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Cilt: 39 Sayı: 2 HAZİRAN 2023

Araştırma Makalesi / Research Article

Olgu Sunumu / Case Report

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YAZARA AÇIKLAMA

Selçuk Tıp Dergisi (Selcuk Med J) Necmettin Erbakan Üniversitesi, Meram Tıp Fakültesi Dekan'lığının yayın organıdır. Dergimize yazı hazırlanırken aşağıdaki açıklamaları lütfen bütünüyle okuyunuz.

Selçuk Tıp Dergisi (Selcuk Med J) tıp bilimine ve akademik çalışmalara katkısı olan, klinik ve deneysel çalışmaları, editoryal yazıları, kısa raporları, klinik olgu bildirimlerini, teknik ve eğitici derlemelerini, tıp konusundaki son gelişmeler ile orijinal görüntü raporlarını, görüntülü hastalık tanımlama sorularını ve editöre mektupları yayınlar. Ayrıca daha önce yayınlanmış makale ve deneysel çalışmalarla ilgili okuyucu soru ve katkıları kısaca yayınlanır. Yayına kabul edilme, editöryal komite ile en az iki hakem kararı ile alınır. Bir hakem, hakemlik talebini kabul etmeye karar vermeden önce, hakem değerlendirme süreci ve gözden geçirmenin nasıl yapılacağı hakkında daha fazla bilgi edinmek isteyebilir.

Hakemler, Selçuk Tıp Dergisi'nin gereklerine, önceden tanımlanmış kriterlere ve sunulan araştırmanın kalitesine, eksiksizliğine ve doğruluğuna dayanarak makale gönderimini değerlendirir. Hakemler makale hakkında geri bildirimde bulunur, iyileştirmeler önerir ve makalede yapılan değişiklikleri kabul edip etmeme, talep etme veya reddetme konusunda editöre tavsiyede bulunur. Nihai karar her zaman baş editöre aittir, ancak hakemler sonucu belirlemede önemli bir rol oynamaktadır. Bir hakemin makaleyle çıkar çatışması varsa, editöre bildirmesi gerekir. Hakemler, hakem gözden geçirme sistemine katılarak bilimsel sürecin katı standartlarını sağlamalıdır. Ayrıca, geçersiz araştırmaları tespit ederek ve derginin kalitesini korumaya yardımcı olarak derginin bütünlüğünü korumalıdırlar. Hakemler, intihal, araştırma sahtekarlığı ve diğer sorunları tespit ederek etik konuların ihlal edilmesini önlemeye gönüllü olmalıdır.

Yayına kabul edilen yazıların her türlü yayın hakkı dergiye aittir. Bu hak özel düzenlenmiş yayın hakkı devir formu ile bütün yazarların imzası ile tespit edilir. Dergi 3 ayda bir, yılda 4 kez yayınlanır. Derginin yayın dili Türkçe ve/veya İngilizcedir. Gönderilen yazılar daha önce herhangi bir dergide yayınlanmamış olmalıdır (Bilimsel kongrelerde sunulan sözlü bildiri ve posterler bildirmek kaydı ile hariçtir). Dergide yayımlanan yazıların her türlü sorumluluğu (etik, bilimsel, yasal vb.) yazarlara aittir. Yazım kurallarına uygun olarak hazırlanmamış olan yazıların incelenmeye alınıp alınmaması Yayın Kurulu'nun insiyatifindedir.

Makalelerin daha önce hiçbir yerde yayınlanmamış ve yayın için başka bir dergiye gönderilmemiş olması gerekir. Selçuk Tıp Dergisi'nde intihal programı (iThendicate) kullanılmaktadır. Akademik atıf sınırını aşan benzerlik taşıyan makaleler ve yayın kurallarına uygun olarak hazırlanmamış makaleler değerlendirmeye alınmayacaktır. Tüm çalışmalarda etik kurul onayı gerekmektedir ve bu onamın belgelendirilmesi yazıların yayınlanmasında esas teşkil edecektir.

Tüm çalışmalarda yazarların çalışmaya katkı düzeyi ve onayı bildirilmelidir. Çalışmada veri toplanması, deney aşaması, yazım ve dil düzenlemesi dahil olmak üzere herhangi bir aşamasında finansal çıkar çatışması olmadığı bildirilmelidir. Çalışmada varsa ticari sponsorluk bildirilmelidir.

Derginin editöryal ve yayın süreçleri International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE) ve National Information Standards Organization (NISO) organizasyonlarının kılavuzlarına uygun olarak biçimlendirilmiştir. Selçuk Tıp Dergisi'nin editöryal ve yayın süreçleri, Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice) ilkelerine uygun olarak yürütülmektedir. Yayın Kurulu, dergimize gönderilen çalışmalar hakkındaki intihal, atıf manipülasyonu ve veri sahteciliği iddia ve şüpheleri karşısında COPE kurallarına uygun olarak hareket edecektir.

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

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

YAZIM KURALLARI

Yayına gönderilen yazılar Microsoft Word programında yazılmalıdır. Yazı, şekil ve grafilerin tamamı elektronik ortamda https://www.selcukmedj.org word ve pdf formatında gönderilmelidir.

Tüm yazılar:

1. Başlık sayfası,
2. Türkçe özet,
3. İngilizce özet,

4. Makale kısmı,
5. Kaynaklar,
6. Tablolar,
7. Şekiller ve resimler,
8. Alt yazılar şeklinde dizilmelidir.

Araştırma inceleme yazılarının makale kısmı (özet, referanslar, tablo, şekil ve alt yazılar hariç) toplam 4000 kelimeyi, özet kısmı 400 kelimeyi, referanslar 60'ı, tablo ve şekil sayısı 10'u geçmemelidir. Özet amaç, gereç ve yöntemler, bulgular ve sonuç bölümlerini içermelidir.

Olgu bildirileri şu bölümlerden oluşmalıdır: Başlık, İngilizce başlık, Türkçe ve İngilizce özet, giriş, olgunun/olguların sunumu, tartışma ve kaynaklar. Olgu sunumları toplam 8 sayfayı geçmemeli ve 3 resimden fazla olmamalıdır. Özet 200 kelimeyi geçmemeli ve tek bir paragraf şeklinde olmalıdır.

Derlemeler İngilizce ve Türkçe özet içermeli ve özet kelime sayısı 300'ü aşmamalıdır. Tablo sayısı ve şekiller (veya resimler) toplam 6 adedi aşmamalıdır. REferanslar 80'i geçmemelidir. Özet tek bir paragraf şeklinde olmalıdır. Editöre mektup, kısa raporlar, görüntü raporları, teknik ve tıp alanındaki gelişmelere ait yazılar ve orijinal konulara ait görüntü sunumları 2 sayfayı geçmemelidir. Kısa bir (100 kelime) İngilizce ve Türkçe özet içermelidir.

YAZILARIN HAZIRLANMASI

Yazının başlığı hem İngilizce hem de Türkçe olarak yazılmalıdır. Yazıda çalışmaya katkısı olan yazarların ad ve soyadları açık olarak yazılmalı. Yazıların altına çalışmanın yapıldığı kurumun açık adresi yazılmalıdır. Çalışma daha önce herhangi bir kongrede sunulmuş ise kongre adı, zamanı (gün-ay-yıl olarak) belirtilmelidir. Başlık sayfasının en altına iletişim kurulacak yazarın adı, soyadı, açık adresi, posta kodu, telefon ve faks numaraları ile e-posta adresi yazılmalıdır.

Özetler

Ayrı bir sayfa olarak verilmelidir. İngilizce özetin başında İngilizce başlık bulunmalıdır. Araştırma inceleme yazılarında 400, olgu sunumlarında 200 kelimeyi geçmemelidir. Araştırma makalelerinde özet amaç, gereç ve yöntemler, bulgular ve sonuç bölümlerini içermelidir. Araştırma ve inceleme yazılarında özetlerden sonra Türkçe ve İngilizce anahtar kelimeler verilmelidir. Anahtar kelime sayısı 5'i geçmemelidir. Anahtar Kelimelerin İngilizcesi Index Medicus'daki Medical Subjects Headings'e uygun olmalı, Türkçe Anahtar kelimeler ise Türkiye Bilim Terimleri'nden (http://www.bilimterimleri.com) seçilmelidir. Özetlerde kısaltma olmamalıdır.

Makale

Yazı Giriş, Gereçler ve Yöntem, Bulgular ve Tartışma bölümlerinden oluşur.

Giriş: Konuyu ve çalışmanın amacını açıklayacak bilgilere yer verilir.

Gereçler ve Yöntem: Çalışmanın gerçekleştirildiği yer, zaman ve çalışmanın planlanması ile kullanılan elemanlar ve yöntemler bildirilmelidir. Verilerin derlenmesi, hasta ve bireylerin özellikleri, deneysel çalışmanın özellikleri ve istatistiksel metotlar detaylı olarak açıklanmalıdır. Çalışma klinik bir çalışma ise başlık 'Hastalar ve Yöntem' şeklinde olmalıdır.

Bulgular: Elde edilen veriler istatistiksel sonuçları ile beraber verilmelidir.

Tartışma: Çalışmanın sonuçları literatür verileri ile karşılaştırılarak değerlendirilmelidir.

Tüm yazımlar Türkçe yazım kurallarına uymalı, noktalama işaretlerine uygun olmalıdır. Kısaltmalardan mümkün olduğunca kaçınılmalı, eğer kısaltma kullanılacaksa ilk geçtiği yerde ( ) içerisinde açıklanmalıdır. Kaynaklar, şekil tablo ve resimler yazı içerisinde geçiş sırasına göre numaralandırılmalıdır. Metin içerisindeki tüm ölçüm birimleri uluslararası standartlara uygun biçimde verilmelidir.

Kaynaklar

Kaynaklar iki satır aralıklı olarak ayrı bir sayfaya yazılmalıdır. Kaynak numaraları cümle sonuna nokta konmadan ( ) içinde verilmeli, nokta daha sonra konulmalıdır. Kaynak yazar isimleri cümle içinde kullanılıyorsa ismin geçtiği ilk yerden sonra ( )
içinde verilmelidir. Birden fazla kaynak numarası veriliyorsa arasına ",", ikiden daha
fazla ardışık kaynak numarası veriliyor ise rakamları arasına ",-" konmalıdır [ör.(1,2), (1-3)gibi]. Kaynak olarak dergi kullanılıyorsa: yıl, cilt, başlangıç ve bitiş sayfaları verilir. Kaynak olarak kitap kullanılıyorsa: sadece yıl, başlangıç ve bitiş sayfaları verilir. Kaynaklarda yazarların soyadları ile adlarının baş harfleri yazılmalıdır. Dergi isimleri Index Medicus'a göre kısaltılmalıdır. Kaynak yazılma şekli aşağıdaki örnekler gibi olmalıdır. Yazar sayısının üçten fazla olması durumunda ise ilk üç yazarın ismi yazılmalı, sonrasında "et al." eklenmelidir.

Dergiler için

1) Kocakuşak A, Yücel AF, Arıkan S. Karına nafiz delici-kesici alet yaralanmalarında rutin abdominal eksplorasyon yönteminin retrospektif analizi. Van Tıp Dergisi 2006;13(3):90-6.

2) Vikse BE, Aasard K, Bostad L, et al. Clinicalprognostic factors in biopsy-proven benign nephrosclerosis. Nephrol Dial Transplant 2003;18:517-23.

Kitaplar için

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

Kitaptan Bölüm İçin

1) Soysal Z, Albek E, Eke M. Fetüs hakları. Soysal Z, Çakalır C, ed. Adli Tıp, Cilt III, İstanbul Üniversitesi Cerrahpaşa Tıp Fakültesi Yayınları, İstanbul, 1999:1635-50.

2) Davison AM, Cameron JS, Grünfeld JP, et al. Oxford Textbook of Clinical Nephrology. In: Williams G, ed. Mesengiocapillary glomerulonephritis. New York: Oxford University Press, 1998: 591- 613.

Tablolar

Tablolar ayrı sayfaya iki satır aralıklı yazılmalı, her tablonun üzerinde numara ve açıklayıcı ismi olmalıdır. Tabloda kısaltmalar varsa tablonun altında alfabetik sıraya göre açılımları yazılmalıdır. Örnekler: PS: pulmoner stenoz, VSD: ventriküler septal
defekt. Tablolar yazı içindeki bilgilerin tekrarı olmamalıdır.

Şekil ve Resimler

Şekil ve resimler mutlaka isimlendirilmeli ve numaralandırılmalıdır. Resimler minimum 300 dots per inch (dpi) çözünürlüğünde ve net olmalıdır. Resimler makaleden ayrı bir şekilde makale gönderimi esnasında elektronik olarak JPEG formatında gönderilmelidir.

Makale içerisinde geçen resimler kabul edilmeyecektir. Renkli resimlerin basımı ancak yazarın basım ücretini kabul etmesi ve bu ücreti ödemesi halinde mümkün olacaktır. Aksi takdirde resim siyah-beyaz olarak basılır. Şekil ve resim altlarında kısaltmalar kullanılmış ise, kısaltmaların açılımı alfabetik sıraya göre alt yazının altında belirtilmelidir. Mikroskobik resimlerde büyütme oranı ve tekniği açıklanmalıdır. Yayın kurulu, yazının özünü değiştirmeden gerekli gördüğü
değişiklikleri yapabilir.

HAKEM RAPORU SONRASINDA DEĞERLENDİRME

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

SON KONTROL

1. Yayın hakkı devir ve yazarlarla ilgili bildirilmesi gereken konular formu gereğince
doldurulup imzalanmış,
2. Özet makalede 400, olgu sunumunda 200 kelimeyi aşmamış,
3. Başlık Türkçe ve İngilizce olarak yazılmış,
4. Kaynaklar kurallara uygun olarak yazılmış,
5. Tablo, resim ve şekillerde bütün kısaltmalar açıklanmış olmalıdır.

ONLİNE YÜKLEME BASAMAKLARI

https://www.selcukmedj.org sayfasında
1. Makale şekli *
2. Türkçe ve İngilizce başlık *
3. Kısa başlık *
4. Türkçe ve İngilizce özet*
5. Türkçe ve İngilizce anahtar kelimeler *
6. Yüklenmesi gerekli bölümler (word makale dosyası, makale dosyasının pdf
formatı, Başlık sayfası, copyright formu, ek dosyalar (resim, şekil ve tablolar) şeklinde
5 basamakta tamamlanmalıdır.

INSTRUCTIONS FOR AUTHORS

Selcuk Medical Journal (Selcuk Med J) is a scientific publication of Necmettin Erbakan University, Meram Faculty of Medicine.

Please entirely read the instructions discussed below before submitting your manuscript to the journal. Selcuk Medical Journal publishes original articles on clinical or experimental work, case histories reporting unusual syndromes or diseases, brief reports, technical and educative reviews, recent advancement of knowledge of the medical sciences with original images, questionnaires of defining disease, and letters to the editor.

Final recommendation for publication is made by the editorial board and at least two independent reviewers. Before reviewers decide to accept a request to review, they might want to know more about the peer review process and how to conduct a review. Reviewers evaluate article submissions to journal based on the requirements of Selcuk Medical Journal, predefined criteria, and the quality, completeness and accuracy of the research presented. Reviewers provide feedback on the paper, suggest improvements and make a recommendation to the editor about whether to accept, reject or request changes to the article. The ultimate decision always rests with the chief editor but reviewers play a significant role in determining the outcome.

If a reviewer has conflict of interest regarding article, he should inform it to the editor. Reviewers should ensure the rigorous standards of the scientific process by taking part in the peer-review system. Also they should uphold the integrity of the journal by identifying invalid research, and helping to maintain the quality of the journal. Reviewers should be volunteer to prevent ethical breaches by identifying plagiarism, research fraud and other problems by dint of their familiarity with the subject area. The copyrights of articles accepted for publication is belonged to journal. This is determined by the assignment of copyright statement, signed by all authors.

The journal is published four times in a year. The language of the journal is Turkish and/or English. Manuscripts submitted to the journal should not be published before or not under consideration elsewhere (in the case of previous oral or poster presentation of the paper at scientific meetings author should inform the journal). The full responsibility of the articles (ethic, scientific, legal, etc.) published in the journal belong to the authors. If the paper is not prepared in conformity with the writing instructions, decision for its evaluation will be made by the members of the editorial board. The articles have not been published anywhere before and no other journal has been sent for the publication. Plagiarism program (iThendicate) is used in Selcuk Medical Journal. Articles with similarities that exceed the academic citation limit and/or articles that are not prepared in accordance with the publication rules will not be evaluated. All studies require approval of the ethics committee and certification of this certificate will be the basis for the publication of the manuscripts.

All studies should be informed of the level of authors' contribution and approval. The study should also be informed that there is no financial conflict of interest at any stage, including data collection, experimentation, writing and language editing. Commercial sponsorship should be reported if it is in work.

The editorial and publication processes of the journal include the International Committee of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME), the Council of Science Editors (CSE), the Committee on Publication Ethics (COPE), the European Association of Science Editors (EASE) and the National Information Standards Organization (NISO). The editorial and publishing processes of the Selcuk Medical Journal are conducted in accordance with the principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

The Editorial Board will act in accordance with COPE rules in the face of claims and suspicions of plagiarism, citation manipulation and data forgery of works submitted to our journal. The Editorial Board of the journal handles cases of objections and complaints within the scope of COPE guidelines. Authors can contact the Chief Editor or Editorial Board directly for objections and complaints. When necessary, an impartial representative is appointed for issues that the Editorial Board cannot solve within itself. The Chief Editor will make the final decision in the decision-making process for appeals and complaints.

Publisher and editor always be willing to publish corrections, clarifications, retractions and apologies when needed.

The all correspondences (manuscript submission, follow up, reviewers reports, revision files, ac- ceptance form and other forms about publications) about Selcuk Medical Journal should be made online at https://www.selcukmedj.org. The rules about the manuscripts that would be submitted are given below.

WRITING INSTRUCTIONS

Submitted manuscripts should be prepared using Microsoft Word program. All manuscripts, figures and pictures must be submitted electronically as word and pdf format h https://www.selcukmedj.org . Authors should ensure that (apart from the title page) the manuscript should contain no clues about the identity of authors and institution where the study was performed.

All papers should be arranged on the basis of following sequence: 1. Title page, 2. Turkish abstract, 3. English abstract, 4. Text of the article, 5. References, 6. Table(s), 7. Figure(s) and illustration(s), 8. Figure legend(s). In the original (research) articles number of words should not exceed 4000 (except abstract, references, tables, figures and legeds) for the text of article and 400 for the abstract. Upper limit for reference number is 60, and this limit is 10 for tables and figures. The abstracts should include objec- tive, materials and methods, results and conclusion sections. Case reports should be composed of Turkish and English title, Turkish and English abstracts, introduction, case report, discussion and references. The number of typewritten pages should not exceed 8 pages and 3 picture in case reports. In abstract, numbers of words should not exceed 200 and should be written as a paragraph.

Reviews should be composed of Turkish and English title. In abstract, numbers of words should not exceed

400. In reviews number of tables and figures (or pictures) should not exceed 6. Upper limit for reference number is 80. Abstracts should be written as a paragraph.

Letter to editor, brief report, image report, advancements in technical and medical topics and questionnaires of original issues should not exceed 2 typewritten pages. Its should be composed of Turkish and English abstracts (100 words).

PREPARATION OF MANUSCRIPT

Title Page

Title of the article should be written both in English and Turkish. The first and last names for all contributors designated as author should be written clearly. Subsequently, address of the institution where the study was performed should be written clearly. If the study was previously presented in any scientific meeting, name and date (as day-month-year) of the organization should be written. The name and mailing address of the corresponding author, accompanied by telephone and fax numbers, and e-mail should be written at the bottom of title page.

Abstracts

Abstracts should be given in separate sheets. English title should be used for English abstracts. The abstracts should not exceed 400 words in original articles and 200 words in case reports. The abstract should include objective, materials and methods, results and conclusion sections in research articles. Turkish and English key words should be listed at the bottom of the abstract page in original articles and should not be more than 5 words. In selecting key words, authors should strictly refer to the Medical Subject Headings (MeSH) list of the Index Medicus. Turkish key words should be selected from Turkish Science Term ((http://www.bilimterimleri.com). The abbreviations should not be used in the abstract.

Text

Text is composed of Introduction, Materials and Methods, Results and Discussion.

Introduction: The matter and purpose of the study is clearly defined.

Materials and Methods: This should include the date and design of the study, the setting, type of participants or materials involved, a clear description of all interventions and comparisons, and the statistical analysis. If the study is a clinical study the title should be 'Patients and Methods'.

Results: Collected data and results of statistical analysis should be outlined in this section.

Discussion: The discussion section should include interpretation of study findings and results should be considered in the context of results in other trials reported in the literature. All written content should be prepared in conformity with grammar and punctuation rules. Avoid abbreviations whenever possible; in case of necessary, it should be given in parentheses when they are first used. References, figures, tables and illustrations should be consecutively numbered in the order in which they have been cited in the text. All measurement units in the text should be used in accordance with international standards for units of measurement.

References

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Journal references:

1) Kocakuşak A, Yücel AF, Arıkan S. Karına nafiz kesici-delici batın yaralanmalarında rutin abdominal eksplorasyon yönteminin retrospektif analizi. Van Tıp Dergisi 2006;13(3):90-6.

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1) Danovitch GM. Handbook of kidney transplantation. Boston: Little, Brown and Company (Inc.), 1996: 323-8. Chapter in book references:

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2) Davison AM, Cameron CS, Grünfeld CF, et al. Oxford textbook of clinical nephrogology. In: Williams G, ed. Mesengiocapillary glomerulonephritis. New York: Oxford University Press, 1998: 591- 613.

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NEÜ YAYINEVİ

Neurodevelopmental Outcomes of Moderate/Late Preterm Infants At 11-12 Years of Age

Orta/Geç Preterm Bebeklerin 11-12 Yaş Arası Nörogelişimsel Prognozu

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Öz

Amaç: Orta/geç preterm doğan 11-12 yaşındaki çocukların nörogelişimsel sonuçlarını ve okul başarısını araştırmayı ve prognozu etkileyen risk faktörlerini belirlemeyi amaçladık.
Hastalar ve Yöntem: Yenidoğan yoğun bakım ünitesinde Ocak 2004-Aralık 2004 tarihleri arasında izlenen orta ila geç preterm bebekler çalışmaya dahil edildi; çocuklar 2016 yılında hastanemiz pediatri polikliniğinde muayene edildi. Perinatal ve neonatal dönem öyküleri hastane veri tabanından elde edildi. Somatik büyüme özellikleri yorumlandı. Nörogelişim, Wechsler Çocuklar İçin Zeka Ölçeği (WISC-R) ölçeği kullanılarak değerlendirildi. Pediatrik Semptom Kontrol Listesi (PSC) uygulandı. Sosyoekonomik düzeyin nörogelişimsel sonuç üzerindeki etkisi incelendi. Okul performansı karne notları kullanılarak değerlendirildi.
Bulgular: Yaş ortalaması 11.6 olan 41 çocuk değerlendirildi. Somatik büyüme ile ilişkili risk faktörleri anne yaşı (>35 yaş), fetal distres ve patent duktus arteriyozus idi. Sepsis, sözel zekada bir azalma ile ilişkilendirildi; periventriküler lökomalazi hem sözel hem de performans zekası üzerinde olumsuz etkilere sahipti. Sosyoekonomik düzey, performans ve tam ölçekli zeka ile orta düzeyde bir korelasyon gösterdi. PSC puanı pozitif olan çocukların zeka bölümü anlamlı olarak daha düşüktü.
Sonuç: Orta ila geç preterm bebekler, beynin tam olgunlaşmaması ve doğum sorunları nedeniyle hem nörolojik hem de gelişimsel olarak geride kalmaktadır. Erken prematüre bebeklere benzer şekilde, bu çocuklar uzun süre izlenmelidir; aile desteği, rehabilitasyon ve özel eğitim ihtiyaçları karşılanmalıdır.

Anahtar Kelimeler: Ergen sağlığı, okul performansı, zeka, nörolojik prognoz

Abstract

Aim: We aimed to investigate the neurodevelopmental outcomes and school success of 11- to 12-year-old children born as moderate/late preterm infants and identify risk factors affecting prognosis.
Patients and Methods: Moderate/late preterm infants followed in the neonatal intensive care unit between January 2004 and December 2004 were included, and the children were examined again in our pediatrics outpatient clinic in 2016. Perinatal and neonatal histories were obtained from the hospital database. Physical growth characteristics were interpreted. Neurodevelopment was evaluated using the revised Wechsler Intelligence Scale for Children (WISC-R). The Pediatric Symptom Checklist (PSC) was also applied. The effect of socioeconomic level on neurodevelopmental outcome was examined. School performance was evaluated using report card grades.
Results: Forty-one children with a mean age of 11.6 years were evaluated. Risk factors associated with physical growth outcomes were maternal age of >35 years, fetal distress, and patent ductus arteriosus. Sepsis was associated with a decrease in verbal intelligence while periventricular leukomalacia had negative effects on both verbal and performance intelligence. Socioeconomic level showed a medium correlation with performance and full-scale intelligence. The intelligence quotients of the children with positive PSC scores were significantly lower.
Conclusion: Moderate/late preterm infants lag both neurologically and developmentally due to incomplete maturation of the brain and natal problems. Similar to early preterm infants, these children should be monitored for extended periods, and family support, rehabilitation, and special education needs should be met.

