessment - Short Form, MST: Malnutrition Screening Tool, SNAQ: Short Nutritional Assessment Questionnaire

high-risk group were obtained from the NRS, SGA, and MNA-SF.

The NRS and MNA-SF exhibited statistically significant decreases for FFMI between the low and high-risk groups. The lowest FFMI value for the high-risk group was observed for the MNA-SF.

Discussion

This study investigated the value of six different nutritional screening tools in patients scheduled for surgical treatment for gastric cancer. The validity of the tools in assessing nutritional status was analyzed using the ESPEN malnutrition criteria as the reference standard. Additionally, parameters reflecting nutritional status, including albumin, lymphocyte count, and FFMI, were used for the evaluation of these screening tools. The MNA-SF emerged as the most effective tool since it demonstrated the strongest association with the diagnosis of malnutrition. Only the MNA-SF and NRS high-risk groups exhibited a distinctive effect for both albumin and FFMI compared to the low-risk groups. NRS risk groups also exhibited a distinctive effect for lymphocyte counts.

All tools exhibited some degree of association with malnutrition in patients undergoing gastric cancer surgery. A statistically significant association was observed between all screening tools and malnutrition. Additionally, the lower limits of the confidence intervals for AUC values exceeded 0.5 for all tools. However, the main objective of this study was to determine the best screening tool for assessing nutritional status, and the MNA-SF emerged as the most effective. The strongest association (large effect size) and agreement (moderate agreement) were determined between the MNA-SF and the ESPEN malnutrition criteria. All test values in our study were higher than those of a previous study which compared three screening tools for geriatric gastric cancer patients (18). This discrepancy was probably due to differences in the selected patient population and the use of FFMI values, which formed part of the ESPEN malnutrition criteria in the present study.

Although screening tools share correlative items, the question that needs to be answered is what makes the MNA-SF superior to other tools in the present study population. The MNA-SF was designed as a comprehensive assessment tool in addition to its screening purpose. Its ability to assess nutritional status more deeply makes it superior to other tools, particularly to those designed only for screening purposes (26). On the other hand, the tools mainly designed as nutritional risk screening tools (such as NRS and MST) exhibited poor correlation with the ESPEN malnutrition criteria. This finding may confirm that tools designed for the assessment of nutritional status should also be recommended as screening tools owing to their diagnostic potential.

One of the most important findings of this study was the fact that the NRS, a commonly used and recommended screening tool, particularly in the in-hospital setting, lagged behind the other tools, correctly classifying only 57% of patients (7,17,27,28). The source of the difference was identified as the relatively low specificity of the NRS. In other words, the NRS was less capable of correctly identifying patients without malnutrition. This may be due to the NRS being intended only for screening purposes, not for assessment. The crucial clinical manifestation of this overestimation would be inaccurate identification of individuals requiring nutritional therapy.

Perioperative nutrition support is a component of standard treatment protocols in the high-risk patient

Table 2. Screening tools and albumin, lymphocyte, and FFMI values with changes according to the risk groups

Tool	Albumin			Lymphocyte†			FFMI		
	Low-risk	High-risk	Change (95% CI)	Low-risk	High-risk	p	Low-risk	High-risk	Change (95% CI)
NRS	3.94±0.39	3.56±0.61	-0.38 (-0.54, -0.20)	1965 (1422 - 2302)	1670 (1098 -2028)	0.013	19.35 ± 2.17	18.49 ± 2.83	-0.86 (-1.71, -0.01)
MUST	3.89±0.43	3.50±0.62	-0.39 (-0.58, -0.21)	1800 (1400 - 2230)	1710 (1075 - 2120)	0.092	19.1 ± 2.52	18.58 ± 2.64	-0.52 (-1.39, 0.34)
SGA	3.90±0.41	3.50±0.64	-0.40 (-0.59, -0.21)	1900 (1400 - 2230)	1670 (1075 - 2120)	0.065	18.94 ± 2.49	18.8 ± 2.71	-0.15 (-1.02, 0.72)
MNA-SF	3.8±0.53	3.57±0.58	-0.23 (-0.43, -0.03)	1800 (1370 - 2230)	1670 (1145 - 2255)	0.506	19.37 ± 2.45	17.77 ± 2.54	-1.60 (-2.49, -0.71)
MST	3.93±0.46	3.63±0.57	-0.30 (-0.47, -0.12)	1735 (1380 - 2165)	1795 (1192 - 2278)	0.986	19.15 ± 2.73	18.74 ± 2.49	-0.41 (-1.31, 0.50)
SNAQ	3.87±0.46	3.61±0.6	-0.26 (-0.44, -0.08)	1790 (1380 - 2220)	1790 (1170 - 2255)	0.564	19.08 ± 2.73	18.71 ± 2.44	-0.37 (-1.23, 0.48)

FFMI: Fat-free mass index, NRS-2002: Nutritional Risk Screening-2002, MUST: Malnutrition Universal Screening Tool, SGA: Subjective Global Assessment, MNA-SF: Mini Nutritional Assessment - Short Form, MST: Malnutrition Screening Tool, SNAQ: Short Nutrition Assessment Questionnaire, CI: Confidence intervals
†: Because the lymphocyte count showed nonparametric distribution, it was presented as median (1^{st}-3^{rd} quartile) and presenting changes was not available

group. However, the most important problem is to determine which patients should be defined as high-risk. The ESPEN defines the high-risk group as meeting at least one of the following four criteria: >10-15% weight loss within six months, BMI <18.5, albumin <3, and SGA grade C or NRS>5 (1). Although the use of the SGA and NRS is effective in various patient groups, the use of these screening tools did not elicit a satisfactory assessment of nutritional status in the present study, which included patients undergoing gastric cancer surgery (11,26,29-31). Moreover, although 76 (54.29%) patients had NRS scores of 3 and over in our study, only one had an NRS score >5 (data not presented). This may suggest that the use of the NRS >5 as a cut-off value should be questioned. Although

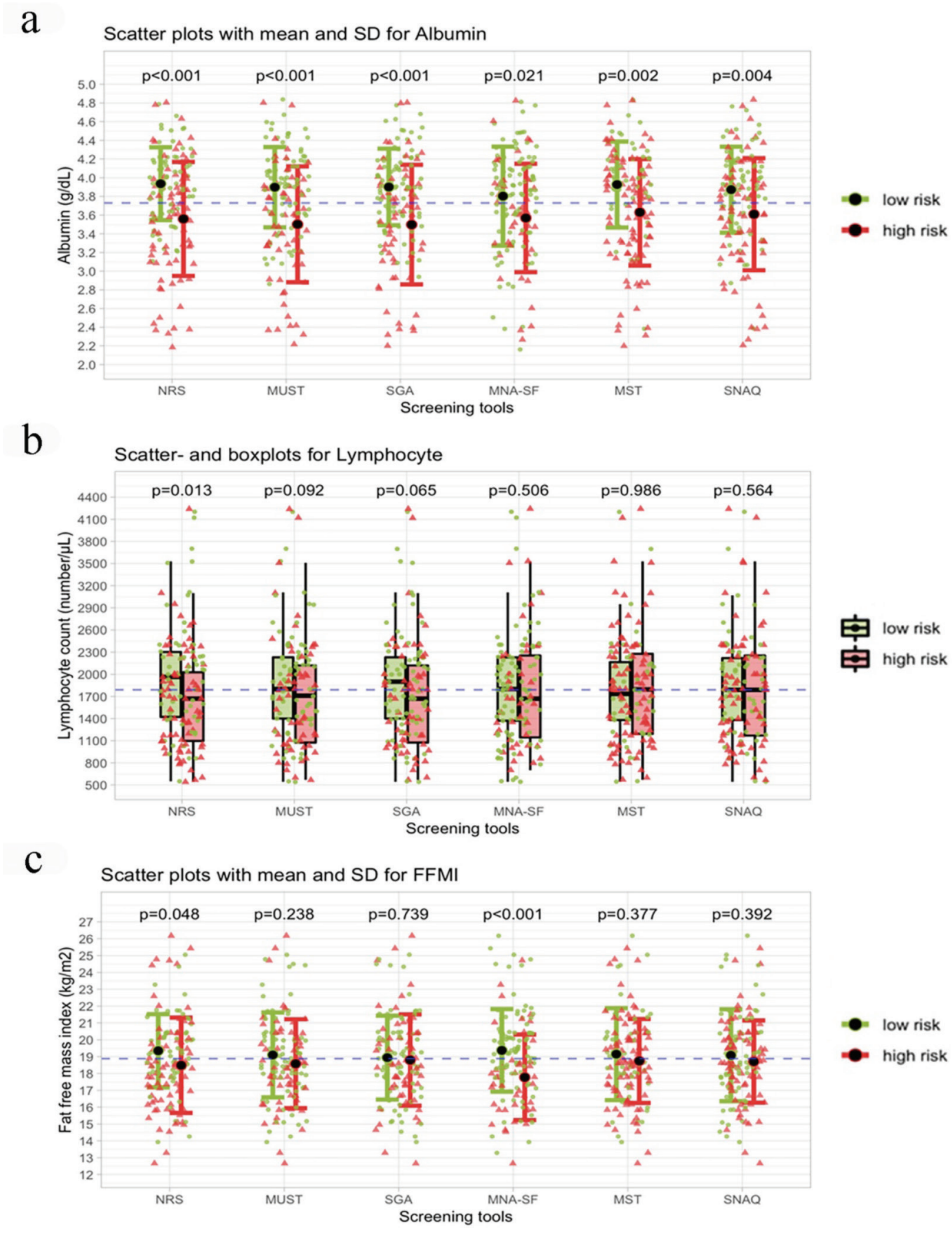

Figure 3. Albumin **(a)**, lymphocyte **(b)** and fat-free mass index **(c)** values for the low- and high-risk groups of the screening tools
Green circles represent low nutritional risk patients, and red triangles represent high nutritional risk patients. Blue horizontal lines represent mean (albumin, FFMI) or median (lymphocyte) values

FFMI: Fat-free mass index, SD: Standard deviation, NRS: Nutritional Risk Screening-2002, MUST: Malnutrition Universal Screening Tool, SGA: Subjective Global Assessment, MNA-SF: Mini Nutritional Assessment - Short Form, MST: Malnutrition Screening Tool, SNAQ: Short Nutritional Assessment Questionnaire

previous studies have demonstrated that regrouping the NRS may be more effective, we believe that a standardized grouping system is vital for the universal use of the screening tools, rather than the use of different cut-off values (32,33). We also strongly agree that the development of new tools is redundant since dozens of screening tools are already available (10,14). Instead, a change in perspective is needed. Investigation of the tools in a single patient population will lead to new insights for researchers.

In addition to comparing the tools, we also evaluated their ability to differentiate risk groups using nutrition-related parameters, including albumin, lymphocyte count, and FFMI (24). Albumin levels, a parameter commonly used to assess nutritional status in surgical oncology, differed significantly between the low- and high-risk groups for all screening tools (23,34). However, only some screening tools demonstrated a difference in terms of lymphocytes and FFMI. Rather than indicating an inadequacy in the screening tools, this finding may suggest that each tool has the potential to reveal a different aspect of nutritional status, regarded as a multifactorial phenomenon (7).

Study Limitations

The present study has some limitations. First, although we used a prospectively maintained comprehensive database, this is a retrospective study from a single center. The NRS is a routinely collected variable of the dataset; however, we used existing data to calculate the scores for other tools. Second, we did not analyze the time required to apply the tool, which represents a key feature of screening tools in terms of applicability. Third, we did not evaluate clinical outcomes because this was not within the scope of the study. In the light of these limitations, we recommend that a future study be performed evaluating the value of screening and assessment tools in a specific gastric cancer patient population, including patients undergoing either curative or palliative surgery, and patients with metastatic or non-metastatic disease. Short-term clinical outcomes, including postoperative complications and operative mortality, should be the primary outcomes. Well-designed prospective trials are now needed to verify our results.

Conclusion

Nutritional assessment should be considered a natural component of surgical treatment in gastrointestinal cancer patients. Screening tools are commonly used, not only for risk stratification, but also to assess nutritional status. All screening tools exhibit a certain degree of association with malnutrition. Although the success of the different screening tools varies based on the reference standard used, in terms of the ESPEN malnutrition criteria, the MNA-SF emerged as the most effective tool for assessing nutritional status in patients with gastric cancer. Studies comparing short- and long-term clinical outcomes are now warranted to confirm the validity of the screening tools.

Authorship Contributions

Surgical and Medical Practices: R.Y., B.C., M.A.U., O.E., S.T., A.G. Concept: A.G., R.Y., B.C., O.E. Design: A.G., R.Y., S.T. Data Collection or Processing: R.Y., A.G., O.E. Analysis or Interpretation: A.G., M.A.U., S.T. Literature Search: R.Y., B.C., M.A.U. Writing: R.Y., B.C., A.G.

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Original Article / *Özgün Araştırma*

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Evaluation of the Proliferation Markers in Interstitial Cystitis/Bladder Pain Syndrome: An Experimental Model

İnterstisyel Sistit/Mesane Ağrısı Sendromu Deneysel Modellerinde Proliferasyon Belirteçlerinin Değerlendirilmesi

Rashad Sholan

Republican Centre of Diagnosis and Treatment, Department of Kidney Diseases and Transplantology, Baku, Azerbaijan

Abstract

Aim: The aim of this study was to determine the level of nerve growth factor (NGF) and epidermal growth factor (EGF) in an animal model of interstitial cystitis/bladder pain syndrome (IC/BPS) and identify correlations between them.

Methods: IC/BPS modeling was performed on white New Zealand female rabbits divided into six groups depending on the methods of modeling. Urine and blood levels of NGF and EGF were determined by the ELISA method. The correlation between the indicators was calculated by the Pearson correlation coefficient.

Results: An increase in the concentration of NGF and EGF was detected in animals of all experimental groups; a significantly high level of these factors in blood and urine was determined in a toxic model created with the introduction of urine into the submucosal layer of the bladder wall. The correlation between NGF and EGF tended to increase by 14 days. There was a strong correlation between these indicators in the urine of animals with IC/BPS created by a 70% alcohol solution ($p<0.01$). A strong correlation between these indicators was observed in blood of animals with IC/BPS created by the administration of protamine sulfate and in animals of toxic model ($p<0.01$).

Conclusion: An increase in the levels of proliferation markers is likely due to chronic inflammation process and urine toxicity. The presence of a correlation between NGF and EGF and their strengthening as the disease progresses indicates an increase in the proliferation processes. Proliferation markers can be used in the diagnosis and monitoring of IC/BPS.

Keywords: Interstitial cystitis/bladder pain syndrome, proliferation, nerve growth factor, correlation, epidermal growth factor, animal models, experimental model

Öz

Amaç: Bu çalışmanın amacı, interstisyel sistit/mesane ağrısı (IC/BPS) sendromu deneysel modellerine sahip hayvanlarda sinir büyüme faktörü (NGF) ve epidermal büyüme faktörü (EGF) düzeylerini belirlemek ve aralarındaki ilişkileri tespit etmektir.

Yöntemler: Modelleme yöntemlerine bağlı olarak altı gruba ayrılan beyaz Yeni Zelanda dişi tavşanlarında IC/BPS modellemesi yapıldı. Sinir büyüme faktörü ve epidermal büyüme faktörü ELISA yöntemi ile kanda ve idrarda belirlendi. Göstergeler arasındaki korelasyon Pearson katsayısı ile hesaplandı.

Bulgular: Tüm deney gruplarındaki hayvanlarda sinir büyüme faktörü ve epidermal büyüme faktörü konsantrasyonunda bir artış tespit edildi; Kan ve idrarda bu faktörlerin anlamlı derecede yüksek olduğu, idrarın mesanenin submukoz tabakasına katılmasıyla toksik bir modelde tespit edildi. Sinir büyüme faktörü ve epidermal büyüme faktörü arasındaki korelasyon 14 gün artma eğilimindeydi. Bu göstergeler arasında hayvanların idrarında %70 alkol solüsyonunun oluşturduğu IC/BPS ile güçlü bir korelasyon vardı ($p<0,01$). Bu göstergeler arasında kuvvetli bir korelasyon, protamin sülfat uygulaması ile yaratılan IC/BPS'li hayvanların kanında ve toksik modeli olan hayvanlarda gözlendi ($p<0,01$).

Sonuç: Proliferasyon belirteçlerinde artış, kronik enflamasyon süreci ve idrar toksisitesinden kaynaklanıyor olabilir. Sinir büyüme faktörü ile epidermal büyüme faktörü arasında bir korelasyonun varlığı ve hastalık ilerledikçe güçlenmesi proliferasyon süreçlerinde bir artışa işaret eder. Proliferasyon belirteçlerinin belirlenmesi, tanı kriterleri ve IC/BPS'nin izlenmesi olarak kullanılabilir.

Anahtar Sözcükler: İnterstisyel sistit/mesane ağrısı sendromu, proliferasyon, sinir büyüme faktörü, korelasyon, epidermal büyüme faktörü, hayvan modelleri, deneysel model

Address for Correspondence/Yazışma Adresi: Rashad Sholan, Republican Centre of Diagnosis and Treatment, Department of Kidney Diseases and Transplantology, Baku, Azerbaijan
Phone: +994 50 212 5357 E-mail: drrashad@hotmail.com ORCID: orcid.org/0000-0002-1047-167X
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Introduction

Interstitial cystitis/bladder pain syndrome (IC/BPS), being a chronic pathology, is accompanied by debilitating pain and discomfort in the bladder. The nature of IC/BPS is not fully understood. However, it was established that this disease has a polyetiological origin as evidenced by its characteristic variety of symptoms (1-3). There are several proposed theories for the etiology of IC/BPS and each of them has its pros and cons: neuropathy, immunological theory, stagnation in the lymphatic system, influence of the infectious factors, corrosion of the mucous layer of the bladder, psychosomatic theory (formation of the disease against the background of psychological conditions), failures in the exchange of nitric oxide, influence of toxins, etc. None of these theories have been proven or completely refuted, but researchers considered the most possible last of these: toxic substances from urine seep through the mucous membrane to the bladder wall and cause inflammation (4-7).

Due to the insufficient number of studies on this pathology, the exact causes of its development are still problematic. However, researchers have number of reasons to distinguish some key triggering factors including chronic infectious and inflammatory processes of the genitourinary system (the local immune response is able to produce certain chemicals for a long time which ultimately provoke the development of the IC/BPS), certain elements released during urination irritate the mucous layer, *manifestation of autoimmune reactions, concomitant* pathology of other organs and systems affecting the urogenital system, and morbid diseases of the central nervous system (5,8,9).

Researchers are currently focusing on the role of urinary factors including urinary nerve growth factor (NGF) and epidermal growth factor (EGF). The role of these factors as diagnostic biomarkers has not been sufficiently studied. In this regard, it is important to study the level of these markers in the blood and urine in *IC/BPS*. Hence, the purpose of this study was to determine the level of NGF and EGF in animal models of *IC/BPS* and to determine the correlation between them.

Methods

This research has been approved by the Ethics Committee of the Republican Medical Diagnostic Center (protocol no: 136, date: 02.09.2018). The study was performed in accordance with the Guide for the Care and Use of Laboratory Animals (NRC - The National Research Council) (10). Experimental models of IC/BPS were created in white New Zealand female rabbits weighing 1500-2000 g.

Pathophysiology of IC/BPS is complex and animal models are used to understand the underlying mechanisms (11-13). The animals were exposed to chemical and toxic substances in order to induce bladder inflammation and were divided into six groups: IC/BPS was provoked by instillation of a 70% alcohol solution, protamine sulfate (10 mg) and HCl (0.2 mL 0.5%) into the bladder of the 1st (n=8), 2nd (n=7) and the 3rd group (n=8) of animals, respectively. In animals of the 4th group, IC/BPS model was created on the basis of one of the etiological theories of the IC, according to which uric toxicity leads to damage of the glycosaminoglycan layer (14,15). A suprapubic section was done and after which a urine sample taken from the bladder using a 0.5 cm^3 syringe with 30-gauge needle inserted through the mucous layer of the bladder. 10 mL of NaCl were injected into the bladder wall of the animals of the 5th group (n=7); animals from 6th group (n=8) formed a control group–they were not given anything.

NGF was determined by enzyme-linked immunosorbent assay (ELISA) using a set of NGF Emax® apparatus Medispec 6000M (Israel). Urine and blood levels of EGF were determined by ELISA using an EGF kit (Cusabio Biotech Co., Ltd., China). Marker concentrations were determined in blood and urine. The measurements were carried out 1st and 14th days after the creation of the experimental model.

Statistical Analysis

Statistical processing of the obtained data was carried out using the programs "Statistical for Windows 8.0" and "Microsoft Excel". Average value and standard deviation of the mean were calculated. A p value of less than 0.05 was considered statistically significant. The correlation between blood and urine biomarkers was calculated by the Pearson's correlation coefficient.

Results

The levels of the NGF in experimental groups are presented in Table 1. Analysis of NGF concentration on the first day of the study revealed a statistically significant increase in the biomarker level in the blood of all groups compared to the control group;, in the 1st group, the NGF level exceeded that in the control group by 46.0% ($p<0.05$), in the 2nd group–by 41.0% ($p<0.05$), in the 3rd group–by 60.4% ($p<0.01$), in the 4th and 5th groups–by 71.3 ($p<0.01$) and 44.0% ($p<0.05$), respectively. The level of NGF in the blood was statistically significantly higher in the 4th group than in other groups ($p<0.05$). In the same period, the urine level of NGF was significantly higher in the 4th group than in the control group (by 68.2%, $p<0.01$); in other groups, the difference in the level of NGF in the urine compared with the control group was insignificant. After 14 days increased blood biomarker level remained

in all groups relative to the control group ($p<0.05$), but the difference was most evident in the 4th group–90.1% ($p<0.001$). The urine concentration of NGF in all groups was higher than in the control group, but a statistically significant difference an increase in the level of NGF in the urine compared with the control group was observed in group 4; 85.0% ($p<0.001$).

There was a wide variation of NGF values in the blood for the first day among the 3rd, 4th and the 5th animal groups and, in urine–in the 4th group. After 14 days, a significant variability in values was observed in the blood of the animals of the 1st and 4th groups, in urine–only in the 4th group.

An intragroup analysis of NGF levels in each of the experimental groups revealed a dissimilar tendency. In the 1st group, when comparing NGF levels at different periods of observation, there was a tendency to increase both in blood and urine. In this group, compared with the initial value, there was an increase in the blood NGF level by 35.1% ($p<0.05$), in the urine–by 8.7% 14 days after creation of the model, On the 14th day of the study, there was a slight decrease in the level of NGF in the 2nd group. The blood concentration of NGF in the 3rd group decreased by 29.3%, while urine concentration of NGF increased by 14.3%. In the 4th group after 14 days of urine injection, compared with the first day, a significant increase in the NGF level in blood and urine was determined, by 65.5% ($p<0.01$) and 52.7% ($p<0.05$), respectively. In the 5th group, a decrease in NGF concentration in the blood and urine was noted: the difference with the initial period of observation in the blood was 30.8%, in the urine–30.5%.

Fourteen days later, compared with the first day after urine injection, a significant increase in the level of NGF blood and urine was determined in group 4, by 65.5% ($p<0.01$) and 52.7% ($p<0.05$) respectively.

The level of EGF in the experimental groups is presented in Table 2.

One day after modeling, there was no significant difference in blood level of EGF between the experimental groups and the control group. A relatively high level of EGF in the blood was detected among animals of the 3rd group, which exceeded that in the intact group by 15.6%; and the blood concentration of EGF in the 1st and the 5th groups was 20.4% and 3.5% lower than in control group, respectively.

One day after modeling, there was no significant difference in the blood level of EGF between the experimental groups and the control group. A relatively high level of EGF in the blood was detected in animals of 3rd group, which exceeded that in the control group by 15.6%, and, the blood concentration of EGF in the 1st and 5th groups was 20.4% and 3.5% lower than in the control group, respectively. Significant differences in EGF in the blood were also noted between the IC/BPS models. At the same time, statistically significant higher level of EGF in urine was revealed in the 3rd and 4th groups compared with the control group–by 48.2% ($p<0.05$) and 34.6% ($p<0.05$).

After 14 days, the highest level of EGF in the blood and urine was determined in animals of the 4th group; the level of EGF in the blood and urine were 63.1% and 49.2% higher than in the control group, respectively ($p<0.01$ and $p<0.05$, respectively). The EGF level in urine was 2.6 times lower in the 5th group than in the control group ($p<0.01$).

