e-ISSN: 2149-8059
ISSN: 1017-6616

SELÇUK TIP DERGİSİ
SELCUK MEDICAL JOURNAL

Selçuk Tıp Dergisi, Türkiye Atıf Dizini, Index Copernicus, International Citation Index, Türk Atıf Dizini (ULAKBİM) ve EBSCO tarafından indekslenmektedir.

Cilt: 39 Sayı: 1 MART 2023

Araştırma Makalesi / Research Article

Olgu Sunumu / Case Report

SELCUK TIP DERGİSİ

Cilt: 39 Sayı: 1 ARALIK 2023

e-ISSN: 2149-8059

ISSN: 1017-6616

Sahibi (Owner)

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Araştırma Makalesi / Research Article

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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.

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

Book references:

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

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 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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Selcuk Med J 2023;39(1): 1-6
DOI: 10.30733/std.2023.01608

NEÜ YAYINEVİ

Boric Acid Shows ER Stress and Apoptosis Mediated Anticancer Activity in Human Pancreatic Cancer MIA PaCa-2 and PANC-1 Cells

Borik Asit İnsan Pankreas Kanseri MIA PaCa-2 ve PANC-1 Hücrelerinde ER Stresi ve Apoptoz Aracılı Antikanser Aktivite Gösterir

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Geliş Tarihi/Received: *3 October 2022*
Kabul Tarihi/Accepted: *13 December 2022*

Öz

Amaç: Bu çalışmada borik asitin insan pankreas kanseri MIA PaCa-2 ve PANC-1 hücrelerinde endoplazmik retikulum (ER) stresi ve apoptoz aracılı antikanser etkisinin araştırılması amaçlanmıştır.
Gereçler ve Yöntem: Borik asitin pankreas kanseri hücrelerinin canlılığı üzerine etkisi ve IC_{50} değeri XTT testi ve CompuSyn version 1.0 yazılımı kullanılarak hesaplanmıştır. Apoptotik, anti-apoptotik ve ER stresi ile ilişkili genlerin ifadesi belirlenmiştir. Borik asitin bu hücrelerin koloni oluşum kapasitesi üzerine etkisi ise koloni oluşum testi ile değerlendirilmiştir.
Bulgular: Borik asit zaman ve doz bağımlı olarak her iki hücre hattında da hücre canlılığını baskılamıştır. XTT testi sonucunda MIA PaCa-2 ve PANC-1 hücrelerinde borik asitin IC_{50} dozlarının sırasıyla 15707,5 ve 14248,8 µM olduğu bulunmuştur. Borik asitin her iki hücre hattında da apoptoz ile ilişkili genlerden *BAX, CASP3, CASP8, CYCS* ve *FAS* genlerinin ifadelerini anlamlı derecede arttırdığı gözlenmiştir. PANC-1 hücrelerinde *CASP9* ve *FADD* genlerinin ifadeleride anlamlı derecede yükselmiştir. Borik asitin her iki hücre hattında da ER stresi ile ilişkili *ATF4, HSP47* ve *XBP1* genlerinin ifadesini istatistiksel olarak anlamlı derecede arttırdığı görülmüştür. Ayrıca borik asit muamelesi sadece PANC-1 hücrelerinde *ATF6, CHOP* ve *EIF2A* ifadelerini anlamlı derecede arttırmıştır. MIA PaCa-2 hücrelerinde ise borik asit *GRP78* gen ifadesinin istatistiksel olarak artmasına neden olmuştur. Koloni oluşum testi sonuçları borik asitin her iki hücre hattında da koloni oluşum kapasitelerinin anlamlı derecede baskılandığını göstermiştir.
Sonuç: Borik asit her iki insan pankreas kanseri hücrelerinde hücre canlılığı ve koloni oluşumunu azaltmış olup apoptoz ve ER stresi ile ilişkili genlerin ifadesini değiştirmiştir. Bu bulgular, borik asitin insan pankreas kanseri hücrelerinde ER stresi ve apoptoz aracılı antikanser etkisini göstermektedir.

Anahtar Kelimeler: Pankreas kanseri, endoplazmik retikulum stresi, borik asit, apoptoz

Abstract

Aim: Objective of this study was to investigate the endoplasmic reticulum (ER) stress and apoptosis mediated anticancer effect of boric acid in human pancreatic cancer MIA PaCa-2 and PANC-1 cells.
Materials and Methods: The effect of boric acid on the viability of pancreatic cancer cells and the IC_{50} value were calculated by XTT test and using CompuSyn version 1.0 software. Apoptotic, anti-apoptotic and ER stress-related gene levels were determined. The effect of boric acid on the colony formation capacity of these cells was evaluated with the colony formation assay.
Results: Boric acid inhibited cell viability in these cell lines as time and dose dependent. As a result of the XTT test, the IC_{50} doses of boric acid in MIA PaCa-2 and PANC-1 cells were found to be 15707.5 and 14248.8 µM, respectively. Boric acid significantly upregulated *BAX, CASP3, CASP8, CYCS* and *FAS* expression, which are the genes associated with apoptosis in both cell lines. *CASP9* and *FADD* gene levels were significantly elevated only in PANC-1 cells. It was observed that boric acid statistically upregulated the expression of *ATF4, HSP47* and *XBP1* genes associated with ER stress in both cell lines. In addition, boric acid treatment significantly increased *ATF6, CHOP* and *EIF2A* expressions only in PANC-1 cells. Boric acid also caused an increase in *GRP78* gene expression in MIA PaCa-2 cells. Colony formation test results illustrated that boric acid significantly suppressed colony formation capacities in both cell lines.
Conclusion: Boric acid reduced cell viability and colony formation in both human pancreatic cancer cells and changed gene levels in apoptosis and ER stress pathways. Findings suggested that boric acid exhibits anticancer activity in human pancreatic cancer cells via ER stress and apoptosis.