Keywords: Adolescent health, school performance, intelligence, neurological prognosis

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INTRODUCTION

In recent years, prematurity has increased by 25% around the world, and moderate/late preterm infants constitute a significant proportion of this group (1). In 2021, the preterm birth rate in the United States was reported as 10.49% and the late preterm birth rate was 7.67%, the highest rate in recent years (2). Some studies have shown that preterm infants have higher rates of motor deficits, cognitive dysfunction, and behavioral problems than those born at full term (3,4).

Neurodevelopmental and behavioral problems detected in moderate/late preterm infants are largely attributed to incomplete brain maturation, as the brain development at 34 weeks is reported to be approximately 65% of that of the full-term brain (5). Although many studies have explored the neurodevelopment of early preterm infants, studies on the long-term follow-up and etiological risk factors of late premature infants are limited, especially at school age.

The present study was therefore undertaken to examine the neurological and developmental outcomes of moderate/late preterm infants and to investigate the factors affecting school performance and neurodevelopment.

PATIENTS AND METHODS

Patient Group

Moderate/late preterm infants who were followed in the Bakırköy Maternity and Pediatrics Hospital Neonatal Intensive Care Unit between January 2004 and December 2004 were included in the study. Those who were born between 32 weeks + 0 days and 33 weeks + 6 days of gestation were moderately preterm, while those born between 34 weeks + 0 days and 36 weeks + 6 days were late preterm. Term infants and infants with congenital malformations, genetic syndromes, or metabolic diseases were excluded. Demographic characteristics and perinatal and neonatal information were obtained from patient files, and follow-up information was obtained from outpatient clinic cards. The families were called via phone and appointments were scheduled with the participants of the research. Families and children were informed about the purpose of the study and informed consent was obtained.

Assessment of Risk Factors

Advanced maternal age, premature rupture of membranes (PROM), chorioamnionitis, placental dysfunction, chronic disease, hypertension, and smoking or substance use were investigated as perinatal risk factors. As neonatal risk factors, gestational week, Ballard score, and intrauterine growth were investigated, and data were also collected about morbidities in the neonatal period, including low Apgar score, respiratory distress syndrome (RDS), need for mechanical ventilation, bronchopulmonary dysplasia, intracerebral hemorrhage (ICH), hydrocephalus, necrotizing enterocolitis, retinopathy of prematurity (ROP), sepsis/meningitis, patent ductus arteriosus (PDA), hypoglycemia, and hyperbilirubinemia. Infants' diets were recorded as having been composed of breast milk and/or formula and mothers were asked about the duration of breastfeeding.

Examinations and Tests

The children were examined in our pediatrics outpatient clinic in 2016. Parents were asked during interviews about the children's health problems and growth characteristics. Information was also collected about the children's preschool education and special education needs. In the evaluation of school performance, grades from Turkish language courses were used for verbal scores and grades from mathematics courses were used for performance scores. Behavioral problems were recorded using the Pediatric Symptom Checklist (PSC) (6). The socioeconomic characteristics of the families were evaluated with a questionnaire form consisting of 13 questions that addressed the parents' monthly income, education, and occupation and the home environment. In total, scores from this questionnaire below 26 points were determined to reflect low, scores of 26-39 medium, and scores above 39 high socioeconomic levels.

Detailed physical and neurological examinations were performed. Height and weight values were situated on percentile curves for Turkish children (7). Cerebral palsy, intellectual disabilities, blindness, and posthemorrhagic hydrocephalus were considered major neurological deficits, whereas minor deficits included disorders of balance, muscle tone changes, speech disturbances, refractive errors, strabismus, and mild hearing deficits. Following these examinations, participants were directed to the psychologist for administration of the revised Wechsler Intelligence Scale for Children (WISC-R) (8).

Statistical Analysis

IBM SPSS Statistics 20 (2011) was used for statistical analysis. Data were analyzed using the chi-square test, Fisher exact test, McNemar test, and independent-samples t-test. Pearson correlation

analysis was used to compare two continuous variables. For statistical significance, $p<0.05$ was accepted at the 95% confidence interval. The ethics committee of our hospital (10.03.2015/15932) approved the study.

RESULTS

A total of 41 children (18 girls and 23 boys) with a mean age of 11.6 years (140.8 months) were evaluated. Thirty-three (80.5%) of these participants were born as preterm infants appropriate for gestational age, while 8 (19.5%) were born as infants with intrauterine growth restriction (IUGR). The mean gestational age was 34.3±1.2 weeks and the mean birth weight was 1780±310 g. When perinatal risk factors were examined, it was seen that 9.8% of mothers had hypertension during pregnancy, 4.9% had gestational diabetes, and 2.4% had PROM; fetal distress was present in 7.3% of the cases. Perinatal and neonatal risk factors are summarized in Table 1.

Table 1. Perinatal and Neonatal Risk Factors of the Cases

Perinatal Risk Factors		
	n	**%**
Maternal age >35 y	39	95.1
IUGR	8	19.5
Hypertension	4	9.8
Fetal distress	3	7.3
Gestational Diabetes	2	4.9
PROM	1	2.4
Placental abruption	0	0.0
Neonatal Risk Factors		
	n	**%**
TTN	20	48.8
Hyperbilirubinemia	18	43.9
Hypoglycemia	7	17.1
Sepsis	6	14.6
ICH	4	9.8
ROP	4	9.8
Hydrocephalus	3	7.3
PVL	3	7.3
Mechanical ventilation	2	4.9
PDA	2	4.9
Apnea	2	4.9
RDS	1	2.4
Meningitis	1	2.4
NEC	1	2.4
Congenital Heart Defect	1	2.4

*Independent risk factors
**IUGR: intrauterine growth restriction , PROM: premature rupture of membranes, TTN: transient tachypnea of the newborn, ICH: intracerebral hemorrhage, ROP: retinopathy of prematurity, PVL: periventricular leukomalacia, PDA: patent ductus arteriosus, RDS: respiratory distress syndrome, NEC: necrotising enterocolitis

ROP was detected in 4 infants (9.8%), and 2 of those cases were classified as Stage 2 and while 2 cases were Stage 3. Four preterm infants (9.8%) had ICH, and 3 of those cases were Stage 1 while 1 case was Stage 3. Regarding nutrition, 6 patients (14.6%) were never breastfed; the average duration of breastfeeding was 10.9 months (range: 1-36 months).

The neurological examinations of 37 children (90%) were within the normal limits. Intellectual disabilities (IQ of <70) in 3 cases and unilateral blindness in 1 case were defined as major neurological sequelae. Among the 3 patients with minor neurological deficits, 2 (5%) were followed for strabismus and 1 patient had gait disturbance (Table 2). Three children (7%) had learning disabilities, 4 (10%) had special educational needs, and 4 (10%) had a diagnosis of autism spectrum disorder (ASD). According to the PSC results, the scores of 4 children were above the threshold of 28, and they were evaluated by a child psychiatrist. The WISC-R scores of children with higher PSC scores were found to be significantly lower ($p<0.001$).

Considering the relationship between perinatal risk factors and WISC-R scores, advanced maternal age (>35 years), hypertension, gestational diabetes, fetal distress, and low Apgar score did not have significant effects on WISC-R scores ($p>0.05$). There was also no significant effect of gestational week or breastfeeding duration on WISC-R scores ($p>0.05$). Of the morbidities in the neonatal period, it was observed that the mean verbal intelligence score of the patients with sepsis was significantly lower ($p=0.019$), while the differences in their performance and full-scale intelligence scores were insignificant ($p=0.788$ and $p=0.197$, respectively). It was found

Table 2. Abnormal Neurological and Behavioral Findings of the Cases

Neurological Findings	**n**	**%**
Intellectual disability	3	7.3
Visual Problems		
Strabismus	2	5
Blindness	1	2.5
Hearing/speech impairment	0	0
Cerebral palsy	0	0
Gait-balance disorder	1	2.5
Behavioral Disturbances	**n**	**%**
PSC positive	4	10
Learning difficulty	3	7.3
Special education	4	10
Autism spectrum disorder	4	10

Table 3. Verbal and Performance Intelligence Scores According to Maternal, Perinatal and Neonatal Risk Factors

Risk Factors	Verbal Intelligence Score Mean ± standard deviation	p[a]	Performance Intelligence Score Mean ± standard deviation	p[a]
Maternal age> 35 yrs	80±4.2	0.329	86.5±7.8	0.499
IUGR	94.8±23.3	0.800	97.1±20.2	0.733
Gestational diabetes	96.5±4.9	0.806	91.5±14.8	0.777
Maternal hypertension	87.8±13.4	0.562	95.8±7.9	0.943
Fetal distress	88.7±15.3	0.680	92.3±11.5	0.787
ROP	77±20.6	0.078	84.8±6.7	0.235
Hydrocephalus	100±6.6	0.532	101±10.8	0.568
Hypoglycemia	90.3±12.8	0.670	98.6±6.2	0.320
Sepsis	84.5±5.5	**0.019**	97±7	0.788
Mechanical ventilation	104.5±16.3	0.402	98±8.5	0.822
ICH	87.8±22	0.562	89.3±11.8	0.504
PDA	101.5±23.3	0.539	116±11.3	0.097
Apnea	105±15.6	0.382	97.5±9.2	0.852
Hyperbilirubinemia	91.3±23.8	0.597	93.2±21.5	0.549
PVL	58.7±19.6	**0.001**	73±30	**0.027**
TTN	89.3±21.2	0.215	93.5±21.8	0.584

[a]**Independent T test**

IUGR: intrauterine growth restriction, ROP: retinopathy of prematurity, ICH: intracerebral hemorrhage, PDA: patent ductus arteriosus, PVL: periventricular leukomalacia, TTN: transient tachypnea of the newborn

that children with periventricular leukomalacia (PVL) had low scores in all areas (p=0.001, p=0.027, and p=0.002, respectively) (Table 3).

The report card grades for Turkish and mathematics were significantly higher among girls than boys (p=0.041 and p=0.037, respectively). On the other hand, no statistically significant difference was found between the full-scale intelligence scores of the genders (p=0.127). Five children (12%) had families with a low socioeconomic level, 32 (78%) had families with a medium socioeconomic level, and 4 (10%) had families with a high socioeconomic level. The high school or university graduation rates of the mothers and fathers were 37% and 51%, respectively; the income level was at the minimum wage in 20% of families and the rate of consanguineous marriage was found to be 15%. Thirty-two percent of the children did not have preschool education. Moderate correlations were found between performance and full-scale intelligence scores and socioeconomic level (p=0.007 and p=0.009, respectively), while no significant relationship was found with verbal intelligence score (p=0.052).

According to the WISC-R results, 1 child (2.5%) had moderate intellectual disability and 2 (5%) had mild intellectual disability. Three (7%) had borderline intelligence, 8 (20%) had low-average IQs, and 21 (51%) had average intelligence. Five (12%) had high-average IQs, and 1 child (2.5%) had very superior intelligence. There was a strong positive correlation of verbal intelligence with Turkish grades and a moderate positive correlation with mathematics grades. There was a moderate correlation of performance scores with Turkish and mathematics grades. On the other hand, a strong relationship was found between full-scale intelligence scores and both Turkish and mathematics grades. Verbal (p<0.001), performance (p=0.002), and full-scale intelligence scores (p<0.001) were found to be significantly lower among children with higher PSC scores.

We observed that 5 of the children (12.2%) were below the 10th percentile for height and 10 (24.4%) for weight. Weight and height percentile values at birth were compared with the current measurements and the change in height growth was found to be significant, while the change in weight values was insignificant (p=0.021 and p=0.189, respectively). Maternal age of >35 years, fetal distress, and PDA were found to be significant perinatal and neonatal risk factors of the children below the 10th percentile for current height (p=0.012, p=0.035, and p=0.012, respectively). No significant relationship was found between current weight and risk factors. There were also no significant relationships between birth height, birth weight, current height, or current weight and IQ.

DISCUSSION

It is suggested in the literature that moderate/late preterm infants are at risk of neurocognitive difficulties and behavioral and attention problems

in childhood, which may result in declines in academic performance and relationships with peers (9,10). Chan et al. reported that at age 7, children born as late preterm infants had poorer academic performance compared to their full-term peers, with a 36% higher risk of achievement below the expected academic level (11). In a recent study performed in Stanford, California, 72,316 students from an urban school district were evaluated for school performance according to their gestational ages and it was reported that moderate/late preterm births were associated with a significantly increased risk of poor academic performance, chronic absenteeism, and suspension (12). In this study, we aimed to examine how certain risk factors experienced by moderate/late preterm infants from the perinatal period up to the age of 11-12 years affected the neurodevelopment, behaviors, and school performance of the children.

When school performance was evaluated in relation to gender, Turkish and mathematics grades were higher among girls than boys, but there was no gender difference between cases with and without cognitive deficits. The cognitive functions of boys born as early preterm infants were previously reported to be delayed in comparison to girls at 6 years of age (13). Looking at anthropometric measurements, 5 (12.2%) of the children were below the 10th percentile for height and 10 (24.4%) for weight. In relation to perinatal risk factors, while advanced maternal age of >35 years, fetal distress, and PDA were significant for height (p=0.012, p=0.035, and p=0.012, respectively), no significant relationship was found between weight and risk factors. Heinonen at al. (14) examined the neurocognitive outcomes of 786 adults born as full term or late preterm infants and reported that late preterm birth may not increase the risk of poorer neurocognitive functioning in adulthood. However, the double burden of being born late preterm and being small for gestational age (SGA) did increase that risk. Another previous study concluded that SGA status may be an additional risk factor for cognitive problems in adulthood among those born late preterm (15). In our study, no relationship was found between IQ scores and gestational week and/or IUGR. This may be due to the small size of our patient group.

It was previously emphasized that there is a significant increase in neurodevelopmental delay in infants with a complicated neonatal course and that late preterm infants without morbidities in the neonatal course exhibit the same neurological performance as term infants (16). In a recent study, neonatal hypoglycemia was found to be an independent factor associated with significant neurodevelopmental impairment (17). We observed that verbal intelligence was significantly lower in premature infants who had a history of sepsis (p=0.019). Akar et al. (18) reported that sepsis is a risk factor for neurodevelopmental delay in both moderate/late and early preterm cases. Van der Ree et al. (19) evaluated the motor, cognitive, and behavioral outcomes of school-aged children with histories of early preterm birth and late-onset sepsis and reported that preterm children with late-onset sepsis tended to have more motor problems, lower IQs, and impaired memory and attention compared to controls matched for gestational age.

ICH is a morbidity that causes cognitive, sensory, and behavioral problems. It was reported that children with ICH experienced more neurodevelopmental problems, and the rate of sequelae was higher in those with stage III-IV hemorrhage and those with PVL (20). In our study, ICH was identified in 4 patients. We found no significant difference in the IQs of these patients, which may be related to the small number of cases in the present study. On the other hand, verbal, performance, and full-scale intelligence scores of patients with PVL were found to be significantly lower, consistent with the findings in the literature (21).

In our study, major neurological sequelae were identified in 4 children (10%), 3 patients had intellectual disabilities (IQ of <70), and 1 patient had unilateral blindness because of retinal detachment. Interestingly, that case of blindness was seen without a history of ROP. Our patients with cognitive problems were preterm infants with families of middle or low socioeconomic status and with morbidities such as low birth weight, ICH, PVL, and autism. All of them were receiving special education. None of our patients had a diagnosis of cerebral palsy. Martínez-Nadal et al. (10) emphasized the influence of social factors such as maternal education and socioeconomic status on the processes of catching up in late preterm populations. It was also stated in the literature that lower gestational age in association with low socioeconomic status had a synergic effect, worsening the neurodevelopmental outcomes of late preterm infants (22). In our study, a moderate positive correlation was found between socioeconomic status and both performance and full-scale intelligence scores, and no significant correlation was found with verbal intelligence scores.

Three children (7%) in our study group had learning disabilities, 4 (10%) had special educational

needs, and 4 (10%) had a diagnosis of ASD. The risk of ASD for late preterm infants was reported to be 2 to 4 times greater than that for term infants (23). In a study from Sweden, 4,061,795 infants born in 1973-2013 were evaluated for ASD and ASD prevalences by gestational age at birth were found to be 6.1% for extremely preterm, 2.6% for very to moderately preterm, 1.9% for late preterm, 2.1% for all preterm, 1.6% for early term, and 1.4% for term infants (24).

It was previously reported that late preterm infants whose behavioral problems became evident from school age were 1.68 times more likely to show attention deficits, 2.04 times more likely to show aggressive disorders, and 3.59 times more likely to show introversive personalities compared to their term peers. In the same study, a significantly higher frequency of attention deficits, aggressiveness, and withdrawal was reported in late preterm children compared to their term peers and a significant difference was found in PSC scores (25). In our study, IQ scores were found to be significantly lower in patients with higher PSC scores. This is an important finding in terms of raising awareness among physicians of the possibility of such outcomes regardless of the education and income levels of the families.

Our study may be criticized for having a limited number of cases. As another limitation, the patient files used were old and in written paper form; therefore, it could be argued that there was a slight decrease in reliability. Furthermore, the factors that could be effective in the analyses were not controlled by statistical methods. Studies involving large multicenter case series should be conducted in the future.

In conclusion, the neurodevelopment of moderate/late preterm infants is adversely affected due to incomplete brain development, morbidities in the neonatal period, and nutrition and growth problems. On the other hand, because of low socioeconomic levels and inadequate parental education, lack of multidisciplinary follow-up, and lack of preschool education and rehabilitation support, these children may experience serious neurological and psychological problems both in and out of school. Parental education is important for the detection and management of problems in neurodevelopment. The multidisciplinary follow-up of these children in neurology, ophthalmology, psychiatry, psychology, and physical therapy clinics is of crucial importance, and the detection of developmental delays and early intervention by providing education for parents can improve problems such as school failure, behavioral disorders, depression, aggression, and schizophrenia, which may be seen in late childhood and adolescence.

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NEÜ YAYINEVİ

Prognostic Importance of Tumor Buddings in Larynx Squamous Cell Carcinomas

Larenksin Skuamöz Hücreli Karsinomlarında Tümör Budding'in Prognostik Önemi

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Öz

Amaç: Larenksin skuamöz hücreli karsinomu, güvenilir prognostik belirteçlerin eksikliği nedeniyle yönetimi zor bir hastalıktır. Literatürde, tümör tomurcuklanması (TB) bazı malignitelerde kötü prognozu öngördüğü gösterilmiştir, ancak larengeal kanserde TB'nin prognostik önemi belirsizliğini korumaktadır. Bu çalışmanın amacı, larenksin skuamöz hücreli karsinomlarında TB'nin prognoza etkisini ve diğer prognostik faktörlerle olan ilişkisini değerlendirmektir.
Hastalar ve Yöntem: Kulak Burun Boğaz kliniğinde 2008-2015 yılları arasında larenksin skuamöz hücreli karsinomu tanısı konulan ve cerrahi tedavi veya postoperatif kemoradyoterapi uygulanan 60 olgu incelendi. Olguların yaşları, özgeçmişleri, TNM (tümör, nod, metastaz) sınıflandırmaları, radyolojik görüntülemeleri, uygulanan cerrahi yöntemleri ve patolojik sonuçları dosyalardan elde edildi. Tümörün Hematoksilen&Eozin boyalı preparatlarından immunhistokimyasal PanCK boyası yapılarak tümör tomurcuklanması skorlamaları patoloji bölümünde değerlendirildi. Elde edilen veriler ile klinikopatolojik değişkenler arasındaki ilişki incelendi.
Bulgular: Bu çalışmada, larenksin skuamöz hücreli karsinomunda perinöral infiltrasyon ile tümör tomurcuklanması arasında istatistiksel olarak anlamlı bir ilişki olduğu bulunmuştur (P=0.006). Ayrıca, tümör tomurcuklanması perinöral infiltrasyon ile patolojik lenf nodu tutulumu açısından bağımsız bir risk faktörü olarak görülmüştür (p=0.003). Patolojik lenf nodu tutulumu, lenfovasküler invazyon açısından bağımsız bir risk faktörü olarak belirlenmiştir (p=0.028).
Sonuç: Çalışmamız, larenksin skuamöz hücreli karsinomu için bilinen prognostik faktörler arasında TB ile perinöral infiltrasyon arasında anlamlı bir ilişki olduğunu göstermektedir ve bu nedenle tümörün prognozunu belirlemede önemli bir rol oynayabilir.

Anahtar Kelimeler: Laringeal kanser, prognoz, tümör tomurcaklanması, neoplazi invazivliği

Abstract

Aim: Laryngeal squamous cell carcinoma poses a management challenge due to the lack of reliable prognostic markers. Although tumor budding (TB) has been shown to predict poor prognosis in some malignancies, its prognostic significance in laryngeal cancer remains uncertain in the literature. Therefore, the objective of this study is to evaluate the impact of TB on prognosis and its correlation with other established prognostic factors in laryngeal squamous cell carcinoma.
Patients and Methods: In the department of otolaryngology the files of 60 patients with laryngeal squamous cell carcinoma who underwent surgery, postoperative chemoradiotherapy between 2008 and 2015 were analyzed retrospectively. The patient's history, family history, age, TNM (tumor, node, metastases) classification, radiological imaging, type of surgery performed, and the results of the pathological specimen were evaluated. PanCK immunohistochemical staining was performed on old paraffin block sections containing tumoral tissue, previously stained with Hematoxylin & Eosin. The TB scores were evaluated by the pathology department, and the association between all obtained parameters and clinicopathological variables was analyzed.
Results: Our findings showed a significant association between tumor budding and perineural infiltration, a known prognostic factor for laryngeal carcinoma (P=0.006). TB was found to be an independent risk factor for perineural infiltration and pathological lymph node involvement (p=0.003). Pathological lymph node involvement was also found to be an independent risk factor for lymphovascular invasion (p=0.028).
Conclusion: Our study provides evidence for a significant association between tumor budding and perineural infiltration, which are established prognostic factors in laryngeal carcinoma. This suggests that tumor budding may be an important factor in determining tumor prognosis.

Keywords: Laryngeal cancer, prognosis, tumor budding, neoplasm invasiveness

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INTRODUCTION

Squamous cell carcinomas account for over %95 of all cases of laryngeal cancer, the form with the highest incidence of head and neck cancer (1). The incidence of laryngeal cancers mostly happens during the fifth and seventh decades of life, with males being affected at a ratio of 3.83 times more than females (2). Smoking and alcohol habits are considered as major risk factors the development of laryngeal cancers (3). The presence of cervical metastases is one of the leading factors defining the prognosis of laryngeal cancer. The localization of the primary tumor, tumor size, degree of differentiation, and the time of onset of symptoms are the factors that affect the frequency of cervical metastases (4). It is thought that factors such as TNM (tumor, node, metastasis) classification and histological grading, which are accepted as prognostic factors, are insufficient to determine the prognosis, due to the presence of laryngeal carcinomas with different clinical courses despite similar clinical and histomorphological appearances. Predicting this clinical course difference would be possible with the use of additional prognostic parameters. Therefore, in recent years, studies on many tumor suppressor genes, oncogenes, and proliferation rate determinants have been done for finding new prognostic factors in laryngeal carcinomas (5). Higher rates of a disease-free lifetime, survival, and organ preservation can be achieved in laryngeal cancers by applying the treatment protocol according to these prognostic factors. Tumor budding which is stated as a negative prognostic factor in many cancer types is considered one of the determinants. The presence of small groups of cells or isolated single cells scattered from the invasive tumor area to the stroma is defined as tumor budding which is likely to be a prognostic factor in laryngeal cancers as well (6,7).

The aim of this study is to state the effect of tumor budding on the prognosis of laryngeal carcinoma cases and to analyze its relevance with other well-known prognostic factors.

PATIENTS AND METHODS

This study is approved by the Local Ethics Committee (Approval number: 2015-245). The files of 60 patients who got a diagnosis of laryngeal squamous cell carcinoma in a third-class reference health center between 2008 and 2015 were reviewed retrospectively.