A comparative analysis of the EGF level among groups after 1st and 14th days showed a decrease in the EGF concentration in the blood and urine in the 2nd group after

Table 1. The level of nerve growth factor in blood and urine in the examination groups during the experiment

Groups	1 day		14 days	
	Blood, ng/mL	Urine, ng/mL	Blood, ng/mL	Urine, ng/mL
1 (n=8)	12.95±2.34 (8.5,15.4)	9.71±0.51 (8.5,10.5)	19.95±7.47* (14.1,47)	10.64±0.37 (10.1,11.4)
2 (n=7)	11.84±1.33 (9.4, 15.3)	11.36±2.78 (8.1, 21.1)	11.34±0.49 (10.3, 12.2)	9.34±0.55 (8.1,10.5)
3 (n=8)	17.64±8.43 (10.2, 48.3)	10.51±1.06 (8.5,13.2)	13.64±0.86 (12.1,15.3)	12.26±1.83 (9.8,17.4)
4 (n=15)	24.33±16.30* (6.9, 68)	30.39±27.46* (9.6,155.1)	70.62±21.63*,** (42.5,125.8)	64.26±22.84*,** (26.4,155.1)
5 (n=7)	12.47±5.02 (4.7, 21.8)	13.3±1.91 (9.6,16.6)	9.53±0.95 (7.8,10.9)	10.19±1.01 (8.5,12.0)
6 (n=8)	6.99±1.84 (4.3, 9.4)	9.65±0.6 (8.5,10.7)	-	-

n: Number
*Statistical significance of differences with the six group (control),
**Between study periods ($p<0.05$-0.001)

14th days by 16.2% and 35.5%, respectively (p<0.05). In the 4th group, EGF level increased both in blood (by 61.2%, p<0.01) and in urine (by 28.7%). In the 5th group, the dynamics of EGF values showed a decrease in blood (by 60.0%, p<0.01) and in urine (by 11.0%).

Multidirectional relationships were revealed when determining the correlation coefficient between the NGF values in blood and urine (Table 3). A statistically significant positive correlation was observed between blood and urine NGF values in the 2nd group on the 14th day of the study. In the control group, there was a weak positive correlation between the values of NGF in the blood and urine.

When determining the correlation coefficient in experimental animal models between the values of EGF in blood and urine, multidirectional relationships were revealed (Figure 1). The study showed that a high direct correlation, which was detected in group 1 a day after modeling, to average after 14 days; in the 2nd group was reduced to very weak, in the 3rd and 4th groups the relationship was also weakened while the correlation in the 5th group decreased from moderate to noticeable. In animals of control group, EGF in the blood and urine were moderately correlated.

Determination of the correlation between the NGF and EGF during the study revealed a tendency to increased correlation between these markers on the 14th day (Figure 2). In the control group, the average indirect correlation was determined between NGF and EGF in blood and urine. Among the 1st, 4th and 5th groups the correlation between NGF and EGF in the blood and urine was increased. One day after modeling, a strong indirect correlation was noted in the 2nd group between the blood indices, but on 14th day the correlation decreased to moderate and became direct. On the contrary, in the urine, the correlation increased and also became direct on day 14.

Discussion

It is known that the etiology of IC/BPS includes inflammatory, autoimmune processes, neurotoxicity

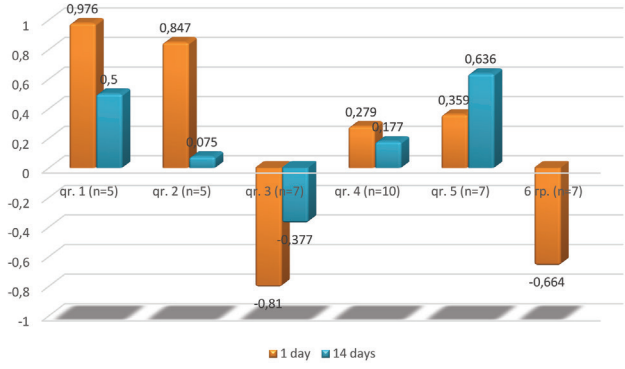

Figure 1. Correlation coefficient (r) between indicators of epithelial factor in blood and urine of study groups

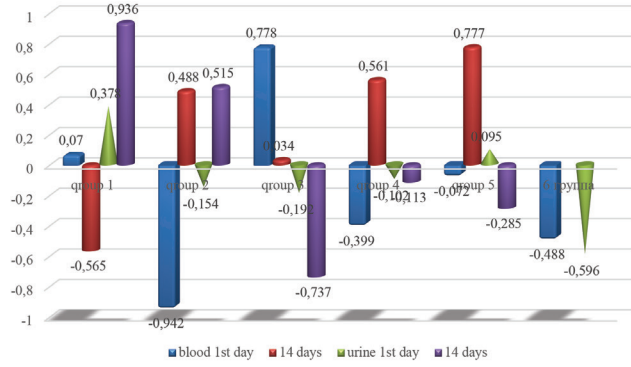

Figure 2. The correlation between nerve growth factor and epidermal factor in the blood and urine

Table 2. The level of epithelial growth factor in the blood and urine of the survey groups during the experiment

Groups	1 day		14 days	
	Blood, pg/mL	Urine, pg/mL	Blood, pg/mL	Urine, pg/mL
1 (n=5)	23.78±11.86 (7.9,36.6)	15.92±1.58 (13.7,18.0)	34.64±1.98 (29.7,37.0)	15.80±0.75 (15.0,17.0)
2 (n=5)	29.1±8.0 (15.4,43.2)	19.96±3.61 (13.8,23.4)	24.38±4.46 (17.2,35.0)	12.88±0.78** (11.6,14.1)
3 (n=7)	33.93±9.79 (12.4,56.0)	25.68±3.19* (17.7,29.1)	35.03±5.99 (25.9,56.0)	16.06±2.23 (13.7,19.4)
4 (n=10)	30.08±11.56 (8.7,56.2)	20.35±4.47* (11.6,33.0)	77.48±62.26*,** (37.9,388.8)	26.2±2.78* (20.8,33.0)
5 (n=7)	27.67±12.06 (9.2,44.2)	12.76±2.42 (6.7,16.5)	11.06±1.62*,** (8.7,15.4)	11.36±2.47 (6.7,14.7)
6 (n=7)	28.63±13.24 (4.6,41.5)	13.31±5.59 (5.6,24.5)	-	-

n: Number
*Statistical significance of differences with the six group (control),
**Between study periods (p<0.05-0.001)

Table 3. Correlation coefficient between indicators of nerve growth factor in blood and urine		
Experimental groups	**Study period**	
	after 1 day	**14 days**
1 (n=8)	+0.163	-0.088
2 (n=7)	-0.219	+0.715 p=0.05
3 (n=8)	+0,294	+0.415
4 (n=15)	+0.215	+0.216
5 (n=7)	+0.691	-0.330
6 (n=8)	-0.059	+0.880 p=0.01
n: Number		

and vascular components. Besides, urinary toxicity and disappearance of the glycosaminoglycan layer from the superficial urothelium have been proposed to be pathophysiological mechanisms (14). In our study, there was a statistically significant increase in blood concentration of NGF in all experimental groups, but a particularly a high level of NGF concentration both in blood and urine was determined in the 4th group. Due to the penetration of urine through the affected areas, interstitial tissues were irritated resulting in a decrease in the protective properties of the mucous membrane. Chronic inflammation leads to fibrosis of the bladder wall and decrease in the bladder capacity–as a result, wrinkled tissues do not function at full strength, their accumulative and excretory functions are impaired eventually (3,16). Our results are comparable with data from the study by Steers and Tuttle (14).

Our data regarding elevated NGF are consistent with the results of other studies (17-19). NGF was discovered as a secreted protein necessary for the development of sympathetic and peripheral sensory neurons (20,21). A direct relationship has been shown between painful inflammatory conditions in the lower urinary tract and elevated levels of urine NGF level (21). It is believed that urinary NGF plays a key role in the correlation between inflammation and pain stimulation since it is produced by urothelial cells, smooth and fat cells while activating their degranulation and proliferation (17,22).

Many researchers consider NGF as a potential IC/BPS biomarker. It has been established that the level of NGF in the urine can be used as a biomarker for the diagnosis of IC/BPS, for the differential diagnosis of this disease and hyperactive bladder and also may serve as a prognostic factor (18,23). Reports showing that inflammation increased the expression of NGF arouse interest in NGF as an important indicator of IC/BPS inflammation. It has been shown that inflammation caused neuroplasticity leading to increased urinary NGF level and thus IC/BPS (18). Some authors supposed that NGF played an important role in the pathogenesis of IC/BPS (18,22). The results obtained in this study also indicated an increase in the concentration of another marker–EGF. At the same time, the most marked changes in the level of EGF in blood and urine were identified in the group of the toxic model of IC/BPS that was created by injecting urine into the bladder wall. It should be noted that EGF that belongs to the group of growth factors (cytokines) and is a polypeptide with a molecular weight of 6000, was first isolated in 1975 (24). It has been established that EGF stimulates cell growth, proliferation and differentiation by binding to its receptor; it is a powerful mitogen and stimulates the synthesis of mRNA, DNA and proteins of epithelial cells (25). We suggest that elevated EGF level is a response to bladder damage caused by chemicals and toxic substances. Taking into consideration that EGF is urothelial and smooth muscle cell mitogen and increases proliferation and also product of many epithelial cells (26-28), it can be assumed that damage to the glucosaminoglycan layer of the bladder by introducing urine into the bladder wall will increase the level of EGF leading to stimulation of cell proliferation. The duration of this condition of the bladder, further enhances the correlation between the level of EGF in the blood and urine, as evidenced by the correlation coefficient in the second experimental group after 14 days of the study. The duration of this condition of the bladder further intensify the correlation between the levels of EGF in the blood and urine as evidenced by the correlation coefficient after 14 days of research in the 2nd group of the experimental model.

Study Limitations

The results allow us to expand modern ideas about the role of NGF and EGF in IC/BPS and confirm that damage to urothelium can be of a neurogenic nature. Our studies are necessary to study the course of the pathological process, with the goal of subsequent extrapolation of the data for use in human medicine.

Conclusion

Studies of the level of NGF and EGF in animal models of IC/PBS in dynamics showed their increase. The presence of elevated levels of NGF in the blood and urine of animals with IC/BPS is apparently caused by inflammatory components and a significant increase in NGF levels in animals with a model created by the introduction of urine into the bladder wall with chronic inflammation and toxicity of the components of the urine. Changes in EGF levels may be associated with IC/BPS. The revealed correlation indicates the cause-and-effect relationship.

The presence of a correlation between NGF and EGF and its strengthening as IC/BPS progresses indicates an increase in proliferation process in the bladder wall. The determination of proliferation markers can be used in the diagnosis and monitoring of IC/BPS. In further studies, along with the study of the level of NGF and EGF, it is advisable to study the level of mast cells in patients with IC/BPS.

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Original Article / *Özgün Araştırma*

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Comparison of Intramedullary and Extramedullary Fixation of Basicervical Fractures of the Femur in the Elderly: A Prospective Randomized Study

Yaşlanan Populasyonda Bazoservikal Femur Kırıklarında İntramedüller ve Ekstramedüller Fiksasyonunun Karşılaştırılması: Prospektif Randomize Çalışma

Engin Eceviz, Hüseyin Bilgehan Çevik*, Güven Bulut

Kartal Dr. Lütfi Kırdar Training and Research Hospital, Clinic of Orthopedics and Traumatology, İstanbul, Turkey
**Dışkapı Yıldırım Beyazıt Training and Research Hospital, Clinic of Orthopedics and Traumatology, Ankara, Turkey*

Abstract

Aim: Basicervical fractures of the femur are a unique type of unstable hip fracture, usually treated with sliding hip screw (SHS) or cephalomedullary nail (CMN). The aim of this randomized, prospective study was to evaluate the outcomes and complications of the two different treatment methods, SHS and CMN, in the management of basicervical fractures of the femur in the elderly osteoporotic bones.

Methods: Patients aged over 65 years, who presented to our clinic with basicervical fracture of the femur between January 2016 and January 2018, were included in the study. The permuted block randomization method was used to randomize the participants into groups. The patients were allocated to one of two groups treated via CMN (n=29) or SHS (n=27). Functional and radiological evaluations included mobility score, Harris Hip score, modified Barthel index, Singh index, tip-apex distance, and fracture settling.

Results: Continuous improvement in the Barthel index was seen over the 12-month period in either group ($p<0.05$). The average mobility score was found to be decreased at the 6th week follow-up visits in either group ($p<0.05$). The fracture settling measurements in SHS group was higher than in CMN group ($p<0.01$).

Conclusion: The clinical and radiological outcomes of both CMN and SHS groups showed no superiority of one technique over the other in the treatment of basicervical fractures of the femur.

Keywords: Basicervical, cervicobasiler, hip, fracture, prospective randomized

Öz

Amaç: Bazoservikal femur kırıkları genellikle kayan kalça vidası (KKV) veya sefalomedüller çivi (SMÇ) ile tedavi edilen dengesiz bir kalça kırığı tipidir. Bu randomize prospektif çalışmanın amacı, yaşlı osteoporotik kemiklerdeki bazoservikal femur kırıklarının tedavisinde kullanılabilen iki farklı tespit yöntemi KKV ve SMÇ'nin sonuçlarını ve komplikasyonlarını değerlendirmektir.

Yöntemler: Bu prospektif, randomize çalışmaya Ocak 2016'dan Ocak 2018'e kadar bazoservikal femur kırığı olan 65 yaş üstü hastalar dahil edildi. Katılımcılar blok randomizasyon yöntemi ile gruplara ayrıldı. Hastalar SMÇ (n=28) veya KKV (n=28) ile tedavi edilen iki gruba ayrıldı. Fonksiyonel ve radyolojik değerlendirmelere mobilite skoru, Harris Kalça skoru, modifiye Barthel indeksi, Singh indeksi, tip-apeks mesafesi ve kırık hattında çökme mesafesi dahil edildi.

Bulgular: Her iki grupta da Barthel indeksinde 12 aylık dönemde sürekli iyileşme görülmüştür ($p<0,05$). Ortalama mobilite skorunun, her iki grupta 6. hafta kontrolünde azaldığı görüldü ($p<0,05$). KKV uygulanan bazoservikal kırıklarda kırık hattındaki çökme mesafesinin SMÇ uygulanan gruptan daha yüksek olduğu görüldü ($p<0,01$).

Sonuç: SMÇ ve KKV uygulanan grupların klinik ve radyolojik sonuçları, bazoservikal femur kırıkları tedavisinde bir implantın diğerine üstünlüğü olmadığını açıkça göstermiştir.

Anahtar Sözcükler: Bazoservikal, servikobaziler, kalça, kırık, prospektif randomize

Address for Correspondence/Yazışma Adresi: Hüseyin Bilgehan Çevik, Dışkapı Yıldırım Beyazıt Training and Research Hospital, Clinic of Orthopedics and Traumatology, Ankara, Turkey
Phone: +90 312 596 20 00/2253 E-mail: bilgehancevik@gmail.com ORCID: orcid.org/0000-0003-1945-3715
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Introduction

Anatomically, basicervical fractures of the femur are intracapsular (1) but biomechanically, they are classified as extracapsular (2). Therefore, these fractures have been considered to be in the borderline category. However, clinically and biomechanically, these fractures behave like intertrochanteric hip fractures, which are usually treated with sliding hip screw (SHS) or cephalomedullary nail (CMN) instead of hemiarthroplasty (2-5).

Some studies have reported better results with the use of SHS in intertrochanteric fracture treatment and others have stated that CMN give better results (6-8). However, the current approach to the treatment of unstable intertrochanteric fractures is the use of CMN (9-11). It has been reported that basicervical fractures could also be evaluated as unstable fractures (12).

There are very little data in the literature regarding the comparison of clinical outcomes of CMN and SHS used in basicervical fractures of the femur (10). There have been several studies of the biomechanical properties of CMN and SHS, which have not shown any clear superiority of either implant in the treatment of these fractures (4,9,13). The objective of this randomized, prospective study was to evaluate the rate of basicervical fractures of the femur and to compare outcomes and complications of the two different treatment methods of SHS and CMN.

Methods

Study Design

Approval for the study was granted by the Local Ethics Review Board of Dr. Lütfi Kırdar Training and Research Hospital and all the procedures were performed in accordance with the principles of the Declaration of Helsinki (1964). The study was registered at ClinicalTrials.gov (NCT04240743). Patients with a basicervical femur fracture were identified on admission to the emergency department of our tertiary hospital from January 2016 to January 2018. Patients, who were scheduled for surgery, met the inclusion criteria and provided written informed consent, were included in the study.

The patients were randomly allocated to a study group by permuted blocks of randomly mixed sizes and stratification according to the type of surgery (CMN or SHS). Randomization was applied using pre-prepared randomization cards, which were placed in opaque, sealed envelopes and given to the surgeons to open just prior to surgery, and the designated procedure was then performed.

Preoperative Assessment

Eligible patients were prospectively enrolled over a two-year period. The inclusion criteria were a basicervical fracture, age ≥65 year, an isolated fracture, ability to walk independently (with or without an aid) before fracture, and a fracture that had occurred less than one week prior to admission. The exclusion criteria were a history of ipsilateral femoral fracture, a fracture due to malignancy, limited life expectancy due to medical comorbidities, any contraindication to surgery, diagnosed dementia, or any other traumatic fracture on admission.

Surgical Procedure

Closed reduction of the fracture was performed under fluoroscopic guidance on a traction table. Then the relevant surgical procedure was applied for implantation of CMN or SHS. Two senior surgeons (E.E, G.B.) with more than 10 years of surgical experience in treating basicervical fractures of the femur were familiar with both surgical techniques.

Cephalomedullary Nail Technique

For patients in the CMN group (Figure 1), an incision was made in the gluteal area from the tip of the greater trochanter in proximal orientation. A guidewire was placed into the medullary canal from slightly medial to the exact tip of the greater trochanter. The entry point of the greater trochanter and proximal medullary canal were reamed. The CMN was then inserted and fixed to the femoral head with a two lag screws. The nail was then locked distally using a guide arm. These CMNs were not locked proximally to maintain dynamization and to allow compression across the basicervical fracture line. In this

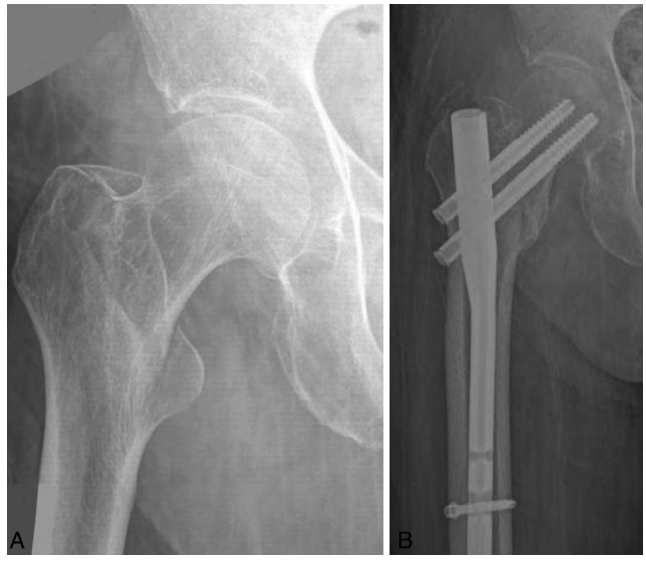

Figure 1. Anteroposterior X-ray view of a 72-year-old female patient showing a basicervical femur fracture of the right hip **(A)**. A postoperative 6th month anteroposterior X-ray view of the same patient undergoing insertion of CMN fixation of an osteoporotic basicervical femoral fracture of the right hip **(B)**

study, all patients were treated with short nails (Profin®, TST).

Sliding Hip Screw Technique

For patients in the SHS group (Figure 2), a lateral incision was made over the lateral proximal aspect of the femur. Under fluoroscopic guidance, the lag screw was placed centrally in the femoral head over the guidewire. A side plate with three holes was then attached to the hip screw (DHS plate, TST).

Postoperative Follow-up

Postoperatively, all patients were allowed immediate weight-bearing as tolerated, regardless of the method of fixation. All patients were followed up clinically and radiologically for a period of 12 months. The clinical and radiological evaluations were performed immediately postoperatively, then at 6 weeks, 3, 6 and 12 months.

Functional outcomes were evaluated with a Mobility score (range: 0-9) (14), and the Harris Hip score (15). Functional independence in daily living (10 basic activities of daily living) was evaluated with the modified Barthel index (range: 0-100) (16). The clinical follow-up evaluations were performed by two independent orthopaedic surgeons who had access to all the patients' files and documents. They were also blinded to the preceded treatment.

The Singh index was used to grade preoperative osteoporosis in the contralateral proximal femur from anteroposterior (AP) radiographs (17,18). AP and lateral radiographs were evaluated for any change in implant position, using the tip-apex distance, and fracture settling (19,20). The quality of reduction was evaluated on the postoperative radiographs, and graded as good (<10˚ varus/valgus and/or ante- or retroversion), acceptable (5-10˚ varus/valgus and/or ante- or retroversion), or poor (>10˚ varus/valgus and/or ante-or retro- version) (21). The radiographic evaluation was performed by two different independent orthopaedic surgeons.

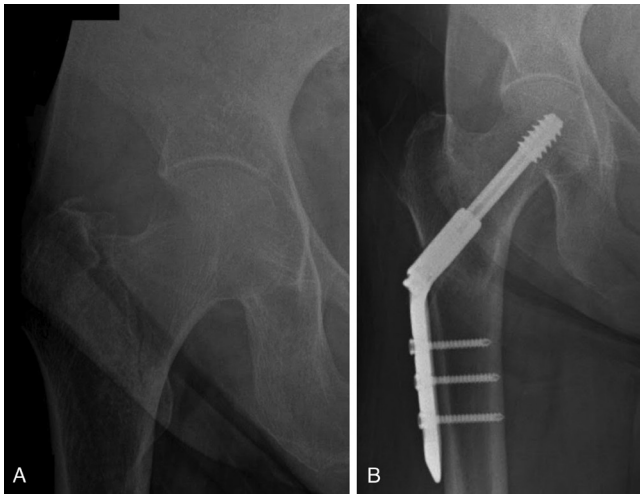

Figure 2. Anteroposterior X-ray view of a 66-year-old female patient showing a basicervical femur fracture of the right hip **(A)**. A postoperative 6th month anteroposterior X-ray view of the same patient undergoing insertion of a SHS **(B)**

Data Management

The convenient clinical and radiological data were collected. The data were entered into a Microsoft Office Excel sheet. All participants in the study were assigned a four-digit number and the radiographs were placed in digital folders. No personal data on the individual patients was transmitted.

Statistical Analysis

The statistical analysis was performed using the SPSS for Windows (SPSS Inc, Chicago, Illinois). Odds ratios and means were compared between the groups, with 95% confidence intervals and a p value of less than 0.05 was considered statistically significant.

In order to calculate the sample size, a comparison of proportions method was used. A total sample size of 100 patients was calculated to show a difference on the order of 25% between the treatment groups at 12 months follow-up, with 80% power and 5% significance level. Twenty-five percent was the predicted difference in functional and radiological outcomes between the two fixation methods, and based on the previous unit data, an attrition rate of 10% was assumed.

Results

A total of 64 patients, who met the inclusion and exclusion criteria, and were willing to participate, were included in the study. The flow diagram of the study according to the Consolidated Standards of Reporting Trials 2010 is presented in Figure 3 (22). Registration in the study was terminated when the planned sample size was reached. Of the 64 patients, 32 were treated with SHS and 32 with CMN. Eight patients, who did not attend the 12-month follow-up examination, were excluded as clinical information was not available. The reasons for loss to follow-up were mortality in six (unrelated to hip surgery) and non-attendance at the final follow-up in two. Thus, final evaluation was made of 56 patients. Implant removal/revision surgery was not required in any patient during the study period.

The total 56 patients comprised 13 male and 14 female patients in the SHS group, and 15 male and 14 female patients in the CMN group, with a mean overall age of 80.75±7.54 years. The detailed distributions of preoperative features are given in Table 1.

The average tip-apex distance was 19.2 mm in the CMN group and 17.9 mm in the SHS group. A statistically significant difference was found between tip-apex distance

measurements according to the implant type (p<0.05); the tip-apex distances in patients who underwent CMN were longer than in the SHS group. There was a loss of neck length (fracture settling) in both groups of average 10.6 mm in the SHS group and 6.7 mm in the CMN group. A statistically significant difference was found between the fracture settling measurements according to the implant type (p<0.01); the measurements of SHS were higher than those of CMN. Fracture settling was determined in the first 6 weeks postoperatively but not thereafter.

The average Singh grade was 4 in the SHS group and 3.9 in the CMN group. Moderate or poor quality of reduction was determined in 22.2% of patients in the SHS group and in 13.9% of patients in the CMN group.