Key words: Pancreatic cancer, endoplasmic reticulum stress, boric acid, apoptosis

Cite this article as: Eroglu Gunes C. Boric Acid Shows ER Stress and Apoptosis Mediated Anticancer Activity in Human Pancreatic Cancer MIA PaCa-2 and PANC-1 Cells. Selcuk Med J 2023;39(1): 1-6

Disclosure: *Author has not a financial interest in any of the products, devices, or drugs mentioned in this article. The research was not sponsored by an outside organization. Author has agreed to allow full access to the primary data and to allow the journal to review the data if requested.*

INTRODUCTION

Pancreatic cancer is the seventh in cancer-related deaths in both genders. Because of poor prognosis in pancreatic cancer, cases and deaths rates are very close to each other. It is estimated that pancreatic cancer can be the third cause of cancer-related deaths by 2025 in a study conducted in 28 European countries (1). Total five-year survival rate of pancreatic cancer is 11% but it is 42% in patients diagnosed with local disease. Because pancreatic cancer is usually diagnosed after the tumor has spread, only less than 20% of patients are eligible for surgery (2). Studies continue for the research of new agents for pancreatic cancer treatment and the development of new treatment strategies.

The endoplasmic reticulum (ER) has critical properties in cellular processes. ER homeostasis can be disturbed due to stress factors such as hypoxia, oxidative insult, hypoglycemia, calcium and ATP depletion. These stress factors affect the correct folding of proteins and can eventually lead to misfolding or accretion of unfolded proteins, resulting in ER stress (3). In the early phase of ER stress, the cell initiates the UPR (unfolded protein response) in response to survive and restore ER homeostasis. PERK (protein kinase RNA-like ER kinase), IRE1 (inositol requiring enzyme1) and ATF6 (activating transcription factor-6) ER transmembrane sensors detect stress and initiate three different UPRs (4). If the time for resolve the unfolded protein event is prolonged, the UPR can stimulate apoptosis pathways via ATF6, PERK and IRE1 signaling pathways (5). Previous literature suggested that ER stress has critical role in antiproliferative properties of many natural compounds having anticancer activity (6, 7). Therefore, inducing ER stress in cancer cells seems to be a potential therapeutic strategy.

Boron is a natural element (8) and Türkiye has approximately 73% of the world's boron reserves and ranks first in the total boron reserves (9). Boron is the ninth most abundant (414 μM) element in seawater (10). Natural boron compounds are used in pharmaceutical formulations due to their antiviral, antibacterial and anticancer effects (11). The anticancer effects of some of the boron derivatives have been demonstrated (12). Mahabir et al. (13) reported that boron intake decreased the incidence of lung cancer in women. Boron can be completely absorbed in the body and passes into all tissues as boric acid (14). Boric acid is a natural component of drinking water and dietary plant products (10). Various studies have shown that boric acid has antibacterial (15), antioxidative (16), anti-inflammatory (17), anticarcinogenic (18), antimutagenic (19), antiinvasive and antiangiogenic (20) properties. Barranco et al. (21) showed that increased boron concentrations in groundwater reduced incidence and mortality of prostate cancer in state of Texas. Also, boric acid (100 and 500 μM for 8 day) showed anti-proliferative effect and increased sensitivity to ionizing radiation in DU-145 human prostate cancer cells (21). This situation is an important reason for elucidating the effect on pancreatic cancer of boric acid at the molecular level.

The effects of boric acid on pancreatic cancer cells were investigated in the context of ER stress, apoptosis and cell proliferation status in this study. For this purpose, XTT, qRT-PCR and colony formation methods were used. Expression levels of genes encoding key proteins in ER stress (*ATF4, ATF6, CALR, CHOP, EIF2A, GRP78, HSP47, IRE1, PERK* and *XBP1*) and apoptosis (*P53, BCL2, BAX, CASP3, CASP7, CASP8, CASP9, CYCS, FAS* and *FADD*) pathways were evaluated using qRT-PCR analysis.

MATERIALS AND METHODS

Chemicals, cell lines and culture conditions

All experimental procedures were approved by the Ethics Committee of N.E.U. Non-drug and Non-Medical Device Research, (2022/3950). Boric acid was commercially obtained from Etimaden and dissolved in DMEM. XTT kit and PBS was purchased from Biological Industries. The human pancreatic cancer cells MIA PaCa-2 (ATCC®CRM-CRL-1420™) and PANC-1 (ATCC®CRL-1469™) were obtained from ATCC and cultured with DMEM (Gibco), 5% FBS (Capricorn Scientific) and 1% penicillin/streptomycin (Biological Industries). These cells were proliferated at 37°C in an incubator including 5% CO_2 and humidified 95% air. QIAzol (Qiagen), cDNA (Bio-Rad) and EvaGreen Supermix (Solis BioDyne) were purchased.

Cytotoxicity

Cells were treated with 10, 50, 250, 1250, 2500, 5000, 15000 and 25000 μM boric acid for 24, 48 and 72 h. The cytotoxic effect of boric acid in these cells was determined by XTT assay using protocol described elsewhere (22). IC_{50} doses of boric acid in MIA PaCa-2 and PANC-1 cells were calculated using CompuSyn Version 1.0 software.

Apoptosis and ER stress-related genes expressions

Total RNAs were isolated using QIAzol and the

Table 1. The Primer Sequences of Studied Apoptosis, ER Stress and Reference Genes in qRT-PCR Analysis.