Demographic characteristics of the patients, personal and family history, age, TNM stages, radiological imaging, types of surgery, and specimen findings were evaluated. In order to make the histopathological evaluation of the study and to determine the immunohistochemical antibodies, paraffin-embedded blocks of all cases' pathologies were extracted from the pathology laboratory archive of the hospital. Appropriate cases with pathologies that have clearly observable lower borders and tumor depth were taken in this study. Sections of 4mμm thickness obtained from paraffin-embedded tissues are stained with hematoxylin&eosin. For immunohistochemical analysis, sections were taken to special poly-L-Lysine slides. PanCK (Mouse anti-Cytokeratin Clone AE1/AE3, LOT 51024681, South San Francisco, CA, USA) antibodies were used for the immunohistochemical examination. Ventana Benchmark XT automatic immunostaining device was used for the staining procedure. All prepared and stained materials were evaluated with the Olympus BX41 light microscope.

Evaluation of Tumor Budding

Immunocytochemical staining with PanCK was also used to provide a more reliable evaluation of TB in addition to H&E-stained preparations. Cytoplasmic staining was accepted as a positive reaction for PanCK. All tumoral and epithelial cells show positive reactions with PanCK. Cell groups containing 1-5 tumoral cells at the lower border of the tumor and around large masses close to the border were evaluated as TB, provided that they were in at least 3 areas in immunohistochemical PanCK stained preparations under X400 magnification with Olympus BX41 microscope. Although there are many different classifications for this assessment, the TB classification, which is commonly used in head and neck cancers, was used (8-11).

The TB classification defined by Wang et al. (8) in head and neck cancers was modified by the addition of a non-TB group in our study and graded as follows;
-non-TB group (grade 0): 0 TB
-Low-risk group (grade 1); less than 5 TB
-High-risk group (grade 2); 5 or more TB
These TB groups were classified as low-grade (grade 0 and grade 1) and high-grade (grade 2) (Figure 1).

Statistical Analysis

The data collected from the cases were coded and transferred into a computer program. SPSS (Statistical Package for Social Science, Worldwide Heaquarters SPSS Inc.) 16.0 Windows package program was utilized for statistical evaluation. The results were presented as either mean ± standard

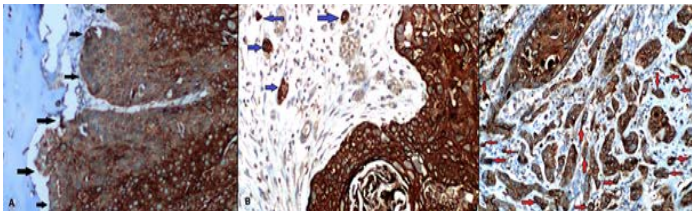

Figure 1. Modified TB Classification for Head and Neck Cancers. (Immunohistochemical PanCK stained preparations under Olympus BX41 microscope at X400 magnification. A: non-TB group (grade 0), B: Low-risk group (grade 1), C: High-risk group (grade 2))

deviation or median (minimum-maximum) related to the nature of the data.

Chi-Square Independence Test (PearsonChi-Square) was used to evaluate if there was an individual association between the dependent variables (TB) and independent risk factors. Also, the related odds ratios and 95% confidence intervals of odds ratios based on these values were obtained with this test. Logistic regression analysis is performed using a forward stepwise method to identify the most significant independent risk factors, and odds ratios were calculated for the significant factors with their corresponding 95% confidence intervals. The statistical significance level is accepted as $p < 0.05$.

RESULTS

58 of the cases (97,7%) are male, and 2 (2,1%) are female; the mean age of cases is 59.81±8.98 years (47-83). Total laryngectomy was performed in 32 cases and partial laryngectomy was performed in 28 cases.

Bilateral functional neck dissection was performed in 51 cases, 4 cases were treated with unilateral radical neck dissection and unilateral functional neck dissection, 4 cases were undergone unilateral modified radical neck dissection and unilateral neck dissection, and 1 case was treated with unilateral functional neck dissection. In the postoperative period, chemoradiotherapy was given to 32 patients, and radiotherapy was given to 6 patients as an additional treatment. In 22 cases, no additional treatment was needed. The mean follow-up time of the cases was 29.65 ± 23.09 months (1-79). Squamous cell carcinoma was present in all 60 cases. In 14 cases (23.3%) good differentiation, in 25 (41.6%) cases moderate differentiation, and in 21 cases (35%) less differentiation was observed. 49 (81.6%) of the tumors were supraglottic, 8 (13.3%) of the cases were glottic, and 3 (5%) were subglottic. In 2 cases (3.3%) T1, in 19 cases (31.6%) T2, in 17 cases (28.39%) T3, in 19 cases (31.6%) T4a and in 3 cases (5%) T4b was observed. Stage 1 was present in 2 cases (3.3%), Stage 2 in 15 cases (25%), stage 3 in 18 cases (30%), stage 4a in 21 cases (35%), and Stage 4b was present in 4 cases (6.6%). Pathological lymph node metastasis was detected in 23 (38.3%) cases. A preoperative nodule was detected in 14 (23.3%) cases.

According to tumor size, there were 11 (18.3%) cases between 0-2 cm, 38 cases (63.3%) between 2-4 cm, and 11 cases (18.3%) larger than 4 cm. According to tumor depth, there were 13 (21.6%) cases under 1 cm, 29 (48.3%) cases between 1-2 cm, and 18 (30%) cases larger than 2 cm. Tumor stroma was mild in 15 (25%) cases, moderate in 23 (38.3%) cases, and severe lymphocyte infiltration in 22 (36.6%) cases. 24 cases (40%) had perineural invasion, and lymphovascular invasion was detected in 13 cases (21.6%).

According to the TB classification (Wang et al.) that we adapted from head and neck cancers for our study, there were 13 cases (21.6%) with no TB at all (8). There were 27 (45%) cases in grade 1, 14 (23.3%) cases in grade 2, and 6 (10%) cases in grade 3.

Throughout the follow-up period of the cases, it was discovered that 10 cases (11.9%) had experienced a recurrence of laryngeal cancer within the local or regional area. The results could not be reached to evaluate the distant metastasis in 2 cases. Distant metastasis was detected in 11 cases of the remaining 58 patients (6.3%). During the follow-up, a secondary primer tumor was diagnosed in 3 cases (5%).

According to our findings, there was a significant

Table 1. Correlation between Tumor Budding and Perineural Infiltration in Patients

Tumor Budding	Perineural Infiltration		N	P
	Positive (n=24)	Negative (n=36)	60 (100%)	
Low Grade	11 (45.80%)	29 (80.60%)	40 (66.70%)	0.006
High Grade	13 (54.20%)	7 (19.40%)	20 (33.30%)	

X^2 (Pearson Chi-Square)

association between tumor budding and perineural infiltration (P=0.006) (Table 1), but no significant correlation was observed between tumor budding and other clinicopathological prognostic factors, except for perineural infiltration (p=0.643, 0.375, 0.776, 0.348, 0.624, 0.576, 0.975; tumor depth, lymphovascular invasion, tumor stage, pathologic lymph node, local recurrence, tumor diameter, distant metastasis respectively). When the risk factors regarding lymphovascular invasion, local recurrence, metastasis, perineural invasion, and pathologic lymph were researched, pathological lymph node involvement for the lymphovascular invasion is an independent risk factor (p=0.028, Odds rate=4.53, %95 confidence interval= 1.181-17.404).

We found that preoperative lymph node involvement was an independent risk factor for local recurrence (p=0.035, Odds ratio=4.66, 95% confidence interval=1.113-19.569). Additionally, tumor budding was identified as an independent risk factor for perineural infiltration (p=0.003, Odds ratio=19.86, 95% confidence interval=2.71-145.11). In addition, preoperative lymph node involvement was found to be an independent risk factor for perineural infiltration (p=0.007, Odds ratio=8.40, 95% confidence interval=1.78-39.47).

Furthermore, we observed that tumor budding was also an independent risk factor for pathological involvement of the lymph nodes (p=0.003, Odds ratio=19.86, 95% confidence interval=2.719-145.112). In summary, our results suggest that tumor budding and perineural infiltration are strongly associated and may serve as important prognostic factors for patients with certain types of cancer.

DISCUSSION

Although the location, size, cervical metastasis, and cellular differentiation of the primary lesion are established as the primary factors influencing the prognosis of laryngeal carcinomas, the need to identify new prognostic factors arises from the observation of different clinical outcomes in cases with similar characteristics. The effect of TB on the prognosis of laryngeal carcinomas and their relation with other known prognostic factors were investigated in our study.

Tumor budding is a histopathological feature seen in the progressive border of neoplasia (12). Today, tumor budding is recognized as a significant histopathological feature associated with lymph node metastasis, recurrence, distant metastasis, and reduced survival rates in different types of cancers, including colorectal cancer, esophageal carcinoma, anal carcinoma, pancreatic carcinoma, lung carcinoma, and ampulla carcinoma (13-16). Moreover, the "International Union Against Cancer" has included tumor budding as an additional prognostic factor (12, 17-21). However, literature has shown that in the studies in which TB was examined, different methods have been utilized for the TB rating, and there is no common rating methodology in this issue. For that reason, in this study, the rating method used by Wang et al. was used to evaluate TB's relationship with other known prognostic factors (8). According to this rating, it was found that perineural infiltration had a meaningful relationship with TB.

This study evaluated tumor budding (TB) as an independent risk factor for both pathological lymph node involvement and perineural invasion. In a previous study by Sarioglu et al., which investigated tumor budding in laryngeal carcinoma, it was reported that tumor budding and pathological lymph node involvement were independent risk factors for distant metastasis, leading to the description of tumor budding as a prognostic factor for laryngeal carcinoma (22).

In our study, we found that tumor budding was an independent risk factor for pathological lymph node involvement, even though it was not found to be a risk factor for metastasis due to the short follow-up period in our cases. However, our study demonstrated that tumor budding is a significant independent risk factor for perineural infiltration, which suggests that tumor budding can be considered a prognostic factor.

In this study, pathological lymph node involvement is an independent risk factor for lymphovascular involvement. The spread of laryngeal carcinomas from the primary site occurs as a result of permeation into vascular, neural, or lymphatic vessels or as a process of embolization (23). Tumor cells passing through the lymphatic vessels reach lymph nodes. Tumor cells may remain in the lymph node due to various factors or may migrate to extracapsular spread and neighboring lymph nodes. Extracellular extension occurs when the tumor cells extravasate out of the lymphatic vessels into soft tissues (23). Taking all of this data into account, pathological lymph node involvement as an independent risk factor for lymphovascular invasion can be considered quite reasonable.

Preoperative lymph node involvement is an independent risk factor for perineural invasion in the study. In the literature, the perineural invasion has

generally been found to be a related factor to loco-regional recurrence (21). Furthermore, it has been reported that perineural invasion affects the prognosis negatively as an effective factor in overall survival and disease-specific survival (24, 25). Therefore, the result obtained in our study would be compatible with the literature. In addition, when the tumor spread pattern described above is considered, it is logical to say that preoperative lymph node involvement for perineural infiltration is an independent risk factor.

In head and neck cancers, one of the most substantial independent prognostic factors is lymph node involvement (26, 27). Lymph node involvement significantly reduces both disease-specific survival and overall survival, especially when combined with vascular invasion in head and neck cancer cases. The existence of metastatic lymph nodes in the cervical region, independent of the primary site, in squamous cell carcinomas of the upper respiratory-digestive tract, reduces the 5-year survival rate by 50%. Therefore, for local recurrence preoperative lymph node involvement is considered as an independent risk factor like the literature.

The tumor stage regarding distant metastasis is an independent risk factor in this study. The T-phase of the primary lesion was reported to affect the risk of cervical metastases (28, 29). In a study, cervical metastasis was reported in 15-40% of tumors in the T1 stage, 35-42% in the T2 stage, 50-65% in the T3 stage, and 65% of tumors in the T4 stage (30). Therefore, we think that tumor staging as an independent risk factor regarding metastasis is consistent with the literature.

Limitations of our study include its retrospective design. Additionally, the sample size was relatively small, which may have limited the statistical power of our findings. Finally, our study was conducted in a single center, which may limit the generalizability of our results to other populations.

CONCLUSION

Our study highlights a significant relationship between tumor budding (TB) and perineural infiltration, both of which are established prognostic factors in laryngeal squamous cell carcinoma. TB also emerges as an independent risk factor for perineural infiltration and lymph node involvement based on pathological analysis. This underscores the importance of TB in determining the prognosis of laryngeal carcinoma and may necessitate changes in postoperative adjuvant therapy. Furthermore, our results confirm the independent risk factors for lymphovascular invasion, perineural invasion, local recurrence, and metastasis as established in previous literature. These findings align with the current understanding of tumor spread patterns and disease progression in head and neck tumors.

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NEÜ YAYINEVİ

Effects of Contact Lens Wearing Habits on Ocular Surface and Microbial Flora

Kontakt Lens Kullanım Alışkanlıklarının Oküler Yüzey ve Mikrobiyal Floraya Etkileri

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Öz

Amaç: Kontakt lens kullanım alışkanlıklarının, bakım önerilerine uyumun ve hijyen alışkanlıklarının sorgulanması ve bunların oküler yüzey ve mikrobiyal kontaminasyon üzerindeki etkilerini incelemek
Hastalar ve Yöntem: Bu prospektif çalışmada 2021-2022 yıllarında kontakt lens bölümümüze başvuran, takipli 108 yumuşak kontakt lens kullanıcısına, kontakt lens kullanımı için riskli kabul edilen davranış ve kullanım alışkanlıkları soruldu. Hastaların son kullandıkları kontakt lensler saklama kapları ile beraber mikrobiyolojik çalışma için laboratuvara gönderildi. Oküler yüzey analizi için oküler yüzey boyanması, göz yaşı kırılma zamanı ve oküler yüzey hastalık indeksi skorlaması yapıldı. Mikrobiyal kontaminasyon ve oküler yüzey analizi ile kontakt lens kullanım alışkanlıkları arasındaki ilişki analiz edildi.
Bulgular: Kliniğimize başvuran kontakt lens kullanıcılarından sadece %6.4 ü tüm kontakt lens kullanım önerilerine uyduğunu bildirdi. Kullanıcıların 72 sinde (%66.7) kültürlerde en az bir üreme olurken, oküler yüzey bozukluğu görülen kullanıcı sayısı ise 60 (%55.6) idi. Kontakt lens ile uyuma (p=0.003) ve lensi belirtilen süreden uzun süre kullanma (p=0.016) oküler yüzey problemlerini arttırıyorken; el hijyenine uymama (p ≤0.001), lens kabı ve solüsyonu belirtilenden uzun süre kullanma (p=0.038) ve solüsyon yerine su kullanmanın (p=0.001) mikrobiyolojik kontaminasyonu arttırdığı gösterilmiştir.
Sonuç: Bu ve bundan önceki çalışmalar göstermiştir ki riskli lens kullanım davranışları oküler yüzey-gözyaşı bozuklukları ve mikrobiyal kontaminasyonu arttıran önemli bir problemdir. Toplumda artan lens kullanımıyla beraber yüksek riskli lens kullanım oranları ciddi oküler patolojileri beraberinde getirebilir.

Anahtar Kelimeler: Kontakt lens, kornea, mikrobiyal kontaminasyon

Abstract

Aim: To question contact lens wearing habits, compliance with care recommendations and hygiene habits and to examine their effects on ocular surface and microbial contamination.
Patients and Methods: In this prospective study, Between 2021-2022, 108 soft contact lens wearers under our follow-up who applied to our contact lens department were asked about their risky contact lens-wearing habits. The last used contact lenses of the patients were sent to the laboratory for microbiological study together with their containers. Ocular surface staining, tear break-up time and ocular surface disease index scoring were used for ocular surface analysis. The relationship between microbial contamination and ocular surface analysis with contact lens wearing habits was analyzed.
Results: Only 6.4% of contact lens wearers who applied to our clinic reported that they followed all contact lens wear recommendations. While there was at least one growth in cultures in 72 (66.7%) of the users, the number of users with ocular surface disorders was 60 (55.6%). While sleeping with a contact lens (p=0.003) and wearing the lens longer than the specified time (p=0.016) increases ocular surface problems; non-compliance with hand hygiene (p ≤0.001), using the lens container and solution for longer than recommended (p=0.038), and using tap water instead of solution (p=0.001) have been shown to increase microbiological contamination.
Conclusion: This and previous studies have shown that risky contact lens wearing habits are an important problem that increases ocular surface-tear film disorders and microbial contamination. With the increasing use of contact lenses in the community, high-risk lens use rates may lead to serious ocular pathologies.

Keywords: Contact lens, cornea, microbial contamination

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INTRODUCTION

Since the end of the 19th century, when they first came into use, contact lens usage have progressively evolved and gained popularity. They are now essential tools for correcting visual impairments due to refractive errors and have been used for therapeutic or cosmetic purposes, especially in developed societies (1). However, their increased usage has also led to a rise in complications.

In addition to being vectors for microbial agents, contact lenses can have mechanical effects on the ocular surface (2). These alterations on the ocular surface result in severe complications such as infectious keratitis. In this case, the microbial agents are most effectively isolated from contact lenses or their containers (3-5). In addition, the use of contact lenses may cause hypoxic, allergic, toxic, and inflammatory reactions in the cornea (6).

Some risky contact lens wearing habits including sleeping with lenses, swimming in the pool, taking a bath, rinsed the lens with tap water, wearing and removing lenses without providing hand hygiene should be avoided as they have been linked to contact lens-related complications (7,8). However, no study has been published about the direct effects of contact lens wearing habits on microbial contamination.

In this study, we aimed to evaluate the direct effects of risky contact lens wearing habits on ocular surface and microbial contamination.

PATIENTS AND METHODS

108 asymptomatic individuals who had been using soft silicone hydrogel contact lenses for at least 6 months and applied to the contact lens department of our hospital for a routine control between 2021 and 2022 were included in this prospective study. The study was conducted in accordance with the Declaration of Helsinki, and local ethics committee approval was obtained (University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital Ethics Committee, 12/06/2017, 39/21).

All contact lens users included in the study were informed about the nature of the study, and gave their informed consent.

The study was initiated by conducting comprehensive ophthalmological examinations on 18-to-40-year-old users who came for a routine contact lens control. Individuals who did not have any systemic diseases or ocular diseases other than refractive errors and who had not undergone any ocular surgery were included in the study. Patients who were taking systemic or ocular medications were excluded from the study.

Risky Contact Lens Wearing Habits

Patients were asked the following queries, which were determined to be risky contact lens wearing habits, and instructed to select either always, sometimes, or never (7)

1. Do you ever sleep with your contact lenses, including at noon?
2. Are there times when you don't wash your hands while putting on or removing your contact lenses?
3. Do you use your contact lens containers or solutions longer than recommended?
4. Do you wear your contact lenses for longer than recommended?
5. Have you ever put your contact lenses in tap water instead of solution or rinsed them with tap water?
6. Do you ever swim or take a bath while wearing your contact lenses?

The questions were asked orally by the same physician. Those who responded "sometimes" or "always" to the questions were considered risky in terms of that habit (7).

Microbiology

The last used contact lenses of the patients along with their containers, were sent to microbiology department. The remaining liquid was completely drained from the lens containers. Cotton swabs soaked with sterile brain heart infusion solution were used to collect samples from inside of the containers and the concave surface of the contact lenses (9). The samples were inoculated on blood agar, chocolate agar, Mac Conkey agar, and Sabouraud dextrose agar, kept under the appropriate conditions for the appropriate amount of time, and then the growths were observed (9).

Ocular Surface-Tear Film Disorders

Ocular surface pathologies were evaluated using the Ocular Surface Disease Index (OSDI), tear break-up time (TBUT), and ocular surface staining, recommended by the Tear Film and Ocular Surface Society (TFOS) as diagnostic tests (10).

OSDI is a test that evaluates various aspects of dry eye symptoms, including severity, impact on daily activities, and impact on quality of life. It consists of 12 questions that assess dry eye symptoms, environmental triggers, and vision-related functions. Each question receives a score between 0 and 4, inquiring about the frequency. A score of 13 or higher is considered significant in terms of dry eye and ocular surface disorders.

For the evaluation of TBUT and ocular surface staining, a drop of saline is dripped onto a paper infused with fluorescein, and the paper is then placed to the lower fornix. Participants are told to blink three times and then look directly ahead without blinking. The time is started immediately after the blink of an eye, and the time of the first tear film break is recorded. The test is administered multiple times, and the average duration was recorded. If the TBUT is less than 10 seconds, it is meaningful. In addition, areas stained with fluorescein are also investigated. Significant staining is defined as five or more spots on the cornea, nine or more spots on the conjunctiva, or stainings longer than 2 mm at the eyelid margin or with a width of at least 25 percent on the eye lid (10).

All tests were performed by a single experinced ophthalmology specialist. A positive result in any of these three tests was considered significant for the participant, in terms of ocular surface-tear film disorders (10).

Statistical Analysis

SPSS (Statistical Package for Social Sciences Inc. Chicago, IL, USA) version 22.0 was used for statistical analysis. The number of participants was determined by a confidence level of 95% (1-α), a test power of 95% (1-β) and an effect size of d=0.5 according to one-tailed independent samples t-test analysis. Logistic multiple regression analysis was used for the risk analysis of the chi-square test results that were significant for the relationship between risky habits and ocular surface problems and the relationship between risky behaviors and growth in microbial culture. A p value of $p \leq .05$ was considered as statistically significant.

RESULTS

Of the contact lens users participating in the study, 44 (40.7%) were male and 64 (59.3%) were female with a mean age of 26.7±9.8 years. The mean lens wearing time was 34.7±9.5 (6-120) months. Only 6.4% (n=7) of the users who participated in the study stated that they avoided risky habits completely (Table 1). While there was at least one growth in cultures in 72 (66.7%) of the users, the number of users with ocular surface disorders was 60 (55.6%). In cultures, Staphylococcus (23.1%; n=25), Bacillus (17.5%; n=19), and Micrococcus (10.1%; n=11) were the most common species (Figure 1).

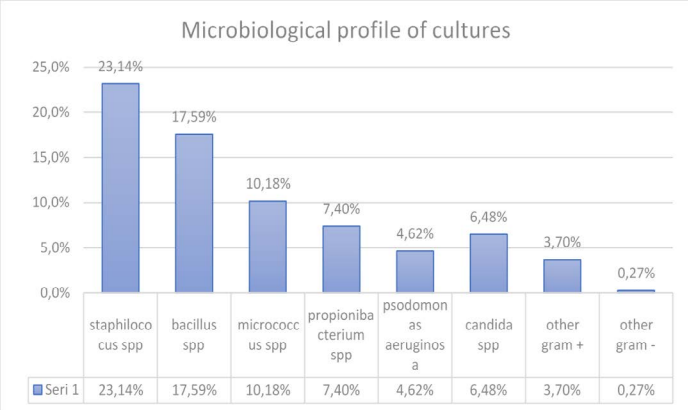

Figure 1. Species and ratio of microorganisms isolated from culture

While sleeping with a contact lens and wearing the contact lens longer than the recommended time are considered risks for ocular surface disorders; Non-compliance with hand hygiene recommendations, using the contact lens solution or container for longer than the recommended time, replacing the solution with tap water or rinsing the lens in tap water were determined to be risky in terms of microbial contamination (Table 2-4).

DISCUSSION

In this study, we evaluated the compliance of contact lens wearers who applied to our clinic to the lens wearing rules, as well as the effects of this compliance on the ocular surface and microbial biological load. In our study, more than 90% of

Table 1. Prevalence of risk behaviors for contact lenses

Risk factor/Behavior	Participant Ratio
Sleeping with contact lenses	%55.6 (n=60)
Non-compliance with hand hygiene recommendation	%47.2 (n=51)
Using the lens container-solution longer than recommended	%61.1 (n=66)
Replacing lenses at intervals longer than recommended	%66.6 (n=72)
Storing lenses in tap water or rinsing lenses in tap water	%59.3 (n=64)
Swimming or taking a shower in contact lenses	%34.2 (n=37)
Any risk behavior	%93.6 (n=101)

Table 2. The relationship between risk behaviors for contact lenses and ocular surface disorders and microbial contamination

	Ocular surface disorders (p* value)	Microbial contamination (p* value)
Sleeping with contact lenses	0.003	0.146
Non-compliance with hand hygiene recommendation	0.083	≤0.001
Using the lens container-solution longer than recommended	0.22	0.038
Replacing lenses at intervals longer than recommended	0.016	0.082
Storing lenses in tap water or rinsing lenses in tap water	0.123	0.001
Swimming or taking a shower in contact lenses	0.096	0.53

* chi-square test

Table 3. Regression Analysis: Relationship between bacterial growth and risk behaviors for contact lenses

	Odds ratio	95% confidence interval	p
Non-compliance with hand hygiene recommendation	3.5	3.1-3.8	≤0.001
Using the lens storage case-solution longer than recommended	1.3	1.1-1.7	0.02
Storing lenses in tap water or rinsing lenses in tap water	2.1	1.6-2.7	≤0.001

Table 4. Regression Analysis: Relationship between ocular surface disorders and risk behaviors for contact lenses

	Odds ratio	95% confidence interval	p
Sleeping with contact lenses	2.5	2.1-3.1	0.001
Replacing lenses at intervals longer than recommended	1.7	1.1-1.7	0.016

participants reported doing at least one risky contact lens wearing habits, whereas in the study by Cope et al. (7) this rate was approximately 85%. In another study conducted by Ibrahim et al., the rate of sleeping with contact lenses was approximately 10%, compliance with hand hygiene regulations was approximately 17%, and other risky habits were comparable to our findings (11). Additionally, the rates were lower in an Australian study compared to ours (12). Considering the "sometimes" option as risky in our research and Cope's study may have affected these rates. In other studies, the responses to the questions were interpreted as "yes" or "no"; however, we think that people do not always adhere to such strict boundaries when it comes to following the rules.