The pre-fracture Modified Barthel index was 94.5 in the SHS group and 93.0 in the CMN group, with no significant difference determined between the groups. The index was applied at every visit. Continuous improvement in the Barthel index was seen over the 12-month period, but the index did not return to pre-fracture values in either group (p<0.05). No difference was determined in Barthel index values between the groups at any of the measured time points. The average mobility score decreased from 8.4 points to 6.0 in the SHS group and from 8.5 points to 7.1 in the CMN group at the 6th week follow-up visit. These values were considered statistically significant (p<0.05). The average Harris Hip score of all the patients was 70.59±9.50. The preoperative Harris score was regained in the post-operative 3rd month by 40% of patients and by 80% at the final follow-up (12 months). The detailed distributions of functional and radiological outcomes are given in Table 2.

In one patient of the SHS group, implant cut-out developed despite adequate initial reduction and implant position. No implant cut-out was seen in the CMN group. No surgery-related infections or wound complications developed in any patient in this study.

Discussion

Proximal femoral fractures are one of the most commonly treated conditions by orthopaedic trauma surgeons. However, one of the least encountered fractures

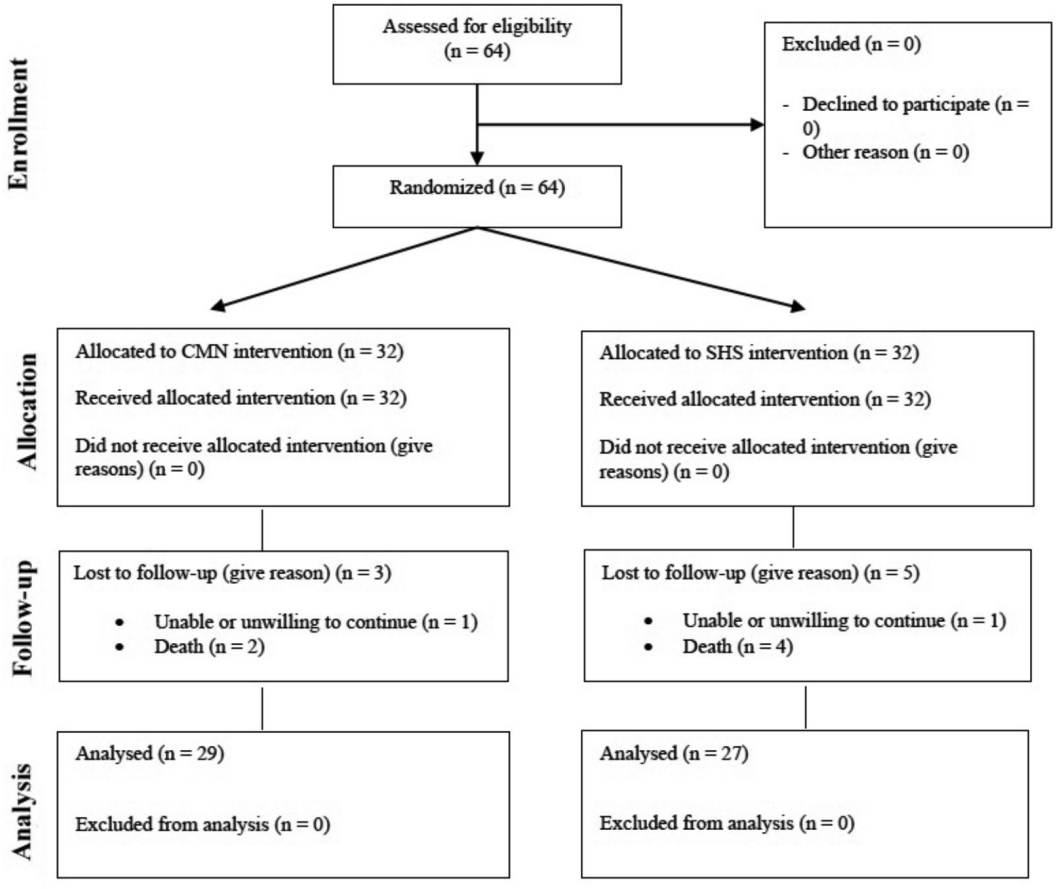

Figure 3. Consolidated Standarts of Reporting Trials (CONSORT) 2010 flow diagram depicting fracture allotment in both groups
CMN: Cephalomedullary nail, SHS: Sliding hip screw, n: Number of the patients

is basicervical fracture. These relatively rare fractures account for 1.8%-3.5% of all proximal femur fractures (2,4,23). In a 2-year period, 2207 patients were treated for proximal femur fractures in our institution and only 2.9% (n=64) had real basicervical fractures. This finding is comparable with the data of previous studies.

Many implants have been used to reconstruct the hip anatomy after basicervical fractures (10,24-26). Fixation of basicervical fractures is difficult, because of the unique nature of the anatomy and the best fixation implants are probably fixed-angle implants, such as SHS and CMN (24,27).

For many types of hip fractures, SHS is the preferred implant. Some authors have stated that a basicervical fracture should be treated as an extra-capsular fracture and they have recommended osteosynthesis with SHS (26,28). However, others have suggested that SHS alone would not provide sufficient rotational stability and it would be necessary to apply a derotation screw (26,29). Saarenpää et al. (2) retrospectively reviewed 1624 hip fractures in an 8-year period and found 30 (1.8%) basicervical fractures, of which 16 were treated as intracapsular hip fractures and 14 as extracapsular. The authors reported that when treated with SHS (like an extracapsular hip fracture), better results were obtained than with hemiarthroplasty (like an intracapsular hip fracture). However, some authors have stated that basicervical fractures were unstable extracapsular fractures and could show good outcomes when treated with CMN (30,31).

Statistically, modified Barthel index, Mobility score and Harris Hip score would seem to be age- and time-dependent, independent of other parameters and implant type.

Table 1. Distributions of preoperative features

		SHS n=27 (48.2%)	CMN n=29 (51.8%)	p
Age (year)	Min-Max (median)	66-96 (81)	66-92 (81)	[a]0.545
	Av. ± SD	80.11±8.23	81.34±6.92	-
Gender	Female	16 (59.3)	14 (48.3)	[c]0.410
	Male	11 (40.7)	15 (51.7)	-
Singh index	Min-Max (median)	2-6 (4)	1-6 (4)	[a]0.594
	Av. ± SD	4.07±1.07	3.90±1.37	-
	Av ± SD	93.15±5.57	92.41±6.63	-
Side	Right	16 (59.3)	15 (51.7)	[c]0.571
	Left	11 (40.7)	14 (48.3)	-
Time interval between trauma to operation (day)	Min-max (median)	2-12 (5)	2-17 (5)	[b]0.823
	Av. ± SD	5.37±2.80	5.76±3.47	-

SHS: Sliding hip screw, CMN: Cephalomedullary nail, Min-max: Minimum-maximum; Av. + SD: Average + standart deviation, n: Number
[a]Student's t-test, [b]Mann-Whitney U test, [c]Pearson chi-square test, [d]Fisher Freeman Halton test, *p<0.05, **p<0.01

Table 2. Distributions of functional and radiological outcomes

		SHS n=27 (48.2%)	CMN n=29 (51.8%)	p
Harris Hip score	Min-max (median)	61-93 (68)	60-96 (68)	[b]0.621
	Av. ± SD	70.81±8.99	70.38±10.10	-
Barthel index	Min-max (median)	80-100 (95)	80-100 (95)	[a]0.657
	Av. ± SD	93.15±5.57	92.41±6.63	-
Tip-apex distance (mm)	Min-max (median)	14-22 (17)	16-23 (19)	[a]0.010*
	Av. ± SD	17.85±1.99	19.21±1.78	-
Fracture settling (mm)	Min-max (median)	7-14 (10)	1-5 (3)	[b]0.001**
	Av. ± SD	10.63±2.20	3.07±1.19	-
Reduction quality	Poor	2 (7.4)	1 (3.5)	[d]0.664
	Acceptable	4 (14.8)	3 (10.3)	-
	Good	21 (77.8)	25 (86.2)	-

SHS: Sliding hip screw, CMN: Cephalomedullary nail, Min-max: Minimum-maximum; Av. + SD: Average + standart deviation, n: Number
[a]Student's t-test, [b]Mann-Whitney U test, [c]Pearson chi-square test, dFisher Freeman Halton test, *p<0.05, **p<0.01

The results of this study showed that treatment with CMN (average fracture settling 6.7 mm) caused significantly less femoral neck shortening, with almost 5 mm more shortening in the SHS group (average fracture settling 10.6 mm). However, this finding was not significantly correlated with any functional impairment in this study. Reindl et al. (20) reported similar results for unstable intertrochanteric fractures in a comparison between CMN and SHS.

A serious complication of these fractures is implant cut-out, for reasons including low bone density, unstable fracture, and insufficient reduction (32). Although the average Singh index value was 3.98±1.23 in the current study patients, no serious collapse or complications were detected. Furthermore, no implant cut-out or protrusion occurred in any patient. There was no significant difference between the SHS and CMN groups in respect of the Singh index and fracture settling.

Early post-operative mobilization of patients after hip fractures is fundamental to enable a return to normal life and to prevent medical complications (33). All the patients in the current study were mobilized on postoperative day 1 and weight-bearing was allowed as tolerated. Although radiological fracture healing was seen at mean 10 weeks in all patients, clinical healing was achieved in mean 6 weeks. There were no cases of implant cut-out, peri-implant femur fracture, and no wound site infections, despite weight-bearing permitted in the early post-operative period.

Study Limitation

The main limitation of this study was the loss of study participants, primarily because of mortality of almost 10% (n=6/64) within the first year after hip fracture, and a further two patients did not attend the last 2 follow-up visits. Another important limitation was the limited number of patients that preclude any definite conclusions being made.

Conclusion

The results of the current study regarding basicervical fracture treatment do not clearly favor one implant over another. CMN resulted in significantly less shortening across the fracture site compared to SHS, but this did not show any significant difference in the extremity or general function as measured with the Harris Hip score, Barthel index and mobility score. It can be considered that basicervical fractures should be classified as unstable intertrochanteric fractures, not as femoral neck fractures, as in the AO classification, because of the implants used and the nature of the fracture in response to treatment.

Authorship Contributions

Concept: E.E. Design: E.E. Data Collection or Processing: H.B.Ç. Analysis or Interpretation: G.B. Literature Search: H.B.Ç. Writing: H.B.Ç., E.E.

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The Relationship Between Serum Vaspin Levels and the Degree of Coronary Involvement in Patients with Stable Angina Pectoris

Stabil Angina Pektorisli Hastalarda Serum Vaspin Düzeyleri ile Damar Tutulum Derecesi Arasindaki İlişki

İlter Bozacı, Oktay Özkan*, Alev Arat Özkan**, Macit Koldaş***, Savaş Öztürk*

Bozyaka Training and Research Hospital, Clinic of Nephrology, İzmir, Turkey

**University of Health Sciences Turkey, Haseki Taining and Research Hospital, Clinic of Nephrology, İstanbul, Turkey*

***İstanbul University Institute of Cardiology, Department of Cardiology, İstanbul, Turkey*

****University of Health Sciences Turkey, Haseki Training and Research Hospital, Clinic of Biochemistry, İstanbul, Turkey*

Abstract

Aim: Vaspin is an insulin-sensitive adipokine secreted from visceral fat tissue, and belongs to the serine protease inhibitor family. The relationship between vaspin level and coronary artery disease is not known yet. We aimed to investigate the relationship between serum vaspin levels and degree of vessel involvement in coronary angiography in patients with stable angina pectoris.

Methods: The patients were chosen from those who had coronary angiography with the diagnosis of stable angina pectoris. Patients with previously diagnosed chronic heart disease, chronic liver disease, renal failure, thyroid dysfunction and any systemic infectious or malignant disease, patients receiving immunosupressive treatment and those who did not give informed consent were excluded from the study. Serum vaspin measurements were performed using an East Biopharm enzyme-linked immunoassay (ELISA) kit using the sandwich ELISA method. For determination of the severity of coronary lesions, the modified Gensini score was used.

Results: Eighty-eight patients [34 female (38.6%) and 54 male (91.4%)] were included in the study. Vaspin levels were similar in male (1.17±1.54 ng/L) and female (1.09±1.23 ng/L) patients (p=0.46). There was no correlation between vaspin levels and the number of vessels involved (p=0.75). Vaspin levels were similar in diabetic and nondiabetic patients.

Conclusion: Vaspin may not be a sensitive marker of the degree of vascular lesions in patients with stable angina pectoris. The underlying cause is probably lack of significant changes in inflammatory cascade and oxidative stress in the involved group of patients.

Keywords: Vaspin, angina pectoris, Gensini score

Öz

Amaç: Vaspin, serin proteaz ailesinden, insulin duyarlı bir adipokin olup visseral yağ dokudan salınmaktadır. Serum vaspin düzeyi ile koroner arter hastalığı arasında ilişki olup olmadığı henüz bilinmemektedir. Çalışmamızda, serum vaspin düzeylerinin koroner anjiografi yapılmış stabil anjina pektorisli hastalarda damar tutulum dereceleri ile ilişkisini araştırmayı amaçladık.

Yöntemler: Hastalar, stabil anjina pektoris tanısı ile koroner anjiografi yapılmış hastalar arasından seçildi. Bilinen kronik kalp hastalığı, kronik karaciğer hastalığı, böbrek yetmezliği, tiroit disfonksiyonu olan, immünsüpresif tedavi alan, herhangi bir sistemik enfeksiyöz veya malign hastalığı olanlar ile onam formu vermeyenler çalışmaya alınmadı. Serum vaspin ölçümleri sandviç enzim bağlı immün ölçüm (ELISA) metodu kullanarak east biopharm ELISA kiti ile yapıldı. Koroner lezyonların şiddeti modifiye Gensini skoru kullanılarak belirlendi.

Bulgular: Çalışmaya seksen sekiz hasta [34 kadın (%38,6) ve 54 erkek (%91,4)] dahil edildi. Vaspin düzeyleri erkeklerde (1,17±1,54 ng/L) ve kadınlarda (1,09±1,23 ng/L) benzerdi. (p=0,46). Vaspin düzeyleri tutulan damar sayısı ile korele değildi (p=0,75). Vaspin düzeyleri diyabetik ve diyabetik olmayan hastalarda benzer seviyelerde idi.

Sonuç: Vaspin, stabil anjina pektorisli hastalarda damar tutulum derecesi için hassas bir belirteç olmayabilir. Bunun nedeni dahil edilen grupta enflamatuvar kaskatta ve oksidatif streste belirgin değişim olmaması olabilir.

Anahtar Sözcükler: Vaspin, anjina pektoris, Gensini skoru

Address for Correspondence/Yazışma Adresi: İlter Bozacı, Bozyaka Training and Research Hospital, Clinic of Nephrology, İzmir, Turkey
Phone: +90 232 240 50 50/2136 E-mail: ilterbozaci@gmail.com ORCID: orcid.org/0000-0001-7535-9403
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Introduction

Atherosclerotic coronary artery disease is the leading cause of death in developed countries. The defined risk factors for cardiovascular diseases are positive family history, lipid metabolism disorders, advanced age, male gender, Diabetes Mellitus, insulin resistance and Metabolic syndrome, hypertension, sedentary life style, smoking, heavy alcohol use, elevated homocysteine, C-reactive protein (CRP) and fibrinogen levels and low estrogen levels (1-4). Interventions against these factors have led to significant improvements in morbidity and mortality in these patients.

Recent studies have revealed that adipose tissue had some endocrine functions besides being a fat store (5). Vaspin is an insulin-sensitive adipokine secreted from visceral fat tissue, and belongs to the serine protease inhibitor family (6). Serum vaspin level is positively correlated with obesity and insulin resistance (7,8). Aust et al. (6) reported a correlation between low vaspin levels and ischemic events within the last three months in patients with carotid artery stenosis due to atherosclerosis; although there was no relationship between vaspin levels and data related with the severity of stenosis.

The relationship between vaspin level and coronary artery disease is not known yet. Vaspin level has been found to be low in patients with coronary artery disease in some of the studies while it was reported to be elevated in others. We aimed to investigate the relationship between serum vaspin levels and the degree of vessel involvement in coronary angiography in patients with stable angina pectoris.

Methods

Patients

Patients were chosen from those who had coronary angiography with the diagnosis of stable angina pectoris. The study was started after obtaining approval from the local ethics committee (decision no: 2011-714198). All patients provided written informed consent. Demographics and clinical data of the patients including age, gender, smoking history, past medical history (Diabetes Mellitus, hypertension, dyslipidemia), family history and the treatment schedule were recorded. Weight and height of the patients were recorded. Body Mass index (BMI) was calculated with the formula–BMI=weight (kg)/height^2 (meters).

Exclusion Criteria

Patients with a previous diagnosis of chronic heart disease (previous myocardial infarction and/or coronary artery by-pass surgery, prominent valvular heart disease), chronic liver disease, renal failure, thyroid dysfunction and any systemic infectious or malignant disease, patients receiving immunosupressive treatment and those who did not gave informed consent were excluded from the study.

For the determination of risk factors for coronary artery disease, the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPI III), and the guideline for prevention and treatment of coronary artery disease published by the Turkish Society of Cardiology in 2002 were used (9,10). With this guidance, risk factors were defined as follows:

Age >45 years in males and >55 years in females was regarded as advanced age. Postmenopausal women were accepted as at risk without considering age. According to the definitions by international guidelines, patients with a BMI of 25-29.9 were considered overweight, and those with a BMI of ≥30 were regarded as obese (11). Positive family history was defined as a history of coronary artery disease in the first degree male relatives before the age of 55, and in the first degree female relatives before the age of 65 years. Patients with a previous diagnosis of type 2 Diabetes Mellitus or fasting blood glucose level higher than 125 mg/dL were recorded as diabetic. Hypertension was defined as a blood pressure of more than 140/90 mmHg; or being on antihypertensive treatment. Patients with a low-density lipoprotein (LDL) cholesterol (or total cholesterol) and triglyceride level greater than 130 mg/dL (or 200 mg/dL) and 150 mg/dL were accepted as hypercholesterolemic and hypertriglyceridemic, respectively. HDL cholesterol levels were regarded as low if below 40 mg/dL. Patients who smoke actively, or have been smoking until the last two years were accepted as smokers.

Laboratory Analysis

Blood samples were collected after 10-12 hours of fasting before coronary angiography for determination of creatinine, glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, high sensitive CRP (hs-CRP), interleukin-6 (IL-6), reactive oxygen species (ROS), nitric oxide (NO) and vaspin levels. Serum samples were stored at -20 °C until analysis. Serum vaspin and IL-6 measurements were performed by an East Biopharm ELISA kit using the sandwich enzyme-linked immunoassay (ELISA) method. The reference range was 0.05-10 ng/mL for vaspin and 2-600 ng/L for IL-6. Serum ROS and NO levels were measured by a cusabio ELISA kit using the sandwich ELISA method. The ranges were 0.16-10.00 ng/mL and 0-20 ng/mL for ROS and NO, respectively. Hs-CRP levels were studied by the turbidimetric method using a Siemens Advia 2400 auto analyzer (normal limits: 0-5 mg/dL).

Angiography Technique

After clinical and laboratory evaluation for risk factors, all patients had coronary angiography by the same operator. Coronary angiography was performed with the judkins technique after twelve hours of fasting. The right and left coronary arteries were viewed at multiple projections. The evaluation of angiographies and scoring of the lesions were performed by two operators.

Calculation of the Gensini score

For determination of the severity of coronary lesions, the modified Gensini score was used (11). The major parameters for this scoring system were the artery involved, the location and the degree of narrowing of the vessel. Coronary arteries were divided into 27 segments with each segment given scores between 0.5 and 5.0. Percentage of narrowing was scaled in the range of 2-64. Gensini score was obtained by multiplying these numbers. The patients were divided into two groups according to the degree of their vascular lesions in angiography.

Group 1: Those with lesions not causing critical stenosis in angiography and/or those with a Gensini score of less than 50.

Group 2: Those with lesions causing critical stenosis in angiography and/or those with a Gensini score of more than 50.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows 16.0. Numerical values were expressed as mean ± standard deviation. For intergroup comparisons, the paired samples t-test and Mann-Whitney U test were used when necessary. For nonnumeric values (gender, presence of diabetes mellitus, etc.), Fisher's exact test and Yates' chi-square test for 2x2 contingency tables were used when appropriate. Correlation analysis related with vaspin levels was performed with Spearman's rho, as vaspin had abnormal distribution. Linear regression analysis was used to determine factors affecting vaspin level. The dependent parameter was vaspin, while independent parameters were age, gender, the study group and hs-CRP levels. A p value of less than 0.05 was considered statistically significant.

Results

Eighty-eight patients [34 female (38.6%) and 54 male (91.4%)] were included in the study. The mean age was 61.2±9.8 years (33-77). Smoking history was positive in 44 patients (50%). The major comorbidities were hypertension (n=61; 69.3%), Diabetes Mellitus (n=36; 40.9%) and hyperlipidemia (n=30; 34.1%). Thirty-four patients (38.6%) had a family history of coronary artery disease.

The number of vessels involved was one in 21 patients (23.9%), two in 20 (22.7%), three in 13 (14.8%) and none in 34 patients (38.6%). The mean Gensini score was 43.67±51.01 (0-268). The patients were grouped according to Gensini score as described before. There were 50 patients in (57%) in group 1, and 38 patients (43%) in group 2. Demographic data, comorbidities and laboratory results of the groups are presented in Table 1. The levels of the cytokines studied are presented in Table 1 and Figure 1.

Vaspin and IL-6 levels were similar in male (1.17±1.54 ng/L and 151±276 ng/L, respectively) and female (1.09±1.23 ng/L and 119±251 ng/L, respectively) patients (p=0.46 and p=0.61, respectively), while NO level was significantly higher in female patients (4220±2038 ng/L vs 3354±1762 ng/L, p=0.024). ROS level was higher in female patients although the difference did not reach statistical significance (0.43±0.26 ng/L vs 0.35±0.24 ng/L, p=0.054). Vaspin, ROS, NOS, NO and IL-6 levels were not correlated with the number of vessels involved (p=0.75, p=0.27, p=0.43 and p=0.52, respectively). The patients were grouped according to the presence of Diabetes Mellitus and it was found that ROS level was significantly higher in the diabetic group (0.45±0.30 ng/L vs 0.33±0.20 ng/L, p=0.035), while vaspin, NO and IL-6 levels were similar in diabetic and nondiabetic patients.

Discussion

Cardiovascular diseases are the leading causes of death. Many risk factors have been recognized, and studies have been concentrated on early recognition and treatment of individuals with these risk factors. One of the most important risk factors is metabolic syndrome. Metabolic syndrome is the combination of obesity, cardiovascular diseases, type 2 Diabetes Mellitus, hypertension and hyperlipidemia (12). Metabolic syndrome is closely related with abdominal obesity (13). The imbalance between caloric intake and consumption causes hyperplasia and hypertrophy of the adipocytes and adipose tissue dysfunction (14). These processes lead to hyperlipidemia, elevated blood pressure, hypercoagulability and inflammation (15-17). Visceral adipose tissue has more important roles in production of proinflammatory cytokines and adipokines compared to other adipose tissues (18). One of these cytokines is vaspin which belongs to the serine protease inhibitor family and has insulin-sensitizing effects (19). Vaspin is produced by mature adipocytes, but is not expressed in stromal endothelial or vascular cells. Vaspin levels in the peripheral blood and in adipocytes have been found to be elevated in obese rats with insulin resistance (19). It was also found to be high in type 2 diabetics with or without obesity (20).

Inflammation is thought to have roles in the development of atherosclerosis and its chronic complications (1,21,22). Many studies have been performed to evaluate the relationship between atherosclerotic coronary artery disease and inflammation. Many bioactive substances, including vaspin, adiponectin, leptin, tumor necrosis factor-alpha, plasminogen activator inhibitor-1, interleukin-6, resistin and various growth factors, that are synthesized and secreted to the circulation by visceral adipose tissue have been defined recently. They were shown to have local and endocrine roles in the development of atherosclerosis (23-25). They are claimed to be responsible for early and accelerated atherosclerosis in obese individuals (26). The relationship between vaspin and coronary artery disease has not been evaluated yet. Inflammation is known to play a role in the development and progression of atherosclerosis, but the biochemical and cellular mechanisms have not been fully elucidated (27).

This study designed to investigate the relationship between serum vaspin levels and the degree of vessel involvement in coronary angiography in patients with stable angina pectoris.

It was performed in patients with stable angina pectoris who had mild (group 1) or severe (group 2) lesions in coronary angiography. Patients with a history of more severe cardiac disease (previous myocardial infarction and/or coronary artery bypass surgery, prominent valvular heart disease) were not included in the study. The mean Gensini score was 43.67±51.01 (0-268). Hypertension (n=61, 69.3%), Diabetes Mellitus (n=36, 40.9%) and hyperlipidemia (n=30, 34.1%) were the major comorbidities. Thirty-four patients (38.6%) had a family history of coronary artery disease, and 44 patients (50.0%) were smokers. Comparison of the groups regarding demographic and biochemical parameters yielded a higher frequency of female gender and smoking history in group 1 (Table 1).