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	PCR product size (bp)
ATF4	TTCGACCAGTCGGGTTTG	GGAGAACCCATGAGGTTTGA	93
ATF6	GAAGGGATCACCTGCTGTTAC	GTCCATCACCTGACAGTCAATC	152
CALR	CGGCTACGTGAAGCTGTT	ACGTTCTTGCCCTTGTAGTT	144
CHOP	AACGGAAACAGAGTGGTCAG	GGTCAGGCGCTCGATTT	137
EIF2A	GGTTTCTTGGCAGCCATTT	TGCAACTTTAGGCTCCTCAC	100
GRP78	TGGTATTCTTCGAGTGACAGC	GACCATCCTTTCAATTTCTTCAGG	108
HSP47	AGATGCAGAAGAAGGCTGTT	GTTCTTGTCGATGGCCTCA	113
IRE1	GCGCATCACAAAGTGGAAGTA	ACATACAGAGTGGGCGTCA	75
PERK	CAAAGTAGATGACTGCAATTACGC	TCCAGCCACGCATTGAAATA	141
XBP1	CCAGAACATCTTCCCATGGAT	GGGTCCAACTTGTCCAGAAT	89
BAX	GGAGCTGCAGAGGATGATTG	GGCCTTGAGCACCAGTTT	151
BCL2	GTGGATGACTGAGTACCTGAAC	GAGACAGCCAGGAGAAATCAA	125
CASP3	GAGCCATGGTGAAGAAGGAATA	TCAATGCCACAGTCCAGTTC	162
CASP7	CGAAACGGAACAGACAAAGATG	TTAAGAGGATGCAGGCGAAG	169
CASP8	GCCCAAACTTCACAGCATTAG	GTGGTCCATGAGTTGGTAGATT	160
CASP9	CGACCTGACTGCCAAGAAA	CATCCATCTGTGCCGTAGAC	153
CYCS	GGAGAGGATACACTGATGGAGTA	GTCTGCCCTTTCTTCCTTCTT	102
FADD	TGACCGAGCTCAAGTTCCTATG	CCAGGTCGTTCTGCTCCAG	108
FAS	GTGATGAAGGACATGGCTTAGA	GCCCAAACTTCACAGCATTAG	156
P53	GAGATGTTCCGAGAGCTGAATG	TTTATGGCGGGAGGTAGACT	129
ACTB	AGCACGGCATCGTCACCAACT	TGGCTGGGGTGTTGAAGGTCT	179

cDNA synthesize was performed by using a Bio-Rad iScript™ cDNA synthesis kit. Primers sequences of apoptosis and ER stress-related genes designed by an online program (https://eu.idtdna.com/site) and were shown in Table 1. qRT-PCR was conducted using the protocol in my previous study (23). *ACTB* was used as an internal control and normalization of qPCR data.

Colony formation assay

The cells were seeded in six-well plates at 10^3 cells/well density. Completed DMEM is refreshed every 3 days for 10 days until colonies become visible. Resulting colonies were methanol fixed for 10 minutes. After 5% crystal violet staining, number of colonies were determined using a light microscope.

Data analyses

All experimental procedures were triplicated. qRT-PCR data were analyzed according to $2^{(-\Delta Ct)}$ method. The comparisons of the groups were assessed using the independent samples t-test in SPSS 26.0 statistical analysis program. In all experiments, $p<0.05$ was accepted as statistically significant.

RESULTS

Boric acid has anti-proliferative effects on MIA PaCa-2 and PANC-1 cells

Effects of boric acid on proliferation of cells was determined using XTT assay. Boric acid suppressed viability of these pancreatic cancer cell lines (Fig. 1).

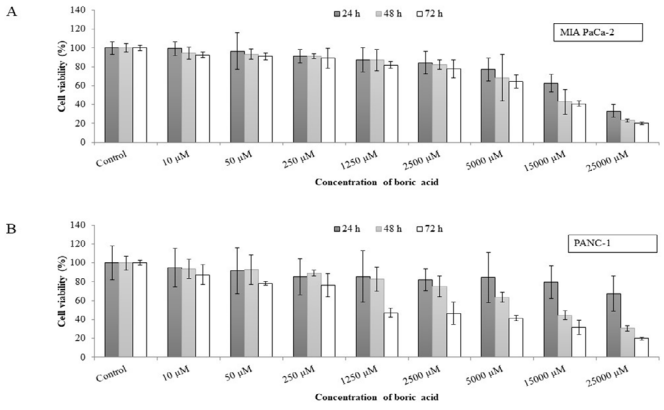

Figure 1. Effects of boric acid on the cell viability in (A) MIA PaCa-2 and (B) PANC-1 cells.

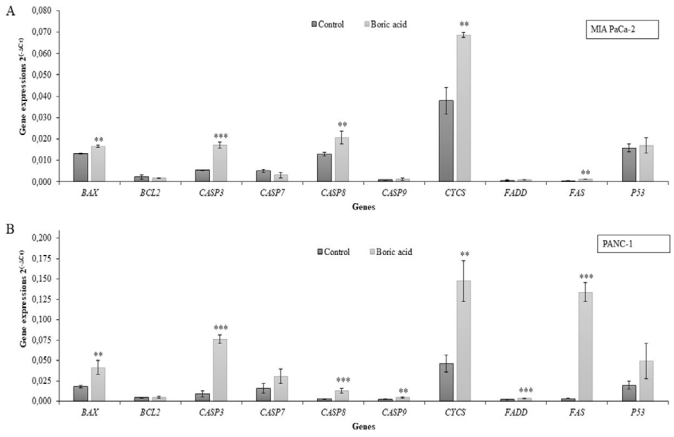

Figure 2. Effects of boric acid on the apoptosis-related genes in (A) MIA PaCa-2 and (B) PANC-1 cells. *P<0.05; **P<0.01; ***P<0.001.

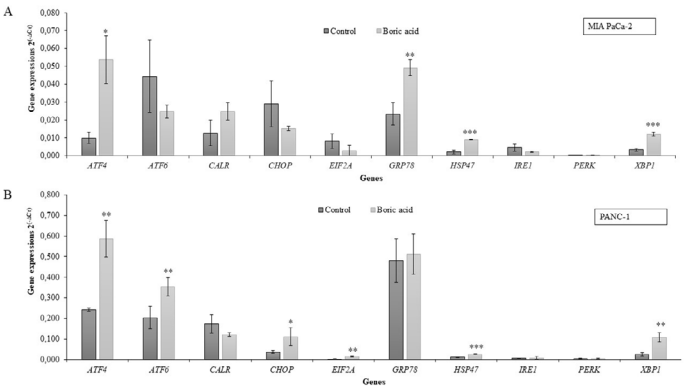

Figure 3. Effects of boric acid on the ER stress-related genes in (A) MIA PaCa-2 and (B) PANC-1 cells. *P<0.05; **P<0.01; ***P<0.001.