When we look at the growths in culture, microbiological agents that often grew in the samples obtained from contact lenses and their containers compatible with the normal flora. Although their reproduction rates and rankings are different in studies, staphylococcus, bacillus, and micrococci are the most frequently obtained microorganisms as in our study and are the organisms that detected mostly on the soft contact lenses (13). The corneal surface is normally regarded as sterile, whereas the conjunctival flora contains the majority of microorganisms. Colonization of contact lenses is also important because it has been linked to lens-related infiltrates and inflammatory conditions besides keratitis (14). In similar studies, the contamination rates as a result of cultures taken from contact lenses and their containers are close to 80% (13). Our research revealed that poor hand hygiene and improper use of lens solution are directly related to the colonization of microorganisms on contact lenses and their containers. Wu et al. (15) demonstrated that contact lens and container contamination occurs when the containers and hand hygiene recommendations are neglected. Yung et al. (16) also drew attention to the risks of contamination, such as using the lens solution for longer than the recommended time or replacing the lens solution with tap water.

Contact lens-related ocular surface disorders are important in terms of contact lens compliance and contact lens-related complications, and they can lead to contact lens discomfort and even contact lens dropout (17). In our study, we noticed that the incidence of ocular surface problems increased

significantly when contact lenses were worn overnight and for longer than recommended. The sleeping with contac results in corneal hypoxia, and prolonged lens wear causes corneal surface problems due to the accumulation of deposits (18). Tear changes and increased inflammatory cytokines in patients who slept with contact lenses have also been shown in previous studies (19). As a result, risky contact lens wearing habits create a suitable environment for keratitis, one of the most serious complications, and threatens the ocular surface, which serves as a barrier against these complications.

Risky contact lens wearing habits are quite common and their prevalence must be decreased. Despite the fact that many factors influence these habits, education is one of the most essential tools for changing them, which was demonstrated by Lam et al. (8) When prescribing contact lenses in ophthalmology clinics with a high patient volume, informing the patient about the use of contact lenses can be skipped. At least once every six months, contact lens wearers should visit an ophthalmologist, who should enlighten and encourage them to avoid risky contact lens wearing habits.

The study has numerous limitations. First, the study was based on the patients' self-reports, and there were no risky habits frequency categories. Second, there were no groups of reproductive severity by culture. In addition, the growths in the contact lenses and their containers were assessed as a single entity and not separately classified and analyzed. The absence of data analyzing the type of microorganism and the associated risky habit is another limitation of the study.

In conclusion, the study revealed that risky contact lens wearing habits increase the likelihood of contact lens complications and ocular surface disorders. In order to reduce contact lens-related complications, patient education and correction of risky habits are crucial.

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NEÜ YAYINEVİ

Investigation of Toll-Like Receptor Family Expression in Glioblastoma: A Comparative Analysis of qPCR and Cell Culture

Glioblastomada Toll-Benzeri Reseptör Ailesi Ekspresyonunun Araştırılması: Karşılaştırmalı Bir qPCR ve Hücre Kültürü Analizi

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Öz

Amaç: Glioblastoma yetişkinlerde en sık görülen ölümcül beyin kanseridir. Toll-benzeri reseptörler, patojen tanıma ve doğal bağışıklığın aktivasyonu ile ilişkili 10 reseptörden (Toll-benzeri reseptör 1-10) oluşan hücre yüzey reseptörleridir. Ancak, çelişkili sonuçlar içermekle birlikte bazı çalışmalar Toll benzeri reseptör ekspresyonunun, glioblastoma dahil bazı tümörlerde, kanser hücresi proliferasyonu ve ilerlemesi ile ilişkili olabileceğini göstermiştir. Bu nedenle, bu çalışmada literatürde ilk kez glioblastomada Toll-benzeri reseptörlerin on üyesinin tamamının ekspresyon profilinin araştırılması amaçlandı.
Hastalar ve Yöntem: 2018 yılı Ocak ve Aralık ayları arasında tanı alan 25 glioblastoma hastasına ait formalinle fikse edilmiş parafine gömülmüş dokularda Toll-benzeri reseptörlerin mRNA ekspresyonu kantitatif gerçek zamanlı polimeraz zincir reaksiyonu kullanılarak değerlendirildi. Ayrıca her bir Toll-benzeri reseptörün ekspresyonu, beş farklı insan glioblastom hücre hattı (T98G, U87-MG, U373, LN18 ve A172) kullanılarak hücre kültürü analizi ile araştırıldı.
Bulgular: Glioblastoma grubunun formalinle fikse edilmiş parafine gömülmüş dokularında kantitatif gerçek zamanlı polimeraz zincir reaksiyonu analizi ile Toll-benzeri reseptör 1, Toll-benzeri reseptör 6 ve Toll-benzeri reseptör 7 mRNA düzeyleri anlamlı olarak arttığı (her biri, $p<0.001$), Toll-benzeri reseptör 4 ve Toll-benzeri reseptör 10 düzeylerinin ise kontrol grubu ile kıyaslandığında anlamlı olarak azaldığı görüldü (sırası ile, $p=0.023$, $p<0.001$). Ek olarak, Toll-benzeri reseptör mRNA ekspresyon profilleri farklı hücre hatları arasında farklılık sergiledi.
Sonuç: Çalışmamızda birçok Toll-benzeri reseptör üyesi glioblastoma mikroçevresinde farklı ekspresyon düzeyi gösteriyor ve onu farklı şekilde etkiliyor gibi görünüyordu. Glioblastomada her bir Toll-benzeri reseptörün endojen protein seviyesini doğrulamak, glioblastomanın patogenezi ve prognozu üzerindeki kesin rollerini netleştirmek ve yeni hedef tedavilere ışık tutmak için daha kapsamlı çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Glioblastoma, hücre hatları, hücre kültürü, real-time PCR, toll-benzeri reseptör

Abstract

Aim: Glioblastoma is the most frequent, and fatal brain cancer in adults. Toll-like receptors are cell surface receptors comprised of 10 receptors (Toll-like receptor 1−10) related to triggering innate immunity by recognizing pathogens. However, some studies suggested that the expression of Toll-like receptors might be related to cancer cell proliferation and progression in some tumors including glioblastoma with some contradictory results. Thus, we aimed to investigate all ten members of the Toll-like receptor expression profile in glioblastoma for the first time in the literature to contribute additional data to the literature.
Patients and Methods: Quantitative real-time polymerase chain reaction was applied to formalin-fixed paraffin-embedded tissues of 25 glioblastoma patients, diagnosed between January and December 2018, to evaluate the mRNA expression of Toll-like receptors. Also, the expression of each Toll-like receptor was investigated by cell culture analysis using five different cell lines of human glioblastoma (T98G, U87-MG, U373, LN18, and A172). The results were compared statistically.
Results: Toll-like receptor 1, Toll-like receptor 6, and Toll-like receptor 7 mRNA levels were significantly increased in the formalin-fixed paraffin-embedded tissues of the glioblastoma group ($p<0.001$, each) whereas the expression of Toll-like receptor 4 and Toll-like receptor 10 was downregulated compared to the control group ($p=0.023$, $p<0.001$, respectively), by quantitative real-time polymerase chain reaction analysis. Additionally, Toll-like receptor mRNA expression profiles differed among the cell lines.
Conclusion: In our study, many Toll-like receptor members seemed to display different expression level in the glioblastoma microenvironment and affect it diversely. Further comprehensive studies are required to confirm the endogenous protein level of each Toll-like receptor in glioblastoma, to clarify their precise role in the pathogenesis and prognosis of glioblastoma, and to shed light on new target therapies.

Keywords: Glioblastoma, cell lines, cell culture, real-time PCR, toll-like receptor

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INTRODUCTION

Glioblastoma (GBM) is a grade 4 glioma according to World Health Organization (WHO) classification that is the most frequent and lethal primary central nervous system cancer in adults (1-3). It usually shows recurrence and resistance to the current therapies of surgery, radiotherapy, and chemotherapy (4-5). Therefore, in terms of discovering more effective therapies to prolong survival, targeting some molecules mainly in the tumor microenvironment that may act in the pathogenesis and the prognosis of GBM are being investigated intensively in the literature. Immunotherapy, targeting Toll-like receptors (TLRs) and using their receptor agonists is one of the most attractive treatments for GBM.

TLRs are cell surface receptors that are fundamentally related to pathogen recognition and activation of innate immunity. The human TLR family comprises 10 receptors (from TLR1 to TLR10, numerically) (6). TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10 are present on the cell membrane and bind to particles of microorganisms. TLR3, TLR7, TLR8, and TLR9 are located in endosomes and activated by nucleic acids of dying cells and microorganisms (6).

In addition to the main functions of immunity, some studies have claimed that expression of various members of the TLRs family might be associated with tumor cell proliferation and progression in many neoplasms (7-9). There are only a few studies in the literature regarding the expression of TLRs, mostly focused on TLR2, TLR4, and TLR9, that have reported GBM initiates the innate immune system by activating TLRs (6, 10). Through binding its ligand, TLRs have been displayed to activate intracellular cascades to stimulate downstream signaling that plays contradictory roles as either tumor progression or suppression (6). Thus, we aimed to investigate the expression profile of each member of TLRs in GBM in a single study for the first time in the literature and contribute additional data about TLRs in GBM to the literature, to assist the development of novel targeted immunotherapies.

PATIENTS AND METHODS

FFPE GBM tumor samples

After obtaining ethics committee approval from the Institutional Review Board of Bozok University (2019/04), 25 formalin-fixed and paraffin-embedded (FFPE) tumor samples of GBM diagnosed in the Pathology Department of Istanbul Yeni Yuzyil University Gaziosmanpasa Hospital and Pathology Department of Bozok University between January and December 2018 were examined. Clinicopathologic parameters were obtained from patients' records for each sample. Non-tumoral tissue samples of 8 patients diagnosed with hematoma were used as a control group. Confirmation of histopathological diagnosis and grading was performed according to the WHO classification.

Initially, FFPE tissue block samples were stained with Hematoxylin-Eosin (H&E) and evaluated under a light microscope to select samples. Tissue containing more than 90% tumor was selected. FFPE tissue samples for each patient and control were cut into 10-µm sections. Necrotic and bleeding areas were not included in the study.

Cell Culture

Human GBM cell lines of T98G, U87-MG, U373, LN18, and A172 (ATCC, USA) and Wi38 human fibroblast cell line (ATCC, USA) were used for this study. All cells were cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA), 2mM L-glutamine (Gibco, USA), penicillin (20 units/ml) and streptomycin (2 µg/ml) (Gibco, USA) at 37^{O}C in a humidified CO_2 incubator (NuAire, USA) containing 95% air and 5% CO_2. Cells were monitored daily and passaged when they reached 80% confluency.

RNA isolation and complementary DNA (cDNA) synthesis

All cells were passaged with trypsin, stained with Trypan Blue Dye, and counted with a Neubauer cell counting chamber. Cells were seeded at 3x105 cells/well in a 6-well tissue culture plate and incubated for 24 hours in a 2 ml medium. At the end of the incubation, the medium was removed, and cells were washed with cold PBS for 1 minute. After PBS removal, 500 µl Trizol reagent was added to cells in each well, and suspension cells were transferred to a microcentrifuge tube. Total RNA isolation from cells was performed via Trizol reagent (Roche Diagnostic, GmBH, Mannheim, Germany) according to the manufacturer's instructions.

Total RNA isolation from tumor and control tissues was carried out by using a High Pure FFPET RNA isolation kit (Roche Diagnostic, GmBH, Mannheim, Germany) by the manufacturer's protocol. The quality and concentration of isolated RNAs from both cells and tissues were determined with Nanodrop (Thermo, USA). RNAs were kept at -80^{O}C until cDNA synthesis. cDNA synthesis from tissue, control, and cells was

performed by using the "OneScript Plus cDNA synthesis kit" (Abmgood, Canada) according to the manufacturer's instructions. cDNAs were stored at -80^{O}C until a quantitative real-time polymerase chain reaction (qPCR) experiment.

Determination of TLR gene expression with quantitative real-time polymerase chain reaction (qPCR)

The gene expression profiling of TLR 1-10 was performed by using specific primers and Universal Probe Library probes on Light Cycler 480 real-time PCR system (Roche, Germany). Relative gene expression profiles of TLR 1-10 were calculated using the β-actin housekeeping gene as a reference. All primer and probe set for TLR 1-10, and β-actin were summarized in Table 1. Totally, 20 µl reaction mixture was prepared for TLRs and β-actin as follows: 1 µl from primer-probe set, 4 µl from TaqMan Master Mix, 2 µl cDNA, and 13 µl molecular grade water. PCR reaction conditions were set as 1 cycle for 10 minutes at 95^{O}C, 45 cycles for 10 seconds at 95^{O}C, for 30 seconds at 60^{O}C, and for 1 second at 72^{O}C. For each run, DNase- and RNase-free water included mixture was loaded for no template control (NTC). Each reaction was performed in triplicate. TLR1-10 expressions were calculated via Light Cycler 480 software after normalized to β-actin.

Statistical Analysis

The ΔΔCT method was applied to detect the expression profiles of TLRs. The statistical analysis was examined by "Statistical Package for Social Sciences" (SPSS) version 22 (SPSS Inc., Chicago, IL). The Shapiro–Wilk test was used to check the normal distribution. For comparing the two groups

Table 1. Primers and UPL probes used for real-time gene expression analysis (5'→3')

Primer sequences	UPL number
TLR1	
CCTAGCAGTTATCACAAGCTCAAA (Forward)	
CCTTGGGCCATTCCAAATA (Reverse)	#79 (04689020001)
TLR2	
GGCCAGCAAATTACCTGTGTG (Forward)	
AGGATCAGCAGGAACAGAGC (Reverse)	#56 (04688538001)
TLR3	
GTGGCCCTTAAAAATGTGGA (Forward)	
GTGTTTCCAGAGCCGTGCTAA (Reverse)	#151 (04694376001)
TLR4	
TCATTGTCCTGCAGAAGGTG (Forward)	
TCC CAC TCC AGG TAA GTG TT (Reverse)	#62 (04688619001)
TLR5	
TGAGGGACTTTCTCATCTTCAAGT (Forward)	
CCTTAATGCAGTCAGATGGCTA (Reverse)	#31 (04687647001)
TLR6	
TTTGGATTTATCTCATAATCAGTTGC (Forward)	
GATCTAAATGCCTGAAACTCACAA (Reverse)	#121 (04693558001)
TLR7	
GTCTAAAGAACCTGGAAACTTTGG (Forward)	
TCTCAGGGACAGTGGTCAGTT (Reverse)	#102 (04692209001)
TLR8	
CAGAATAGCAGGCGTAACACATCA (Forward)	
TGTTGTCATCATCATTCCACAA (Reverse)	#59 (04688562001)
TLR9	
CTGGGACCTCTGGTACTGCT (Forward)	
CTGCGTTTTGTCGAAGACCA (Reverse)	#98 (04692152001)
TLR10	
TGTCACCATTGTGGTTATTATGC (Forward)	
GCAGATCAAAGTGGAGACAGC (Reverse)	#76 (04688996001)
β-actin	
ATTGGCAATGAGCGGTTC (Forward)	
CGTGGATGCCACAGGACT (Reverse)	#11 (04685105001)

UPL: Universal Probe Library

Mann-Whitney U was used. Continuous variables were given as mean and standard deviation (mean ± SD). p<0.05 was considered statistically significant.

RESULTS

Subjects

The mean age of the GBM patients was 53±13 (38-83) and the control group was 52.75±9.98 (42-63). While 11 (44%) of GBM patients were female and 14 (56%) were male, 3 (37.5%) of the individuals constituting the control group were female and 5 (62.5%) were male.

TLR1–10 mRNA expression patterns

TLR1, TLR6, and TLR7 mRNA levels were significantly increased in the GBM group (p<0.001, each) whereas TLR4 and TLR10 mRNA levels were significantly decreased in the patients' group (p=0.023, p<0.001, respectively) (Figure 1). In this study, human

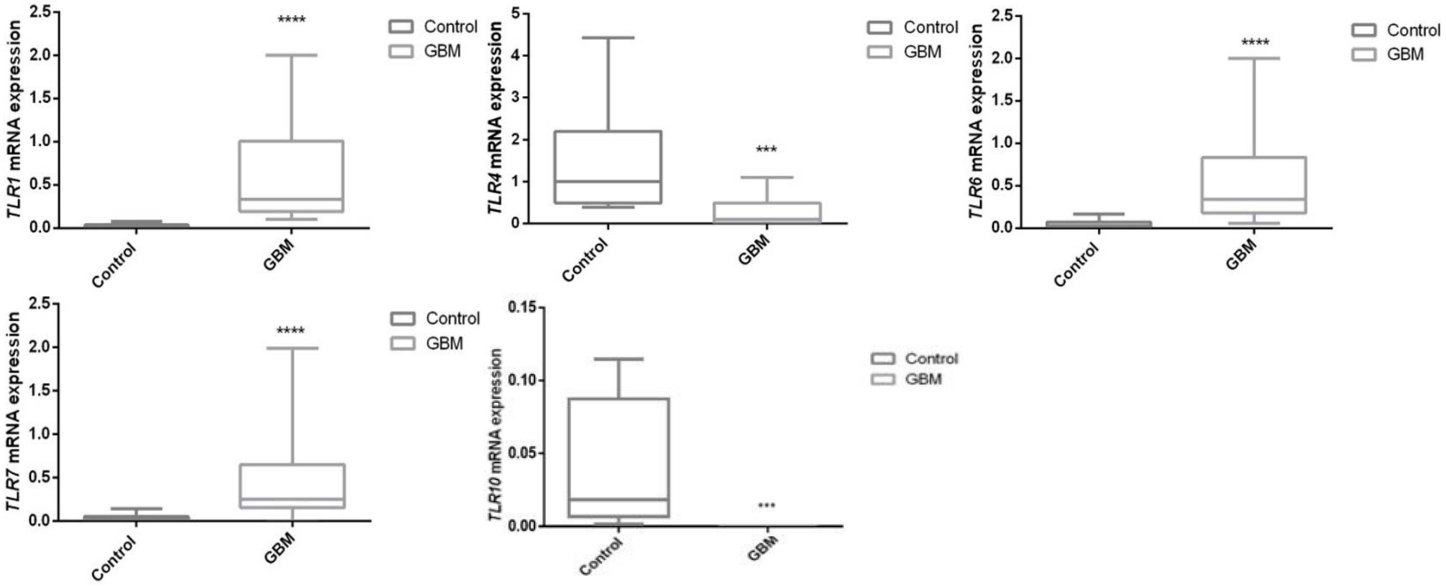

Figure 1. Comparison of TLR1, TLR4, TLR6, TLR7, and TLR10 mRNA expression levels between GBM and the control groups.

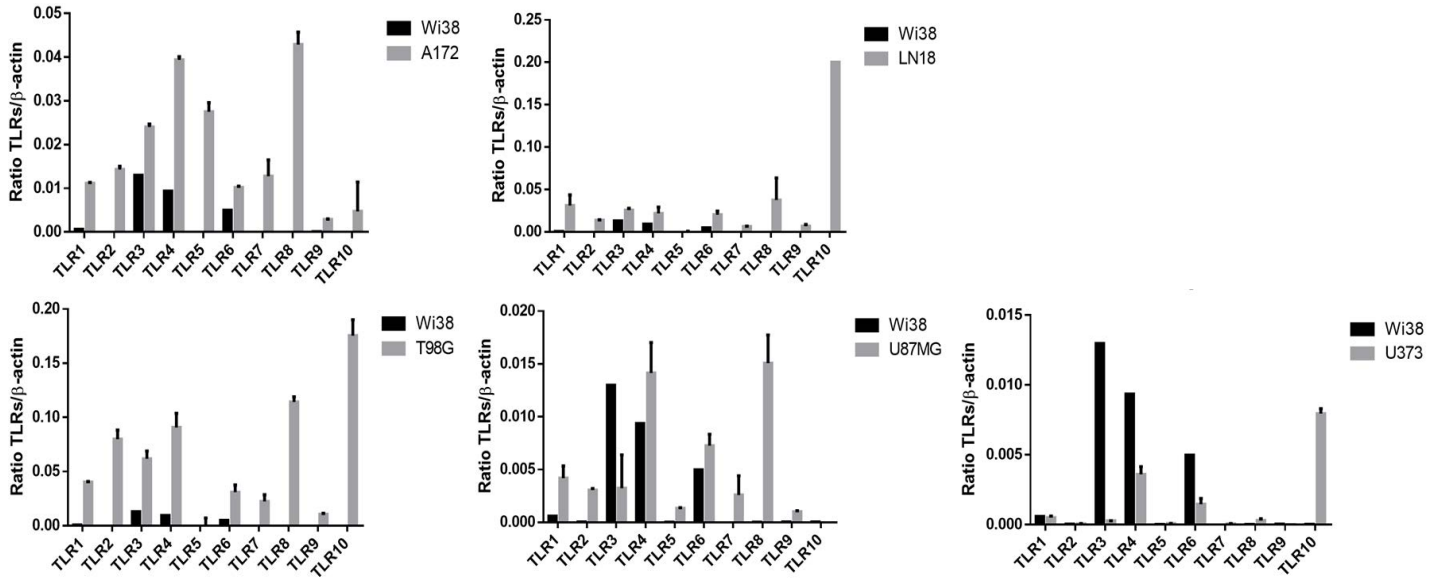

Figure 2. Comparison of TLR 1-10 expression levels between glioblastoma cell lines A172, LN18, T98G, U87-MG, U373, and the control group (Wi38).

GBM cell lines of T98G, U87-MG, U373, LN18, and A172, and for the control group Wi38 fibroblast cells were used. TLR mRNA expression profiles differed according to the cell lines. mRNA expression of all TLRs, from TLR1 to TLR10, were significantly increased in human GBM cell lines of A172, LN18, and T98G ($p<0.001$, each). TLR3 and TLR10 showed downregulation in the U87-MG cell line, while the others were upregulated ($p<0.001$, each) (Figure 2). TLR3, TLR 4, and TLR6 downregulation and TLR10 upregulation were demonstrated in the U373 cell line ($p<0.001$, each) (Figure 2).

DISCUSSION

TLR1, TLR2, and TLR6 are similar receptors that are present in cells of immunity as well as neoplastic cells (6). TLR2 heterodimerizes with TLR1 or 6. They have a significant function to identify bacterial and viral ligands and signals from nonviable cells (6). It has been demonstrated that the upregulation of TLR2 in tissues and cell lines of glioma displayed a negative correlation with the survival time in GBM by Li et al. (6, 11). In addition, overexpression of TLR2 mRNA and higher protein levels are claimed to enhance tumor cell activity and progression that is directly correlated with higher histological grades and shorter survival (11-12). We have found overexpression of TLR1 and TLR6 in the FFPE tissues of GBM patients. Whereas TLR2 expression did not exhibit a difference between the groups.

Overexpression of TLR1 and TLR6 in U87-MG and A172 cell lines, and overexpression of TLR2 in U87-MG, A172, and GL261 cell lines have been reported in the literature, (6). While TLR1, TLR2, and TLR6 mRNA expression was significantly increased in the present study in GBM of human A172, LN18, T98G, and U87-MG cell lines, TLR6 expression has been decreased in the U373 cell line.

TLR3 has a crucial role in the recognition of viral RNA to activate adaptive immunity that is targeted for antiviral and antitumor therapies. Compared with the nonneoplastic tissues, TLR3 expression has been significantly elevated in GBM in a recent study reported by Zou et al. (13). However, TLR3 expression did not display a significant difference between the tumoral and nontumoral groups in our study. In the literature, TLR3 overexpression has been established in human glioma tissues, as well as human GBM cell lines of U87-MG, U251, A172, and LN229 (6). Similarly, TLR3 mRNA expression was significantly increased in human GBM cell lines of A172, LN18, and T98G in the present study. On the contrary, TLR3 downregulation has been exhibited in U87-MG and U373 cell lines.