Vaspin and IL-6 levels were similar in both genders, while NO and ROS levels were higher in women although only the difference regarding ROS level reached statistical significance. There was no correlation of the number of involved vessels with vaspin, ROS, NO and IL-6 levels. Vaspin, IL-6 and NO levels in diabetic and non-diabetic patients were similar. Thus, there was no correlation between the severity and the number of coronary arteries involved and markers of inflammation and oxidative stress. This fact may be due to patient selection criteria. Besides, diabetes mellitus was not a factor affecting vaspin levels.

Kadoglou et al. (28) reported lower vaspin levels in patients with coronary artery disease compared to healthy individuals. In their study including 40 patients with stable angina pectoris without Diabetes Mellitus, morbid obesity, history of coronary artery disease and/or coronary revascularization and 40 healthy controls, Kobat

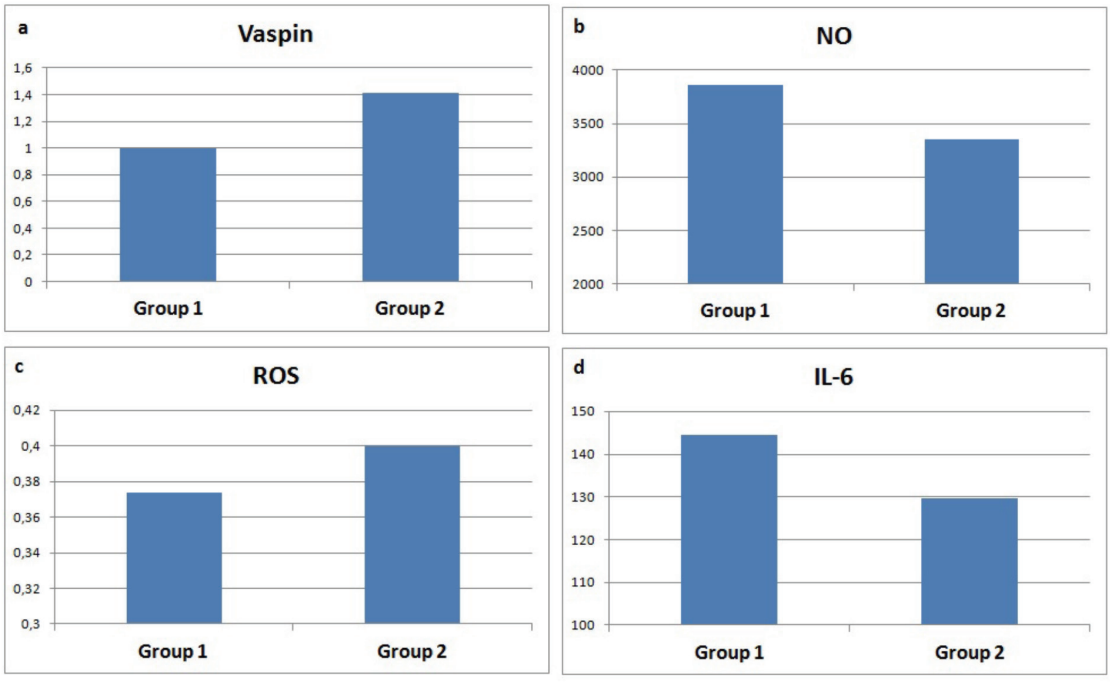

Figure 1. The comparison of the cytokine levels between groups

NO: Nitric oxide, ROS: Reactive oxygen species, IL-6: Interleukin

et al. (29) found that the mean vaspin level in the patient group was significantly lower than in the control group. Zhang et al. (30) reported the lowest values of vaspin in patients with acute myocardial infarction followed by in those with unstable angina pectoris, stable angina pectoris and no coronary artery disease, in ascending order. Aust et al. (6) evaluated serum vaspin concentrations in patients with carotid stenosis who underwent carotid endarterectomy. They reported that vaspin level was not correlated with the severity of carotid lesions, but vaspin level was significantly lower in patients with carotid artery stenosis with a history of ischemic stroke within the last three months. They detected no difference in vaspin levels before and after endarterectomy.

There is no clear explanation for different data in the literature including the presented study. However, it can be said that it is difficult to evaluate vaspin level in all patients with coronary artery disease. Besides, there are many factors that may interfere with vaspin levels including obesity, Diabetes Mellitus and many pharmacological and non-pharmacological factors affecting levels of both cytokines and adipokines. Weight loss, exercise, thiazolidinediones, metformin, salicylates, angiotensin II receptor blockers, angiotensin converting enzyme inhibitors, statins, fibrates and beta blockers are among other factors affecting vaspin level (31,32).

The other important finding in the presented study is the similar vaspin levels in patients with and without diabetes mellitus (0.80±0.41 ng/L vs 1.38±1.80 ng/L, p=0.03). It has been reported that vaspin level decreased in the presence of diabetic microvascular complications, although the issue is not clear because the proteases acting on vaspin release in diabetes mellitus have not been elucidated yet (32). On the other hand, vaspin level increases as the Hemoglobin A1c (HbA1c) level rises, possibly due to compensatory effect of vaspin (33). Thus, change in vaspin level may be regarded as a factor associated with chronic vascular disease besides its relationship with metabolic and vascular changes.

Study Limitations

The major limitation of our study is lack of patients with more severe coronary artery disease. Inclusion of another group composed of patients with unstable angina pectoris or myocardial infarction would more clearly delineate differences in vaspin levels and the role of other markers. Moreover, availability of data regarding medications given to study participants would allow more

Table 1. Distribution of demographic and biochemical analysis according to the patient groups

	Group 1 (n=58)			Group 2 (n=30)			p
	Mean	Min	Max	Mean	Min	Max	
Age	59.8±10.1	33.0	77.0	63.7±8.8	47.0	77.0	0.077
Gender (F/M)	30/28			4/26			<0.001
Family history (%)	43.1			30.0			0.23
Smoking (%)	55.2			40.2			<0.001
DM (%)	43.1			36.7			0.56
HT (%)	70.7			66.7			0.70
Hyperlipidemia (%)	31.0			40.0			0.40
BMI (kg/m^2)	29.5±5.6	17.9	45.0	28.6±3.3	22.9	36.3	0.42
Glucose (mg/dL)	123±49	80	419	121±45	77	265	0.865
HDL (mg/dL)	45±14	27	86	44±10	23	64	0.645
hsCRP (mg/dL)	2.43±1.90	0.10	9.40	2.71±2.1	0.10	6.80	0.52
Creatinine (mg/dL)	0.95±0.14	0.60	1.40	0.96±0.19	0.21	1.40	0.88
LDL (mg/dl)	121±33	43	194	130±43	64	238	0.31
Leukocyte (10^3/mm^3)	6.82±1.26	4.58	9.80	7.30±1.5	4.80	10.60	0.118
IL6 (ng/L)	144±282	3	1331	129±238	15	1098	0.814
Vaspin (ng/L)	0.99±1.03	0.49	6.29	1.41±1.97	0.51	8.21	0.19
NO (ng/L)	3861±2043	858	11049	3357±1602	1248	7864	0.243
ROS (ng/L)	0.37±0.23	0.06	1.49	0.40±0.3	0.06	1.22	0.654

Min: Minimum, Max: Maximum F: Female, M: Male, DM: Diabetes Mellitus, HT: Hypertension, BMI: Body mass index, HDL: High density lipoprotein, hsCRP: High sensitive C-reactive protein LDL: Low density lipoprotein, IL-6: Interleukin -6, NO: Nitric oxide, ROS: Reactive oxygen species

precise comparison. Inclusion of HbA1c in the statistical analysis would reveal another relationship.

Conclusion

Vaspin may not be a sensitive marker of the degree of vascular lesions in patients with stable angina pectoris. The underlying cause is probably lack of significant changes in inflammatory cascade and oxidative stress in the involved group of patients. Further controlled studies in diabetic and non-diabetic patients with more severe coronary artery disease are warranted.

Authorship Contributions

Concept: İ.B., S.Ö., A.A.Ö., M.K., O.Ö. Design: S.Ö., O.Ö., İ.B., A.A.Ö. Data Collection or Processing: İ.B., S.Ö., A.A.Ö., M.K. Analysis or Interpretation: S.Ö., İ.B., M.K. Literature Search: İ.B., O.Ö. Writing: İ.B.

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C-reactive Protein to Albumin Ratio as A Novel Inflammatory Biomarker for Postoperative Delirium in Patients Undergoing Transcatheter Aortic Valve Replacement

Transkateter Aort Kapak Replasmanı Uygulanan Hastalarda Postoperatif Deliryum İçin Yeni Bir Enflamatuvar Biyobelirteç Olarak C-reaktif Protein/Albümin Oranı

Muhsin Kalyoncuoğlu, Halil İbrahim Biter, Gündüz Durmuş, Birgül Baştan*, Mehmet Mustafa Can

University of Health Sciences Turkey, Haseki Training and Research Hospital, Clinic of Cardiology, İstanbul, Turkey
**University of Health Sciences Turkey, Haseki Training and Research Hospital, Clinic of Neurology, İstanbul, Turkey*

Abstract

Aim: We aimed to investigate whether C-reactive protein-to-albumin ratio (CAR) predicts postoperative delirium (POD) development in patients undergoing transcatheter aortic valve replacement (TAVR) procedure.

Methods: Data of 78 patients with the mean age of 76.3±8.4 years, who underwent TAVR, were retrospectively analyzed. The CAR, neurophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio values were calculated in all patients. Presence of delirium was determined by using the Richmond Agitation-sedation scale and the Confusion Assessment Method for the Intensive Care Unit.

Results: As compared with the nondelirious group, delirious patients had significantly higher EuroSCORE II (p=0.03) and TAVR score (p=0.009) and more frequent major bleeding (p=0.005), major vascular complications (p=0.018) and acute kidney injury (AKI) (p=0.002). The main independent predictors of POD were CAR value (p=0.02), AKI (p=0.02), major bleeding (p=0.005), and TAVR score (p=0.04). The area under the curve of CAR for POD development was 0.718 (95% confidence interval: 0.605-0.814; p=0.002) with 82% sensitivity and 56% specificity.

Conclusion: CAR is a promising inflammatory parameter in predicting POD and may help identify subgroups of individuals at risk for POD.

Keywords: Transcatheter aortic valve replacement, inflammation, C-reactive protein, serum albumin, delirium

Öz

Amaç: Transkateter aort kapak replasmanı (TAVR) uygulanan hastalarda C-reaktif protein albumin oranının (CAO) postoperative deliryum (POD) gelişimini tahmin edip etmediğini araştırmayı amaçladık.

Yöntemler: TAVR uygulanan 78 hastanın (yaş ortalaması: 76,3±8,4 yıl) verileri retrospektif olarak incelendi. Tüm hastaların CAO, nörofil lenfosit oranı ve trombosit/lenfosit oranı değerleri hesaplandı. Deliryum varlığı, Richmond Ajitasyon-Sedasyon skalası ve Yoğun Bakım Ünitesi skalası için Konfüzyon Değerlendirme Yöntemi skalası kullanılarak değerlendirildi.

Bulgular: Deliryum olmayan grupla karşılaştırıldığında, POD hastaları anlamlı derecede daha yüksek Euroscore II (p=0,03) ve TAVR skorlarına (p=0,009) sahip idi. POD hastalarında daha sık majör kanama (p=0,005), majör vasküler komplikasyonlar (p=0,018) ve akut böbrek hasarı (ABH) geliştiği gözlemlendi (p=0,002). CAO değeri (p=0,02), ABH (p=0,02), major kanama (p=0,005) ve TAVR skoru (p=0,04) POD gelişiminin bağımsız öngördürüleri idi. POD gelişimi için CAO eğrisi altındaki alan %82 duyarlılık ve %56 özgüllük ile 0,718 (%95 güven aralığı: 0,605-0,814; p=0,002) olarak bulundu.

Sonuç: CAO, POD'yi tahmin etmede umut verici bir enflamatuvar parameter olup POD riski taşıyan bireylerin identifiye edilmesine yardımcı olabilir.

Anahtar Sözcükler: Transkatater aort kapak replasmanı, enflamasyon, C-reaktif protein, serum albümin, deliryum

Address for Correspondence/Yazışma Adresi: Muhsin Kalyoncuoğlu, University of Health Sciences of Turkey, Haseki Training and Research Hospital, Clinic of Cardiology, İstanbul, Turkey
Phone: +90 212 531 59 47 E-mail: mkalyoncuoglu80@gmail.com ORCID: orcid.org/0000-0002-6552-0942
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Introduction

Nowadays, transcatheter aortic valve replacement (TAVR) has emerged as an alternative to surgical aortic valve replacement (SAVR) for the treatment of patients with symptomatic severe calcific aortic valve disease (CAVD) who are not candidates for surgery or who are considered to be at a high surgical risk (1). Delirium is a neuropsychiatric behavioural syndrome characterised by acute changes and fluctuations in attention, thinking, and consciousness (2,3). Postoperative delirium (POD) is frequently observed after TAVR and proposed to be associated with several adverse outcomes including prolonged hospital stay, readmission, and mortality after TAVR (3). Since it is an important source of clinical concern, attempting to determine independent risk factors for POD and establishing an early diagnosis are of great importance in improving the outcome.

Despite the knowledge about the epidemiology of POD, its pathophysiology remains unclear. Inflammation is one of the various proposed mechanisms and there is growing evidence that a systemic inflammatory process may play a role in the development of POD (4,5). Previous reports have also proposed that CAVD represents an inflammatory state of the valve interstitium similar to that seen in atherosclerosis and, even after TAVR, the inflammatory state persists in almost half of the patients (6,7). As such, identifying accurate biomarkers for POD may shed light on the pathophysiology and potentially improve delirium recognition and prediction after TAVR. Several inflammatory markers, such as proinflammatory cytokines, chemokines, and the neutrophil-to-lymphocyte ratio (NLR), have been proposed to be associated with POD in various clinical conditions including cardiac surgery (8-10). In addition, although there are conflicting results, delirium has been found to be associated with proteins involved in the stress response, including the markers of systemic inflammation such as positive acute-phase reactant C-reactive protein (CRP) and negative acute-phase reactant albumin (2,4,5,11). Recently, a novel inflammatory marker defined as the CRP-to-albumin ratio (CAR) has been proposed as more valuable than either CRP or albumin alone in predicting inflammatory status and prognosis in various clinical settings (12,13). To our knowledge, there are no data available regarding the association between CAR and POD in patients undergoing TAVR. Therefore, in this study, we sought to investigate the predictive value of CAR in determining POD development.

Methods

We performed a retrospective analysis of collected data of 81 consecutive patients, who underwent TAVR between January 2015 and March 2018, from the records of the cardiology department of our hospital. Data on patient demographics, comorbidities, primary cardiac acute illness, medical treatment, electrocardiogram, chest X-ray, echocardiogram, multislice computed tomography of the aorta and branches, cine coronary angiography, length of hospital stay, clinically significant in-hospital acute adverse events, Richmond agitation-sedation scale (RASS) score, Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) score and mortality data were obtained using a computerised system and or patient file records or by way of follow-up visits of the patients. The exclusion criteria of the present study were as follows: patients undergoing combined procedures such as concurrent percutaneous coronary intervention, patients with an estimated life expectancy of less than one year, patients with a significant mental impairment, and concomitant inflammatory conditions (i.e., active infection, inflammatory arthritis, inflammatory bowel disease, or connective tissue disease) or malignancies, or those who had recent (<2 months) surgery or major trauma. Of 81 patients, three patients were excluded; one (1.3%) due to insufficient data two for having a history of cognitive impairment. Consequently, the study population consisted of 78 patients with a life expectancy of at least one year who were considered to be at high surgical risk for SAVR on the basis of clinical assessments by a multidisciplinary heart team (2,3). Thirty-day and 37-month clinical follow-up data were obtained via outpatient visit or telephone contact. One hundred percent of patients adhered to the follow-up schedule after the index operation.

Severe aortic stenosis is defined as a valvular orifice area of less than 1.0 cm^2 or less than 0.6 cm^2/m^2 and/or a mean pressure gradient of more than 40 mmHg and/or a jet velocity of more than 4.0 m/s. The heart team used a guideline that was based on a risk model developed by the EuroSCORE II (ES II) to estimate the risk of death after the index TAVR procedure. ES II and Society of Thoracic Surgeons (STS)/American College of Cardiology (ACC) transcatheter valve therapy TAVR (TAVR score) scores were calculated using online tools (www.euroscore.org and https://tools.acc.org/tavrrisk).

The primary endpoint of this study was the presence of delirium on any day during the in-hospital stay after TAVR. Other clinical outcomes, such as all-cause mortality within 30 days, all-cause, cardiovascular, and noncardiovascular mortality after 30 days during follow-up, myocardial infarction, stroke, acute kidney injury (AKI), pacemaker requirement, life-threatening or disabling major bleeding, and major vascular complications, were adjudicated according to the Valvular Academic Research Consortium (VARC) criteria (14).

The default access for TAVR was transfemoral and valve choice was decided at the discretion of the heart team. The procedure was performed in the cardiac catheterization laboratory under conscious sedation (mostly) or general anaesthesia with transesophageal echocardiography guidance. All patients received aspirin (81 mg) and clopidogrel (≥300 mg) before the procedure and heparin during the procedure; the patients continued to take aspirin indefinitely and clopidogrel for a minimum of one month. After the index procedure, all patients were followed up at 30 days, six and 12 months, and yearly thereafter. Written informed consent was not obtained from the participants due to retrospective nature of the study, but our study protocol conformed to the principles of the Declaration of Helsinki and was approved by the University of Health Sciences of Turkey, Haseki Training and Research Hospital Ethics Committee (no: 116/09.05.2019).

Laboratory Measurements

Routine complete blood cell count and blood evaluations for determining the blood glucose, creatinine, albumin, and CRP levels were performed using the admission blood samples. Serum albumin and CRP levels were measured by using a Roche Diagnostics Cobas 8000 c502 analyser (Roche Holding AG, Basel, Switzerland). CAR was calculated as the ratio of serum CRP level (mg/L) to serum albumin level (mg/L) multiplied by 100 for easy interpretation, as done in previous studies (12). NLR was calculated by dividing the neutrophil count by the lymphocyte count. The estimated glomerular filtration rate (eGFR) was calculated by using the Modification of Diet in Renal Disease formula.

Delirium Evaluation

In our institution as part of routine care, all TAVR patients were screened for the presence of delirium using the RASS at admission and then once daily by coronary intensive care unit (CICU) physicians and cardiovascular nurses (15). Presence of delirium was determined according to the CAM-ICU at admission and then if the RASS was−3 or greater to avoid inclusion of comatose/unconscious patients (16).

The CAM-ICU value was considered positive for delirium if patients had a RASS score of -3 or greater and had an acute change or fluctuation in mental status plus inattention and either disorganized thinking or an altered level of consciousness. The patients were divided into two groups according to delirium status, i.e., delirious and non-delirious.

Statistical Analysis

Categorical variables are given as frequencies and percentages. The chi-square (χ^2) test was used to compare the categorical variables between the groups. Continuous variables were given as mean ± standard deviation (if normal distribution) and medians (interquartile ranges) (if not normal distribution). The Kolmogorov-Smirnov test was used to assess whether the variables were normally distributed. The student's t-test or Mann-Whitney U test was used to compare continuous variables between the groups according to whether they were normally distributed or not. In order to identify the independent preprocedural risk factors for POD, univariate and backward stepwise multivariable logistic regression analyses were performed. Only the variables with a p value of less than 0.1 in univariate analysis were incorporated in the multivariate logistic regression analysis. Variables already included in the ACC/STS TAVR score and which exhibited excellent correlation with CAR and NLR were not considered separately in multivariable analysis, independently of their significance in univariable analysis. The receiver operating characteristics (ROC) curve analysis was used to evaluate the sensitivity and specificity of the CAR and its cut-off value for predicting POD. The association between POD and mortality was analysed using Kaplan-Meier curves created by delirium status and the long-rank test. The results were evaluated within a 95% confidence interval (CI) and at a significance level of $p<0.05$. All statistical analyses were carried out using the Statistical Package for the Social Sciences version 24.0 (IBM Corp., Armonk, NY, USA).

Results

Baseline Characteristics

The median follow-up period was 18 [interquartile range (IQR): 10-25] months with a maximum of 37 months. The mean age was 76.3±8.4 years and 46 (59%) patients were female**.** The recorded admission diagnosis was heart failure in 53 (67.9%), angina or angina-equivalent symptoms in 20 (25.6%), and presyncope or syncope in five (6.4%); none of the patients suffered sudden cardiac death. The mean aortic valve area (AVA) was 0.61±0.12 cm^2 and mean transvalvular gradient was 50.6±6.1 mmHg. Twenty-three (29.5%) patients had severe symptoms defined as New York Heart Association (NYHA) classes III/IV. Additionally, the mean ES II was 5.2±2.1% and TAVR risk score was 3.7±1.7%.

The procedure was performed by using conscious sedation in 64 (82.1%) patients or general anaesthesia in 14 (17.9%) patients. Urgent procedures were performed in 18 (23.1%) patients. Fifty-one (65.4%) patients received a self-expandable valve, while 27 (34.6%) received a balloon-expandable valve. Complications included new pacemaker insertion in 13 (16.7%), any VARC2-defined major vascular injury in seven (9%), major bleeding in 14 (17.9%), and AKI in 10 (12.8%) patients.

Three (3.7%) patients required dialysis treatment. None of the patients experienced myocardial infarction or permanent stroke or required surgical intervention peri-TAVR or post-TAVR. Detailed baseline demographic, and clinical, echocardiographic, pre- and post-procedural characteristics of the population are summarised in Table 1 and 2.

Factors Associated with Postoperative Delirium

In the present study, POD diagnosed using the CAM-ICU was observed in 17 (22%) patients. Delirious patients had higher frequency of heart failure, severe chronic obstructive pulmonary disease (COPD), and NYHA classes III and IV than did non-delirious patients (p=0.08, p=0.05, and p=0.07, respectively), although not in a statistically significant fashion. Regarding blood examinations, serum CRP level (p=0.007) and neutrophil count (p=0.04) were higher in the delirious group, while serum albumin level was lower (p=0.04). As compared with that in the non-delirious group, delirious patients also had significantly higher values of CAR (p=0.006). NLR value was also found to be higher in delirious patients, but this finding had borderline statistical significance (p=0.06). According to echocardiographic examination, AVA and mean aortic valve gradient were not different between the two groups. Besides that, left ventricular ejection fraction (LVEF) was lower (p=0.03), whereas pulmonary artery systolic pressure was higher (p<0.001) in delirious patients than in the others. eGFR was also found to be lower in those suffering from POD (p=0.04). When considering the procedural risk assessment scoring systems, ES II and TAVR score were significantly higher in the delirious group than in the non-delirious group (p=0.03 and p=0.009, respectively). Additionally, major bleeding, major vascular complications, and AKI were more frequent in patients with POD (p=0.005, p=0.018, and p=0.002, respectively).