The IC_{50} doses of boric acid were 15707.5 µM (MIA PaCa-2) and 14248.8 µM (PANC-1) for 48 h. These doses were used in all subsequent experiments.

Boric acid effects apoptosis-related genes expressions

The effects of boric acid on apoptosis-related genes in pancreatic cancer cells were evaluated using qRT-PCR analysis. It was observed that boric acid caused an upregulated *BAX, CASP3, CASP8, CYCS* and *FAS* gene expressions in MIA PaCa-2 (Fig. 2A, P<0.05). In PANC-1 cell line, boric acid treatment elevated *BAX, CASP3, CASP8, CASP9, CYCS, FADD* and *FAS* gene levels (Fig. 2B, P<0.05).

Boric acid upregulates expression of ER stress-related genes

Expression levels of *ATF4, ATF6, CALR, CHOP, EIF2A, GRP78, HSP47, IRE1, PERK* and *XBP1* that are important in ER stress were evaluated. Boric acid application resulted in *ATF4, HSP47* and *XBP1* upregulation in these cancer cells. Also, a significant upregulation was observed in *ATF6, CHOP* and *EIF2A* gene levels only in PANC-1 cells and in *GRP78* level only in MIA PaCa-2 cells (Fig. 3A, 3B, P<0.05).

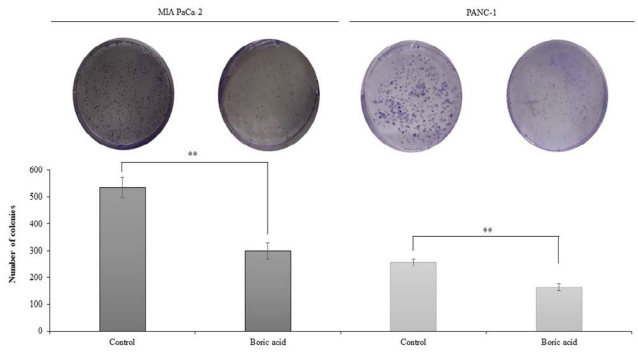

Figure 4. Effects of boric acid on the colony formation in MIA PaCa-2 and PANC-1 cells. **P<0.01.

Boric acid decreases colony formations of MIA PaCa-2 and PANC-1 cells

Effect of boric acid on cells colony formation was determined by using colony assay. The mean number of colonies in MIA PaCa-2 cells were determined as 535±38 in control group and 298±11 in boric acid treated group. On the other hand, mean numbers of colonies in PANC-1 cells were 256±11 in control group and 164±14 in boric acid treated group (Fig. 4, P<0.05).

DISCUSSION

Cancer continues to be a serious health problem for years and the second leading reason of death after heart diseases. Since there are no sufficient treatment options are available, the need for new treatments continues. Scientists are still searching for novel treatments for pancreatic cancer. Scientific interest in boric acid has increased after it was seen that boric acid has anticarcinogenic properties (24, 25). In the present study, the possible anticancer effect of boric acid on human pancreatic cancer cells was investigated by examining cell proliferation, colony formation and expression of genes having critical roles in apoptosis and ER stress pathways.

In a study, researchers reported that boric acid inhibited DU-145 prostate cancer cell proliferation 30% at 100 µM concentration, 60% at 250 µM, and 97% at 1000 µM (26). Hacioglu et al. showed that the IC_{50} and IC_{75} values of boric acid on DU-145 cells were 10.77 and 16.15 mM at 24 hours, respectively. Moreover, boric acid caused cell growth inhibition, apoptosis, decrease in antioxidant levels in dose-dependent manner in these cells (24). In a previous study, IC_{50} value of boric acid was found to be 17 mM in the 48 h in U-87 MG glioblastoma cells (27). It was reported in a study that boric acid decreased the proliferation of DU-145 and LNCaP prostate cancer cells. Eight day 250-1000 µM boric acid treatment inhibited >50% proliferation of DU-145 and LNCaP cells (28). In the present study, the results of the XTT test showed that the IC_{50} doses of boric acid were 15707.5 µM in MIA PaCa-2 cells and 14248.8 µM in PANC-1 cells at 48 h (Fig. 1).

Apoptosis pathway is one of the most important targets in cancer therapy. This pathway must be triggered for the programmed death of cancer cells.

P53, BCL2, BAX, CASP3, CASP7, CASP8, CASP9, CYCS, FAS and *FADD* genes play an important role in this pathway. For this reason, in order to evaluate ER stress-mediated apoptosis, the expressions of *ATF4, ATF6, CALR, CHOP, EIF2A, GRP78, HSP47, IRE1, PERK* and *XBP1* were also investigated. As a result of qRT-PCR analysis, it was observed that *CASP3, CASP8, BAX, FAS* and *CYCS* were upregulated in MIA PaCa-2 cells with boric acid treatment. In addition, boric acid caused increased expression of *CASP3, CASP8, CASP9, BAX, FAS, FADD* and *CYCS* genes in PANC-1 cells (Fig.2). In ER stress-related genes, boric acid increased the levels of *ATF4, HSP47* and *XBP1* genes in both cell lines. Moreover, boric acid caused increased gene expression of *ATF6, CHOP* and *EIF2A* only in PANC-1 cells, and *GRP78* gene expressions only in MIA PaCa-2 cells (Fig. 3).