TLR4 expression has been revealed to be notably more upregulated in GBM than in anaplastic astrocytoma, associated with higher grades and poorer prognosis in the literature (14-16). TLR4 overexpression has been thought to downregulate Wnt/Claudin signaling to prevent apoptosis and assist the progression of GBM (17). Similarly, TLR4-mediated signals have been claimed to induce immune evasion, migration, proliferation, and survival (15). Reducing TLR4 expression has been suggested to abolish GBM invasiveness, and induce apoptosis (17). Nevertheless, contradictory effects have been reported by Alvarado et al and Cruz et al. (18-19). They have presented parallelly that glioma cells cause TLR4 downregulation, leading them to survive due to immune evasion (18-19). Similar to those reports, we have shown downregulation of TLR4 in GBM. In the literature, TLR4 upregulation has been demonstrated in human GBM cell lines of U87-MG, LN229, A172, SF126, U118, U251, U87, and GI261, and glial stem cells (6). Similarly, we have found increased TLR4 mRNA expression in U87-MG, A172, LN18, and T98G cell lines. In addition, we showed decreased TLR4 mRNA expression in the U373 cell lisimilararly to our GBM tissue samples. These conflicting results might be attributed to the self-renewal cycle arrest and receptor-restricted tumor growth. Evaluation of those controversial results attentively is crucial to clarify the role of TLR4 in GBM.

TLR5 has been claimed to be related to various neurological disorders such as neurodegenerative diseases. However, its expression in GBM is not widely explored in the literature. Ifuku et al. (20) have recently declared that TLR5 has no impact on the growth of an ex vivo GL261 glioma mouse model. Moretti et al. (14). reported higher expression of TLR5 along with TLR1, TLR2, TLR4, and TLR6 in astrocytomas and GBM than in non-neoplastic brain tissue. However, we have not demonstrated any statistically significant difference between the expression of TLR5 in GBM cells and the control group in FFPE tissues. In the literature, upregulation of TLR5 has been reported in human GBM cell lines of U87-MG, and A172 (6). Similarly, our results of cell lines T98G, U87-MG, LN18, and A172 about TLR5 have confirmed the literature.

TLR7 and TLR8 represent similar biological functions and architectures, that respond to

cytokines. Substantially, Buonfiglioli et al (21) have reported recently that binding TLR7 to its ligand let-7 miRNA triggers the release of cytokines and antigen presentation that inhibits migration and growth of glioma cells. In addition, two main agonists of TLR7/8 exhibited antitumoral response by diminishing cell proliferation and prolonging survival (6). To the best of our knowledge, overexpression of TLR7 and TLR8 were not demonstrated in glioma cell lines up to date (22). Remarkably, we found TLR7-8 mRNA overexpression in T98G, U87-MG, LN18, and A172 cell lines in the present study. Also, herein, we have revealed overexpression of TLR7 in GBM tissues of FFPE, for the first time in the literature, however, TLR8 expression was not different from the control group.

TLR9 upregulation has been reported to be relevant to a higher grade, disease progression, and poorer survival, due to the interaction with CCL2/CCL5, STAT3, MMP-2, and MMP-9, especially in supratentorial GBM (26). TLR9 expression has been documented in human glioma biopsies, glioma stem cells, and cell lines of U251, and U87, in the literature (6). We have demonstrated TLR9 mRNA overexpression in T98G, U87-MG, LN18, and A172 cell lines, in parallel to the literature. However, the mRNA level of TLR9 in GBM patients' tissues did not show a significant difference from the control group.

TLR10 is one of the least known TLRs in the literature. Even its ligand is still uncertain. TLR10 shares the same locus as TLR1, and TLR6, which are structurally similar to each other (27-28). Unlike TLR1 and TLR6, TLR10 does not lead to a classic downstream signaling pathway (29-30). However, TLR10's biological utility is unclear which is attributed to the complicated modulatory processes, activation of various signaling pathways, competition for ligands, and heterodimerization with other TLRs (27). Furthermore, there is extremely little information about TLR10 expression in GBM in the literature. According to the database of The Cancer Genome Atlas (CGA), TLR10 overexpression in GBM might be relevant to higher tumor grade, poorer overall, and progression-free survival (31). Based on the CGA database, Ge et al. (31) have exposed by gene correlation analysis that GBM shows a high expression level of TLR10 and also confirmed their analysis immunohistochemically. In contrast, we have detected downregulation of TLR10 mRNA in FFPE tissues. On the other hand, we have shown TLR10 overexpression in cell lines of T98G, U373, LN18, and A172, while there is downregulation in U87-MG. To our knowledge, this is the only study in the literature investigating TLR10 expression in cell lines.

Our results comparing the literature of each TLRs from TLR1 to TLR10, consequently, in GBM are stated above. In summary, we have analyzed mRNA levels of the whole TLRs family in both FFPE tissues of GBM patients and compared them with five different GBM cell lines (T98G, U87-MG, U373, LN18, and A172) for the first time in the literature and overview those issues. Additionally, we have used cell lines of U373 and LN18 in our study firstly that were not used in the literature before to investigate TLR expression. We have demonstrated overexpression of TLR1, TLR6, and TL7; and lower expression of TLR4 and TLR10 in FFPE tissues. mRNA expression of all TLRs, from TLR1 to TLR10, were significantly increased in human GBM cell lines of A172, LN18, T98G. TLR3 and TLR10 showed downregulation in the U87-MG cell line, while the others were upregulated. TLR3, TLR4, and TLR6 downregulation and TLR10 upregulation were demonstrated in the U373 cell line.

To conclude, while high expression was detected in some TLR members, low expression was detected in others, in the present study. Thus, all members of TLRs do not seem to have a similar level of expression or affect the GBM microenvironment in the same way. Indeed, we are willing to confirm the present findings by evaluating the endogenous protein level with a larger cohort using fresh tissue samples, to rule out the possible errors due to the FFPE tissue processing steps. Additionally, we claim that conducting more comprehensive studies including a higher number of patients with survival data is crucial to clarify the exact role of each TLRs on the pathogenesis and prognosis of GBM and shed light on observing more effective novel targeted therapies.

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NEÜ YAYINEVİ

Lip Lift With Dermal Suspension Flap Technique: Effect on Patient Satisfaction and Scar Appearance

Dermal Suspansiyon Flebi ile Dudak Kaldırma: Hasta Memnuniyeti ve Skar Görünümü Üzerine Etkisi

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Öz

Amaç: Bu çalışma klasik boğa boynuzu eksizyon paternli dudak kaldırma ameliyat tekniği ile (grup I), aynı paterndeki insizyonla üst dudakta hazırlanan dermal flep kullanılarak gerçekleştirilen dudak süspansiyonu tekniğinin (grup II) , dudak kaldırma ameliyatlarındaki hasta memnuniyeti ve skar görünümüne etkisini karşılaştırmak amacıyla yapılmıştır.
Hastalar ve Yöntem: Yazar tarafından 2016-2023 yılları arasında ameliyat edilen 48-66 yaş arası 28 kadın hasta iki grupta incelendi. Birinci gruba klasik boğa boynuzu eksizyon paternli dudak kaldırma tekniği ile ameliyat edilen 14 hasta, ikinci gruba aynı paterndeki insizyonla üst dudakta hazırlanan dermal flep kullanılarak dudak süspansiyonu tekniği ile ameliyat edilen 14 hasta dahil edildi. Tüm hastalar lokal anestezi altında ameliyat edildi. Altı ay takip edilen her iki guruptaki hastalara 6. ayın sonunda global estetik iyileştirme ölçeği kullanılarak genel estetik sonuçtan memnuniyet düzeyleri soruldu. Ayrıca bir değerlendirici cerrah, Vancouver skar ölçeğini kullanarak ameliyat sonrası oluşan skarı skorladı. Tüm sonuçlar istatistiksel olarak değerlendirildi.
Bulgular: Genel hasta memnuniyeti grup 2'de grup 1'e göre klinik olarak anlamlı olarak daha yüksekti ve grup 2'de grup 1'e göre skar klinik olarak anlamlı olarak daha az bulundu ancak istatistiksel olarak anlamlı fark yoktu (p>0,05).
Sonuç: Hastalarımızda hem genel estetik açıdan hasta memnuniyetinin daha yüksek, hem de skar görünümünün daha az olması nedeniyle, dermal suspansiyon flebi tekniği kullanılarak yapılan dudak kaldırma ameliyatlarının klasik eksizyon yöntemine değerli bir alternatif olarak katkı sağlayabileceği kanaatine varılmıştır.

Anahtar Kelimeler: Flep, cerrahi, dudak, kaldırma

Abstract

Aim: This study was designed to compare the effect of the classical bull horn excision pattern lip lift technique (group I) and the lip suspension technique (group II) which was performed using a dermal flap prepared on the upper lip with the same pattern incision, on patient satisfaction and scar appearance in lip lift surgeries.
Patients and Methods: Twenty-eight female patients aged 48-66 years, operated by the author between 2016 and 2023, were analyzed in two groups. The first group included 14 patients who were operated on with the classical bull horn excision pattern lip lift technique, and the second group included 14 patients who were operated with the lip suspension technique using a dermal flap prepared on the upper lip with the same pattern incision. All patients were operated under local anesthesia. At the end of the 6th month, the patients in both groups, who were followed up for 6 months, were asked about their satisfaction with the overall aesthetic result using the global aesthetic improvement scale. In addition, an evaluator surgeon scored the postoperative scar using the Vancouver scar scale. All results were evaluated statistically.
Results: Overall patient satisfaction was clinically significantly higher in group 2 when compared with group 1 and the scar was found to be clinically significantly less in group 2 when compared with group 1, even though there is no statistical significance (p>0.05).
Conclusion: It has been concluded that lip lift surgeries performed using the dermal suspension flap technique can contribute as a valuable alternative to the classical excision method, due to both higher overall aesthetic patient satisfaction and less scarring in our patients.

Keywords: Flap, Surgical, lip, lifting

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INTRODUCTION

As age progresses, the upper lip loses its youthful appearance, loses its volume and fullness, lengthens vertically, the visibility of the teeth decreases, the philtrum straightens, the vermillion becomes thinner, the cupid's bow disappears, the eversion of the lip decreas-es (1). As a result, the distance between the subnasale and the upper lip increases, and the nasolabial angle decreases (2,3).

Realizing that the upper lips look aged interestingly despite facelift surgeries, Dr. Aus-tin saw that the skin excisions from the vermillion border were far from satisfying the pa-tients due to scarring to solve the problem, and he first defined the lip-lift procedure in 1986 with 83 cases where he made skin excision from the base of the nose in the form of a wavy ellipse (4). This central lip lift was introduced as an upper lip lift, Austin-type lip lift, or sub-nasal lip lift in 1980s (5-10).

Despite all this, although a large number of major surgeries for facial rejuvenation are performed in elderly patients, the lip lift procedure is still adopted and applied by a limited number of surgeons, perhaps due to fear of scarring (9). Contrary to classical bull horn exci-sion pattern lip lift technique, the dermal suspension technique can cause less scarring. There are not enough studies in the literature on the effect of lip lift surgeries with dermal suspension on patient satisfaction and scar formation.

The aim of the study was to compare the effect of the classical bull horn excision pat-tern lip lift technique and the lip suspension technique which was performed using a dermal flap prepared on the upper lip with the same pattern incision, on patient satisfaction and scar appearance in lip lift surgeries.

PATIENTS AND METHODS

Patients admitted to our hospital between 2016 and 2023 years with the request of lip rejuvenation were included in this retrospective study. The patients who underwent surgery for lip rejuvenation and were min. 45 to max. 67 years old were included in the study. Pa-tients who previously underwent surgeries in the upper lip, who had permanent lip fillers and keloid history were excluded from the study. Patients were randomized based on their appli-cation dates. Patients were divided into two groups: (1) those operated with classical lip lift technique (group 1) and (2) those operated with a dermal suspension flap (group 2). Ethics committee approval was obtained (2023/4171), and both verbal and written informed con-sents were acquired from all the patients included.

Once the excision height is marked, the remaining reference vertical markings are drawing with several lines in order not to lose symmetry when closing the skin. In all pa-tients, between 4- and 10-mm excisions were made from subnasale to labiale superius, with the remaining lip length not less than 12 mm. The procedures in both groups were performed under local anesthesia with %2 lido-caine with Jetokain® Ampoule (2 mL ampoule, Adeka A.S., Turkey) containing about 20 mg of lidocaine HCl, 0.125 mg of epinephrine.

Patient demographics, complications, and clinical results were recorded. At the end of the 6th month, all patients were asked about their level of satisfaction of both overall aes-thetic results using global aesthetic improvement scale (GAIS) (Table 1). Also, an evaluator surgeon assessed postoperative scar, using the Vancouver scar scale (VSS) (Table 2). The VSS, which was designed by Sullivan et al. (10) in 1990, rated the scars according to four parameters: vascularity, pigmentation, pliability, and height (Figure 1c).

Surgical techniques;

Group I: Classic the bull's horn excision pattern for subnasal lip lift was used in all pa-tients. After standard deep dermal sutures with 5/0 pds , the incision is closed by interrupted vertical mattress sutures with 6/0 nylon to be removed 6 days later.

Group II: A dermal suspension flap is prepared on the upper lip after the bull's horn in-cision (Figure 1a). Dissection extended 10 mm under the dermal flap and

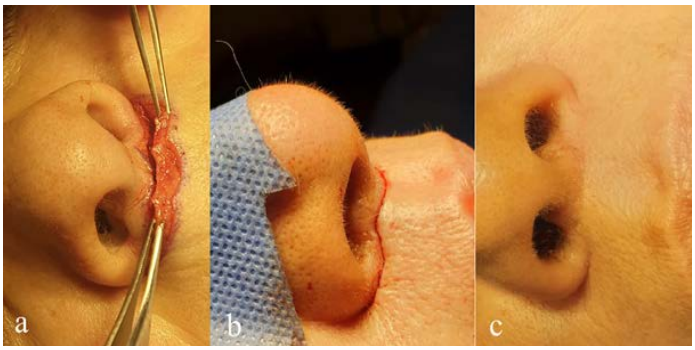

Figure 1. a) dermal suspension flap is prepared on the upper lip after the bull's horn incision, b) The dermal flap is sutured and suspended with multıple 5/0 pds sutures deep to fibrous tissues under the subnasal area that was previously dissected 4 mm c) Evaluator surgeon rated the scars according to four parameters: vascularity, pigmentation, pliability, and height

Table 1. Postoperative Patient's satisfaction measured by global aesthetic improvement scale (GAIS).

Postoperative Assessment	Group-I n (%)	Group-II n (%)	p-value*
Patient's satisfaction overall aesthetic improvement			0.584
Very much improved	6 (%42.9)	9 (%64.3)	0.449
Much improved	4 (%28.6)	3 (%21.4)	1.000
Improved	3 (%21.4)	2 (%14.3)	1.000
No change	1 (%7.1)	-	1.000
Worse	-	-	NA

* Chi-square test.

over the muscle layer, and 4 mm under the columella and subnasale. The dermal flap is sutured and suspended with multiple 5/0 pds sutures deep to fibrous tissues under the subnasal area that was previously dissected 4 mm (Figure 1b). While excising the dermal tissue more than 4 mm on the sides, the entire dermal flap in the middle, which falls under the columella, remains intact, in order to create volume on the nasal spine. After standard deep dermal su-tures with 5/0 pds , the incision is closed by interrupted vertical mattress sutures with 6/0 nylon to be removed 6 days later. All photographs were taken pre-operatively, on the 6th day before the suture removal, and at the 6th months postoperatively (Figure 2)

Patients were advised to use ophthalmic antibiotic ointment (bacitracin) for 2 weeks after the procedure to keep the wound constantly moist. All patients in the series were eval-uated clinically with a standard postoperative follow-up and by surgeon review of photo-graphs.

Statistical Analysis

Data analysis was performed using the Statistics Package for Social Science (SPSS 23.0-IBM, NY, USA). Characteristics of patients, as n (percent) or mean standard deviation (SD) and median (minimum-maximum) for categorical and continuous variables,

Table 2. The Vancouver scar scale postoperative assessment (by evaluator surgeon)

Variables	Score Value	Group-I n (%)	Score	Group-II n (%)	Score	p-value
Vascularity						0.351*
Normal	0	9 (%64.3)	0	12 (%85.7)	0	
Pink	1	4 (%28.6)	4	2 (%14.3)	2	
Red	2	1 (%7.1)	2	-	-	
Purple	3	-	-	-	-	
Pigmentation						0.596*
Normal	0	11 (%78.6)	0	13 (%92.9)	0	
Hypopigmentation	1	3 (%21.4)	3	1 (%7.1)	1	
Hyperpigmentation	2	-	-	-	-	
Pliability						0.472*
Normal	0	11 (%78.6)	0	13 (%92.9)	0	
Supple	1	2 (%14.3)	2	1 (%7.1)	1	
Yielding	2	1 (%7.1)	2	- -		
Firm	3	-	-	-	-	
Ropes	4	-	-	-	-	
Contracture	5	-	-	-	-	
Height (mm)						1.000*
Flat	0	13 (%92.9)	0	14 (%100)	0	
<2	1	1 (%7.1)	1	-	-	
2-5	2	-	-	-	-	
>5	3	-	-	-	-	
Total Score			14.0		4.0	
Score, Mean±SD			1.0±1.79		0.29±0.83	0.183**
Score, Median (Min-Max)			0 (0-6)		0 (0-3)	

* Chi-square test.
** Mann Whitney U-Test.

respectively, and were compared among treatment groups using chi-square or Mann-Whitney tests, as appropriate. The P value was set at <0.05 for statistical significance.

RESULTS

A total of 28 patients aged between 48-66 were included in the study. In group 1 where 14 patients were included, the mean age was 55, with a minimum of 49 and a maximum of 66 years, while in group 2, which included 14 patients, the mean age was 54, with a mini-mum of 48 and a maximum of 65 years. In both groups, average excision length was 7 mm.

9 of 14 patients (66%) stated very much improvement in the group 2 in which the dermal suspension flap was used, while 6 of 14 patients (%42.9) stated very much improve-ment with classical technique in Group 1 (Table 1) in postoperative assessment at 6th month. One patient (%7.1) in group 1 stated that there is no improvement after surgery. Likewise when the scar score performed by the evaluator surgeon was examined in 6th month, The Vancouver scar scale was 14 in group 1 and 4 in group 2, in Group 1, the values of the pa-tients ranged from 0 to 4, and the mean value was 1.0. In Group 2, the values ranged be-tween 0-2 and the mean value was 0.3 (Table 2).

According to these findings, overall patient satisfaction was clinically significantly higher in group 2 when compared with group 1 and the scar was found to be clinically sig-nificantly less in group 2 when compared with group 1, even though there is no statistical significance (p>0.05) between 2 groups in overall patient satisfaction and scar score, proba-bly due to limited number of patients in groups.

No significant complications were observed in patients in either group. One patient in group 1 required revision to obtain further lifting.

Figure 2. All photographs were taken a) pre-operatively , b) on the 6th day before the suture removal, c) and at the 6th months postoperatively

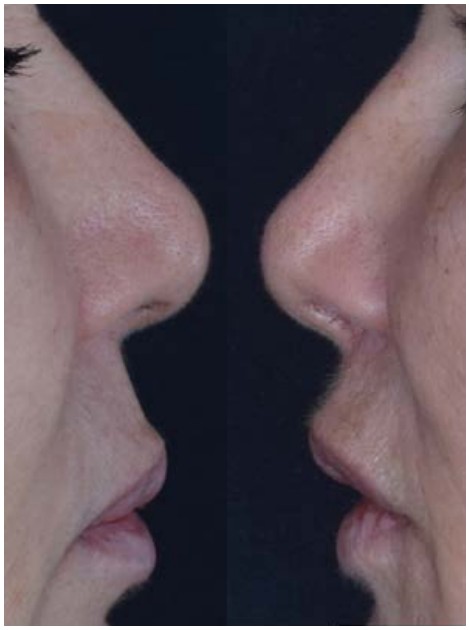

Figure 3. Before and 6 months after photos demonstrates providing volume on the nasal spine and under the base of the nose, which can improve the upper lip convexity that causes causes an aged, simian appearance, and increase the nasolabial angle as well as shortens the lip.

DISCUSSION

With the increase in the average life expectancy in the world and the more accessible aesthetic surgery opportunities against the effects of aging, the number of aesthetic facial surgeries are increasing. Due to the fact that the upper lips continue to look old despite facelift and other rejuvenation surgeries, lip lift surgeries are becoming more common (11). As age progresses, the upper lip loses its youthful appearance, loses its volume and fullness, lengthens vertically, the visibility of the teeth decreases, the philtrum straightens, the ver-million becomes thinner, the cupid's bow disappears, the eversion of the lip decreases. However, the concern of visible scarring and over exaggerated unnatural results still causes surgeons to approach this procedure with caution. With the technique we described in our study, we aimed to reduce the tension in the suture line by suspending the upper lip dermal flap under the subnasale and columella. Our technique is based on the classic bullhorn inci-sion with flap suspansion modification.

Located in the center of the face, philtrum is

related to important landmarks such as the philtrum, vertical facial proportion, upper tooth show. A short philtrum, prominent and symmetrical philtral columns and Cupid's bow has a significant impact on facial expressions (12,13). Although there are various lip lift surgical techniques successfully applied for this purpose, it is particularly important for patients to be informed in detail about the risk of scar formation before the operation, to draw and simulate the area to be excised in front of the mirror, in terms of postoperative patient satisfaction. This process is helpful to evaluate whether the patient's expectations are realistic or not. The most significant disadvantage of this procedure is skin scars, such as hypertrophic, atrophic, invaginated scarring or discol-ored scars (14-17). Therefore, even with a good surgical planning, minimizing scar for-mation is one of the most important goals in this surgery. That's our technique is based on the classic bullhorn incision modified with upper lip based dermal flap for suspension that is focused on reducing tension in the incision line.

In a study, it was claimed that there is a significant relationship between dermis thick-ness and hypertrophic scar (18). Although the thinnest skin on the face is in the eyelid (19,20), it was reported that upper lip dermis was measured thinner than nose region in the male and female patients (21). This data may explain the low scarring and high satisfaction rates in our patients.

It is observed that the nasolabial angle increases, with the increase in the volume on the nasal spine, which is one of the 4 application areas where the dermal filler is injected for the purpose of non-surgical rhinoplasty and contributes to the nasal tip elevation (22). Leav-ing the dermal flap in the upper lip using for suspension, can additionally provide volume on the nasal spine and under the base of the nose, which can improve the upper lip convexity that causes an aged, simian appearance, and increase the nasolabial angle (Figure 3).

Therefore, we can say that the fullness under the subnasale creates an aesthetic im-provement as important as shortening the lip. Some lip lifting techniques (23,24) rely on full thickness skin resection, muscle suspension, excision, or plication to reduce tension on the suture line on the contrary subdermal tissue and muscle resection is not performed in our technique, all tissues are used to create volume.

The main limitations of our study are retrospective design and the limited number of patients. Future investigations that include a large number of patients will contribute pre-cious data to the literature on lip rejuvenation.

CONCLUSION

It has been concluded that lip lift surgeries performed using the dermal suspension flap technique can contribute as a valuable alternative to the classical excision method, due to both higher overall aesthetic patient satisfaction and less scarring in our patients.

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Financial conflict of interest: *Author declares that he did not receive any financial support in this study.*

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NEÜ YAYINEVİ

Phenotypic and Genotypic Identification of Carbapenem-Resistant *Klebsiella Pneumoniae* and Determination of Antibiotic Susceptibility

Karbapenem Dirençli Klebsiella Pneumoniae'nin Fenotipik ve Genotipik Tanımlanması ve Antibiyotik Duyarlılığının Belirlenmesi

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Öz

Amaç: *K. pneumoniae* izolatlarında karbapenemaz genlerinin tanımlanması amaçlanmıştır.
Hastalar ve Yöntem: Bu çalışmaya Temmuz 2016-Aralık 2017 tarihleri arasında örneklerden izole edilen çok ilaca dirençli 95 *K. pneumoniae* suşu dahil edilmiştir. Meropenem, tigesiklin ve kolistinin MIC değerleri Vitek 2, Etest ve Sıvı Mikrodilüsyon Yöntemleri (SMD) ile belirlenmiştir. Kategorik uyum (KU), Çok Büyük Hata (ÇBH) ve Büyük Hata (BH) oranları hesaplanarak değerlendirilmiştir. blaOXA-48, blaOXA-181, blaNDM-1, blaVIM, blaIMP ve mcr-1 kolistin direnç geni araştırılmıştır.
Bulgular: SMD, Vitek 2 ve Etest ile izolatlarda belirlenen meropenem direnci sırasıyla; %70,5, %87,4, %81,1 olarak tespit edilmiştir. SMD yöntemine göre KU, ÇBH ve BH oranları Vitek 2 ve Etest için sırasıyla %69,5, %4,8 ve %20; %70,5, %1,5 ve %0 olarak belirlenmiştir. İzolatlarımızda en yüksek duyarlılık tigesikline karşı tespit edilmiştir. Tigesiklin için KU oranları Vitek 2 ve Etest ile %70,5 ve %95,8 olarak bulunmuştur. Vitek 2 tarafından belirlenen BH oranı, kabul edilebilir %7,6 (<%3) sınırının üzerinde olarak saptanmıştır. Kolistin direnci SMD ile %48,4 olarak tespit edilmiştir. Kolistinin belirlenen KU; ÇBH; BH oranları Vitek 2/Etest için sırasıyla %86,3/%72,6; %17,4/%50; %10,2/%6,1 olarak bulunmuştur. Çalışmamıza dâhil ettiğimiz izolatlarda ağırlıklı olarak blaOXA-48 (%93,7) saptanmıştır. blaOXA-48, izolatların 56'sında (%59) tek başına, 33 izolatta (%34,74) ise blaOXA-181 genleriyle birlikte tespit edilmiştir. İzole OXA-48 pozitif suşlarda, OXA-181 ile birliktelik gösteren izolatlara göre daha düşük kolistin MİK seviyelerine rastlanmıştır. İzolatların %3,2 'sinde blaNDM-1 geni tespit edilmiştir. PZR ile karbapenemaz kombine disk sonuçları izolatların %91,3'ünde uyumlu olarak bulunmuştur.
Sonuç: OXA-48 direnç geni bölgemizde yaygınlığını korumaktadır. Bunun yanında blaOXA-48-benzeri gen bölgelerinin gerçek prevalansının ve direnç dinamiklerinin kapsamlı olarak ortaya konması, ulusal karbapenemaz sürveyans politikalarının gelişimine katkı sağlayacaktır.