Table 1. Baseline demographic, clinical, laboratory parameters of study population

Variables	All population	No Delirious (n=61)	Delirious (n=17)	p
Female gender	46 (59)	37 (60.7)	9 (52.9)	0.57
Age, years	76.3±8.4	75.7±8.7	78.7±6.8	0.19
Hypertension	52 (66.7)	39 (63.9)	13 (76.5)	0.3
Diabetes Mellitus	25 (32.1)	17 (27.9)	8 (47.1)	0.1
Heart failure	36 (46.2)	25 (41)	11 (64.7)	0.08
Vascular disease	38 (48.7)	29 (47.5)	9 (52.9)	0.69
CVA history	7 (9)	4 (6.6)	3 (17.6)	0.16
CKD history	25 (32.1)	16 (26.2)	9 (52.9)	0.04
COPD	26 (33.3)	17 (27.9)	9 (52.9)	0.05
NYHA Class III-IV	23 (29.5)	15 (24.6)	8 (47.1)	0.07
Atrial fibrillation	16 (20.5)	12(19.7)	4 (23.5)	0.7
Presence of BBB	17 (21.8)	13 (21.3)	4 (23.5)	0.9
FBG, mg/dL	141±63.2	138±59.6	151.8±75.9	0.43
eGFR, mL/min/1.73m^2	66.7±24.4	69.6±23.7	56.2±24.4	0.04
CRP, mg/L	5.5 (2.2-11.4)	4.4 (2.0-9.1)	9.55 (5.5-18.5)	0.007
Albumin, g/L	38 (33.3-40)	39 (34.8-41.3)	34 (30.9-39.5)	0.04
CAR, (x100)	14.8 (5.3-32.3)	11.8 (4.8-26.8)	29.5 (14.5-59)	0.006
Hematocrit, %	35.5±4.5	35.9±4.4	34.1±4.7	0.15
Neutrophil, 10^3/µL	4.7 (3.4-7.1)	4.3 (3.3-6.1)	7.1 (3.9-12.1)	0.04
Lymphocyte, 10^3/µL	1.62 (1.2-2.2)	1.61 (1.2-2.2)	1.64 (0.98-2.1)	0.8
NLR	2.9 (2.1-4.8)	2.63 (2.1-3.9)	4.0 (2.4-9.2)	0.06
TAVR score, %	3.7±1.7	3.4±1.5	4.9±1.8	0.009
Euroscore II, %	5.2±2.1	4.9±2.0	6.1±2.1	0.03

CVA: Cerebrovascular accident, CKD: Chronic kidney disease, COPD: Chronic obstructive pulmonary disease, NYHA; New York Heart Association, BBB: Bundle branch block, FBG: Fasting blood glucose, eGFR: Estimated glomerular filtration rate, CRP: C-reactive protein, CAR: CRP to albümin ratio, NLR: Neutropil to lymhocyte ratio, TAVR: Transcatheter aortic valve replacement, n: Number
Values are n (%), median (interquartile range), or mean ± standart deviation

Independent Predictors of Postoperative Delirium

In order to determine the independent predictors of POD, we performed multivariable logistic regression analysis by incorporating variables that showed statistically significant associations in the univariate analysis. CRP and neutrophil were not included in the regression analysis because of excellent correlation between CAR and CRP (r=0.983; p<0.001) and between neutrophil and NLR (r=0.718; p< 0.001), but albumin was included since it exhibited a moderate correlation with CAR (r=-0.467; p<0.001). In addition, ES II was not included in the multivariable regression analysis because it encompasses many POD-related parameters such as creatinine clearance, pulmonary artery pressure, LVEF, NYHA class, and COPD and is also not a TAVR-specific scoring system. Conversely, because the TAVR score contains relatively less POD-related variables and is a TAVR-specific scoring system, it was included in the multivariate regression analysis. In the multivariable analysis, postoperative AKI (p=0.02), major bleeding (p=0.005), TAVR score (p=0.04), and CAR (p=0.02) were found to be independent predictors for the development of POD (Table 3). To test the predictive performance of CAR, we performed ROC curve analysis. The area under the curve of CAR for POD development was 0.718 (95% CI: 0.605–0.814; p=0.002) with a cut-off value of greater than 13 (82% sensitivity and 56% specificity) (Figure 1).

Postoperative Delirium and Overall Survival

At 30 days and 37 months, the mortality rate was higher in the delirious group than in the nondelirious one (p=0.002 and p<0.001, respectively). POD was significantly associated with short-term (≤30 days) [p=0.009; hazard ratio (HR): 0.186; 95% CI: 0.052-0.658] and late mortality (p<0.001; HR: 0.193; 95% CI: 0.088-0.422) after TAVR in univariate Cox regression analysis. The association between POD and mortality is depicted by Kaplan-Meier plots of survival curves in Figure 2.

Discussion

The main findings of the present study are as follows: 1) POD is common after TAVR and occurs in about one-fifth of patients; 2) higher preoperative CAR values, procedure-related major bleeding, postoperative AKI, and TAVR score are independently associated with the

Table 2. Echocardiographic, pre-and post-procedural parameters of study population

Variables	All population	No delirium (n=61)	Delirium (n=17)	p
AVA, cm^2	0.61±0.12	0.63±0.13	0.58±0.12	0.16
Mean AVG, mmHg	50.6±6.1	50.1±6.2	52.4±5.4	0.19
LVEF, %	47.8±8.3	48.9±8	43.8±8.4	0.03
PAPs, mmHg	49±9.8	47.1±9.3	55.8±9.0	<0.001
Urgent procedure	18 (23.1)	10 (16.4)	8 (47.1)	0.008
Conscious sedation	64 (82.1)	51 (83.6)	13 (76.5)	0.5
Type of valve	-	-	-	0.2
Self-expandable	51(65.4)	42 (68.9)	9 (52.9)	-
Balloon-expandable	27 (34.6)	19 (31.1)	8 (47.1)	-
Predilatation	36 (46.2)	27 (44.3)	9 (52.9)	0.5
Postdilatation	14 (17.9)	11 (18)	3 (17.6)	0.97
Major bleeding	14 (17.9)	7 (11.5)	7 (41.2)	0.005
Acute kidney injury	10 (12.8)	4 (6.6)	6 (35.3)	0.002
Major vascular complications	7 (9)	3 (4.9)	4 (23.5)	0.018
Permanant pacemaker	13 (16.7)	12 (19.7)	1 (5.9)	0.18
Myocardial infarction	0 (0)	0 (0)	0 (0)	-
Stroke	0 (0)	0 (0)	0 (0)	-
Hospitalization, days	6 (4-8)	5 (4-7)	10 (6-12.5)	<0.001
Rehospitalization	14 (17.9)	9 (14.8)	5 (29.4)	0.16
Early mortality (≤30 days)	10 (12.8)	4 (6.6)	6 (35.3)	0.002
Late mortality (>30 days)	26 (33.3)	13 (21.3)	13 (76.5)	<0.001

AVA: Aortic valve area, AVG: Aortiv valve gradient, LVEF: Left ventricular ejection fraction, PAPs: Systolic pulmonary artery pressure, n: Number
Values are n (%), median (interquartile range), or mean ± standard deviation

development of POD, while NLR is not; 3) a CAR value of greater than 13 is a predictor of POD development; and 4) POD is significantly related with both early and late overall mortality.

In accordance with the literature, the incidence of POD on the basis of the CAM-ICU criteria was 22% in the current study cohort (3,17,18). In comparison, the incidence of delirium in the existing literature ranges from 12% to 53% after TAVR (18). This variation in the POD incidence may in part be due to the lack of frequent assessments, different assessment methods of delirium (e.g., medical records, diagnosis codes), and a lack of standardised criteria for diagnosis (19).

Although no consensus regarding the risk factors for POD has yet been established, probably due to its complicated pathogenesis, a number of predisposing

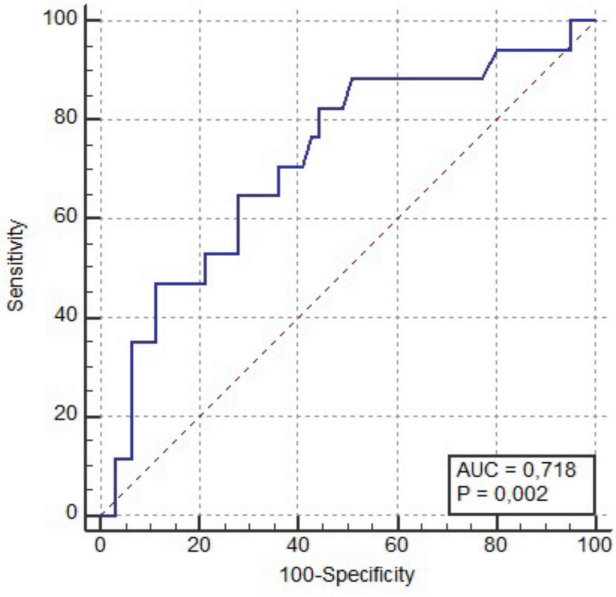

Figure 1. ROC curves of the C-reactive protein to albumin ratio for prediction the postoperative delirium development

ROC: Receiver operative characteristics, AUC: Area under the curve

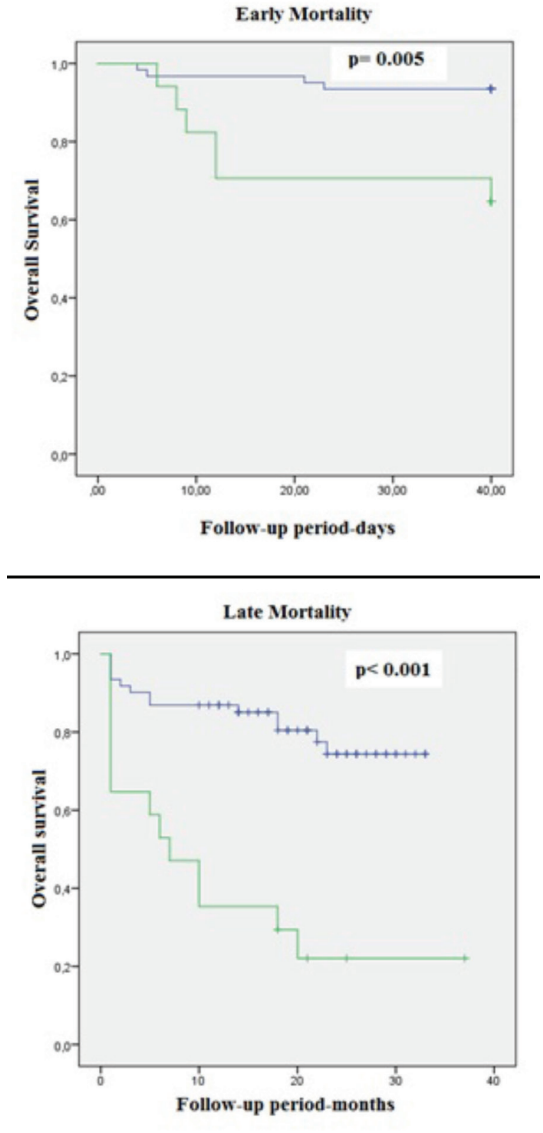

Figure 2. Kaplan-Meier plots of survival curves of deliriuos and no delirious patients

Table 3. Univariable and multivariable regression analysis for determining the predictors of the postoperative delirium development

Variables	Univariable		Multivariable	
	OR (95% CI)	p	OR (95% CI)	p
AKI	0.129 (0.031-0.532)	0.005	0.136 (0.024-0.765)	0.02
Major bleeding	0.185 (0.053-0.644)	0.008	0.080 (0.014-0.466)	0.005
CAR	1.027 (1.005-1.049)	0.015	1.039 (1.006-1.073)	0.02
LVEF	0.928 (0.868-0.994)	0.03	1.041 (0.940-1.154)	0.44
COPD	0.343 (0.114-1.037)	0.06	0.279 (0.064-1.212)	0.09
TAVR score	1.706 (1.219-2.387)	0.002	1.621 (1.033-2.541)	0.04

AKI: Acute kidney injury, CAR: CRP to albumin ratio, LVEF: Left ventricular ejection fraction, COPD: Chronic obstructive pulmonary disease, TAVR: Transcatheter aortic valve replacement, OR: Odds ratio, CI: Confidence interval

and precipitating risk factors have been identified to date (3,11,20). Predisposing factors, such as age, physical status, previous vascular disease, prior stroke, hypertension, diabetes, atrial fibrillation, NYHA classes III and IV, COPD, LVEF, and ES, are generally nonmodifiable and characterise a person's susceptibility to developing delirium, while precipitating factors, like medications, major surgery, TAVR, infectious disease, AKI, bleeding, and metabolic alterations, are modifiable items that trigger the onset of delirium (3,20-22). In the current study, none of these aforementioned predisposing risk factors, such as stroke, hypertension and COPD, were found to be predictors. To the best of our knowledge, there is no evidence to assess the relationship between TAVR score and delirium. In this study, among the predisposing factors, only TAVR score was found to be a predictor of the development of POD. On the other hand, among the precipitating factors, procedure-related AKI and bleeding were found to be independent predictors of POD development, in line with previous reports (20,21). Although there is no established protocol for the prevention of periprocedural AKI, adequate hydration and avoidance of nephrotoxic medications may constitute preventative therapy. In addition, a previous study showed that anemia increases the risk of POD, whereas a blood transfusion reduces its incidence (23). So, the incorporation of blood transfusions might prevent POD in patients after TAVR.

Despite the condition's observed high prevalence and negative effect on outcomes, the pathophysiology of POD remains unclear (4,9,22). Previous reports have proposed that systemic inflammation contributes to the pathogenesis of POD by compromising blood–brain barrier integrity and consequently promoting neuronal injury, causing neurocognitive behavioural abnormalities (4,5,9). Several immune and inflammatory alterations such as increased acute-phase reactants and abnormal levels of inflammatory cytokines detected in both the serum and cerebrospinal fluid of delirious individuals have been reported in the literature (4,8-11,24). Therefore, the identification of inflammatory markers associated with POD can improve our understanding of POD pathophysiology and ultimately leads to new interventions that may improve patient outcomes.

CRP, one of the most common markers for systemic inflammation, has been linked with increased cardiovascular and allcause mortality following TAVR (25,26). In addition, although there are conflicting data, the previous literature sought to examine the relationship between pre- and postoperative elevated CRP levels and the development of POD in patients undergoing cardiac and noncardiac surgery (2,27,28). In the present study, because of its excellent correlation with CAR, we did not analyse the predictive value of preoperative plasma CRP level in determining POD, but did note that baseline CRP levels were higher in delirious patients than in non-delirious ones. The association of preoperative hypoalbuminemia with the development of POD has also been reported in various surgical populations (11,28,29). This relationship can be explained by several mechanisms, such as reduced transport of delirogenic medication with consequent increased risks of side effects and drug activity. Second, hypoalbuminemia is an important indicator of the acute-phase reaction and activation of the immune system. Therefore, patients with POD have a stronger acute-phase reaction and activation of the immune system followed by an increasingly damaging cytokine influence on the transmitter systems of the central nervous system (29). CAR is a novel inflammatory parameter and was first described by Fairclough et al. (12). CAR contains both CRP and albumin parameters and reflects not only the proinflammatory state, but also the nutritional status. Therefore, the combination of albumin and CRP in a single index may be more valuable and provide both inflammatory and nutritional information. In addition, this combination as a prognostic score provides stability between fluctuating CRP and albumin levels in diseases where inflammation plays an important role (30). Considering these aforementioned reasons, the combination of albumin and CRP in a single index has previously been proposed and subsequent studies have shown that the CAR was more sensitive and specific in the prediction of the systemic inflammatory state and prognosis than either serum CRP or albumin level alone in various clinical conditions (12,13,30,31). To our knowledge, this is the first study evaluating the association between CAR and the development of POD in patients who underwent transfemoral TAVR. In the current study, we observed that delirious patients had higher preoperative CAR values, and CAR values of more than 13 were found to be predictive of POD development. We know that CAVD is the most common valvular heart disease in the elderly and inflammation plays an important role in the development and progression of CAVD, with similarities to atherosclerosis (32). In addition, there is also increasing evidence that aging is associated with increased basal neuroinflammation that manifests as increased levels of activated microglia, the immune cells of the brain (33). As a result, higher CAR values may be an indicator of this basal inflammatory condition, which may have a priming effect, leading to an increased response to peripheral stimuli such as surgery (34).

NLR, another inflammatory marker, has been reported to serve as a valuable indicator of systemic inflammation

in comparison with traditional markers such as total white blood cell (WBC) count, individual WBC subtypes, and CRP in various clinical conditions (24,35). NLR has been investigated in the context of neuropsychiatric disorders such as cerebrovascular disease, schizophrenia, and Alzheimer's disease to quantify the systemic inflammation in these disorders (24). Egberts and Mattace-Raso (24) in their study observed increased NLR levels in delirious patients and suggested that an inadequate response of the immune system and oxidative stress may play a role in the pathogenesis of delirium. To the best our knowledge, no previous study has investigated a possible association between NLR and POD in patients who underwent TAVR. Although preprocedural NLR is higher in patients with delirium, contrary to Egberts and Mattace-Raso (24), we have not observed any relationship between NLR and delirium. Furthermore, some previous studies have suggested that CAR was superior to NLR in predicting systemic inflammation and prognosis in several cardiac and noncardiac conditions (36,37).

In line with previous data, in the current study, POD after TAVR was significantly associated with increased early and late mortality (3,38). However, the real question to be asked here is whether or not the increases in mortality and morbidity can really be attributed to POD. Rather than being causally related with mortality, POD may reflect a patient's decreased resilience against noxious stimuli (i.e., their fragility) and thus merely identify the individuals who are already predisposed to worse treatment outcomes.

Nonpharmacological strategies reduce the incidence of delirium by 30% to 40% (3). As such, knowledge of the predictive factors for POD is very important for identifying patients who are at increased risk and who are most likely to benefit from preventive measures such as proactive interdisciplinary collaboration, geriatrics consultation, rehabilitation, nonpharmacologic sleep protocols, early mobilization, sensory aids, minimization of psychoactive medication usage, and intensified postoperative monitoring (3,19).

Study Limitations

Our study has some limitations that should be noted. First, our investigation was a retrospective registry analysis involving a small number of consecutive patients who underwent TAVR at a single centre. Since our patient number was relatively small, this study may be underpowered to detect the predictive value of CAR upon POD development. Second, the retrospective assessment of delirium may have led to an underestimation of the incidence of POD. Third, our analysis is retrospective regarding early and late mortality; in particular, we could not assess long-term cognitive impairment. Fourth, although this study points to CAR as a new risk factor for POD development, the mechanism by which it operates is still not clear. Thus, large-scale prospective studies are needed to validate the predictive value of preoperative CAR in determining POD occurrence after TAVR.

Conclusion

Our findings suggest an independent role of CAR in delirium above and beyond conventional inflammatory markers. From a clinical standpoint, we think that preoperative CAR is a novel and promising predictive inflammatory parameter for the development of POD and may help the physician with identifying subgroups of individuals at risk for POD to guide the deployment of preventive strategies before TAVR. Our findings also support the important role of inflammation in the pathophysiology of delirium and can shed light on development of new pathophysiologically based intervention strategies to prevent and/or treat this clinically important issue.

Authorship Contributions

Concept: M.K. Design: M.K., H.İ.B. Data Collection or Processing: M.K., H.İ.B., B.B. Analysis or Interpretation: M.K., B.B., M.M.C. Literature Search: M.K., H.İ.B., G.D. Writing: M.K.

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Analysis of Relationship Between Imaging Features of Aortic Dissection and Blood Parameters

Aort Diseksiyon Görüntü Özellikleri ile Kan Parametreleri Arasındaki İlişki

Abdullah Algın, Hatice Şeyma Akça, Serdar Özdemir, Mustafa Ahmet Afacan*, Serkan Emre Eroğlu

University of Health Sciences Turkey, Ümraniye Training and Research Hospital, Clinic of Emergency Medicine, İstanbul, Turkey
**University of Health Sciences Turkey, Haydarpasa Numune Training and Research Hospital, Clinic of Emergency Medicine, İstanbul, Turkey*

Abstract

Aim: Aimed to evaluate the relationship of serum biochemical and blood parameters with aortic rupture and dissection types according to the DeBakey classification.

Methods: Forty-three patients, who were admitted to the emergency department between 01.01.2011 and 01.01.2019 with the diagnosis of aortic dissection, were retrospectively evaluated. Gender, age, symptoms at presentation, vital parameters, creatinine and, troponin levels, and hemogram parameters were recorded after blood tests. Neutrophil-to-lymphocyte ratio and monocyte-to-platelet ratio were obtained from these values. These results were compared with the presence of aortic rupture and the DeBakey classification.

Results: Of the 43 patients included in the study, 30 were male (69.8%), and 13 were female (30.2%). Aortic rupture was seen in two of the 30 male patients (6.7%) and none of the females (p=1.000). There was a significant difference only in blood urine nitrogen and monocyte-to-platelet ratio between patients with and without aortic rupture (p=0.035 and p=0.015, respectively).

Conclusion: Most of the routinely evaluated blood and biochemical parameters in aortic dissection cases did not yield significant results in comparison to the DeBakey classification and the presence of rupture. Only blood urea nitrogen and monocyte-to-platelet ratio values were lower in patients with aortic rupture.

Keywords: Aortic dissection, DeBakey classification, aortic rupture, biochemical marker, emergency medicine

Öz

Amaç: Acil servise başvuran ve aort diseksiyonu tanısı alan hastalarda serum biyokimya ve kan parametrelerinin aort rüptür varlığı ile DeBakey sınıflaması diseksiyon tipleriyle ilişkisinin değerlendirilmesi amaçlandı.

Yöntemler: Acil servise 01.01.2011 ve 01.01.2019 tarihleri arasında aort diseksiyonu tanısı ile başvuran 43 hasta retrospektif olarak değerlendirildi. Cinsiyet, yaş, başvuru anındaki semptomlar, sistolik ve diyastolik kan basıncı, nabız, kan üre azotu, kreatinin, troponin, hemoglobin, hematokrit, beyaz kan hücresi sayımı, monosit, nötrofil, lenfosit, trombosit sayısı, ortalama trombosit hacmi ve kırmızı hücre dağılımı kaydedildi. Bu değerlerden nötrofil-lenfosit oranı ve monosit-trombosit oranı elde edildi. Bu sonuçlar aort rüptürü varlığı ve DeBakey sınıflandırması ile karşılaştırıldı.

Bulgular: Çalışmaya alınan 43 hastanın 30'u erkek (%69,8), 13'ü kadındı (%30,2). Aort rüptüründe 30 erkek olgunun ikisinde (%6,7) rüptür görüldü ve kadın olguların hiçbirinde rüptür gözlenmedi (p=1,000). Sadece kan üre azotu ve monosit-trombosit oranı değerleri aort rüptüründe anlamlı farklılık gösterdi (sırasıyla p=0,035 ve p=0,015).

Sonuç: Aort diseksiyonu olgularında rutin olarak değerlendirilen kan ve biyokimya parametrelerinin çoğu DeBakey sınıflandırması ve rüptür varlığı ile karşılaştırıldığında anlamlı sonuç vermedi. Aort rüptürü olan hastalarda düşük olan kan üre azotu ve monosit-trombosit oranı değerleri anlamlıydı.

Anahtar Sözcükler: Aort diseksiyonu, DeBakey sınıflandırması, aort rüptürü, biyokimyasal belirteç, acil tıp

Address for Correspondence/Yazışma Adresi: Abdullah Algın, University of Health Sciences of Turkey, Ümraniye Training and Research Hospital, Clinic of Emergency Medicine, İstanbul, Turkey
E-mail: dralgin@hotmail.com ORCID: orcid.org/0000-0002-9016-9701
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Introduction

Aortic dissection is a cardiovascular disease that has high mortality and morbidity. Typical signs and symptoms include chest pain, back pain, syncope, and neurological symptoms. The rate of mortality within the first 24 hours in untreated cases is 21% (1). Acute aortic dissection results in mortality in 20% of patients before admission to emergency department, in 30% in the hospital, and in 20% within ten years after discharge (1). There are often underlying pathologies, such as arteriosclerosis and hypertension, and the incidence increases due to these entities (1,2). Risk factors for acute aortic dissection include age, hypertension and, Marfan syndrome. Aortic dissection usually occurs at a younger age in patients with Marfan syndrome than in others. In the pathogenesis of aortic dissection, firstly, cystic medial necrosis develops. Cystic medial necrosis is characterized by destruction of elastic tissue and accumulation of proteoglycans and apoptosis of smooth muscle cells in the media layer leading to, aortic dilatation, dissection and aortic wall rupture (3).

It is known that the diagnosis of aortic dissection with imaging methods can be made with 95-98% specificity and 98-100% sensitivity (3,4). Besides, some biochemical tests that can be used for diagnostic purposes as a contribution to these imaging methods have been reported in the literature (3,5). Smooth muscle myosin heavy chain, creatinine kinase BB isoenzyme, calponin, elastin, and transforming growth factor (TGF)-beta are some of the biomarkers that may be used (3,4). It is claimed that these markers can be used for triage, in the distinction of acute-chronic dissection, or to rule out acute myocardial infarction that has a similar presentation (3).

Biochemical markers have been evaluated as diagnostic or mortality indicators for aortic dissection in the literature. On the other hand, the relationship between biochemical markers and clinical conditions, such as location of dissection and development of aortic rupture, that may affect prognosis have not been reported in the literature. In this study, it is aimed to evaluate the relationship between routine biochemical markers and such clinical conditions in patients admitted to the emergency department and diagnosed with aortic dissection.

Methods

In this study, 47 patients, who were admitted to the emergency department between 01.01.2011 and 01.01.2019 with the diagnosis of aortic dissection established by computed tomography (CT) angiography, were retrospectively evaluated. Ethics committee approval dated 04.09.2019 and numbered 170 was obtained from the University of Health Sciences Ümraniye Training and Research Hospital Ethics Committee (September 4th 2019,170) before the study. Four patients whose images of the CT angiography or biochemical parameters could not be obtained were excluded, and the remaining 43 patients were included in the study.

Demographic data, such as gender and age, symptoms at presentation, systolic and diastolic blood pressure, and pulse were recorded. In addition, blood urine nitrogen (BUN), creatinine, troponin, hemoglobin (Hb) and, hematocrit (Hct) levels, white blood cell count (WBC), monocyte, neutrophil, lymphocyte and platelet (Plt) counts, mean platelet volume (MPV), and red cell distribution width (RDW) values were recorded after blood tests. Neutrophil-to-lymphocyte ratio (NLR) and monocyte-to-platelet ratio (MPR) were obtained from these values.