In a previous study, boric acid upregulated ATF4, ATF6 and eIF2α protein levels in prostate cancer DU-145 cells (29). In a study, it was shown that boric acid induced ER stress in kidneys of rats with cisplatin nephrotoxicity (25). Boric acid has induced ER stress by activating eIF2a, GRP78/BiP, and ATF4 in DU-145 prostate cancer cells in the previous study (30). Studies have shown the effect on ER stress of boric acid in prostate cancer DU-145 cells (29, 31) and in rats with cisplatin nephrotoxicity (25). However, studies investigating boric acid effects on ER stress is still quite limited.

A previous study showed increased arrest in the G2/M phase of the cell cycle in boric acid-treated HepG2 human hepatocellular carcinoma cells. Also, boric acid treatment caused an increase in tumor suppressor P53 but a decrease of anti-apoptotic gene *BCL2* level in HepG2 cells (32). Boric acid increased expression levels of *BAX* and *CASP3* apoptotic genes in DMS-114 small-cell lung cancer cells. Expression levels of *BIRC-2, BIRC-5* and *BCL2* anti-apoptotic genes decreased in boric acid treatment groups (12). It was showed that boric acid inhibited proliferation, migration, invasion and colony formation of ovarian cancer MDAH-2774 cells. It was also reported that boric acid increased expression of *BAX, BID, CASP3* and *CASP9* apoptotic genes, and decreased anti-apoptotic genes *BCL2* and *BCL-XL* (33). In a study conducted on DMS-114 small-cell lung cancer cells, boric acid reduced the colony formation abilities of these cells (12) In the present study, boric acid reduced the colony formation capacity of pancreatic cancer cells (Fig. 4).

In conclusion, boric acid showed anticarcinogenic effect by acting expression of apoptosis and ER stress related genes in human pancreatic cancer cells. Further analyzes and in vivo studies are needed to possible application of boric acid in the treatment of pancreatic cancer.

Acknowledgements

This study was supported by Necmettin Erbakan University, Scientific Research Projects (#221218018). I would like to express my deepest gratitude to Dr. Ercan Kurar for his valuable and constructive suggestions for this study.

Conflict of interest: *Author declares that there is no conflict ofinterest between the authors of the article.*

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

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71(3):209-49.
2. American Cancer Society. Cancer Facts & Figures 2022, Atlanta: American Cancer Society 2022.
3. Maurel M, McGrath EP, Mnich K, et al. Controlling the unfolded protein response-mediated life and death decisions in cancer. Semin Cancer Biol 2015;33:57-66.
4. Wang M, Kaufman RJ. The impact of the endoplasmic reticulum protein-folding environment on cancer development. Nat Rev Cancer 2014;14(9):581-97.
5. Urra H, Dufey E, Lisbona F, et al. When ER stress reaches a dead end. Biochim Biophys Acta 2013;1833(12):3507-17.
6. Tan B, Jia R, Wang G, et al. Astragaloside attenuates the progression of prostate cancer cells through endoplasmic reticulum stress pathways. Oncol Lett 2018;16(3):3901-6.
7. Heo JR, Kim SM, Hwang KA, et al. Resveratrol induced reactive oxygen species and endoplasmic reticulum stress-mediated apoptosis, and cell cycle arrest in the A375SM malignant melanoma cell line. Int J Mol Med 2018;42(3):1427-35.
8. Howe PD. A review of boron effects in the environment. Biol Trace Elem Res 1998;66(1-3):153-66.
9. Eti Maden (Eti Maden İşletmeleri Genel Müdürlüğü). Bor sektör raporu, Strateji Geliştirme Dairesi Başkanlığı, Ankara, 2018.
10. Miwa K, Takano J, Omori H, et al. Plants tolerant of high boron levels. Science 2007;318(5855):1417.
11. Scorei RI, Popa R Jr. Boron-containing compounds as preventive and chemotherapeutic agents for cancer. Anticancer Agents Med Chem 2010;10(4):346-51.
12. Cebeci E, Yüksel B, Şahin F. Anti-cancer effect of boron derivatives on small-cell lung cancer. J Trace Elem Med Biol