Anahtar Kelimeler: Karbapenemaz, *K. pneumoniae*, OXA-48

Abstract

Aim: Characterization of carbapenemase genes in *K. pneumoniae* isolates was aimed.
Patients and Methods: In this study, 95 multi-drug resistant *K. pneumoniae* from samples between July 2016 and December 2017, were included. The MIC of meropenem, tigecycline and colistin were determined by Vitek 2, Etest and Broth Microdilution Methods (BMD). The Categorical agreement (CA), Very Major Error (VME) and Major Error (ME) rates were calculated and evaluated. The mcr-1, blaOXA-48, blaOXA-181, blaNDM-1, blaVIM, blaIMP genes were detected.
Results: The meropenem resistance were determined in the isolates by BMD, Vitek 2 and Etest are respectively; as 70.5%, 87.4%, 81.1%. According to the BMD method, the rates of CA, VME and ME were determined by Vitek 2; Etest as 69.5%, 4.8% and 20 %; 70.5%, 1.5 % and 0 % respectively. It was determined that our isolates showed the highest sensitivity to tigecycline. The CA rates were determined by Vitek 2 and Etest for tigecycline as 70.5% and 95.8%. The ME rate determined by Vitek 2 was above the acceptable limit of 7.6%. The colistin resistance was 48.4% via BMD. The CA; VME; ME ratios determined by Vitek 2/Etest were 86.3%/72.6%; 17.4%/50%; 10.2%/6.1% respectively. In the isolates, predominantly blaOXA-48 (93.7%) was detected. blaOXA-48 was detected alone in 56. isolates and together with blaOXA-181 genes in 33 isolates. Lower colistin MIC levels were found in the only OXA-48 positive strains than in isolates together with OXA-181. The blaNDM-1 gene was investigated in 3.2% of the isolates. PCR and carbapenemase combined disc results were found to be compatible in 91.3% of the isolates.
Conclusion: The blaOXA-48 gene region remains prevalent. In addition, comprehensive identification of the real prevalence and resistance dynamics of blaOXA-48-like gene regions will contribute to the development of national carbapenemase surveillance policies.

Keywords: Carbapenemase, *K. pneumoniae*, OXA-48

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INTRODUCTION

Klebsiella pneumoniae, a member of the Enterobacterales family, is found in humans colonizing the skin, nasopharynx, and gastrointestinal flora (1,2). However, the diversity and epidemiology of infections caused by *K. pneumoniae* has changed recently and has become an important nosocomial agent (2,3). *K. pneumoniae* is an opportunistic pathogen that causes a variety of infections, often associated with risk factors such as mechanical ventilation, urinary catheterization and surgical interventions, and prolonged stay in intensive care unit (3). The increasing use of carbapenems in clinical practice has accelerated the emergence of Carbapenem Resistant *K. pneumoniae* (CRKP) worldwide (4-6). The most important well-known mechanism causing carbapenem resistance is carbapenemase enzymes (7). Carbapenemases are beta-lactamases reside belonging to various Ambler classes (A, B, D) that hydrolyze carbapenems (8). Although many of these enzymes have been reported in our country, OXA-48, which was first identified in our country in 2001, is the most common detected carbapenemase (3,9). Because of the ability of OXA-48 producing strains to transmit resistance genes to other bacteria, the prevalence and spread of this resistance pattern is increasing worldwide, particularly in the Mediterranean region. (4). However, there are studies reported from our country in which different enzymes have been identified, particularly metallo beta-lactamases (MBL) such as blaIMP, blaVIM, and blaNDM, and blaKPC, a class A carbapenemase (4,10). Most of the carbapenemase-producing Enterobacterales members are resistant to all agents except tigecycline and polymyxins; Since they pose a serious threat in hospitals, early detection of carbapenem resistance in *K. pneumoniae* will facilitate the rapid spread of these isolates under control (10-11). In microbiology laboratories that do not have the infrastructure and opportunity to show the presence of carbapenemase genes by molecular methods; Although the use of tests related to enzyme hydrolysis is considered phenotypically, it should be taken into account that the specificities and sensitivities differ (10-12). On the other hand, due to the regional variability of carbapenemase species and prevalence; regional surveillance has gained importance with the molecular characterization of these isolates and epidemic studies (4-6,10-11).

Since the options are limited in the treatment of infections with CRKP, it is important to accurately determine the susceptibility tests of last-resort antibiotics such as tigecycline and colistin. Available commercial automated systems and gradient tests greatly facilitate routine routine laboratory operation. However, error rates above the acceptable limits and categorical incompatibilities regarding these methods reduce the response to treatment (11-13).

The aim of this study was to demonstrate the presence of carbapenemase in CRKP strains isolated from various clinical samples by phenotypic and genotypic methods, to screen for plasmid-mediated colistin resistance gene mcr-1 and to evaluate susceptibility to some antibiotics.

MATERIALS AND METHODS

This study was carried out with the approval of the University of Necmettin Erbakan Non-Pharmaceutical and Medical Device Research Ethics Committee (Date: 17.06.2016 and Decision No: 2016/618).

Collecting Bacterial Isolates

In this study, 95 *K. pneumoniae* isolates, which were found to be resistant to at least one carbapenem, isolated from clinical samples (blood n=68, bronchoalveolar lavage n=13, urine n=4, wound n=3, drainage n=3, endotracheal aspirate n=3and catheter n=1) sent to the central microbiology laboratory of Necmettin Erbakan University Faculty of Medicine Hospital from the clinics and intensive care units between July 2016 and December 2017, were included. The strain isolated for the first time was included in the study only once from each patient.

2.2. Identification of Bacterial Strains and Antibiotic

Susceptibility Tests

Vitek-2 automated system (bioMérieux, France) was used to identify bacteria and detect antibiotic susceptibility. Strains were confirmed at species level simultaneously with the MALDI-TOF MS (bioMérieux, France) system. Meropenem (0.002-32 mg/L), tigecycline (0.016-256 mg/L), fosfomycin (0.016-226 mg/L), and colistin (0.016- 256 mg/L) (HiMedia, Mumbai, India) Ezy MIC™ commercial The minimum inhibitory concentration values of these antibiotics were determined by running the strip tests in accordance with the manufacturer's recommendations. For the in house broth microdilution (BMD) antibiotic susceptibility test, powdered active substances (Sigma-Aldirich, USA) of meropenem, tigecycline and colistin antimicrobials were dissolved in accordance with CLSI (standard M100-27; CLSI 2017) (12) document and stock solutions were prepared. The stock solutions were serially diluted (32-0.032 mg/L) in cation-adjusted Mueller-Hinton

broth (Oxoid Ltd., Basingstoke, UK) prepared daily in 96 microplates. After the suspension of all isolates was prepared with a turbidity of 0.5 Mc Farland standard, it was added to the microdilution plates at a final bacterial concentration of 5X105 cfu/ml. The microplates were incubated for 18-24 hours at 35±2 °C. The meropenem and colistin were evaluated according to EUCAST clinical breakpoints (14,15). The tigecycline was interpreted by US Food and Drug Administration (FDA) criteria (Sensitive ≤2, Intermediate sensitive= 4, and Resistant≥8 mg/L) (16,17). In the study, *K. pneumoniae* NCTC 13443 (NDM-1), *E. coli* NCTC 13846 (mcr-1) isolates were positive control; *E. coli* ATCC 25922 was used as negative control. The evaluation was performed using the double disc synergy method (DDST) and E test methods to confirm the ESBL producing strains

Investigation of Carbapenemase Activity Phenotypically

Isolate suspensions with 0.5 McFarland turbidity were prepared for carbapenemase typing in carbapenem resistant isolates. Inoculation was made on Mueller Hinton Agar (MHA) medium (BioMérieux, France) with the help of a sterile swab. Commercial discs of “MASTDISCSTM ID carbapenemase” were placed in the medium using the combined disc method. The isolates were evaluated after 18-24 hours of incubation in accordance with the manufacturer's recommendations.

Polymerase Chain Reaction

DNA extraction from bacterial suspensions for molecular assay was applied with the “GF-1 Bacterial DNA Extraction Kit” (Vivantis brand, Vivantis company, Malaysia). Purified DNA samples of all samples were prepared in house in LightCycler® system (Roche Applied Science, Switzerland) in accordance with the manufacturer's instructions, using the consensus (18-20) primer sequences in Table 1. Real-time PCR method was studied and proliferation curves were evaluated. PCR protocol first denaturation 10 min at 95ºC; denaturation was carried out at 95ºC for 20 seconds, primer bonding at 60ºC for 20 seconds, and elongation at 72ºC for 20 seconds as 40 cycles.

Klebsiella pneumoniae NCTC 13442 (blaOXA-48), *K. pneumoniae* NCTC 13438 *K. pneumoniae* Carbapenemase (blaKPC), *K. pneumoniae* NCTC 13443 Metallo-β-lactamase (blaNDM-1), *E. coli* NCTC 13476 Metallo-β-lactamase (blaIMP), *K. pneumoniae* NCTC 13440 Metallo-β-lactamase (blaVIM) (KWIK-STIK™, MicroBiologics®) and *E. coli* NCTC 13846 (mcr-1) reference strains were used as positive control.

Statistical analysis

ISO 20776-1 standards (21) were used in calculating the categorical agreement (CA), Very Major Errors (VME), Major Errors (ME), and minor error rates of the tests. According to this, a VME is resistant in the reference antibiotic susceptibility test (AST) while giving a sensitive result in the other antibiotic susceptibility method. Major Errors (ME) reference AST is sensitive while the other AST method gives a resistant result; minor error, reference AST is intermediate sensitive; other AST was defined as a sensitive or resistant result.

RESULTS

All strains were found to be MDR. The clinical specimens were obtained from reanimation intensive care unit (n=26), internal intensive care unit (n=15), other medical units (emergency unit, neurosurgery unit, chest diseases unit, thoracic surgery unit, neurology wards (n=9), pediatric intensive wards

Table 1. Primary sequences used in real-time PCR process

Target gen	Primary direction	Primer sequence (5’-3’)	Band size (bp)	Reference
blaOXA-48	OXA-48-F	TGTTTTTGGTGGCATCGAT	177	18
	OXA-48-R	GTAAMRATGCTTGGTTCGC		
blaKPC	KPC-F	TCGCTAAACTCGAACAGG	785	18
	KPC-R	TTACTGCCCGTTGACGCCCAATCC		
blaNDM-1	NDM-F	TTGGCCTTGCTGTCCTTG	82	18
	NDM-R	ACACCAGTGACAATATCACCG		
blaIMP	IMP-F	GAGTGGCTTAATTCTCRATC	120	18
	IMP-R	AACTAYCCAATAYRTAAC		
blaVIM	VIM-F	GTTTGGTCGCATATCGCAAC	382	18
	VIM-R	AATGCGCAGCACCAGGATAG		
blamcr-1	mcr-F	CGGTCAGTCCGTTTGTTC	35-343	19
	mcr-R	CTTGGTCGGTCTGTAGGG		
blaOXA-181	OXA-181-F	ATGCGTGTATTAGCCTTATCG	798	20
	OXA-181-R	AACTACAAGCGCATCGAGCA		

Table 2. Vitek 2 Automated System antibiotic susceptibility results of *K. pneumoniae* isolates (n=95)

Antibiotics	Numbers of Resistant isolates (%)
Ampicillin	95 (100)
Amoxicillin clavulanic acid	95 (100)
Amikacin	72 (75.8)
Ceftazidime	91 (95.8)
Ciprofloxacin	83 (87.4)
Ceftriaxone	95 (100)
Colistin	43 (45.3)
Cefuroxime	95 (100)
Cefuroxime-axetil	95 (100)
Cefazolin	95 (100)
Ertapenem	90 (94.7)
Cefepim	94 (99)
Cefoxitin	89 (93.7)
Gentamicin	87 (91.6)
Meropenem	83 (87.4)
Trimethoprim-sulfamethoxazole	71 (74.7)
Tigecycline	0 (0)
Piperacillin-Tazobactam	87 (91.6)

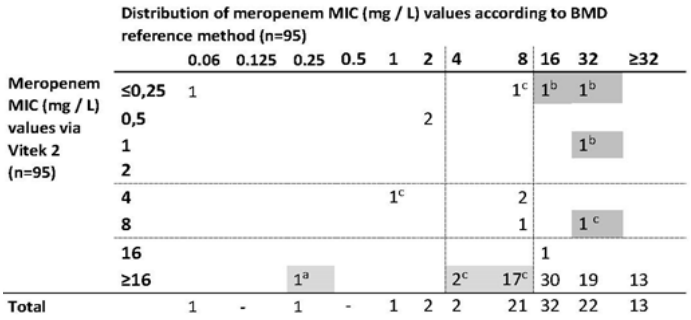

Figure 1.1. Comparison of MIC Values of *K. pneumoniae* strains determined by reference BMD method and Vitek 2 Automated System

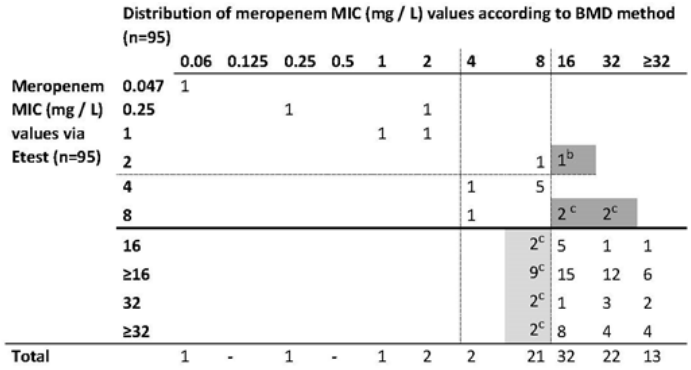

Figure 1.2. Comparison of reference BMD method and Etest Meropenem MIC Values of *K.pneumoniae* strains
a Numbers of isolates with major error,
b Numbers of isolates with very major error,
c Numbers of isolates with minor error
(Meropenem MIC was interpreted as <=2mg /L sensitive and >8mg/L resistant according to EUCAST clinical breakpoints www.eucast.org)

(n=14), internal wards (n=15), pediatric wards (n=15), and surgical wards (n=15) were included. These samples were blood (71.6%), bronchoalveolar lavage (13.7%), urine (4.2%), wound (3.16%), drainage (3.16%), endotracheal aspirate (3.16%) and catheter (1.1%), respectively. Multidrug resistant (MDR) was defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories. All tested isolates were multidrug resistant. The Vitek 2 results of the isolates are given in Table II. In addition that the AST of meropenem, tigecycline and colistin were also tested by Etest and BMD methods. The MIC distributions of the isolates according to the BMD method are given (Table III.). Of all isolates, 74 (77.9%) were isolated from patients in intensive care units. The MIC50 and MIC90 value were given in Table IV. Meropenem resistance was detected with BMD in 67 (70.5%) of 95 patients. The numbers of these resistant isolates for the Vitek 2 automated system and the Etest, respectively; 83 (87.4%) and 77 (81.1%) were determined (Figure 1.1 and Figure 1.2). According to Meropenem AST; VME, ME and minor error ratios of the Vitek 2 system are determined respectively; as 4.8%, 20% and 21.1%. In reference to Meropenem BMD; the VME, ME and minor error ratios of the Etest were 1.5%, 0%, 20%. The categorical agreements were observed for meropenem with Vitek 2 and Etest 69.5% and 70.5%, respectively.

Of the isolates, it was not detected resistant to

Table 3. Distribution of Isolates by MIC Values of Meropem, Tigecycline and Colistin Detected by Broth Microdilution Method

Type of antibiotic	Distribution of Isolates by MIC Values (mg/L)										
	0,064	0,125	0,25	0,5	1	2	4	8	16	32	>=32
Meropenem (n=95)	1	-	1	-	1	2	2	21	32	22	13
Tigecycline (n=95)	-	10	18	40	19	5	3	-	-	-	-
Colistin (n=95)	4	9	16	14	3	3	3	5	12	19	7

Table 4. Comparative Activity of Meropenem, Tigecycline, and Colistin Against *K. pneumoniae* isolates in Patients with and without Intensive Care Unit

	ICU			Non-ICU	
	n	MIC50	MIC90	n	MIC50
Total isolate	74			21	
Meropenem		16	>=32		16
Tigecycline		0,5	1		0,5
Colistin		4	32		0,5

ICU: intensive care unit
MIC: Minimum inhibitor concentration, n:number

tigecycline, only 3.2% of them were "intermediate catagory". According to tigecycline BMD, the detected ME and minor errors rates by Vitek 2 were 7.6 % and 22.1 %, respectively. (Figure 2.1.). VME and ME were not detected by Tigecycline Etest and the minor errors rate was 4.2 % (Figure 2. 2.). The categorical agreements were observed for tigecycline with Vitek 2 and Etest as 70.5% and 95.8%, respectively.

In this study, colistin resistance was found to be 48.4% with BMD. The VME rates were determined as 17.4% and 50% for Vitek 2 and Etest, respectively. The ME rates of Vitek 2 and Etest were found to be 10.2 % and 6.1 %, respectively. The CA between the BMD-Vitek 2 system and BMD-Etest methods for colistin was determined as 86.3% and 72.6%, respectively. (Figure 3.1 ve Figure 3.2).

The resistance rates to fosfomycin, which is an alternative for the treatment of CRE infections, was observed as 16.8%. The MIC50 and MIC90 values for fosfomycin were interpreted as 6 mg/L and 24 mg/L, respectively.

Expanded Spectrum Beta-Lactamase (ESBL) production was observed in 20 (21.05%) of the strains by the combined disc method (Oxoid, Thermo Fisher Scientific, Basingstoke, UK) and only 9 (9.47%) isolates were found to be ESBL positive by the gradient test (HiMedia, India).

According to the PCR results, at least one resistance gene was detected in 92 (96.8%) of the isolates, while no carbapenemase resistance gene was detected in 3 of them. The most common carbapenemase detected in isolates was OXA-48 (93.7%). blaOXA-48 was shown alone in 56 (59%) isolates and together with blaOXA-181 genes in 33 (34.7%) isolates. Although the MIC50 values of colistin were 0.5mg/L in OXA-48 positive isolates (n=56), the colistin MIC50 was determined as 16mg/L coexistence of OXA-48+OXA-181 (n=33). The blaNDM-1 gene was detected in 3 (3.2%) of the isolates. The colistin plasmid-mediated resistance gene (mcr-1), IMP, VIM and KPC carbapenemase resistance genes were also not detected in any of the isolates. In this study, the presence of carbapenemase enzyme in 88 isolates and ESBL+porin loss in 3 isolates were determined by the phenotypic carbapenemase combination disc test. No carbapenemase enzymes were detected with combined discs in 4 isolates. However, the presence of OXA-48 gene was determined by PCR in 2 of these isolates. In 2 of them, no carbapenemase

Distribution of tigecycline MIC (mg / L) values according to BMD method (n=95)

Tigecycline MIC (mg / L) values via Vitek 2 (n=95)	0,06	0,125	0,25	0,5	1	2	4	8	16	32	≥32
≤0,5		2	2	9	3		1[c]				
1		2	3	12	4	1	1[c]				
2		3	8	12	5	1	1[c]				
4		3[c]	4[c]	2[c]	6[c]	3[c]					
≥8			1[a]	5[a]	1[a]						
Total	-	10	18	40	19	5	3				

Figure 2.1. Comparison of tigecycline MIC Values of *K.pneumoniae* strains determined by BMD method and Vitek 2

Distribution of tigecycline MIC (mg / L) values according to BMD method (n=95)

Tigecycline MIC (mg / L) values via Etest (n=95)	0,06	0,125	0,25	0,5	1	2	4	8	16	32	≥32
0,12											
5				1							
0,25			1								
0,5		1	2	8	4						
0,75		4	4	13	5						
1		3	2	9	2	2					
1,5		2	5	7	5	2					
2			4	2	2	1	1[c]				
4					1[c]						
≥8							2[c]				
Total	-	10	18	40	19	5	3				

Figure 2.2. Comparison of tigecycline MIC Values of *K.pneumoniae* strains determined by reference BMD method and Etest

a Numbers of isolates with major error, b Numbers of isolates with very major error, c Numbers of isolates with minor error

(Tigecycline MIC was interpreted accordid to FDA criteria as Sensitive ≤ 2 mg /L, Intermediate sensitive = 4 mg/L, and Resistant ≥ 8 mg/L)

Distribution of colistin MIC (mg / L) values according to BMD method (n=95)

		0,06	0,125	0,25	0,5	1	2	4	8	16	32	≥ 32
Colistin MIC (mg / L) values via Vitek 2 (n=95)	≤ 0,5	4	8	15	12	3	1	1[b]		1[b]	1[b]	1[b]
	1						1	1[b]				
	2									1[b]	2[b]	
	4							1	1	1		
	8				1[a]		1[a]		1	3	1	
	16											1
	≥ 16		1[a]	1[a]	1[a]			3	6	15	5	
Total		4	9	16	14	3	3	3	5	12	19	7

Figure 3.1. Comparison of colistin MIC Values of *K.pneumoniae* strains determined by reference BMD method and Vitek 2 system

Distribution of colistin MIC (mg / L) values according to BMD method (n=95)

		0,06	0,125	0,25	0,5	1	2	4	8	16	32	≥32
Colistin MIC (mg / L) values via Etest (n=95)	0,064	1										
	0,25			1								
	0,5			2	1							
	0,75		1	3	1							
	1	1	4	4	2	2			1[b]	1[b]		2[b]
	1,5	2	3	4	10	1	2	2[b]	3[b]	5[b]	6[b]	
	2			1						2[b]	1[b]	
	4		1[a]						1		2	3
	8			1[a]						1	5	2
	16						1[a]	1		2	3	
	≥ 32									1	2	
Total		4	9	16	14	3	3	3	5	12	19	7

Figure 3.2. Comparison of colistin MIC Values of *K.pneumoniae* strains determined by reference BMD method and Etest
a Numbers of isolates with major error, b Numbers of isolates with very major error, c Numbers of isolates with minor error
(Colistin MIC was interpreted <=2mg/L sensitive, >2mg/L resistant according to
EUCAST clinical break points www.eucast.org)

gene was detected by PCR. In combination tests, it was determined that 83 of the carbapenemase types carried OXA-48, 2 had KPC and 3 had MBL resistance gene.