Radiological images of the patients were retrospectively evaluated by a radiologist with a 7-year-experience in CT interpretation. The patients were also classified according to the DeBakey classification and presence of aortic rupture were noted according to the radiological images.

Statistical Analysis

The IBM SPSS Statistics version 21.0 (New York, United States) software was used for statistical analysis. Categorical data were expressed as numbers and percentages and mean and standard deviation were used for the expression of numerical data with normal distribution, and median, minimum and maximum values were used for nonparametric numeric data with no normal spatial distribution. The distribution of numerical data was evaluated by the Shapiro-Wilk test. A chi-square test was used to evaluate the relationship between categorical data. Student's t-test was used for comparison of the normally distributed numerical data between the two groups and the Mann-Whiney U test was used for comparison of the nonparametric numerical data between the two groups. The Kruskal-Wallis test was used for comparison of nonparametric numerical data if there were more than two groups. A p value of less than 0.05 was considered statistically significant.

Results

Of the 43 cases included in the study, 30 were male (69.8%), and 13 were female (30.2%). The most common symptoms were chest pain in 11 patients (25.6%), dyspnea in seven (16.3%), back pain in five (11.6%), abdominal pain in three (7%), low back pain in two (4.7%), myalgia in two (4.7%), syncope in two (4.7%), and epigastric pain in two patients (4.7%). Also,

symptoms such as aphasia, non-specific pain, foot pain, headache, purple discoloration of the skin, pneumonia, flank pain, and inability to walk were observed in one patient (2.3%). According to the DeBakey classification, type 1 aortic dissection was observed in 16 (37.2%), type 2 aortic dissection in four (9.3%), and type 3 aortic dissection in 23 patients (53.5%). In two cases (4.7%), aortic rupture was observed on CT angiography. Descriptive statistics of the numerical data are summarized in Table 1.

According to the DeBakey classification, type 1 dissection was present in 40% of 30 male patients, type 2 dissection in 6.7% and type 3 dissection in 53.3%. In the 13 female patients, type 1 dissection was present in 30.8%, type 2 dissection in 15.4%, and type 3 aortic dissection in 53.8%. There was no significant relationship between gender and the type of dissection according to the DeBakey classification (p=0.622). Aortic rupture was seen in two of 30 male patients (6.7%), and no rupture was observed in any of the female patients. The relationship between gender and presence of aortic rupture was not statistically significant (p=1.000).

Table 1. Descriptive statistics of the numerical data

Parametric data		
	Mean	**± Standard deviation**
Blood pressure-systole (mmHg)	138.56	±42.02
Blood pressure-diastole (mmHg)	78.81	±19.8
Pulse (/min)	84.51	±22.8
WBC (x10^3/µL)	10.03	±3.6
Monocyte (x10^3/µL)	0.65	±0.28
MPV (fL)	8.29	±1.78
RDW (%)	15.92	±1.83
Nonparametric data		
	Median	**Minimum-maximum**
Age	60	26-88
BUN (mg/dL)	42.8	21.4-228.98
Creatinine (mg/dL)	0.98	0.55-9.11
Troponin (ng/dL)	0.012	0-0.53
Hb (g/dL)	12.8	7.4-35.4
Htc (%)	39.1	10.7-48.2
Neutrophil (x10^3/µL)	6.74	2.18-14.9
Lymphocyte (x10^3/µL)	2.29	0.6-5.16
Platelet (x10^3/µL)	226	104-539
NLR	3.6	0.92-10.82
MPR	0.039	0.01-0.09

WBC: White blood cell, MPV: Mean platelet volume, RDW: Red cell distribution width, BUN: Blood area nitrogen, Hb: Hemoglobin, Htc: Hematocrit, NLR: Neutrophil to lymphocyte ratio, MPR: Monocyte-to-platelet ratio

There was a significant difference between males and females in Hct and creatinine values, which were both lower in the female group (p=0.01 and p<0.0001, respectively). There was no statistically significant difference in other parameters between males and females (Table 2).

In the comparison of aortic rupture with numerical data, the relationship between BUN and aortic rupture was statistically significant (p=0.035). Additionally, a statistically significant relationship between MPR values and aortic rupture was detected (p=0.015, respectively). According to these results, BUN and MPR values were significantly lower in patients with aortic rupture. No significant relationship was found between aortic rupture and the remaining numerical data (systolic and diastolic blood pressures, pulse, WBC, monocyte, MPV, RDW, age, creatinine, troponin, Hb, Hct, neutrophil, lymphocyte, platelet, and NLR) (Table 3).

No significant correlation was found between DeBakey classification and numerical data (systolic and diastolic blood pressures, pulse, WBC, monocyte, MPV, RDW, MPR, age, creatinine, BUN, troponin, Hb, Hct, neutrophil, lymphocyte, Plt, and NLR) (Table 4).

Discussion

Aortic dissection is a potentially lethal clinical entity. Radiological methods, especially CT angiography, are used for diagnosis. CT angiography provides information not only in the diagnosis of cases but also in different features of dissection, such as location, type of dissection, and the status of the affected solid organs (6). In recent years, there have been an increasing number of biochemical parameters that may be useful in the diagnosis of aortic dissection in centers that do not have CT angiography or may be used for triage before imaging even if angiography is available. In this study, biochemical and blood parameters are compared with the DeBakey classification, gender, and presence of aortic rupture in 43 patients with aortic dissection. As a result, no significant relationship was found between the DeBakey classification and any of the numerical parameters, and BUN and MPR values were found to be statistically significantly lower in cases with aortic rupture. In comparison biochemical parameters with gender, creatinine and Hct values were found to be statistically lower in the female group, and no significant correlation was found between gender and other numerical data.

The use of biochemical markers in cardiac pathologies is particularly common in acute myocardial infarction, and its origin dates back to the 1950s (1). For the

diagnosis of aortic dissection, imaging methods are more prominent, and it is known that aortic dissection can be diagnosed by imaging with 95-98% specificity and 98-100% sensitivity. In this context, diagnostic imaging methods are reliable. However, biochemical markers may be used in cases where urgent CT examination cannot be performed, in order to exclude acute myocardial infarction in cases with similar presentation i.e., chest pain, or for triage in the determination of patients requiring urgent imaging. In its pathogenesis, cystic medial necrosis, characterized by destruction of elastic tissue, accumulation of proteoglycans and apoptosis of smooth muscle cells in the media layer is followed by aortic dilatation, dissection, and aortic wall rupture. After the dissection, smooth muscle-derived proteins that arise from apoptosis of smooth muscles in the media layer get into the circulation (3). Smooth muscle myosin heavy chain, creatinine kinase BB isoenzyme and, calponin are among these proteins which can be used as a marker of dissection (3,4). A rapid 30-minute assay of smooth muscle myosin heavy chain has been shown to be successful in the diagnosis of acute aortic dissection with 97% specificity (5). Elastin, which is a crucial component of the aortic wall structure, inflammatory markers, such as CRP, matrix metalloproteinases, TGF-beta, and D-dimer, are also some biomarkers that can be used in the diagnosis (3,4).

Low levels of total cholesterol have been shown to be associated with increased in-hospital mortality in patients with Stanford type A aortic dissection (2). This negative correlation has also been shown in different diseases such as acute coronary syndrome and acute heart failure and is called cholesterol paradox (7,8). In this study, no analysis of mortality was made, and cholesterol level was not evaluated.

Levčík et al. (9) showed that D-dimer levels above 0.5 mg/L could be used in the diagnosis of acute aortic dissection with 100% sensitivity, 100% negative predictive value, 37% specificity, 65% positive predictive value, and 71% accuracy. According to this result, D-dimer level below 0.5 mg/L can be used to exclude aortic dissection. Therefore, D-dimer can be used in the diagnosis of aortic dissection for exclusion and triage purposes (9,10).

Table 2. Statistical analysis results of the relationship between gender and numerical data

	Male		Female		
Parametric data					
	Mean	**± Standard deviation**	**Mean**	**± Standard deviation**	**p**
Blood pressure-systole (mmHg)	140.1	±45.11	135	±35.26	0.719
Blood pressure-diastole (mmHg)	77.27	±20.68	82.38	±17.81	0.443
Pulse (/min)	86.57	±24.1	79.77	±19.52	0.376
WBC (x10^3/µL)	10.52	±3.95	8.91	±2.41	0.18
Monocyte (x10^3/µL)	0.67	±0.29	0.6	±0.24	0.462
MPV (fL)	8.34	±1.95	8.17	±1.33	0.783
RDW (%)	15.94	±15.88	15.87	±1.54	0.911
Nonparametric data					
	Median	**Minimum-maximum**	**Median**	**Minimum-maximum**	
Age	59.5	46-86	65	26-88	0.764
BUN (mg/dL)	42.8	21.4-229	38.5	21.4-57.8	0.522
Creatinine (mg/dL)	1.07	0.77-9.11	0.84	0.55-1	**<0.0001**
Troponin (ng/dL)	0.0135	0-0.53	0.006	0-0.16	0.636
Hemoglobin (g/dL)	13.4	7.4-15.7	12.3	9.69-35.4	0.159
Hematocrit (%)	40.35	24.6-48.2	37.3	10.7-41	**0.01**
Neutrophil (x10^3/µL)	6.74	3.05-14.9	6.17	2.18-10.17	0.158
Lymphocyte (x10^3/µL)	2.1	0.6-5.16	2.5	0.68-3.74	0.63
Platelet (x10^3/µL)	225	106-539	229	104-287	0.744
NLR	3.055	1.08-8.51	2.24	0.92-10.82	0.313
MPR	0.039	0.01-0.09	0.039	0.02-0.07	0.868

WBC: White blood cell, MPV: Mean-platelet volume, RDW: Red cell distribution width, BUN: Blood urine nitrogen, NLR: Neutrophil to lymphocyte ratio, MPR: Monocyte-to-platelet ratio

In a study conducted by Guan et al. (11), it was showed that preoperative low fibrinogen level was associated with neurological complications in patients with acute aortic dissection. It is thought that this may be secondary to large amounts of fibrinogen getting into the circulation and fibrin deposition. Meng et al. (12) found that fructosamine and Hba1c were related to thoracic aortic dissection. These markers have been suggested to be risk factors for the development of thoracic aortic dissection. In a study by Liu et al. (13), low HDL values were shown to be associated with mortality in aortic dissection cases. In their study, Sbarouni et al. (14) found no relationship between ischemia-modified albumin and acute aortic dissection. These biochemical parameters are not among the parameters evaluated in this study.

In previous studies, an increase in NLR level was shown to be associated with increased mortality in patients with Stanford type B and DeBakey type 1 acute aortic dissection (15,16). In this study, although higher NLR values were obtained in DeBakey type 2 cases compared to other types, the difference was not statistically significant (p=0.549). High platelet-to-lymphocyte ratio has also been shown to be associated with mortality in patients with Stanford type B aortic dissection, which was not evaluated in this study (17).

Study Limitations

This study has some limitations. First, the role of parameters in the diagnosis of aortic dissection could not be evaluated because there was no control group without aortic dissection. Besides, the limited number of patient groups constitutes the weakness of the study, and only two cases of aortic rupture limit the evaluation of biochemical parameters especially in the diagnosis of rupture. Another limitation is the retrospective design of the study.

Conclusion

Most of the routinely evaluated blood and biochemical parameters in aortic dissection cases did not yield significant results in comparison to the DeBakey classification and the presence of rupture. The only significant parameters were BUN and MPR values which were lower in patients with rupture. Further large-scale prospective multicenter studies are warranted.

Table 3. Statistical results of comparison of aortic rupture and numerical data

	Aortic rupture absent		Aortic rupture present		
	Median	**Minimum-maximum**	**Median**	**Minimum-maximum**	**p**
Blood pressure-systole (mmHg)	138	65-270	107.5	80-135	0.29
Blood pressure-diastole (mmHg)	78	40-122	62	40-84	0.223
Pulse (/min)	80	45-150	109	95-123	0.121
WBC (x10^3/µL)	9.66	4.2-18.7	11.76	7.6-15.9	0.496
Monocyte (x10^3/µL)	0.61	0.03-1.31	0.54	0.35-0.72	0.569
MPV (fL)	8.4	5.25-13.2	7.51	7.1-7.92	0.532
RDW (%)	15.8	12.7-22.1	14.55	14.3-14.8	0.283
Age	60	26-88	58	49-67	0.602
BUN (mg/dL)	42.8	21.4-229	24.9	22-27.8	**0.035**
Creatinine (mg/dL)	0.98	0.55-9.11	0.99	0.84-1.13	0.842
Troponin (ng/dL)	0.0125	0-0.53	-	-	-
Hemoglobin (g/dL)	12.6	7.4-35.4	13.95	13.8-14.1	0.179
Hematocrit (%)	39	10.7-48.2	41.75	40.3-43.2	0.268
Neutrophil (x10^3/µL)	6.53	2.18-14.9	8.09	5.58-10.6	0.5
Lymphocyte (x10^3/µL)	2.29	0.6-5.16	2.76	1.32-4.21	0.683
Platelet (x10^3/µL)	226	104-425	373	208-539	0.465
NLR	2.86	0.92-10.82	3.38	2.52-4.23	0.8
MPR	0.039	0.01-0.09	0.026	0.01-0.04	**0.015**

WBC: White blood cell, MPV: Mean-platelet volume, RDW: Red cell distribution width, BUN: Blood urine nitrogen, NLR: Neutrophil to lymphocyte ratio, MPR: Monocyte-to-platelet ratio

Table 4. Evaluation of relationship between numerical data and DeBakey classification							
DeBakey Classification	**Type 1**		**Type 2**		**Type 3**		
Parametric data							**p**
	Mean	**± Standard deviation**	**Mean**	**± Standard deviation**	**Mean**	**± Standard deviation**	
Blood pressure-systole (mmHg)	133.8	±46.05	120	±36.29	145.01	±40.28	0.474
Blood pressure-diastole (mmHg)	73.5	±19.96	80	±23.85	82.3	±19.11	0.4
Pulse (/min)	81.5	±23	105.3	±43.3	83	±17	0.159
WBC (x10^3/µL)	10.64	±3.68	9.26	±1.87	9.75	±3.82	0.687
Monocyte (x10^3/µL)	0.69	±0.29	0.59	±0.22	0.62	±0.28	0.691
MPV (fL)	8.32	±1.79	7.42	±1.15	8.42	±1.87	0.59
RDW (%)	15.86	±1.47	14.53	±1.3	16.21	±2.06	0.237
Nonparametric data							
	Median	**Minimum-maximum**	**Median**	**Minimum-maximum**	**Median**	**Minimum-maximum**	
Age	57	26-83	73.5	67-88	60	46-86	0.051
BUN (mg/dL)	38.5	23.54-68.5	47.2	22-57.8	44.9	21.4-229	0.607
Creatinine (mg/dL)	1.07	0.55-1.44	0.96	0.84-1.69	0.98	0.63-9.11	0.658
Troponin (ng/dL)	0.0065	0-0.53	0.03	0.03-0.04	0.014	0-0.48	0.129
Hemoglobin (g/dL)	12.7	9.5-15.4	12.6	9.91-14.2	12.8	7.4-15.7	0.997
Hematocrit (%)	38.85	10.7-44.2	37.7	30.8-43.2	39.1	4.2-18.7	0.546
Neutrophil (x10^3/µL)	7.95	2.18-13	5.81	4.85-10.17	6.19	2.6-14.9	0.846
Lymphocyte (x10^3/µL)	2.48	0.97-5.16	1.59	0.94-2.61	2.5	0.6-4.77	0.342
Platelet (x10^3/µL)	222	104-425	266.5	171-539	224	137-393	0.529
NLR	2.81	1.08-7.85	3.74	1.86-10.82	2.7	0.92-8.51	0.549
MPR	0.04	0.01-0.09	0.028	0.01-0.05	0.039	0.02-0.08	0.371

WBC: White blood cell, MPV: Mean-platelet volume, RDW: Red cell distribution width, BUN: Blood urine nitrogen, NLR: Neutrophil to lymphocyte ratio, MPR: Monocyte-to-platelet ratio

Authorship Contributions

Concept: A.A., H.Ş.A. Design: A.A. Supervision: A.A. Data collection and/or processing: A.A. Analysis and/or interpretation: H.Ş.A., S.Ö., S.E.E. Literature searching: A.A., M.A.A. Writing: A.A.

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Efficacy of Presepsin as a Biomarker of Sepsis and Its Prognostic Value for Prediction of Mortality

Bir Sepsis Biyobelirteci Olarak Presepsinin Etkinliği ve Mortalite Öngörüsü için Prognostik Değeri

İbrahim Akkoç, Zümrüt Mine Işık Sağlam*, Öznur Şen, Nurdan Aydın, Macit Koldaş*, Mehmet Toptaş, Sinan Uzman

University of Health Sciences Turkey, Haseki Training and Research Hospital, Clinic of Anesthesiology and Reanimation, İstanbul, Turkey
**University of Health Sciences Turkey, Haseki Training and Research Hospital, Clinic of Medical Biochemistry, İstanbul, Turkey*

Abstract

Aim: We aimed to determine the efficacy of presepsin (PRE) as a biomarker of sepsis and to asssess its prognostic value for prediction of mortality were determined.

Methods: Selected clinical data of 21 patients admitted to intensive care unit for systemic inflammatory response syndrome (SIRS), sepsis and septic shock were collected. APACHE-II score, blood culture results and WBC and CRP measured at baseline, procalcitonin (PCT) and PRE measured at baseline and on days 2, 4, and 7, and mortality of patients were recorded.

Results: Compared to SIRS, sepsis and septic shock caused comparable increases in the APACHE II scores and mortality rates. The PRE values of the septic shock patients were significantly higher as compared to the sepsis and the SIRS patients at baseline and on day 2. Moderate or high correlations among both the PCT and PRE values measured at baseline and on days 2, 4, and 7 were detected. Overall, the diagnostic and prognostic efficacies of PRE were comparable to those of PCT.

Conclusions: During differential diagnosis, follow-up and prediction of mortality risk of patients for SIRS, sepsis and septic shock, findings of current study support that PRE possesses a merit to be used as a biomarker with comparable efficacy with PCT.

Keywords: Systemic inflammatory response syndrome (SIRS), sepsis, septic shock, APACHE-II, presepsin, procalcitonin, C-reactive protein (CRP), white blood count (WBC)

Öz

Amaç: Amacımız bir sepsisbiyobelirteci olarak presepsinin (PRE) etkinliğini değerlendirmek ve mortalite öngörüsündeki prognostik değerini belirlemekti.

Yöntemler: Sistemik enflamatuvar cevap sendromu (SIRS), sepsis ve septik şok ile yoğun bakım ünitesine kabul edilen 21 hastanın seçilen bulguları toplandı. APACHE II skorları, kan kültürü sonuçları ve biyobelirteçler olarak lökosit ve CRP'nin ilk gün değerleri ile prokalsitonin (PCT) ve PRE'nin ilk gün ve gün 2, 4, ve 7 değerleri ve mortalite durumu kaydedildi.

Bulgular: Sepsis ve septik şok hastalarının relatif olarak yükselmiş APACHE-II skorları ve mortalite oranları, SIRS hastalarınınkilerdenfazla idi. Septik şok hastalarının PRE düzeyleri, ilk gün ve ikinci günde sepsis ve SIRS hastalarınınkine göre anlamlı derecede yüksekti. Hem PCT hem de PRE'nin ilk gün ve gün 2, 4, ve 7 değerleri birbiri ile orta-yüksek derecede ilişkili idi. Genel olarak bakıldığında, ilk gün ve izlemde kullanıldığında ve mortalite öngörüsü için dikkate alındığında PRE'nindiagnostik ve prognostik etkinliği PCT'ye yakın bulundu.

Sonuç: Bu araştırmanın sonuçları PCT ile oldukça benzer etkinliğe sahip bir biyobelirteç olarak PRE'nin, SIRS, sepsis ve septik şok bulunan hastaların ayırıcı tanısı, izlemi ve mortalite risk öngörüsü sırasında kullanılabilirliğine işaret etmektedir.

Anahtar Sözcükler: Sistemik enflamatuvar cevap sendromu (SIRS), sepsis, septik şok, APACHE-II, presepsin, prokalsitonin, C-reaktif protein (CRP), lökosit sayısı

Address for Correspondence/Yazışma Adresi: İbrahim Akkoç, University of Health Sciences of Turkey, Haseki Training and Research Hospital, Clinic of Anesthesiology and Reanimation, İstanbul, Turkey
E-mail: ibrakkoc@gmail.com ORCID: orcid.org/0000-0002-3614-5274
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Introduction

Sepsis is a clinical syndrome of life-threatening organ dysfunction caused by a disorganized host inflammatory response to infection. It is among the most important causes of morbidity and mortality in intensive care units (ICUs). The clinical term of systemic inflammatory response syndrome (SIRS) is used to describe the complex pathophysiologic response to infection in patients before the detection of infectious agents, as it has long been used to identify early sepsis. In septic shock, there is a critical reduction in tissue perfusion; acute failure of multiple organs, including the lungs, kidneys, and liver, can occur. Septic shock, a subset of sepsis, has significantly increased mortality due to persistent hypotension (defined as the need for use of vasopressors and high level of serum lactate despite adequate fluid support (1,2).

The cornerstone of proper management in patients with sepsis is the early determination of mortality risk in the ICU. For this purpose, the application of severity scores and serum biomarkers are used at admission and follow-up. The most widely applied score is the Acute Physiology and Chronic Health Evaluation II (APACHE II). However, APACHE II scoring may be misleading in some clinical situations. In younger patients with severe sepsis but without chronic organ failures, the APACHE II score may be relatively low in sepsis patients with a risk of higher mortality. In contrast, in older patients with sepsis but with chronic organ failures, it may be higher even when the mortality risk is relatively low (3).

After early diagnosis of sepsis, goal-directed management may reduce their morbidity and mortality (4). Blood culture is the gold standard for diagnosing sepsis; however, it takes a long time to obtain its result. Depending on the previous antibiotic treatment, 30% of sepsis patients tend to be diagnosed with bacteremia (5). In sepsis, traditional systemic inflammatory variables include leukocytosis [white blood cellcount (WBC) >12,000/μL] or leukopenia (WBC<4,000/μL) (6,7).

There is a need for successful and new biomarkers with satisfactory sensitivity and specificity in order to achieve early diagnosis and sound estimation of the prognosis for the patients with sepsis. For this purpose, the most frequently studied biomarkers are procalcitonin (PCT), interleukin 6 (IL-6), tumor necrosis factor, C-reactive protein (CRP), and WBC (8).

Presepsinis a recently defined infection biomarker, which is a 13 kDa fragment of the N-terminal of soluble CD14. It is secreted into the blood after the activation of monocytes in response to several types of infections (9-11). Presepsinappeared to be comparable to other inflammatory biomarkers, including CRP, PCT, and IL-6, in the diagnosis of sepsis and its diagnostic performance superior to conventional markers and blood culture (11,12). However, it is being proposed that presepsin, one of the newly exploited biomarkers, not only has a higher sensitivity and specificity but also assists in predicting the severity and the outcome more reliably (13,14).

Presepsin needs further study to have a place in the armamentarium of intensive care physicians. When presepsin added among the diagnostic tests performed in the diagnosis and management of patients with sepsis types, it is possible to obtain an increase in the survival of these patients. We aimed (1) to determine the efficacy of presepsin as a biomarker of sepsis relative to those of WBC, CRP, and PCT in SIRS, sepsis, and septic shock; (2) to test its correlation with the APACHE-II scores; and (3) to assess its prognostic value for prediction of mortality were determined.

Materials and Methods

This retrospective study was conducted at the Haseki Training and Research Hospital with 21 adult patients who were treated at the ICU with diagnoses of SIRS, sepsis and septic shock. The approval of the Human Research Ethics Committee of our institution was obtained (decision no: 242, date: 26.09.2018).

The exclusion criteria were: recent cardiac resuscitation; severe head trauma; nephrotic syndrome; cirrhosis; burns or having been diagnosed with New York Heart Association class III-IV cardiac failure; HIV infection; presence of neutropenia; defined as <1000 neutrophils/mm^3; constantly use of antihypertensives related to angiotensin; recent use of heparin medications; long-term corticosteroid use; requirement of enteral nutrition; and women with pregnancy or breast-feeding. After the informed consent of patients or relatives, from the electronic hospital records of patients, selected clinical variables of the patients were collected. The patients were selected in this study on the basis of the criteria defined by The American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (15-17). Hence the selection criteria consisted of detection of two or more of the following SIRS symptoms within the latest 6-24 hours of a known or suspected infection, such as having a core temperature of >38 °C or <36 °C; heart rate of <90 beats/min; respiratory rate of <20 breaths/min; arterial partial pressure of carbon dioxide <32 mmHg or the requirement for mechanical ventilation for an acute pathological process; a white blood cell count >12,000/μL or <4,000/μL or observation of more than 10% immature neutrophils. SIRS patients with positive blood cultures were included in patients with sepsis.