2022;70:126923.
13. Mahabir S, Spitz MR, Barrera SL, et al. Dietary boron and hormone replacement therapy as risk factors for lung cancer in women. Am J Epidemiol 2008;167(9):1070-80.
14. Nielsen FH, Meacham SL. Growing evidence for human health benefits of boron. J Evid Based Complementary Altern Med 2011;16(3):169-80.
15. Lang PA, Parkova A, Leissing TM, et al. Bicyclic boronates as potent inhibitors of AmpC, the class β-lactamase from Escherichia coli. Biomolecules 2020;10(6):899.
16. Çelikezen FÇ, Turkez H, Togar B, et al. DNA damaging and biochemical effects of potassium tetraborate. EXCLI J 2014;13:446-50.
17. Acaroz U, Ince S, Arslan-Acaroz D, et al. Bisphenol-A induced oxidative stress, inflammatory gene expression, and metabolic and histopathological changes in male Wistar albino rats: protective role of boron. Toxicol Res (Camb) 2019;8(2):262-9.
18. Maslah H, Skarbek C, Pethe S, et al. Anticancer boron-containing prodrugs responsive to oxidative stress from the tumor microenvironment. Eur J Med Chem 2020;207:112670.
19. Turkez H, Tatar A, Hacimuftuoglu A, et al. Boric acid as a protector against paclitaxel genotoxicity. Acta Biochim Pol 2010;57(1):95-7.
20. Türkez H, Arslan ME, Sönmez E, et al. Toxicogenomic responses of human alveolar epithelial cells to tungsten boride nanoparticles. Chem Biol Interact 2017;273:257-65.
21. Barranco WT, Hudak PF, Eckhert CD. Evaluation of ecological and in vitro effects of boron on prostate cancer risk (United States). Cancer Causes Control 2007;18(1):71-7.
22. Dodurga Y, Seçme M, Eroğlu C, et al. Investigation of the effects of a sülfite molecule on human neuroblastoma cells via a novel oncogene URG4/URGCP. Life Sci 2015;143:27-34.
23. Eroğlu Güneş C, Güçlü E, Vural H, et al. Knockdown of lncRNA ZEB2NAT suppresses epithelial mesenchymal transition, metastasis and proliferation in breast cancer cells. Gene 202;805:145904.
24. Hacioglu C, Kar F, Kacar S, et al. High concentrations of boric acid trigger concentration-dependent oxidative stress, apoptotic pathways and morphological alterations in DU-145 human prostate cancer cell line. Biol Trace Elem Res 2020;193(2):400-9.
25. Hazman Ö, Bozkurt MF, Fidan AF, et al. The effect of boric acid and borax on oxidative stress, inflamation, ER stress and apoptosis in cisplatin toxication and nephrotoxicity developing as a result of toxication. Inflammation 2018;41(3):1032-48.
26. Barranco WT, Kim DH, Stella SL Jr, et al. Boric acid inhibits stored Ca2+ release in DU-145 prostate cancer cells. Cell Biol Toxicol 2009;25(4):309-20.
27. Aydin HE, Gunduz MK, Kizmazoglu C, et al. Cytotoxic effect of boron application on glioblastoma cells. Turk Neurosurg 2021;31(2):206-10.
28. Barranco WT, Eckhert CD. Boric acid inhibits human prostate cancer cell proliferation. Cancer Lett 2004;216(1):21-9.
29. Kobylewski SE, Henderson KA, Yamada KE, et al. Activation of the EIF2α/ATF4 and ATF6 pathways in DU-145 cells by boric acid at the concentration reported in men at the US mean boron intake. Biol Trace Elem Res 2017;176(2):278-93.
30. Henderson KA, Kobylewski SE, Yamada KE, et al. Boric acid induces cytoplasmic stress granule formation. Biometals 2015;28(1):133-41.
31. Yamada KE, Eckhert CD. Boric acid activation of eIF2α and Nrf2 is PERK dependent: a mechanism that explains how boron prevents DNA damage and enhances antioxidant status. Biol Trace Elem Res 2019;188(1):2-10.
32. El-Hefny IM, Al Senosy NK, Hozayen WG, et al. Evaluation of the cytotoxicity and apoptotic induction in human liver cell lines exposed to three food additives. Recent Pat Food Nutr Agric 2020;11(3):193-201.
33. Cabus U, Secme M, Kabukcu C, et al. Boric acid as a promising agent in the treatment of ovarian cancer: Molecular mechanisms. Gene 2021;796-7:145799.

SELÇUK TIP DERGİSİ
SELCUK MEDICAL JOURNAL

Araştırma Makalesi / Research Article

Selcuk Med J 2023;39(1): 7-11
DOI: 10.30733/std.2023.01607

NEÜ YAYINEVİ

The Effect of Rib-Sparing Internal Mammarian Vascular Approach with in Breast Reconstruction on Postoperative Pain Management

Meme Rekonstrüksiyonunda Kosta Koruyuculu İnternal Mammaryan Damar Yaklaşımının Ameliyat Sonrası Dönemde Ağrı Yönetimine Etkisi

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Geliş Tarihi/Received: *20 November 2022*
Kabul Tarihi/Accepted: *11 February 2023*

Öz

Amaç: Otolog meme rekonstrüksiyonunda postoperatif dönemde kosta kıkırdağı müdahalesine bağlı olarak alıcı bölgede ağrı olur. Bu çalışmada meme rekonstrüksiyonunda alıcı saha hazırlanırken kosta koruyucu cerrahi yaklaşımının postoperatif ağrı üzerine etkisini kostal koruyucu olmayan yaklaşımla karşılaştırarak ortaya koymayı amaçladık.
Hastalar ve Yöntem: Çalışmaya 2018-2022 yılları arasında opere edilen 25 hasta dahil edildi. Gruplar internal mammarian arter (IMA) izole etme tekniklerine göre ayrıldı. Grup 1: Kosta koruyucu cerrahi uygulanan hastalar(n=9), Grup 2: Kosta kıkırdak rezeksiyonu uygulanan hastalar(n=16). Postoperatif dönemde her iki grupta da Hasta Kontrollü Analjezi pompası (HKA) kullanım süresi, kullanılan morfin dozu, erken ve geç ağrı skorları kaydedildi.
Bulgular: Hastaların yaş ortalaması 49,8 idi. Postoperatif erken ve geç dönemde herhangi bir komplikasyonla karşılaşılmadı. Tüm flep transferleri başarılı oldu. Ortalama HKA süresi grup 1'de 24±1,41 saat, grup 2'de 26,31±1,62 saat idi. Grup 1'de morfin dozu 9,67±1 mg, grup 2'de 23,93±3,02 mg idi. Erken ağrı skoru grup 1'de 2,89±1,16, grup 2'de ise 5,18±1,22 idi. Geç ağrı skorları grup 1'de 2,11±0,98 ve grup 2'de 2,75±0,77 idi. Grupların morfin dozu ve erken ağrı skorları arasında istatistiksel olarak anlamlı fark vardı (p<0,01). HKA kullanım süresi(p=0,3) ile geç ağrı skorları(p=0,07) arasında anlamlı fark yoktu (p>0,05).
Sonuç: Sonuç olarak, otolog meme rekonstrüksiyonunda alıcı bölge hazırlığında erken dönemde kostal koruyucu cerrahinin ağrıyı önemli ölçüde azalttığını düşünüyoruz.