Comparing the carbapenemase combined disc and PCR results, the agreement was found at the rate of 91.3% (n=84). OXA-48 enzyme was also detected with carbapenemase combined disc in 82 of 89 isolates whose presence of OXA-48 gene was detected by PCR, and the sensitivity of the combined discs for OXA-48 enzyme was found to be 92.1%. Combination disc tests were detected as 'metallo-betalactamase enzyme positive' in 2 of 3 isolates with positive NDM-1 gene by PCR. In one isolate, the OXA-48 enzyme was positive. The sensitivity of

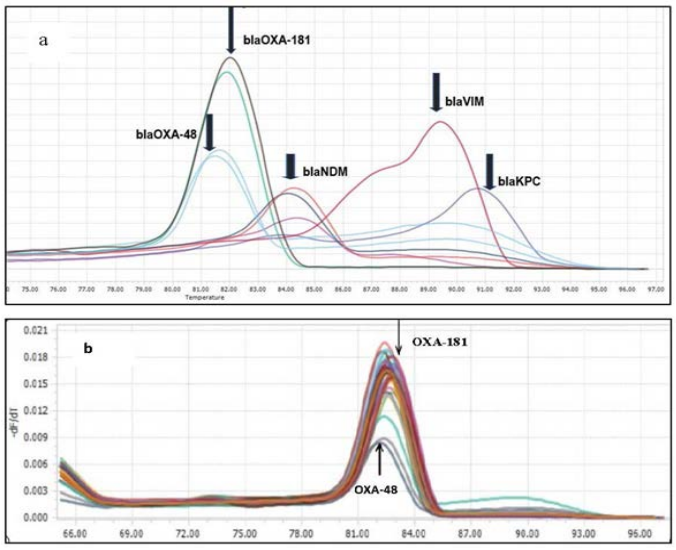

Figure 4a. Evaluation of melting curves by in-house real-time PCR. The detected Tm values of blaOXA-48 (81.65 °C), blaOXA-181 (82.04 °C), blaNDM (84.07 °C), blaVIM (89.410C) and blaKPC (90.69 °C) primers are shown. **Figure 4b.** There are fluctuations that deviate from the clustering. The Tm degree of OXA-181 variants was higher than that of OXA-48 containing strains.

combined discs in detecting the presence of NDM-1 was 66.7%. The real PCR melting temperature (Tm) is specific for each product, so the method can be used reliably when two unknown DNA sequences are compared with this technique (22). The specific Tm grades detected in the primers are given in Figure 4a. In our study, the Tm degree of the OXA-48 variant OXA-181 was found to be higher than the strains containing the OXA-48 gene in PCR melting curve analysis (Figure 4a-b).

DISCUSSION

In this study, 77.9% of all strains were isolated from patients in ICUs where colonization and infections due to resistant pathogens are common. OXA-48 carbapenemase was detected most frequently. However, OXA-181 was also determinated as remarkable. High colistin MIC value detected in the coexistence of OXA-48 and OXA-181 were noteworthy.

Carbapenem susceptibility may vary in isolates containing carbapenemase. Although the isolate is a carbapenemase producer, it can be detected within the carbapenem sensitivity limits. In this study, isolates with reduced carbapenem susceptibility with meropenem MIC>0.12mg/L were included. In

one isolate meropenem and ertapenem MICs were 0.06mg/L. However, it was included in the study because of the detected high level the colistin MIC (32mg/L). Only by PCR could it be determined that it carried the blaOXA-48 carbapenemase gene. It was thought that the presence of carbapenemase could not be detected completely phenotypical methods due to the weak carbapenemase expression of the related strain. There are inadequate data on the response of patients infected with such a strain to carbapenem therapy. It should be kept in mind that in our country, where OXA-48 type carbapenemase production is common, such isolates may be encountered in patients who do not respond to treatment (23).

Broth microdilution is a reference AST methods for many antibiotic groups. In addition, it is required intensive labor and experience. Thus, alternative commercial methods are being researched. Morover there is an increasing need for studies comparing performances between methods in order to ensure the necessary standardization.

In this study, the susceptibilities of meropenem, tigecycline and colistin were tested by Vitek 2, Etest and BMD methods. The detected meropenem resistance by BMD, Vitek 2 and Etest was 70.5%, 87.4% and 81.1%, respectively. The determined CA rates were below the acceptable limit, 69.5% and 70.5% for Vitek 2 and Etest, respectively. The VME ratios for meropenem were detected by Vitek 2 and Etest as 4.8%, 1.5%, respectively. The ME rates were observed almost 20% by Vitek 2. None major error was not detected via Etest. Haldorsen et al. (24) found meropenem MICs via BMD for 50%, 61% and 25% higher than >2 fold dilution levels in Vitek 2 for class A, B, D carbapenemase producing bacteria, respectively. In the study, the rates of CA, ME and minor error between BMD-Vitek2 methods were determined as 56%, 26% and 18%, respectively. The CA between BMD-Etest was 73%; ME and minor error rates were reported as 7% and 20%, respectively. Similar to our results, high ME rates detected with Vitek 2 is a major problem, that limits the use of meropenem, which is still an important agent in combination therapies in CRE infections (25).

Tigecycline shows promise in the treatment of infections caused by MDR gram-negative bacteria, including CRKP (26). Previous studies have shown that tigecycline results can be affected by the method used (27). In addition, the lack of clinical breakpoints and a recommended method for Enterobacterales including CLSI and the limited number of data from literature studies reveal the need for accurate and reliable tigecycline AST (17, 26). In the study of Yin et al. (27), in which they compared the effectiveness of tigecycline on 372 CRKP strains with disc diffusion, modified disc diffusion, Vitek 2 system, Etest methods and BMD method. The CA between these methods were found 78.5%, 96.5%, 69.9% and 96.7%, respectively. These investigators found that 96.8% of strains with BMD were susceptible to tigecycline. Li et al. (17) reported that the CA between agar dilution method, disk diffusion method, Etest, MicroScan, Vitek 2 Compact, BD Phoenix 100 methods and SMD method were determined as 96%, 53%, 88%, 92%, 74%, and 93%, respectively. The rate of ME detected only with Vitek 2 (9%) was above the acceptable limit of 3%. Etest has been reported to have a higher BMD agreement compared to Vitek 2.

In this study, the tigecycline resistance was not detected, similar to the study of Yin et al. (27). Tigecycline was determined to be the most effective antibiotic against MDR *K. pneumoniae* among the antibiotics in our study. The CA rates for tigecycline BMD-Vitek 2 and BMD-Etest were determined as 70.5% and 95.8%, respectively. These results are in agreement with the data of Yin et al. (27). The CA rate (95.8%) determined for Etest was higher than the study of Li et al. (17) (88%). In our study, VME was not found in any of the tigecycline AST methods. Consistent with previous studies, the rate of ME via Vitek 2 was determined to be 7.6% above the acceptable limit (<3%). This result suggests that the isolates found resistant with Vitek 2 should be confirmed with an alternative method. In line with our data, it was determined that tigecycline Etest had higher compatibility with BMD and lower error rates compared to Vitek 2.

In the study evaluating Sensitire, Vitek 2, Etest, MicroScan and BMD methods on 76 multi-resistant Enterobacterales, 21 of which were mcr-1 positive, by Chew et al. (28). It has been reported that colistin MIC values determined by Sensititre and Vitek 2 have a higher agreement with BMD compared to Etest. Despite the high CA detected in Vitek2-BMD and Etest-BMD, it was reported that the VME rates were also found to be above acceptable limits (12-36%). In our study, colistin resistance was 48.4% with BMD. The CA, VME, ME ratios for Vitek 2/Etest were determined as 86.3%/72.6%, 17.4%/50%, 10.2%/6.1%, respectively. Although Vitek 2 showed higher CA ratio than Etest, the detected VME rates for both tests were highly above the acceptable limit.

In our isolates, resistance to fosfomycin was found to be 16.8%, For this reason, it should be considered as one of the alternative options in combination protocols with other drugs in the treatment of CRE.

Although it is known that various carbapenemases are encountered in different parts of the world, it is known that the blaOXA-48 gene is mainly responsible for resistance in our country. This enzyme (4), which was reported in our country for the first time in the world, was predominantly detected (93.7%) in our study. blaOXA-48 was detected alone in 56 (59%) isolates and together with blaOXA-181 genes in 33 (34.74%) isolates. blaOXA-181 is a variant carbapenemase strain derived from blaOXA-48 with similar carbapenemase activity, first identified in Enterobacterales isolates in India. Colistin resistance in *K. pneumoniae* isolates producing blaOXA-181 has been reported worldwide (29-31). It is currently known that inactivation of mgrB gene is associated with colistin resistance and is the most common mechanism responsible for polymyxin resistance in *K.pneumoniae*. It has been reported in previous studies that the mobile genetic element, which becomes functional with the insertion of the blaOXA-181 carbapenemase gene, causes colistin resistance by inactivating the mgrB gene (29). This study finding were found compatible with previous data. Although colistin MIC values were found as MIC50:0.5mg/L only in OXA-48 positive isolates (n=56); in isolates with OXA-48+OXA-181 association (n=33), colistin MIC50 was determined as 16mg/L. This data suggests that revealing the resistance genetics carried by the blaOXA-181 gene in detail will be useful in elucidating the factors that facilitate the development of resistance to existing antibiotics. In this study, the blaNDM-1 gene was detected in 3.2% of the isolates. In a multicenter study reported from our country, it was stated that the predominant carbapenemase in the country was blaOXA-48, but the increase in blaNDM-1 was remarkable (10). blaIMP, blaVIM and blaKPC carbapenemase resistance genes were not detected in any of the isolates. The mcr-1 could not be determined. Moreover, there is also a need to investigate other mechanisms and genes in colistin-resistant strains. The agreement between our carbapenemase combined disc and PCR results was found in 91.3% of the isolates. The sensitivity of the combined discs in detecting the presence of blaOXA-48 and blaNDM-1 was as 92.1% and 66.7% respectively. The sensitivities in phenotypic methods may differ according to the type of carbapenemase carried by the predominantly isolates in the investigated region. Considering the relatively low sensitivity of the combined disk test for the blaNDM-1 enzyme and the low number of samples. It is highly recommended that optimized studies involving more bacterial numbers and species are needed.

Parlak et al. (32) found the sensitivity of the temocillin disc to detect the presence of OXA-48 as 88%, and the specificity as 89%. In the present study, temocillin disc and Etest sensitivities were 95.6% / 94.4% respectively. The specificities were determined as 66.7% for both methods. Temocillin Etest and discs are considered to be strong indicators for detecting the presence of OXA-48, but the tests are not sufficient to exclude it alone. In addition, since our sensitivity and specificity results with temosilin disc and Etest are similar; cost-effective disk method can be recommended.

According to the data obtained from our study, blaOXA-48 producing isolates maintain their frequency in our region. Among the OXA-48 variants, the prevalence of the blaOXA-181 gene, its association with the bla-OXA-48 gene, and the high colistin MIC values detected in these isolates are noteworthy. Although the sensitivity of phenotypic tests varies according to the carbapenase type, our results indicated that the OXA-48 producing isolates are still dominant in our country, these tests can be used for screening purposes in routine laboratories but it should be supported by molecular methods.

Conflict of interest: *Authors declare that there is no conflict of interest between the authors of the article.*

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NEÜ YAYINEVİ

The Effects of Topiramate on Methotrexate-Induced Pancreatic Injury

Topiramatın Metotreksat İlişkili Pankreas Hasarı Üzerine Etkisi

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Öz

Amaç: Bu çalışmada metotreksat ilişkili pankreas hasarında topiramatın pankreasın Langerhans adacık hücreleri üzerine olan etkisinin araştırılması amaçlandı.
Gereçler ve Yöntem: Çalışma 04 Temmuz- 24 Temmuz 2022 tarihinde Recep Tayyip Erdoğan Üniversitesi Hayvan Deneyleri ünitesinde gerçekleştirilmiştir. Çalışmada Spraque-Dawley erkek sıçanlar her grupta 8 hayvan olacak şekilde 3 gruba ayrıldı. Kontrol grubuna ait sıçanlara sadece %0.9'luk serum fizyolojik intraperiteonal (i.p.) olarak uygulandı. Metotreksat (MTX) grubuna tek doz 20mg/kg MTX uygulandı. Topiramat (TPM) tedavi grubuna MTX uygulamasından 7 gün önce ve 7 gün sonra olmak üzere toplam 14 gün süreyle günde tek doz TPM 100 mg/kg/gün oral gavaj yoluyla uygulandı. TPM uygulamasından 7 gün, son dozdan 16 saat sonra tüm denekler 50 mg/kg ketamin HCL ve 20 mg/kg ksilazin i.p. uygulanarak uyutuldu.
Bulgular: MTX grubunda Langerhans adacıklarında yaygın ödematöz alanlar ve nekrotik hücreler gözlemledik. TPM grubunda MTX grubuna kıyasla nekrotik hücrelerin ve ödematöz alanların azaldığını saptadık. MTX grubunda insulin pozitivitesi gösteren β hücrelerinin kontrol grubuna kıyasla azalmış olduğunu saptadık ($p<0.05$). TPM grubundaki pankreas dokusuna ait kesitlerde MTX grubuna kıyasla insülin pozitivitesi gösteren β hücrelerinde anlamlı düzeyde artış olduğunu gözlemledik ($p<0.05$). MTX grubunda kontrol grubuna kıyasla glukagon pozitivitesi gösteren hücrelerin sayısında anlamlı olarak azalma olduğunu izledik ($p<0.05$). TPM grubunda MTX grubuna kıyasla glukagon pozitivitesini gösteren α hücrelerinin sayısının artmış olduğunu gözlemledik ($p<0.05$). Histopatolojik Hasar Skoru kontrol grubunda 0(0-1), MTX grubunda 6(6-8)'ya yükseldiğini saptadık ($p<0.05$). TPM grubunda HHS 2(2-3) olarak gözlemlendi ($p<0.05$).
Sonuç: Çalışmamız topiramatın pankreas adacık hücreleri üzerinde koruyucu bir etkiye sahip olduğunu göstermektedir.

Anahtar Kelimeler: Metotreksat, pankreas hasarı, sıçan, topiramat

Abstract

Aim: The present study aimed to investigate the effects of topiramate on pancreatic islets of Langerhans cells in Methotrexate-related pancreatic injury.
Materials and Methods: The study was conducted between 04 July - 24 July 2022 in the Animal Research Unit of Recep Tayyip Erdogan University. Male Spraque-Dawley rats were divided into 3 groups with 8 animals in each group. Control group (C) (Only 0.9% saline was administered). A single dose of 20mg/kg Methotrexate was administered to the MTX group (MTX). The TPM group (TPM) was administered 100 mg/kg/day Topiramate by oral gavage for a total of 14 days, 7 days before and after Methotrexate administration. All subjects were euthanized by anesthesia 16 hours after the last Topiramate dose.
Results: We observed widespread edematous areas and necrotic cells in the islets of Langerhans in the MTX. We found that necrotic cells and edematous areas were decreased in the TPM compared to the MTX. We found fewer β cells showing insulin positivity in the MTX compared to the controls ($p<0.05$). We observed an increase in insulin-positive β-cells in the TPM compared to the MTX ($p<0.05$). We observed a significant decrease in the number of cells showing glucagon positivity in the MTX compared to the controls ($p<0.05$). We observed an increased number of α cells showing glucagon positivity in the TPM compared to the MTX ($p<0.05$). We found that the HDS increased from 0(0-1) in the control group to 6(6-8) in the MTX ($p<0.05$). It was found as 2(2-3) in the TPM ($p<0.05$).
Conclusion: Our study shows that topiramate has a protective effect on pancreatic islet cells.

Keywords: Methotrexate, pancreatic injury, rat, topiramate

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INTRODUCTION

Topiramate (TPM) is an anti-epileptic drug indicated for the treatment of partial and generalized epileptic attacks in adults and children that possess different mechanisms than other antiepileptic drugs (1). TPM, which is accepted as an antiepileptic and neuroprotective agent, has found an area of use either as a single agent or in combination with other drugs in a multitude of conditions including TPM, migraine episodes, essential tremors, alcohol dependence, neuropathic pain, Lennox-Gastaut syndrome, bipolar disorder, and schizophrenia, as well as obesity in combination with phentermine. (2)

Although antiepileptic drugs are typically associated with weight gain, TPM differs from other antiepileptics as it is known to be associated with weight loss and a decrease in appetite (3). TPM was observed to exert an effect similar to that of antidiabetic drugs by achieving improved glycemic control in animal and human studies conducted to date (4-7). The molecular mechanism underlying the effects of TPM on glycemic control has not been clarified; however, this effect is independent of its effects that promote weight loss and decreased appetite (3).

Studies on the effects of TPM on glycemic control have proposed several mechanisms. One of these suggests that TPM reduces blood glucose levels by increasing glucose-stimulated insulin secretion (6). However, this hypoglycemic effect of TPM was not observed in healthy subjects and it did not alter glucose-stimulated insulin secretion in these individuals (8, 9). Therefore, the strongest mechanism that has been proposed is that the increase in insulin secretion might be linked to the protective effects of TPM on islet beta cell function (4, 8, 10). Published data suggest that the improvement of b-cell function in lipid and/or glucotoxicity-related models may explain the antidiabetic effects of TPM (4, 7, 8).

Studies have shown that TPM has neuroprotective and antiapoptotic effects (11). It has been suggested that the reduction of apoptotic activity might have favorable effects on pancreatic injury as well as on the central nervous system (12). Accordingly, TPM may be improving pancreatic tissue injury and beta cell function through its direct trophic effects in addition to its metabolic effects on beta cells. Almost all studies conducted on pancreatic islet beta cell function with TPM have included either obese or diabetic patients or animal models (4, 9, 13). It is known that TPM has no effect related to glucose regulation or pancreatic beta cell function in healthy individuals with normal weight (7, 8, 10). To our knowledge, no more than one study investigated the protective effects of TPM on pancreatic injury related to causes other than obesity and diabetes (12).

Methotrexate (MTX) is a chemotherapeutic drug that essentially takes effect by inhibiting the synthesis of thymidylate and the folic acid cycle and results in the impairment of nucleic acid synthesis, which leads to cell death (14). Toxicity studies on methotrexate, which is an antimetabolite used in the treatment of many cancers and autoimmune diseases, have mostly included the liver, kidneys, and the lung (15-19). However, MTX-based chemotherapy often results in various toxicities that require dose reduction or treatment cessation (16). There are very few studies in the literature showing that MTX may induce pancreatic toxicity.

Based on the neuroprotective and antiapoptotic effects of TPM, we hypothesized that it could have a protective effect on pancreatic injury and islet cells. Another aim of our study is to investigate the potential damage in endocrine pancreatic tissues that methotrexate exposure causes using histopathological and immunohistochemical methods.

MATERIALS AND METHODS

This study included 24 male, 3-4-month-old Sprague-Dawley rats weighing 300±30g. Subjects were obtained from the Animal Research Unit of Recep Tayyip Erdogan University. The study was approved by the local ethics committee for animal research at Recep Tayyip Erdogan University (ID24, Date: 30/06/2022). The study was conducted at Recep Tayyip Erdogan University Animal Research Unit between July 4-July 24, 2022. The subjects were kept under optimal conditions with constant temperature (21±2°C) and photoperiod (12:12 hour light-darkness cycle). All animals were allowed ad-libitum access to water and food. The sample size was calculated based on the method described by Charian et al. (22). With the use of a numerator software, the subjects were randomly assigned to one of three groups: control group (n=8), methotrexate group (n=8), and methotrexate + topiramate group (n=8).

Chemicals and Medications

Methotrexate “EBEWE (50 mg/5 ml Ebewe Pharma GmbH Nfg. KG Mondseestrasse 11 A–4866 Unterach, Austria). Topiramate (TOPAMAX 25 mg, Johnson and Johnson Medical Products Industry and Trade Ltd., Cilag AG - Schaffhausen/Switzerland). Ketamine HCL (Ketalar 500 mg, Pfizer Pharmaceuticals Ltd.

Co. Ortakoy, Istanbul, Turkey). Xylazine (Rompun 2%, Bayer, Turkey, Istanbul, Turkey).

Experimental Procedure

Rats in the control group were only given a single intraperitoneal (i.p.) dose of 1 mL of 0.9% physiological serum (Biofleks, Osel Pharmaceuticals Industry and Trade Inc., Beykoz, Istanbul, Turkey). The MTX group was given a single dose of 20mg/kg MTX (19, 23). The TPM treatment group was given a single dose of 100 mg/kg/day by oral gavage over 14 days; 7 days before and 7 days after the administration of MTX (24, 25). All subjects were euthanized with 50 mg/kg Ketamine HCL and 10 mg/kg Xylazine HCl seven days from the TPM application and 16 hours from the final dose.

Histopathological Analysis

Pancreatic tissue specimens excised from the rats were trimmed and fixed in 10% formalin for 48 hours. Following the fixation procedure, pancreas specimens were transferred to a tissue processing cassette (Isolab GmbH, Germany) and passed through ethanol (Merck GmbH, Germany) series of increasing concentration (50%, 70%, 80%, 90%, 96%, 100%, 100%) using a tissue processor (Shandon Citadel 2000, Thermo Sceintific Inc., Germany). In the next stage, pancreatic tissue specimens were kept in two series of xylol (Merck GmbH, Germany) and subjected to an embedding procedure with soft (Merck GmbH, Germany) and hard paraffin (Merck GmbH, Germany). Pancreatic tissue specimens that were kept overnight in paraffin were then embedded in tissue embedding cassettes (Isolab GmbH, Germany) using a paraffin embedding station (Leica Biosystems, EG1160, Germany). From the resulting paraffin blocks of pancreatic tissue, 4-5-micrometer sections were obtained using a rotary microtome (RM2525, Leica Biosystems, Germany). The resulting pancreatic tissue sections were stained with Harris hematoxylin (Merck GmbH, Germany) and Eosin G (Merck GmbH, Germany) using a staining device (Leica 5020ST, Leica Biosystems, Germany)

Immunohistochemical Analysis

2-3-micrometer sections obtained from the paraffin blocks (RM2525, Leica Biosystems, Germany) of pancreatic tissue were transferred onto positively charged slides. The primary insulin antibody kit (ab15147, Abcam, United Kingdom) was used to identify the insulin-secreting β cells in the islets of Langerhans and the primary glucagon antibody kit (ab92517, Abcam, United Kingdom) was used to identify the glucagon-secreting α-cells. Secondary antibody kits corresponding to the primary antibodies (Goat Anti-Rabbit IgG H&L (HRP), ab97051, Abcam, United Kingdom) were used. Primary and secondary antibody kits were incubated using an ICH/ISH staining device (Leica Bond Max, Leica Biosystems, Germany) according to the instructions manual of the manufacturer. Harris Hematoxylin (Merck GmbH, Germany) was used for counter-staining.

Semi-quantitative Analysis

A Histopathological Damage Score (HDS) was developed for the histopathological scoring of pancreatic tissue according to the study by Schmidt et al. (26) as shown in Table 1. Twenty randomly selected fields per preparation were scored by an experienced histopathologist. The histopathologist was blind to the study groups.

Immunopositive cells in the pancreatic tissues incubated with the primary antibodies were scored using immunohistochemical methods as shown in Table 2. Twenty randomly selected fields per preparation were scored by an experienced histopathologist. The histopathologist was blind to the

Table 1. The pancreatic Histopathological Damage Score (PHDS) was modified based on the Damage Score by Schmidt et al.

Score	Findings
Necrotic Cells	
0	≤5%
1	Between 6%-25%
2	Between 25%-50%
3	≥50%
Edematous Areas	
0	≤5%
1	Between 6%-25%
2	Between 25%-50%
3	≥50%
Cells with Pyknotic Nuclei	
0	≤%5
1	Between 6%-25%
2	Between 25%-50%
3	≥50%

Table 2. Immunohistochemical Positivity Scoring

Score	Findings
0	≤5%
1	Between 6%-25%
2	Between 25%-50%
3	≥50%

Table 3. Pancreatic Histopathological Damage Score (PHDS) Results (median-(25%-75% values)).

Gruplar	Necrotic Cells	Edematous Areas	Cells with Pyknotic Nuclei	PHDS
Control	0(0-0)	0(0-0)	0(0-0)	0(0-1)
MTX	2(2-2)*	2(2-2)*	2(2-2)*	6(6-7)*
MTX+TPM	1(0-1)**,***	0(0-1)***	1(1-1)**,***	2(1-3)**,***

* p<0.001; Between the control group and the MTX group,
** p<0.001; Between the control group and the MTX+TPM group,
***p<0.001; Between the MTX group and the MTX+TPM group,
Kruskal Wallis/Mann Whitney U test

study groups.

Statistical Analysis

Data obtained using histopathological and immunohistochemical methods were subjected to Shapiro-Wilk, Q-Q plot, and Skewness-Kurtosis analyses using the SPSS 20 (IBM Corp., USA) computer software. For non-parametric data; median (25% and 75% interquartile range) values were calculated. The differences between the groups were subjected to the Kruskal-Wallis test and then to the Bonferroni-corrected Mann-Whitney U test. P <0.05 was accepted as statistically significant.

RESULTS

Histopathological Analysis

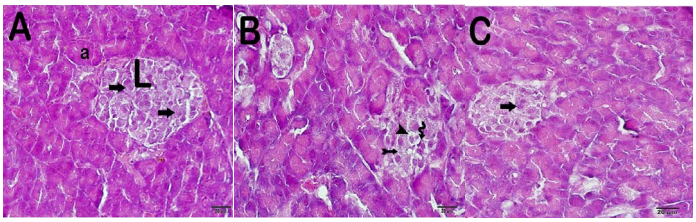

Figure 1. Light microscopic image of H+E-stained pancreatic tissue.
Langerhans Islets (L), Acinus (a)
A(x20) Control Group: Pancreatic islet of Langerhans (L) showing normal cells (arrow). B (x20) MTX treatment group: Pancreatic islet of Langerhans (L) showing extensive loss of cytoplasm accompanied by necrotic cells (tailed arrow) with vacuolizations. In addition, numerous necrotic cells are observed to form edematous regions in the islets of Langerhans (spiral arrow). Also, pancreatic cells with pyknotic nuclei can be observed at places (arrowhead). C(x20) Topiramate Treatment Group: Islets of Langerhans (L) showing extensive typical cells (arrow) with fewer necrotic cells and decreased edematous areas (arrowhead).