Sepsis patients were required to have signs of more than one organ dysfunction (15,16,18). The following

anomalies were identified with severe sepsis: (1) cardiovascular anomalies as arterial systolic blood pressure less than 90 mmHg or mean arterial pressure less than 70 mmHg that responds to administration of intravenous fluid; (2) renal dysfunction with oliguria of less than 0.5 mL/kg per hour despite adequate fluid resuscitation; (3) respiratory anomaly of PaO_2/fraction of inspired oxygen being less than 250; (4) hematological anomalies as platelet count of less than 80,000/L or a decline of 50% in platelet count from highest value recorded over the previous 3 days; (5) unexplained metabolic acidosis with a pH of 7.30 or base deficit of 5.0 mEq/L and plasma lactate level exceeding 1.5 times upper limit of normal for the reporting laboratory; (6) pulmonary artery wedge pressure being less than 12 mmHg or central venous pressure of less than 8 mmHg with adequate fluid resuscitation; and (7) acute alteration of mental status.

Septic shock was defined as sepsis with hypotension, in addition to signs of organ dysfunction, when recording a systolic blood pressure of <90 mmHg or its reduction by 40 mmHg or more from the baseline in the absence of other causes for hypotension, despite adequate fluid resuscitation; or when the use of inotropics or vasopressors were needed to maintain a systolic blood pressure of more than 90 mmHg or a mean arterial pressure of more than 70 mmHg (19,20).

Blood specimens were collected immediately after admission to the ICU for blood culture and assessment of the levels of biomarkers (WBC, CRP, PCT, and presepsin), which was repeated on days 2, 4, and 7 after the admission of patients to the ICU. Hemodynamic parameters, arterial blood gases, ventilation parameters, fluid balance, vasoactive agent usage, antibiotic therapy, and standard laboratory tests were followed up daily. APACHE-II score was determined within the first 24 hours after admission to the ICU. The APACHE-II scores and the mortality and survival incidences at the ICU were evaluated on the basis of the three diagnostic groupings of SIRS, septic shock and sepsis.

Biomarker Measurements

presepsin measurements were performed in whole blood specimens collected into EDTA tubes, using PathfastPresepsin Assay Kit with Pastfast Analyzer (Mitsubishi Chemical, Japan). Measurements of PCT and CRP were carried out in SST serum (BD, USA); with the PTC assay (DiaSorin, Italy), and CRP Assay (Backman, USA). All of the procedures were conducted according to the manufacturer's instructions.

Statistical Analysis

SPSS software (IBM SPSS, Version 22.0, IBM Corporation, Armonk, NY, USA) was used for the statistical analyses on the data. Descriptive statistics consisted of the counts for the categorical variables and median with interquartile range and maximum and minimum for the numerical variables. During statistical analyses, Mann-Whitney, Kruskal-Wallis ANOVA, and Spearman correlation tests were performed. The p value of <0.05 was accepted as statistical significance.

Results

In the study population, the rates of non-survivors were 1/11 (9.1%), 2/6 (33.3%), and 2/6 (33.3%) in patients with SIRS, sepsis, and septic shock. As a total, there were five non-survivors and 16 survivors in the study population. The median age of participants was 64 (33-93) and male/female ratio of them was 14/7. With sepsis and septic shock, the rate of non-survivors was increased but this difference was not reached statistical significance (p>0.05). As presented in Table 1, the rates of culture positivity in the blood, tracheal, and urine samples of the study population were 38.1%, 47.6%, and 33.3%, respectively. There was no significant difference among these rates (p>0.05).

Figure 1 presents the median APACHE II scores of patients with SIRS (n=9), sepsis (n=6), and septic shock (n=6). The median APACHE II score of patients with SIRS was found significantly lower than those of the patients with sepsis and septic shock [15 (12.5-21.5) vs 23 (22.7-28.5) and 24 (22-27), respectively); p<0.05]. The median APACHE II scores of patients with sepsis and septic shock were found similar [23 (22.7-28.5) and 24 (22-27), respectively; p>0.05].

Table 2 displays the association of the APACHE II score with studied biomarkers (n=21). There was no significant correlation of the APACHE II score with the CRP and

Table 1. Blood, tracheal and urine culture results of participants

Culture type	Patients (n)
Blood culture (positive/negative)	8/13 (38.1%)
Methicillin resistant staphylococcus aureus	4
Candida albicans	3
Pseudomonas aeruginosa	1
Tracheal culture (positive/negative)	10/11 (47.6%)
Pseudomonas aeruginosa	8
Klebsiella pneumoniae	1
Acinetobacter	1
Urine culture (positive/negative)	7/14 (33.3%)
Escherichia coli	4
Acinetobacter	2
Candida albicans	1

n: Number

WBC measured at baseline and PCT and presepsin values measured at baseline and on days 2, 4, 7 in the study population.

Figure 2 shows the median WBC and CRP values of patients with SIRS, sepsis, and septic shock. In the patients with SIRS, sepsis, and septic shock, the median WBC [18 (12.2-25.2), 12.8 (9.5-21.8), and 23 (10.3-33.1), respectively) and CRP [24.4 (9.1-68.0), 92 (19.8-136), and 31.4 (118.5-93.3), respectively] values were found comparable (p>0.05). As seen in the presentation, these parameters did not follow similar pattern according to the severity of disease and in addition, there was no significant correlation between them in the study population (p>0.05).

Figure 3 presents the PCT and presepsin values of non-survivors and survivors measured at baseline during admission and 2, 4, and 7 days later after admission. Kruskal-Wallis ANOVA test revealed no significant difference among the PCT values measured at baseline and on days 2, 4, and 7 of both the non-survivors and survivors (p>0.05). When the PCT values of non-survivors and survivors were compared with the Mann-Whitney test, there was no significant difference (p>0.05). The Kruskal-Wallis ANOVA test demonstrated no significant difference among the presepsin values measured at baseline and on days 2, 4, and 7 of both the non-survivors and survivors (p>0.05). The Mann-Whitney test revealed no significant difference between the non-survivors and survivors regarding the presepsin

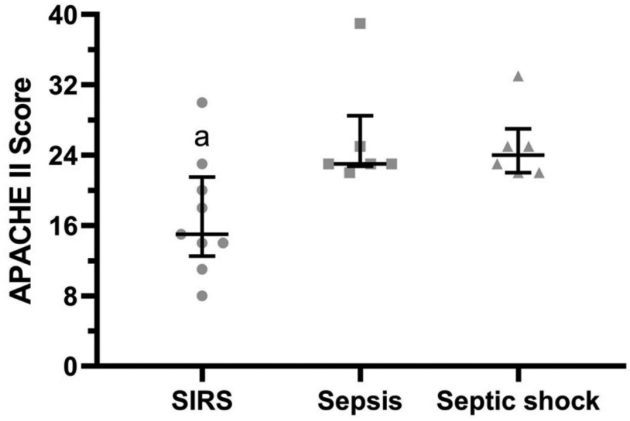

Figure 1. APACHE II scores of patients with SIRS (n=9), sepsis (n=6), and septic shock (n=6). Data were presented as median with interquartile range of 25-75%. The median APACHE II score of patients with SIRS was found significantly lower (a) than compared to the patients with sepsis and septic shock (p<0.05)

APACHE II: Acute Physiology and Chronic Health Evaluation II, SIRS: Systemic inflammatory response syndrome

Table 2. Correlation coefficients between APACHE II and studied sepsis markers according to time points in the study population (n=21)

	Time points	Correlation coefficient of APACHE II score	Significance
CRP	Baseline	0.066	NS
WBC	Baseline	0.252	NS
PCT	Baseline	0.023	NS
-	Day 2	0.104	NS
-	Day 4	0.080	NS
-	Day 7	-0.086	NS
PRE	Baseline	0.181	NS
-	Day 2	0.128	NS
-	Day 4	0.162	NS
-	Day 7	0.256	NS

CRP: C-reactive protein, WBC: White blood count, PCT: Procalcitonin, and PRE: Presepsin, APACHE II: Acute Physiology and Chronic Health Evaluation II, n: Number NS; not significant at a p value of less than 0.05.
There were no significant correlations of the APACHE II score and studied biomarkers.

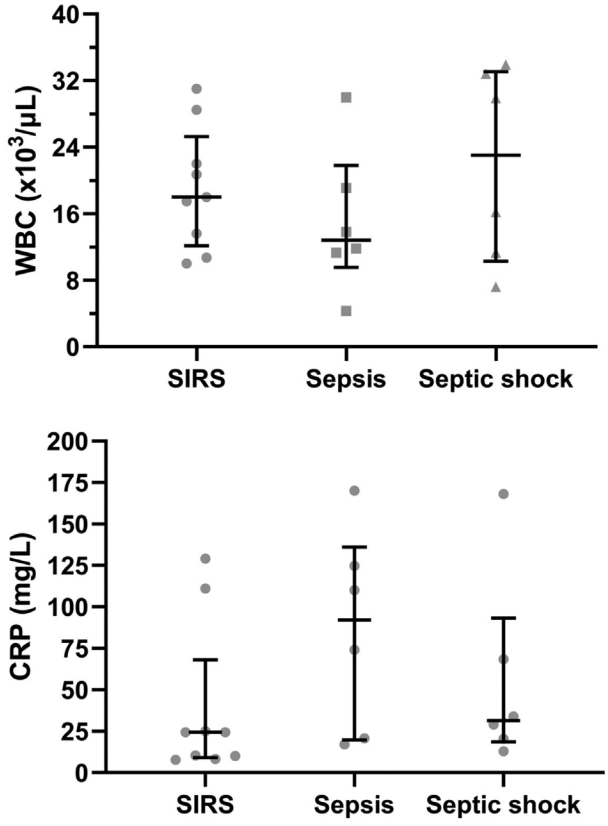

Figure 2. WBC and CRP values of patients with SIRS (n=9), sepsis (n=6), and septic shock (n=6). Data were presented as median with interquartile range of 25-75%. The median WBC and CRP values of patients with SIRS, sepsis, and septic shock were found comparable (p>0.05)

WBC: White blood cell, CRP: C-reactive protein, SIRS: Systemic inflammatory response syndrome

values measured at baseline and on days 2, 4, and 7 (>0.05).

In Figure 4, the PCT and presepsin values of patients with SIRS (n=9), sepsis (n=6), and septic shock (n=6) were displayed. Although there were, overall, decreases in the PCT and presepsin levels from baseline to day 7, these differences were not reached statistical difference (p>0.05) except the presepsin values of patients with SIRS at baseline and on day 2 were significantly higher compared to those of septic shock (p<0.05).

After Spearman correlation analyses (Table 3), we found that moderate or high correlations among both the PCT and presepsin values measured at baseline and on days 2, 4, and 7 (p<0.05). There was a significant correlation between the PCT and presepsin values measured only on day 7 (r=0.56, p<0.05); however, there was no significant correlation between these parameters measured on other time points.

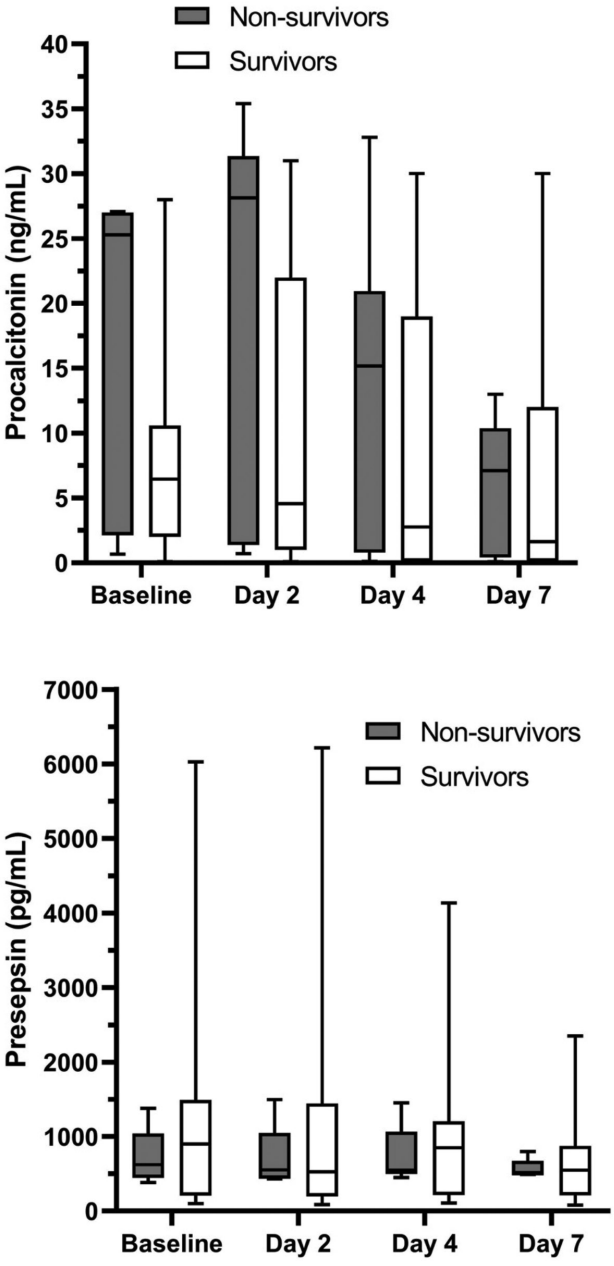

Figure 3. PCT and presepsin values of non-survivors (n=5) and survivors (n=16) measured at baseline during admission and 2, 4, and 7 days later after admission. Data were presented as median with interquartile range of 25-75% and min-max values. Overall, there was no significant difference between the study subjects regarding the PCT and presepsin values (>0.05)

PCT: Procalcitonin, min: Minimum, max: Maximum

Discussion

In the current study, we assessed the admission and follow-up findings of patients with SIRS, sepsis, and septic shock in the ICU. In moderate percentage of patients, the positivity of microbiological culture was detected. Relative

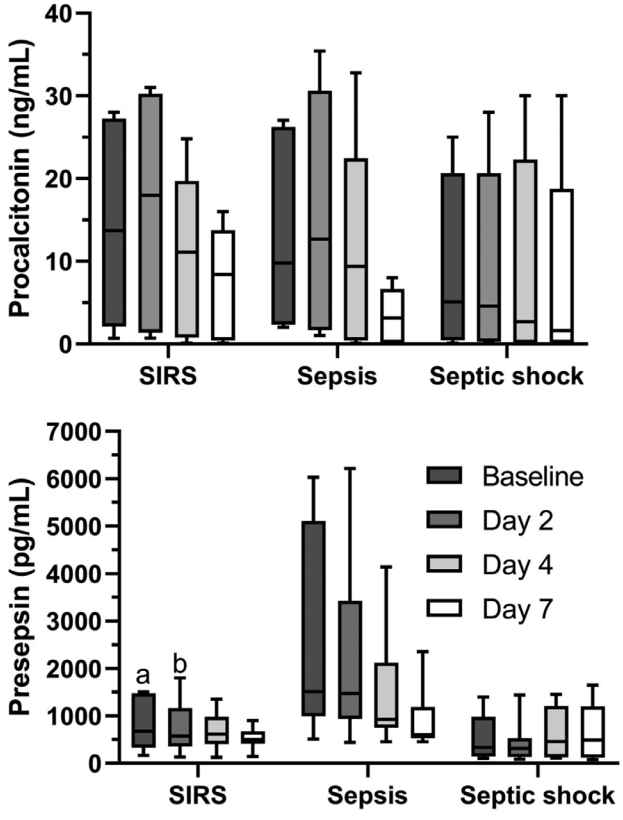

Figure 4. PCT and presepsin values of patients with SIRS (n=9), sepsis (n=6), and septic shock (n=6). Data were presented as median with interquartile range of 25-75% with min-max values. There were overall decreases without significance in the PCT and presepsin values (p>0.05) except the presepsin values of patients with SIRS at baseline (a) and on day 2 (b) were significantly higher compared to those of septic shock (p<0.05)

PCT: Procalcitonin, SIRS: Systemic inflammatory response syndrome

Table 3. Correlation coefficients of the PCT and PRE values measured at baseline and on days 2, 4, and 7

		PCT			
		Baseline	**Day 2**	**Day 4**	**Day 7**
PCT	Baseline	-	0.87	0.63	0.63
	Day 2	-	-	0.88	0.83
	Day 4	-	-	-	0.90
	Day 7	-	-	-	-
		PRE			
		Baseline	**Day 2**	**Day 4**	**Day 7**
PRE	Baseline	-	0.94	0.69	0.60
	Day 2	-	-	0.76	0.58
	Day 4	-	-	-	0.89
	Day 7	-	-	-	-

PCT: Procalcitonin, PRE: Presepsin

to conventional biomarkers of sepsis (WBC, CRP, and PCT), the efficacy of presepsin at baseline and on days 2, 4, and 7 was examined and its prognostic value for prediction of mortality was determined. Compared to SIRS, sepsis and septic shock caused comparable increases in the APACHE II score of patients. The APACHE II score had no meaningful association with the studied sepsis biomarkers of CRP and WBC measured at baseline and PCT and presepsin values measured at baseline and on days 2, 4, and 7. Regarding the WBC and CRP parameters, SIRS, sepsis, and septic shock caused comparable changes.

Regarding mortality of studied participants, non-survivors had higher PCT values from baseline to day 7 but it was not reached statistical significance and some of the survivors had considerably high presepsin values from baseline to day 7 although this was not revealed as significant. Considering the type of disease from SIRS to sepsis and septic shock, there was a tendency to decrease in the PCT and presepsin values from baseline to day 7 although overall without any significance except a meaningful decrease in the presepsin values at baseline and on day 7 compared to SIRS. Correlation analyses revealed that both the PCT and presepsin values measured at baseline and on days 2, 4, and 7 had moderate-high associations, however, there was no similar association between the PCT and presepsin values measured on study time points except on day 7. We considered this as the sign of a difference in the mechanism of elevation of PCT and presepsin biomarkers. After further studies considering mortality and subtypes of sepsis, these biomarkers can gain new prognostic value in the management of sepsis in the ICUs.

Sepsis remains a medical emergency and a major challenge in critical care. Early diagnosis and treatment with appropriate antimicrobial therapy is the most important factor for reducing its morbidity and mortality associated with sepsis (21). Blood culture is the gold standard for detecting microorganisms in the bloodstream but it has limited usefulness and requires several days for obtaining its results. Therefore, there isa need for biomarkers enabling early diagnosis of sepsis and the prediction of the prognosis (22). In the 1980s CRP, and in the 1990s PCT were discovered to be increased in the blood of bacterially infected patients (23). In recent years, presepsin has started to become a popular biomarker, with reports of higher sensitivity and specificity in the diagnosis of sepsis as compared to other biomarkers (13,14,24).

Regarding APACHE II scoring to determine severity, Behnes et al. (25) have also reported an elevated first-day APACHE-II score in septic shock patients. In the study by Kweon et al. (26) significant variations in the APACHE-II scores of different patient groups were not observed. On the other hand, Shozushima et al. (27) have reported a correlation between the APACHE-II scores and presepsin levels of the 107 patients they studied.

Kweon et al. (26) also reported that mortality rates did not correlate with the presepsin levels on the first day. Masson et al. (28) have argued that the elevated presepsin levels but not the PCT levels were associated with the negative outcome during the hospital stay. Ulla et al (29) demonstrated a correlation between high presepsin values and hospital mortality. Also, Behnes et al. (2) demonstrated that presepsin levels were significantly higher in the non-survivors than the survivors.

Study Limitations

There are several limitations in the present study. The first limitation of the study was its retrospective nature. Secondly, this study included a limited number of patients with sepsis and septic shock. Thirdly, there were no daily measurements of the study parameters. Fourth, this study did not consider the importance of the type of microbiological agent. Fifth, because of the limited number of patients we couldn't investigate the relationship between the severity of sepsis, type of microorganism isolated from cultures and presepsin. Moreover, this study was performed in a single center with a small number of patients. It is not possible to reach solid conclusions on the diagnostic and prognostic values of presepsin on the basis of the severity of sepsis and mortality related to the SIRS, sepsis, and septic shock. Sepsis related definitions has been changed rapidly in recent years. However, there is a need for further studies according to the implications of the current study according to the new definitions. Presepsin reached several times increase in some patients and during management, their levels relatively decreased until day 7. Interestingly, its levels somewhat lower in the non-survivors and in patients with septic shock. presepsin

levels considerablyhigher with sepsis compared to SIRS and septic shock.

Conclusion

During differential diagnosis, follow-up, and determination of mortality risk of patients for SIRS, sepsis and septic shock, findings of the current study support that presepsin possesses merit to be used as a biomarker after further studies investigating how presepsin increases and its relationship other clinical parameters.

Authorship Contributions

Concept: İ.A., Ö.Ş., M.T. Design: İ.A., Ö.Ş., M.T. Data Collection or Processing: İ.A., Z.M.I.S., Ö.Ş., N.A., M.K. Analysis or Interpretation: İ.A., Z.M.I.S., M.K., M.T., S.U. Literature Search: İ.A., Z.M.I.S., M.K., M.T., S.U. Writing: İ.A., Ö.Ş., M.T.

Conflict of Interest: The authors declared that there was no conflict of interest.

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Case Report / *Olgu Sunumu*

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A Novel Frameshift Mutation in Two Siblings with Merosin-deficient Congenital Muscular Dystrophy

Merozin Negatif Müsküler Distrofi Tanılı İki Kardeşte Yeni Tanımlanmış Bir Çerçeve Kayma Mutasyonu

Senem Ayça, Hamide Betül Gerik Çelebi*, Sırrı Çam*, Muzaffer Polat

Celal Bayar University Faculty of Medicine, Department of Pediatric Neurology, Manisa, Turkey

**Celal Bayar University Faculty of Medicine, Department of Medical Genetics, Manisa, Turkey*

Abstract

We present two siblings with elevated serum creatine kinase concentrations, developmental delay, muscle weakness, and contractures of the lower limbs. Cranial magnetic resonance imaging revealed diffuse white matter hyperintensity in both siblings. In the older sister, muscle biopsy was performed; immunohistochemical studies showed a dystrophic pattern and merosin deficiency. With the diagnosis of merosin-deficient congenital muscular dystrophy (MDC1A), *LAMA2* gene mutation analysis revealed an NM_000426.3:c.163_163delA; (p.N55Mfs*16) homozygous frameshift mutation in the siblings. This mutation leads to a premature stop codon and has not been reported previously in the literature.

Keywords: Merosin, muscular dystrophy, white matter hyperintensity

Öz

Artmış serum kreatin kinaz seviyesi, gelişim geriliği, kas güçsüzlüğü ve alt ekstremitede kontraktürlerin olduğu iki kız kardeş sunulmuştur. Büyük kardeşte yapılan elektromiyogram miyopatik bulgular ile uyumluydu. Her iki kardeşteki kranial manyetik rezonans görüntülemede yaygın beyaz cevher sinyal artışı izlendi. Büyük kardeşe kas biyopsisi yapıldı ve immüno histokimyasal çalışmalar distrofik patern ve merozin negatifliği ile uyumluydu. Merozin negatif müsküler distrofi (MDC1A) ön tanısıyla yapılan *LAMA2* gen mutasyon analizinde NM_000426.3:c.163_163delA; (p.N55Mfs*16) homozigot çerçeve kayma mutasyonu görüldü. Bu mutasyon prematür stop kodona yol açmaktadır ve daha önce literatürde saptanmamıştır.

Anahtar Sözcükler: Merozin, müsküler distrofi, beyaz cevher sinyal artışı

Introduction

Merosin-deficient congenital muscular dystrophy (MDC1A, #607855) is a disorder characterized by muscle weakness and hypotonia at birth due to mutations in the laminin α-*2 gene (LAMA2)* mapped to the 6q22-23 chromosomal location. *LAMA2 gene* encodes merosin. LAMA2-related muscular dystrophies are a clinically homogeneous group typically presenting with hypotonia and brain white-matter hyperintensity (1,2). In this study, we present a case of MDC1A in two siblings with a novel homozygous frameshift mutation c.163_163delA; (p.N55Mfs*16) in the *LAMA2 gene*. This mutation leads to a premature stop codon, which has not been reported in the literature.

Case Reports

Case 1

A 3-year-old girl was the first child of second-degree consanguineous parents (first cousins). The parents were healthy and there was a family history of two cousins with unknown neuromuscular disease. The girl was born at 39 weeks of gestation and the pregnancy was uneventful, but she was hospitalized for ten days in the neonatal unit because of hypotonia and feeding difficulties.

On physical examination, the muscle strength of her lower limb muscles was grade 3/5 of the Medical Research Council (MRC) scale and the upper limb muscle strength was grade 4/5 with contractures of the lower limbs.