Anahtar Kelimeler: Meme rekonstrüksiyonu, ağrı, kosta, internal mammarian damarlar

Abstract

Aim: In autologous breast reconstruction, there is pain in the recipient site due to rib cartilage intervention in the postoperative period. In this study, we aimed to reveal the effect of the rib-sparing internal mammarian vessel approach on postoperative pain in breast reconstruction by comparing it with the non-costal-sparing approach.
Patients and Methods: Between 2018 and 2022 twenty five patients underwent surgery were included in the study. Groups were divided according to internal mammary artery(IMA) exposure techniques. Group 1: Patients who underwent rib-sparing surgery(n=9), Group 2: Patients who underwent rib cartilage resection(n=16). Patient Controlled Analgesia(PCA) pump usage time, morphine dose used, and early and late pain scores were noted in both groups in the postoperative period.
Results: The mean age of the patients was 49.8 years. No complications were encountered in the early and late postoperative period. All flap transfers were successful. Mean PCA duration was 24±1.41 hours in group 1 and 26.31±1.62 hours in group 2. The dose of morphine was 9.67±1 mg in group 1 and 23.93±3.02 mg in group 2. The early pain score was 2.89±1.16 in group 1 and 5.18±1.22 in group 2. Late pain scores were 2.11±0.98 in group 1 and 2.75±0.77 in group 2.
There was a statistically significant difference between the morphine dose and early pain scores of the groups (p<0.01). There was no significant difference between the duration of PCA use (p=0.3) and late pain scores (p=0.07) (p>0.05).
Conclusion: In conclusion, we think that costal sparing surgery significantly reduces pain in the early period during recipient site preparation in autologous breast reconstruction.

Key words: Breast reconstruction, pain, rib, internal mammarian vessels

Cite this article as: Ercin BS. The Effect of Rib-Sparing Internal Mammarian Vascular Approach with in Breast Reconstruction on Postoperative Pain Management. Selcuk Med J 2023;39(1): 7-11

Disclosure: *Author has not a financial interest in any of the products, devices, or drugs mentioned in this article. The research was not sponsored by an outside organization. Author has agreed to allow full access to the primary data and to allow the journal to review the data if requested.*

INTRODUCTION

Autologous breast reconstruction is a frequently used reconstruction method in breast cancer treatment. Pain due to the costal approach used in this method is a common complaint in the early and late postoperative periods. Management of postoperative pain is vital, as pain relief can minimize perioperative opioid use, shorten hospital stay, speed up patient recovery, and reduce healthcare costs (1-5). Furthermore, several studies have shown that inadequate acute postoperative pain control is linked to persistent postoperative pain (6). Furthermore, 5% of autologous breast reconstruction patients who had not used opioids before their surgery continued to use opioids three months afterward, increasing the risk of long-term addiction and sequela (7).

From our literature review, we found it noteworthy that, although the positive effect of the rib-sparing internal mammary artery approach on postoperative pain has been emphasized, no statistical comparison has been performed. In this study, we reveal the effect of using a costal sparing approach while preparing the recipient area during breast reconstruction surgery on postoperative pain by comparing the costal sparing approach with a non-costal sparing approach.

PATIENTS AND METHODS

Study Protocol

This is a retrospective study conducted between March 2018 and April 2022. Ethics committee approval was obtained for the study, and informed consent was obtained from each patient. (Protocol number:23-24 Istinye University Ethical Commitee). The inclusion criteria were as follows: breast cancer patients undergoing immediate and unilateral autologous breast reconstruction using the deep inferior epigastric perforator (DIEP) flap technique. The exclusion criteria were as follows: patients undergoing delayed or bilateral autologous breast reconstruction. Twenty-five patients were recruited to the study and divided into two groups based on the internal mammary artery (IMA) isolation technique used in the surgery. Group 1 comprised patients who underwent rib-sparing surgery, while Group 2 comprised patients who underwent costal cartilage resection. During the postoperative period, morphine dose, early and late postoperative pain scores, and patient-controlled analgesia (PCA) pump usage time were recorded for patients in both groups.

Surgical Technique

All surgical procedures were performed by the same surgeon using a ×4.5 magnification loop. In preparing the recipient area, the pectoral muscle fibers on the third rib were first separated using electrocautery, exposing the third costal cartilage. In the non-costal sparing approach, the perichondrium on the rib was opened, and the perichondrium of the posterior aspect of the rib was elevated with a blunt dissection; approximately 4 cm of cartilage segment was resected, and the internal mammary vessels were reached via perichondrium resection. In the costal sparing approach, the internal mammary vessels were accessed via the third intercostal space. The internal mammary arteries were exposed in all participating patients, typically in less than 15 minutes. After the DIEP flap was temporarily placed in a stable position, arterial and vein anastomoses were performed under a microscope using the back-wall-first technique and 9-0 nylon sutures in each anastomosis. The anastomoses were performed first between the internal mammary vein and the flap veins and then between the arteries.

Postoperative Period

All participants in this study were given 20 mg of intravenous morphine and 1 g of paracetamol prior to extubation. Subsequently (i.e., postoperatively), they were allowed to self-regulate the amount of morphine they needed via a PCA system. During the postoperative period, flaps were checked every two hours during the first 24 hours. The early assessment pain score was measured at the 24th hour post operation. In contrast, the pain score for the late postoperative period was measured at the 168th hour post operation to account for the effect of anesthesia on pain in the chest area where the breast reconstruction was performed. The pain scores were evaluated on a scale of 0 to 10. All assessments were performed by the same clinician. The total time each patient used the PCA pump to self-administer analgesia for pain relief (labeled PCA duration) and the morphine dose used was noted.

Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Mac, version 21.0 (IBM Corp., Armonk, NY, USA). The mean, standard deviation, minimum/maximum values, and the first/third quartile (Q1/Q3) values of the two groups were calculated. An independent t-test was used for continuous parameters, while the Mann–Whitney U test was used for ordinal parameters. A p value of less than 0.05 was considered statistically significant.