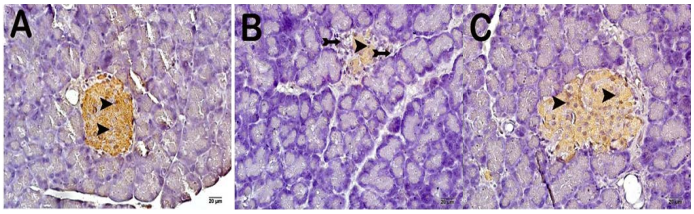

Figure 2. Light microscopic image of pancreatic tissue incubated with insulin primary antibody.
A(x20) Control Group: β cells (arrowhead) showing extensive insulin positivity in normal islets of Langerhans B(x40) MTX Group: Fewer insulin-positive β cells (tailed arrow) observed in degenerative islets of Langerhans. C(x40) MTX+TPM Treatment Group: More β cells showing insulin positivity (tailed arrow) in typical islets of Langerhans.

On examination of H+E stained sections under a light microscope, we observed extensive edematous areas and necrotic cells in the islets of Langerhans in the MTX treatment group (Figure 1a-b). In contrast, we determined a decrease in necrotic cells

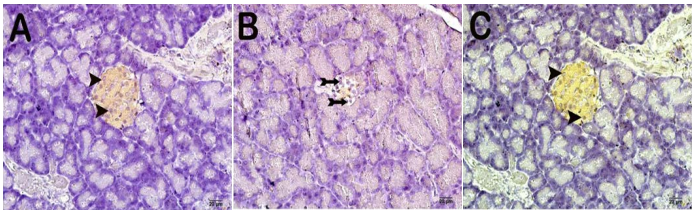

Figure 3. Light microscopic image of pancreatic tissue incubated with glucagon primary antibody.
A(x20) Control Group: Normal α cells (arrowhead) showing extensive insulin positivity in the islets of Langerhans B(x40) MTX Group: Atypical islets of Langerhans showing reduced insulinp ositivity in α cells (tailed arrow). C(x40) MTX+TPM Treatment Group: An increased amount of typical α cells (tailed arrow) showing insulin positivity in the islets of Langerhans

Table 4. Semi-quantitative analysis (median (values between 25%-75% quartiles)

Groups	Anti-Insulin Positivity Score	Anti-Glucagon Positivity Score
Control	3(2-3)	2(2-2.5)
MTX	0.5(0-1)*	0.5(0-1)*
MTX+TPM	2(2-2)**	1(1-2)**

* p<0.001; Between the control group and the MTX group,
**p<0.001; Between the MTX group and the MTX+TPM group,
Kruskal Wallis/Mann Whitney U test

and edematous areas in the TPM treatment group compared to the MTX treatment group (Figure 2b-c).

Immunohistochemical Analysis

On examination of the sections of pancreatic tissue incubated with insulin primary antibody under a light microscope, we determined fewer β-cells showing insulin positivity in the MTX group compared to the control group (Figure 2a-b, Table 4, p<0.001). On the other hand, we observed a significant increase in β-cells showing insulin positivity in sections of pancreatic tissue belonging to the TPM treatment group compared to the MTX group (Figure 2b-c, Tablo 4, p<0.001).

On examination of the sections of pancreatic tissue incubated with glucagon primary antibody under a light microscope; we determined significantly fewer cells showing glucagon positivity in the MTX group compared to the control group (Figure 3a-b, Table 4, p<0.05). In contrast, in the TPM treatment group, we observed an increase in α-cells showing glucagon positivity compared to the MTX group (Figure 3b-c, Table 4, p<0.05).

Semi-quantitative Analysis

The HDS score calculated concerning edematous areas, necrotic cells, and cells with pyknotic nuclei were determined as 0 (0-1) in the control group and as 6(6-7) in the MTX group (Figure 1a-b, Table 3; p<0.001). However, the HDS score calculated as 6 (6-7) for the MTX group was found as 2(1-3) in the TPM treatment group (Figure 1b-c, Table 3, p<0.001).

DISCUSSION

In this study, the effects of MTX on pancreatic tissue and the effects of TPM on rats treated with MTX were evaluated histopathologically and immunohistochemically. Methotrexate treatment-induced damage in pancreatic islets of Langerhans cells. According to the results of our literature review, there are very few studies on MTX-induced pancreatic toxicity and these have shown high-dose MTX to result in atypical islets of Langerhans, mild edema, necrotic cells in the islets of Langerhans and acinar cells, and inflammatory infiltration (20). Similarly, we observed extensive edematous areas and necrotic cells in the islets of Langerhans after MTX treatment in the present study. Again, the immunohistochemical analysis performed in the present study determined a decrease in the number of cells showing insulin and glucagon positivity, in line with the literature. There are studies in the literature that report MTX-induced liver, kidney, and lung toxicity and show MTX-induced apoptosis and inflammation in liver and kidney tissues (16, 21). In a case report on MTX-induced pulmonary toxicity, reactive epithelial hyperplasia, as well as focal histiocyte clusters in the alveoli and chronic lymphocytic interstitial inflammation, were found on lung biopsy (15, 23). MTX causes impairment of nucleic acid synthesis, and thus, cell apoptosis by inhibiting thymidylate synthesis and the folic acid cycle (14). This effect is more pronounced in malignant cells. The mechanism by which MTX induces pancreatic injury is likely linked to apoptosis. Our study is one of the very rare studies that have demonstrated pancreatic injury induced by MTX.

Consistent with the literature, our study observed a protective effect of TPM on the islets of Langerhans cells. The recognition that TPM causes decreased appetite, weight loss, and, in diabetic patients, reduced blood glucose in contrast with other antiepileptics has stimulated a great amount of research on its anti-obesity and antidiabetic aspects in addition to its antiepileptic effects. Many studies have been conducted to determine whether the effect of TPM that decreases blood sugar is through its effects that increase insulin sensitivity due to weight loss or through its effects that replace beta cells (5, 6, 8). The few studies showing that TPM reduces blood glucose due to elevated insulin sensitivity in peripheral tissues as a result of weight loss and higher energy consumption were not corroborated by further studies and it was understood that the blood glucose reduction effect was independent of weight

loss and energy consumption (27). Furthermore, the absence of this blood glucose reduction effect in healthy individuals despite its presence in diabetics guided research towards the idea that TPM improves impaired beta cell function (8, 9).

Pancreatic islet cells are quite sensitive to lipotoxicity (10). The exposure of beta cells to lipotoxicity initiates the metabolic processes that lead to lipid accumulation, mitochondrial dysfunction, and ultimately, reduced insulin secretion (10). In addition to being proven by the partial loss of mitochondrial resting membrane potential and reduced hyperpolarization in response to glucose, the lipotoxic effects have also been linked to impaired mitochondrial function (8). TPM increases the beta-oxidation rate by elevating the expression of PPAR alpha and CPT-1, which is a mitochondrial fatty acid carrier (8). The most important mechanism is presumed to be the direct antiapoptotic effect TPM exerts on b-cells by offering protection against lipotoxicity (10).

Certain studies involving animal models of obesity and diabetes have shown that TPM improves insulin sensitivity independently of weight loss (6). The associated mechanisms are uncertain; however, studies have speculated that adipose tissue might be a target. TPM was shown to improve the effects of insulin and the transportation of glucose in fat cells obtained from obese and insulin-resistant rodents, as well as to elevate adiponectin secretion (5, 9). Despite the favorable effects of TPM on weight and glycemia, human studies on type II DM did not yield any evidence suggesting that it alters insulin sensitivity (5, 9).

In addition to hormones, pancreatic islet cells also secrete neurotransmitters. It is known that pancreatic islet cells secrete glutamate decarboxylase and GAMA transaminase (28). GABA, which is released from synaptic vesicles on beta cells, binds to the ionotropic GABA A receptors found on a-cells and leads to the inhibition of glucagon secretion, while it also stimulates insulin secretion by binding to the GABA B receptors found on beta cells (28, 29). Studies are showing that TPM inhibits glucagon secretion through the reinforcement of GABA activity by increasing GABA activity in some GABA A receptor subtypes (1).

Islet cells express ionotropic (iGluRs) and metabotropic (mGluRs) glutamate receptors (29). From ionotropic glutamate receptors, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) modulates the pancreatic secretion of insulin and glucagon ((2). The activation of ionotropic glutamate receptors (iGluRs) leads to an increase in the concentration of cytosolic Ca^{+2} ($[Ca^{+2}]i$). The constant increase in ($[Ca^{+2}]i$) due to the overstimulation of these receptors may result in impaired cell function, and thus, cell apoptosis (30). Therefore, TPM blocks kainate and AMPA receptors from glutamate receptor subtypes (13).

Carbonic anhydrase (CA) VA and CA VB are known to be involved in certain metabolic processes including urogenesis, glucogenesis, and lipogenesis (31). TPM was shown to inhibit many CA isoforms including mitochondrial CA VA and CA VB (1). All of these results support that TPM inhibits mitochondrial CAs, and therefore, de novo lipogenesis (31).

To our knowledge, there are no studies in the literature that have suggested any negative effects on the pancreas by TPM. However, studies that have shown protective and anti-apoptotic effects on neuronal cells led us to think that TPM could exert a protective effect on the pancreas as well. In their experimental study, Niebauer and Gruenthal showed reduced neuronal degeneration in the hippocampal area of the brain after status epilepticus (32). Similarly, Kurul et al. (33) showed by inducing experimental hyperoxic brain injury that TPM yielded a significant decrease in cell death in the hippocampal region. The study by Park et al. (30) has also shown that TPM produces a decrease in cell death-related seizures. However, it has not been elucidated whether this protective effect is caused by the metabolic effects or the trophic effects of TPM or by a combination of these two effects, as well as which effect is more dominant. Apart from its direct trophic effects on islet cells, topiramate may indirectly improve beta cell function through metabolic effects.

In this study, we aimed to investigate whether TPM exerts a protective effect on the islets of Langerhans cells, by inducing pancreatic toxicity through the treatment of rats with MTX. Certain limitations of this study should be considered. Firstly, this is a pilot study conducted by inducing pancreatic injury using MTX. Therefore, there are not enough studies in the literature to perform a comparison. We did not determine the levels of insulin and glucose in this study. The molecular mechanisms of the effects of MTX and TPM on the pancreas need to be supported by future studies.

CONCLUSION

In summary, this study shows that MTX induces cellular damage in pancreatic tissues and that TPM

has a protective effect on pancreatic islet cells. More experimental and clinical studies are needed to investigate the mechanisms underlying the protective effects of various doses of TPM in pancreatic beta cell damage in both type 1 and type 2 diabetes. When TPM therapy is planned for diabetic epilepsy patients on insulin or insulin secretagogues, hypoglycemia should be given consideration, and perhaps, the option of reducing the doses of insulin should be preferred.

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Olgu Sunumu / Case Report

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NEÜ YAYINEVİ

Intestinal Intussusception Seen in Adult Patients: Case Report

Erişkinde Görülen İleoileal İnvajinasyon: Olgu Sunumu

Sumeyra Emine Boluk[1], Salih Boluk[2]

Öz

Bağırsak intusepsiyonları genellikle çocukluk çağında görülen mekanik bağırsak obstrüksiyonu sebeplerinden birisidir.İnvajinasyonların yaklaşık %1-5 i erişkinlerde görülmektedir.Çocukluk çağında görülen vakaların %90 ı idiopatik iken,erişkin intusepsiyonlarında % 90 altta yatan organik bir lezyon bulunmaktadır. Etyolojiye bağlı olarak da tedavi değişmektedir .Sunacağımız 2 olgu aracılığı ile erişkinde görülen intusepsiyon vakaları hakkında bilgi vermeyi amaçladık.

Anahtar Kelimeler: İntusepsiyon, ince bağırsak, polip

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Abstract

Intestinal intussusceptions are one of the causes of mechanical intestinal obstruction usually seen in childhood. Approximately 1-5% of invaginations seen in adults. While 90% of childhood cases are idiopathic, 90% of adult intussusceptions have an underlying organic lesion. We aimed to give information about intussusception cases in adults with 2 cases we present.

Keywords: Intussusception, ileum, polyp

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INTRODUCTION

Intussusception is the invagination of the proximal bowel segment into the adjacent distal segment. It was first described by Barbette in 1674 (1). Known as a childhood disease. However, 1-5% of all intussusceptions are seen in the adult population. The rate of incidence in men and women is similar in adults (2). Ileocolic intussusceptions are mostly seen in childhood. Most of them can be treated with non-surgical reduction procedure. Adult intussusceptions are usually seen in the small bowel and 70-90% has an underlying cause. Therefore, the probability of clinical recovery without surgical intervention is low (3). Causes of adult intussusceptions include benign and malignant tumors, adhesions, inflammatory causes, Meckel's diverticulum, and congenital malformations. Definitive diagnosis is made by pathological examination of the postoperative piece. Nevertheless, radiological scans done in the preoperative period are helpful in making the diagnosis. In our case report, we aimed to discuss the diagnosis and treatment of adult intussusceptions with 2 patients.

CASE

Our first case, a 50-year-old male patient, presented to the emergency department due to increased abdominal pain lasting for 3 days. There were complaints of nausea and vomiting with abdominal pain. There were no features in his CV and family history. On physical examination, there was tenderness in the paraumbilical region. Leukocytosis and high C-reactive protein (CRP) was detected in laboratory values. No pathology was seen in the plain abdominal X-ray. In abdominal ultrasonography (USG), an appearance compatible with gato intestinal loops was observed in the paraumbilical region. Abdominal computed tomography (CT) scan was done for the patient due to suspicion of intussusception. İn CT scan an area that may be intussusception in the ileal loops was observed (Figüre 1). Urgent operation was planned. In the exploration, 20 cm ileal loop invagination was seen at approximately 110 cm proksimal from Treitz ligament .Because of intestinal iscemic and micro perforation foci resection decision was made. Resection and end -to -end anastomosis was done (Figure 2) . The patient was discharged on the 5th postoperative day with surgical recovery. The pathological evaluation showed that intussusception came up due to a juvenile polyp in the ileal loop.

The second case is a 24-year-old female patient who applied to the emergency department with abdominal pain, nausea and vomiting. In addition, she had constipation for 2 days. Her history was unremarkable except for mental retardation. On physical examination, there was distention and tenderness in the abdomen. Leukocytosis and CRP elevation were present. CT scan was done due to air-fluid levels in plain abdominal x-ray. Computed tomography showed an appearance compatible with intussusception at the level of the distal ileal loops and dilatation in the proximal intestinal loops. Urgent exploration decision was made. In the exploration,

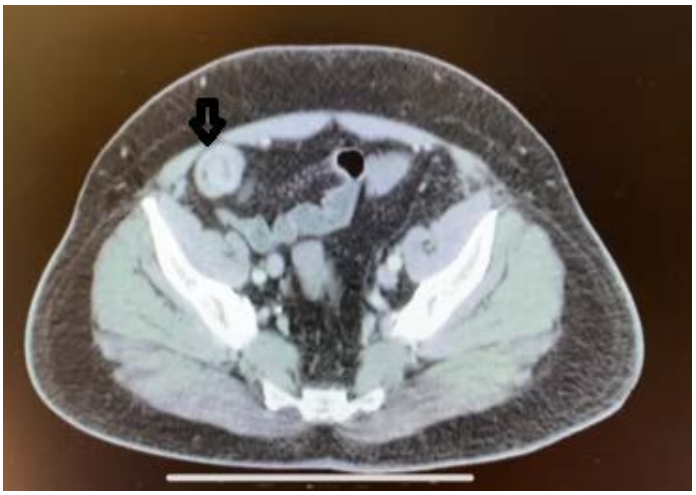

Figure 1. Image of intussusception due to juvenile polyp on tomography

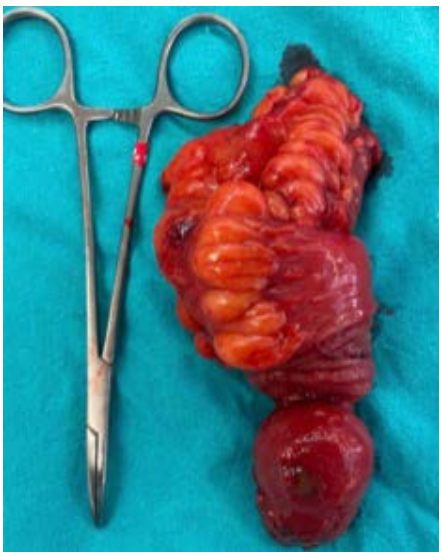

Figure 2. Ileoileal intussusception resection material

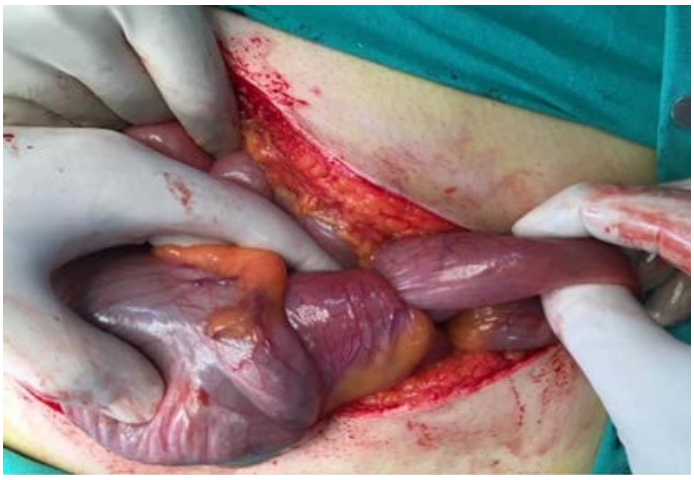

Figure 3. Ileal intussusception seen in exploration

there was invaginationin the ileal loops approximately 80 cm proximal to the ileocecal valve (Figure 3). When the invaginated part of the ileum was opened a 5 cm ileal ans with an inverted lesion? polyp? was seen (Figure 4). Because of the possibility of an inverted malign lesion and the iscemic areas in the ileum resection and anastomosis decision was made.No complications occured peroperatively. The patient was discharged with surgical recovery in the postoperative period. Pathological evaluation of the specimen revealed an inverted Meckel's diverticulum in the ileum.

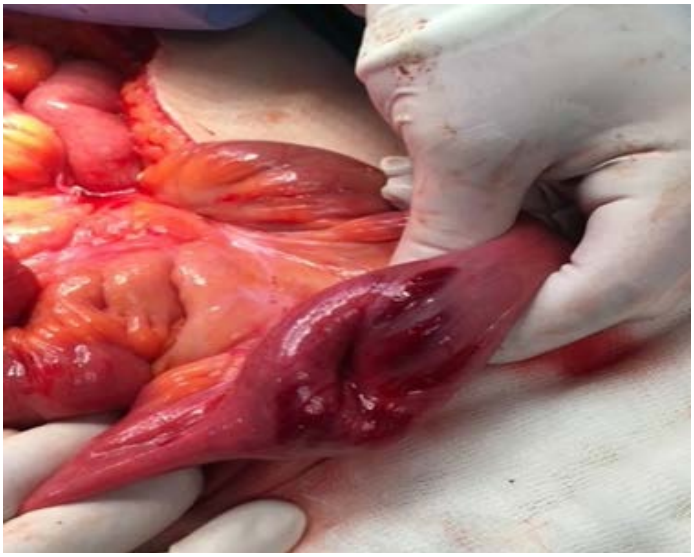

Figure 4. Suspected area of polyp or mass on ileum

DISCUSSİON

Intestinal intussusceptions are frequently seen in childhood. It was first described by Barbette in 1674 as the invagination of the proximal part of the intestine into the distal part of the adjacent intestine (1). In 1789, John Hunter defined 3 more such patients and used the term "intussusception" (4). Sir Jonathan Hutchinson, on the other hand, first described the process of reduction of intussusception in 1871 (5). Adult intussusceptions are a rare condition. The underlying mechanism is not clear. However, it is thought that the presence of a mass or inflammation that may cause irritation in the lumen or its wall may cause intussusception by affecting the peristaltic activity (6). While there is no underlying cause in 90% of childhood intussusceptions, approximately 90% of adult intussusceptions develop due to a lesion. For this reason, the reduction procedure done in childhood intussusceptions gives more results. Causes of adult intussusceptions include benign and malignant lesions, postoperative adhesions, vascular malformations, anatomical variations, intestinal ulcers, and idiopathic conditions. About two-thirds of cases are caused by benign and malignant lesions. In a literature review covering 1214 patients, it was observed that 63% of adult intussusceptions were due to tumoral lesions and 50% of this was malignant lesions. Intussusception is more common at the small intestine level in adults. It has been determined that 48% of colonic intussusceptions are due to malignant masses, and 17% of small intestinal intussusceptions are malignant. In addition, while most of the lesions detected in the colon are primary malignancies, most of the small intestinal malignant masses are metastatic masses (7).

Abdominal pain is the most common symptom of intussusception in adults. In addition, loss of appetite, nausea, vomiting, gastrointestinal bleeding may also occur. Diarrhea may also occur if necrosis due to intussusception is present. On physical examination, decreased bowel sounds, abdominal distension according to the level of intussusception, and if perforation has developed, diffuse abdominal tenderness, defense, rebound may be seen (8). One of our patients had distension due to distal obstruction, and the other one had tenderness due to perforation.

Diagnosis of bowel intussusception is easier in children than adults. Target sign seen in abdominal USG in children is diagnostic with symptoms. In addition, in the treatment, there are rates of up to 80% of hydrostatic reduction done with USG in appropriate

cases (9). In adults, findings related to intestinal obstruction can be detected by direct radiography and ultrasonography. However, contrast-enhanced abdominal CT will give clearer information about the etiology (10). Both of our patients had CT scan to search the ethiology.

Adult intussusceptions can be classified into 4 groups according to the region of origin: Enteric, ileocolic, ileocecal and colonic. Enteric ones are only in the small intestine, colonic ones are only in the colon. It is difficult to distinguish between ileocolic and ileocecal intussusceptions. In the ileocolic type, the small intestine passes through the ileocecal valve and invaginates to the colon. In ileocecal intussusception, the ileocecal valve can not be passed (11).Enteric intussusception was present in the 2 patients we presented.

Treatment varies according to the patient and clinical characteristics. Hydrostatic reduction is often beneficial, as the cause is idiopathic in 90% of children with symptoms not exceeding 3 days. However, surgery should be considered in patients with unsuccessful reduction and acute abdomen findings in the first evaluation. Reduction is not the first choice, as there is an underlying mass cause in approximately 90% of intussusception in the adult population. It is stated that peroperative reduction in adults may cause iatrogenic injury, and if there is an underlying malignancy, implantation into the abdomen is therefore not recommended. However, it is stated in the literature that if there is no perforation and necrosis, large bowel resection can be prevented by the reduction procedure and time can be gained for malignancy surgery (3,12,13,14).

Benign lesions that cause intestinal intussusception include lipomas, leiomyomas, hemangiomas, Meckel's diverticulum, and polyps. One of the patients presented in our case was due to Meckel's diverticulum and the other was small bowel intussusception due to inflammatory polyp.

Meckel's diverticulum is the most common congenital malformation of the gastrointestinal tract. It occurs as a result of failure of the omphalomesenteric duct to close. It is found in 1-3%. It is a true diverticulum containing all layers of the intestinal wall. The incidence in men and women is equal. The clinical presentation of Meckel's diverticulum varies. Clinical symptoms and presentation can be chronic abdominal pain, bleeding, intestinal obstruction, perforation and diverticulitis (15,16). In our patient , small bowel intussusception due to Meckel's diverticulum and therefore mechanical intestinal obstruction developed. It is debatable whether resection should be performed in case of every bowel intussusceptions. However, if the cause is Meckel's diverticulum, it can be said that resection will be the definitive treatment method (17).

Intussusceptions develop from juvenile polyps are rare. The most common type of polyp in the pediatric age group is isolated juvenile polyp. Juvenile polyps are generally observed in the large intestine and most frequently in the 2-5 age group (18). Adult intussusceptions due to juvenile polyps are less common than in the pediatric age group. In one of the patients we presented, intussusception due to a juvenile polyp was present. The patient underwent segmental small bowel resection and anastomosis.

In conclusion, intestinal intussusceptions are rare disorders in the adult population. It is not easy to diagnose because it can progress with more chronic symptoms compared to the pediatric age group. Computed tomography for diagnosis is the imaging method that can be most helpful in terms of etiology and differential diagnosis. About 90% of them have a pathology. For this reason, hydrostatic reduction, which is frequently used in children, is not the first treatment option in adults. Surgery comes to the fore in treatment. It should be kept in mind that intussusception may be the etiology of acute and chronic abdominal pain in adults.

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