Address for Correspondence/Yazışma Adresi: Senem Ayça, Celal Bayar University Faculty of Medicine, Department of Pediatric Neurology, Manisa, Turkey
E-mail: senemkaleci85@gmail.com ORCID: orcid.org/0000-0001-7486-9655
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Developmental milestones were delayed; by 5 months of age, the patient was able to hold her head up, and the patient was able to sit unsupported at the age of 17 months, but she could not stand. Her deep tendon reflexes in the upper and lower extremities were absent. The cranial nerves and coordination were normal. On sensory examination, tactile, pinprick, and vibration were normal. An elevated serum creatine kinase (CK) concentration of 1690 IU/L was revealed. An electromyogram showed low amplitude polyphasic motor unit potential compatible with a myopathic process. MDC1A was suspected and magnetic resonance imaging (MRI) showed diffuse white matter hyperintensity supporting the diagnosis (Figure 1). At the age of one year, a muscle biopsy showed myofibers in different sizes and shapes with neonatal myosin, proliferated interstitial tissue with Masson's trichrome, and increased immature fiber and irregular myofibrils with nicotinamide adenine dinucleotide tetrazolium reductase and diffuse sarcolemmal merosin deficiency (Figure 2). The patient had MDC1A both with clinical and imaging presentation. We performed *LAMA2* gene sequencing and revealed a novel homozygous frameshift mutation c.163_163delA; (p.N55Mfs*16), which confirmed the diagnosis of MDC1A by molecular test.

Case 2

A 20-month-old girl was the second child of the same family. The pregnancy was uneventful but after birth, axial hypotonia was recognized and she was referred to the pediatric neurology department of our clinic. On physical examination, she had lower limb proximal muscle weakness of grade 3/5 MRC with contractures of the lower limbs. Her deep tendon reflexes in the upper and lower extremities were absent. An examination of the cranial nerves was normal. The serum CK concentration was 2140 mU/mL. MRI revealed diffuse white matter hyperintensity. *LAMA2* gene sequencing of the patient revealed a novel homozygous frameshift mutation c.163_163delA; (p.N55Mfs*16), the same as in her sister.

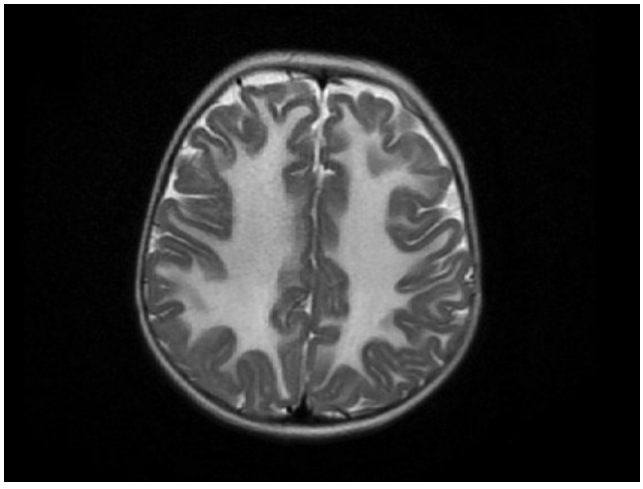

Figure 1. Case 1 MR T2 Flair axial image reveals diffuse T2 hyperintensity

MR: Magnetic resonance

Discussion

MDC1A is one of the most frequently seen congenital autosomal recessive muscular dystrophy. Muscle weakness is slowly progressive and is accompanied by arthrogryposis. In the infantile period, patients with MDC1A usually have respiratory and feeding difficulties that ultimately result in hospitalization, especially in the neonatal period. The older sibling had a history of hospitalization in the neonatal period because of feeding difficulties, but the younger sibling had no such history.

Most patients with MDC1A have the ability to sit unsupported, but cannot stand or walk. They usually have normal intellectual and speech development. Our patients had the ability of sitting unsupported and Denver Developmental Screening Test assessments were in accordance with their age.

The pattern of brain white matter abnormalities on neuroimaging is characteristic and presents in patients with MDC1A aged over 6 months (3). MRI revealed diffuse white matter hyperintensity in both siblings. In MDC1A, high serum CK concentrations are present. With the characteristic MRI pattern and high serum CK values, molecular testing for the *LAMA2* gene should be performed.

Although over 350 causative mutations have been identified for MDC1A, no treatment is yet available. There are many therapeutic approaches in development;

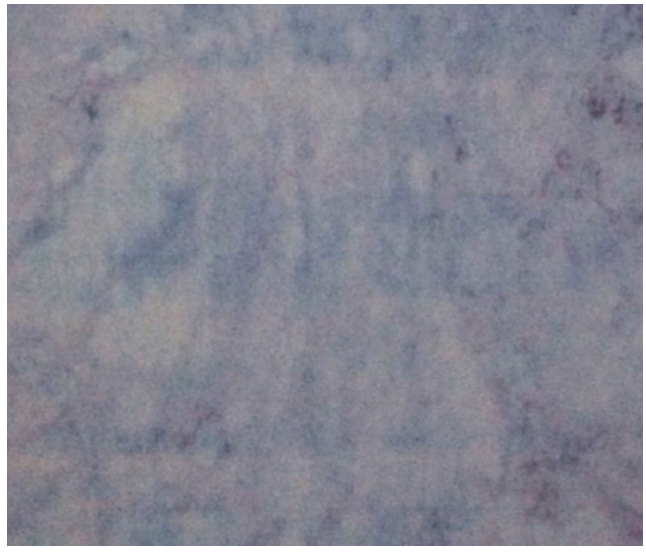

Figure 2. Muscle biopsy case 1 with diffuse sarcolemmal merosin deficiency

a recent study confirmed the recovery of the laminin-α2 chain following skipping of the mutated exon in mice (4). These findings support the future development of phosphorodiamidate morpholino oligomer-mediated therapies for MDC1A (4). Also, the orphan drug Tarix TXA127, which is a pharmaceutical formulation of the naturally occurring peptide angiotensin-1-7, has been effective in MDC1A animal models and has been granted status as a potential treatment for MDCA1 (5).

MDCA1 is inherited in an autosomal recessive manner, further pregnancies carry a one in four risk of bearing an infant with MDCA1, thus molecular diagnosis is highly recommended for parents with an affected child. Fadiloglu et al. (6) performed chorionic villus sampling for prenatal diagnosis of MDCA1 with immunohistochemical studies. They concluded that immunohistochemical studies had high specificity for the diagnosis of merosin-negative muscular dystrophy and may be evaluated as reliable.

Molecular genetic testing was planned but could not be performed for the older sibling and at that time, the parents had the second affected child. Sequencing of the two siblings revealed a novel homozygous frameshift mutation c.163_163delA; (p.N55Mfs*16) within the *LAMA2* gene. *In silico* analysis with MutationTaster, PolyPhen2, SIFT predicted this variant as a disease-causing mutation. Their parents refused to be included in the study for showing familial segregation. This mutation leads to a premature stop codon and has not been reported previously in the literature. Consistent with the phenotype of the patient, this mutation might be responsible for the early-onset disease and severe congenital hypotonia.

The aim of this study was to characterize the clinical and genetic features of the siblings with MDC1A. This study emphasizes that MDC1A should be kept in mind in individuals with congenital hypotonia, muscle weakness, elevated serum CK concentrations, and white matter abnormalities. Molecular diagnosis is essential for genetic counselling and prenatal diagnosis when required, especially in our country because consanguineous marriage is frequently seen.

Authorship Contributions

Concept: M.P., S.Ç. Design: S.A., H.B.G.Ç. Data Collection or Processing: S.A., H.B.G.Ç. Analysis or Interpretation: M.P., S.Ç. Literature Search: S.A., H.B.G.Ç. Writing: S.A., H.B.G.Ç.

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Breast Cancer Metastasis Mimicking Osteomyelitis

Osteomiyeliti Taklit Eden Meme Kanseri Metastazı

Deniz Hancı, Onur Üstün*, Şelale Dinç**

Okmeydanı Training and Research Hospital, Clinic of Otorhinolaryngology, İstanbul, Turkey
**University of Health Sciences Turkey, Haseki Training and Research Hospital, Clinic of Otorhinolaryngology, İstanbul, Turkey*
***Memorial Şişli Hastanesi, Clinic of Radiology, İstanbul, Turkey*

Abstract

An 80-year-old woman with a history of breast cancer 16 years ago presented with malnutrition, swelling, and paresthesia of the right cheek. Computed tomography revealed diffuse osteosclerosis without lysis and adjacent soft tissue swelling on the right mandible. These radiological findings were more compatible with primary chronic osteomyelitis than metastasis from breast cancer, and biopsy was necessary for diagnosis. This report describes breast cancer metastasis mimicking primary chronic osteomyelitis 16 years after diagnosis and its differential diagnosis in the mandible.

Keywords: Osteomyelitis, breast cancer metastasis, mandibula

Öz

On altı yıl önce geçirilmiş meme kanseri hikayesi mevcut olan 80 yaşındaki hasta malnütrisyon, sağ yanağın şişmesi ve parestezisiyle başvurdu. Bilgisayarlı tomografide sağ mandibulada lizis bulunmayan osteoskleroz ve komşu dokularda şişlik izlendi. Bu radyolojik bulgular meme kanseri metastazından çok primer kronik osteomiyelit ile uyumluydu. Tanı için biyopsi alındı. Bu olgu sunumunda 16 yıl önce geçirilmiş meme kanserinden mandibulaya primer kronik osteomiyeliti taklit eden metastaz olgusu sunulmuştur.

Anahtar Sözcükler: Osteomiyelit, meme kanseri metastazı, mandibula

Introduction

Metastatic tumors are rare in the oral region. Metastatic lesions may occur in the bones of the jaw, in soft tissues of the oral cavity, or in both osseous and soft tissues. The mandible is one of the most frequent locations for metastases and, the most frequently involved site in the jaw bones is the molar area. This region is susceptible to the accumulation of neoplastic cells due to branching of local blood vessels, slowing of blood flow and the presence of hematopoietic bone marrow. Diagnosis of metastatic lesions is challenging due to their rarity and their atypical clinical and radiographic appearance. Since the most common symptom in the jaw is pain, these lesions can be misdiagnosed as pathological entities of dental origin (1).

Metastatic tumors of the oral cavity do not show a pathognomonic radiographic sign, and radiographic examination is therefore rarely diagnostically important. Although most patients have been previously diagnosed with primary neoplasms and treated, the first clinical sign of the malignancy is seen in the oral region in one third of metastases (2).

Patients with metastases in the jaw show various clinical symptoms and signs, which are swelling, pain, paresthesia of the lip, halitosis, loose or extruded teeth, gum irritation, regional lymphadenopathy, trismus, mandibular nerve involvement and numb chin syndrome (NCS), ulceration, cortical expansion of the jaw bones, and exophytic growth (1). Paresthesia or numbness of the chin and lower lip are important signs of metastatic tumors (2).

The clinical presentation resembles frequent pathological conditions, such as inflammatory hyperplasia, toothache, temporomandibular joint pain, osteomyelitis, periodontal conditions, trigeminal neuralgia, pyogenic granuloma, or giant cell granuloma, and therefore, the diagnosis of such cases can be difficult.

In the early stages of the disease, the lesion may not present a radiographic sign. In the analysis of Hirshberg et al. (2) with 390 metastatic tumors of the jaw, they found

Address for Correspondence/Yazışma Adresi: Deniz Hancı, Okmeydanı Training and Research Hospital, Clinic of Otorhinolaryngology, İstanbul, Turkey
E-mail: dhanci007@hotmail.com ORCID: orcid.org/0000-0002-6644-5059
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that 5.4% of the tumors did not show any important radiographic changes.

Diagnosis is made more difficult compared to cases of acute and secondary chronic osteomyelitis because of the clinical appearance and clinical course of primary chronic osteomyelitis. There is no predisposing event, such as an oral surgical procedure or an infected tooth. Clear signs of infection, such as the occurrence of a pus or fistula, are lacking.

This report describes breast cancer metastasis mimicking primary chronic osteomyelitis 16 years after diagnosis and its differential diagnosis in the mandible.

Case

An 80-year-old female with a history of breast cancer 16 years ago was admitted to our ear nose and throat clinic with the complaints of feeding difficulty, fatigue and subtle pain in the area of right mandibular molar parts. She had numbness for 3 years and swelling on the right side of the cheek for 1 year. The medical anamnesis revealed that the patient was operated (modified radical mastectomy with axillary lymph node dissection) 16 years ago for breast carcinoma of the right breast. After operation, the patient did not take radiotherapy or chemotherapy. The patient did not receive bisphosphatase treatment. She had visited her physician 5 years periodically for annual examination. Her medical history included hypertension and diabetes, and there was no history of alcohol or tobacco use. Clinical examination was difficult because of severe trismus. On intra-oral examination, diffuse swelling that was hard on palpation was observed on the right half of the mandible. Movement of the jaw was restricted. There was severe pain over the right half of the face and paresthesia of the chin and lower lip. Regional lymph nodes were not palpable.

Axial and serial 1 mm-thick cross-sectional images obtained from cone beam computed tomography (CT) showed small radiolucent areas in close proximity to the third molar (Figure 1) that were not diagnostic of metastases. Magnetic resonance imaging (MRI) provided the most accurate view of a chronic osteomyelitis lesion in the right mandible (Figure 2).

Incisional biopsies were taken under local anesthesia from the mandible and sent to the pathology laboratory.

Formalin-fixed, paraffin-embedded five micron-thick tissue sections were stained with hematoxylin and eosin. The neoplastic cells contained abundant eosinophilic cytoplasm and large, darkly stained, pleomorphic nuclei (Figure 3). Several mitoses including atypical forms and minimal lymphoplasmacytoid inflammatory infiltration of the stroma were observed. The diagnosis was consistent with metastatic carcinoma of breast origin. Slides from the primary breast lesion were not available to compare with the metastatic focus.

For further treatment, the patient was referred to an oncologist, but the patient refused treatment. Written informed consent was obtained from the patient. The patient died of her disease 6 month later.

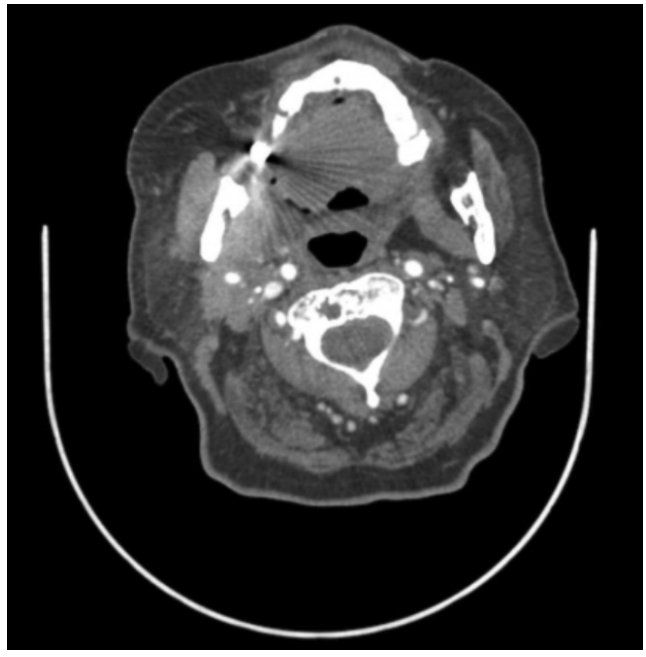

Figure 1. Axial and serial cross-sectional 1-mm thick cone beam computed tomography showed small radiolucent areas in close proximity to the third molar

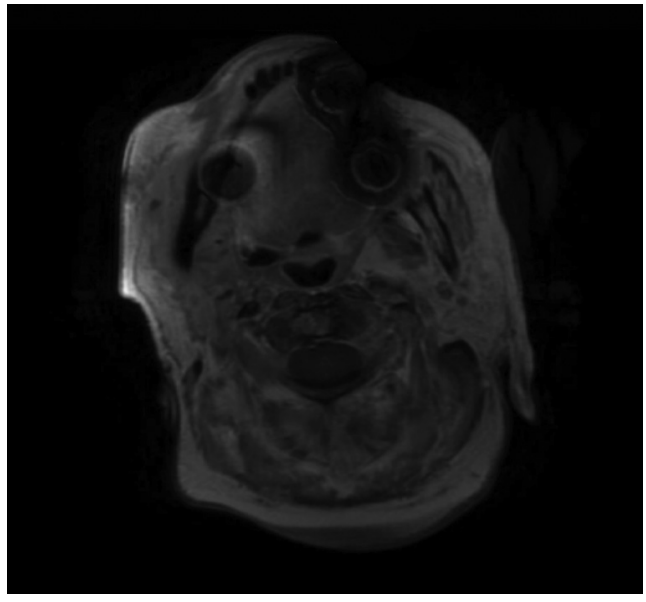

Figure 2. Magnetic resonance imaging provided the most accurate view of a chronic osteomyelitis lesion in the right mandibula

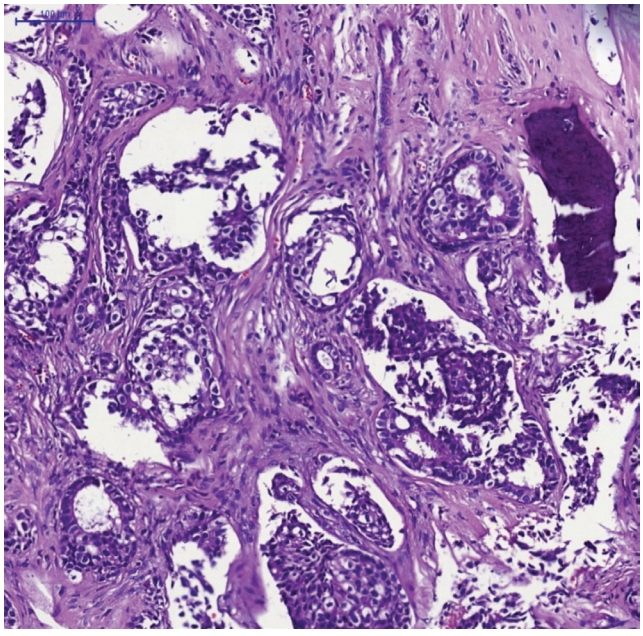

Figure 3. Adenocarcinoma. This tumor forms irregular-shaped and a cribriform glands with cytologically malignant cells exhibiting hiperchormatic nuclei in a fibroblastic stroma

Discussion

Metastasis is the spread of tumor cells from the primary tumor, their invasion of lymphovascular structures and survival in the circulation. The microvascular structure of the target organ provides space for metastatic tumor cells to which they can extravasate, invade and multiply in this target tissue. Angiogenesis is mandatory for tumor cell load of more than 2-3 mm for adequate oxygen and nutrient supply (2).

Diagnosis of oral cavity metastasis is an important challenge for the clinician due to the absence of pathognomonic signs and symptoms.

Breast cancer primarily metastasizes to the regional lymph nodes, bone, lungs, pleura, and liver. Bone is the most common site of recurrence of the breast cancer which can be lytic, sclerotic or mixed. In the present case, the mandibular lesion was of sclerotic type. Sclerotic bone metastases are more common in patients with prostate, bladder, medulloblastoma or bronchial carcinoid tumors.

Late metastasis is usually defined for lesions when they appear more than 5 years after the treatment of a primary malignant tumor. Late recurrence of some of the malignant tumors, such as renal cell carcinoma, breast cancer and malignant melanoma, has been well documented in the literature. Late recurrences are common in estrogen receptor (ER)-positive breast cancers. More than half of the recurrences of ER-positive breast cancers occur 5 years or later after the diagnosis and treatment of the primary tumor, and some cases may recur even more than 20 years after surgery. In contrast to ER-positive breast cancers, ER-negative breast cancer recurrences are more common in the first two years of treatment and these are rare 5 years after the treatment (3).

The present case was an ER-negative breast cancer and her mandibular lesion was diagnosed as late metastasis, occurring 16 years after the treatment of the primary tumor.

Oral region metastatic tumors are rare, comprising 1-3% of all malignant oral neoplasms. Malignant tumors of the breast, lung and kidney are the frequent primary sources. Metastatic tumors may occur in the oral soft tissues, jaw bones or both. Early detection of oral metastasis is critical, because the prognosis is usually poor. Most patients with the diagnosis of oral metastasis die within 1 year, while the 4-year survival rate is around 10%. The survival in patients with lung cancers is longer than in patients with non-lung cancers. Most patients having oral metastasis have already developed generalized metastases; but, in numerous cases, mandibular metastasis may be the first sign of a primary tumor (4).

The mandible is the frequently involved location for metastases. Clinical findings of mandibular metastasis may mimic reactive or benign lesions or sometimes simple odontogenic infections. Pain, bony swelling with tenderness, hemorrhage, ulcer, tooth mobility, paresthesia, trismus and pathological fracture can be seen as clinical findings (5).

According to some meta-analysis about oral metastases, the gender distribution is either predominantly male or nearly equal. The primary location reported in the Western literature is the breast for women and lungs for men (6). In this paper, we reported a female patient.

Metastasis to the jaw bones is caused by the hematogenous pathway of the spread of the malignant tumor, and this needs the presence of a well-connected hematopoetically active bone marrow with the sinusoidal vascular spaces in the accumulation area of malignant cells. The posterior part of the mandible and focal osteoporotic bone marrow defects have been shown to be predisposed areas for metastatic tumor cells (7). Vascular changes in association with inflammatory process are thought to be responsible for oral metastasis. Chronic trauma to oral tissues has been shown to support the metastatic spread of malignant tumors into the oral cavity (8). A lymphatic system does not exist in the jaws and it is thought that metastasis occurs through bloodstream. A metastasis pathway to

the maxillofacial area is Batson's plexus (vertebral venous plexus), which in some cases do not include the lungs, as in this patient. In another study with 55 cases, it was found that tooth extraction predated the detection of the metastasis (9). Therefore, the role of mucosal trauma, especially in particular improper dentures, poor oral hygiene and tooth extraction trauma, sharp teeth or restorations, should be further investigated in causation of oral metastasis.

The symptoms of oral/oropharyngeal metastasis are inequable and not pathognomonic. When oral lesions are present, biopsy is mandatory, especially in patients with known malignant disease, even if a benign condition is considered.

Paresthesia of the chin and lower lip is one of the important symptoms in metastatic disease. In the literature, it is defined as mental nerve neuropathy or NCS (10). Branches of the mandibular nerve, which are the inferior alveolar nerve and its branches like mental nerve, are associated with NCS. Since this nerve does not have motor fibers, NCS is a sensory neuropathy. Numbness of the teeth and mucosa may occur in addition to the lip and chin paresthesia. The main cause of NCS involves compression of nerve tissues by a tumor or perineural spread of a metastatic disease. Neoplasms, which are most frequently associated with NCS, are metastatic carcinomas of the mandible and lymphomas (8). Mostly, NCS is iatrogenic and is commonly caused by dental injury or anesthesia of the inferior alveolar nerve after placement of dental implants improperly. The other reasons are odontogenic or nonodontogenic tumors or cysts, acute or chronic osteomyelitis, and also systemic diseases such as Diabetes Mellitus, multiple sclerosis, sickle cell anemia, and human immunodeficiency virus infections (10). Our patient did not report paresthesia as the initial complaint, but it was observed that there was a change in the sensation of the lips and jaws in both intra-oral and extra-oral examinations. Thus, the presence of NCS should always alert the physician about the possibility of the presence of a primary or recurrent malignant neoplasm, particularly in cases of significant medical history.

NCS may be the first and only manifestation of malignancy. Therefore, it should be seen as malignancy unless proven otherwise and should never be ignored as a minor symptom. In a patient with a known malignancy, a detailed physical examination should be performed in the presence of a jaw, followed by panoramic radiography, CT or MRI, and a full body bone scan for further examination (11).

Osteosclerosis is the most striking radiologic pattern seen in primary chronic osteomyelitis which is usually encountered in adult cases. Osteosclerosis and osteolysis can be seen together and leading to a so called "mixed pattern" which is more frequent in younger patients. Bone expansion and "onion skin" periosteal reaction are some of the radiologic features associated with primary chronic osteomyelitis. Sequestration is not seen any case of primary osteomyelitis. Sclerotic form of primary chronic osteomyelitis may be confused with fibrous dysplasia or Paget's disease (12).

In our case, the sclerotic bone lesion was compatible with late metastasis from breast cancer and primary chronic osteomyelitis. Biopsy and histopathological examination were required because clinical and imaging findings were unspecific for definitive diagnosis. Histopathological examination revealed ER-negative breast cancer metastasis.

Conclusion

Otolaryngologists and dentists should include mandibular metastasis in the differential diagnosis during their general physical examination in patients with atypical symptoms, particularly if there is a history of previous breast cancer even many years after the initial diagnosis.

Authorship Contributions

Concept: D.H., O.Ü. Design: D.H., O.Ü. Data Collection or Processing: D.H., Ş.D. Analysis or Interpretation: O.Ü., Ş.D. Literature Search: D.H., Ş.D. Writing: D.H.

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