Table 1. Parameter values in groups

	Group 1(n:9) (Mean±SD) (Max-Min) (Q1-Q3)	**Group 2(n:16) (Mean±SD) (Max-Min) (Q1-Q3)**	**p value**
PCA Time(hr)	24±1,41 (26-22) (23-25)	26,31±1,62 (30-24) (25-27,25)	0.3
Morphine Dose(mg)	9,67±1 (11-8) (9-10)	23,93±3,02 (28-19) (21-26)	<0.01*
Early Pain Score	2,89±1,16 (5-1) (2-3)	5,18±1,22 (7-3) (6-4,75)	
Late Pain Score	2.11±0.98 (4-1) (2-2)	2.75±0.77 (4-2) (2-3)	0.07

-Mean: Mean Value
-SD:Standart Deviation
-Max:Maximum Value
-Min:Minimum Value
Q1:First quarter
Q3:Third quarter
*:Statistically Significant

RESULTS

A total of 25 patients were included in the study. The mean age of the patients was 49.8 years. No complications were encountered in the early and late postoperative periods, and all flap transfers were successful.

The mean PCA duration was 24 ± 1.41 h for Group 1 and 26.31 ± 1.62 h for Group 2. The average morphine dose was 9.67 ± 1 mg for Group 1 and 23.93 ± 3.02 mg for Group 2. The average early postoperative pain score was 2.89 ± 1.16 for Group 1, and 5.18 ± 1.22 for Group 2. The average late postoperative pain score was 2.11 ± 0.98 for Group 1 and 2.75 ± 0.77 for Group 2. All relevant data are presented in Table 1.

There was a statistically significant difference between the morphine dose and the early postoperative pain scores of both groups ($p < 0.01$). However, there was no significant relationship between the PCA duration ($p= 0.3$) and the late postoperative pain scores ($p= 0.07$) ($p > 0.05$). (Figure 1)

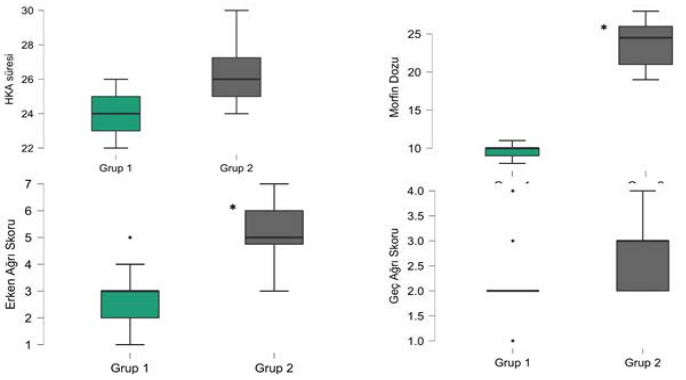

Figure 1. Graphical display of parameters

DISCUSSION

Breast cancer is diagnosed in one out of every eight women, and its incidence is increasing every year. Consequently, the number of patients who are potential candidates for breast reconstruction is increasing at the same rate. Although implants are presently a more commonly used option in breast reconstruction, autologous reconstruction is also performed quite frequently. The most preferred donor site for autologous reconstruction is the abdomen (8). The IMA and internal mammary vein are generally used as recipient vessels for the transfer of flaps taken from the abdomen.

In conventional autologous breast reconstruction, anastomosis is performed with resection of the third costal cartilage for a coastal approach to the internal mammary vessels; however, postoperative pain in the recipient area, chronic pain during the late postoperative period, and contour disorder in chest walls with costal defects are common complications (8).

The importance of postoperative pain control cannot be overestimated as it contributes to faster patient mobilization, shorter hospital stay, lower

costs, and an overall positive patient experience. Opioids are often used to achieve the desired pain control. However, the U.S. government has declared opioid abuse a public health emergency; more than 42,000 people died from opioid abuse in 2016 alone (9). Therefore, approaches to minimizing opioid use for postoperative pain control are crucial. Autologous breast reconstruction is associated with a significant postoperative opioid requirement, and numerous opioid-restricting perioperative strategies have been explored to achieve the desired pain control while reducing opioid consumption. Although pain control is typically aimed at postoperative acute pain, the rate of post-mastectomy pain syndrome has been reported to be up to 56%, and approximately 50% of women continue to experience chronic pain seven to 12 years after surgery (10). The mechanism of post-mastectomy pain syndrome is not yet fully understood, but pain experienced during surgery has been implicated as a possible causative factor (11). Therefore, pain-reducing techniques can help solve problems with both early and late postoperative chronic pain.

In our study, PCA duration, morphine dose, and early and late postoperative pain scores were used as target parameters. In Group 2, the early postoperative pain score and the amount of morphine used during the early postoperative period were significantly higher. It is noteworthy that there was no significant difference in the late postoperative pain scores of Group 1 and Group 2. The absence of a significant relationship between PCA duration and late postoperative pain scores is considered a good indicator because patients can self-adjust the amount they use and are motivated to quit when they no longer need analgesia. However, another theory in the cardiothoracic literature is known as internal breast syndrome due to damage to the intercostal nerves during vessel isolation. Therefore, instead of protecting the rib alone, it may depend on leaving the nerves intact, which is responsible for any reduction in postoperative pain derived from protecting the rib (12). In addition, excessive pain prolongs hospital stay, which increases the cost of treatment. Hence, reducing pain is extremely important for the treatment experience of the patient and economically.

Parret et al. report that using the rib-sparing technique for internal mammary vessel isolation while performing breast reconstruction operations with free flaps reduces postoperative pain (13). We did not find many studies on this approach while conducting the literature review. The biggest disadvantage of the rib-sparing technique is that the area where the anastomoses are to be performed is narrower than with other approaches. This may prolong the anastomosis time and potentially lead to an increase in ischemia, especially for surgeons new to microsurgery. However, no study has reported a statistically significant difference in flap ischemia times (14). Furthermore, for ease of anastomosis, it is desirable that the area to be anastomized is wide, and the vessel stump to be anastomized is long. To suture the posterior wall during anastomosis, it should be possible to rotate the vessel easily using Acland micro clamps. All anastomoses in our study were performed using single micro clamps and a backwall-first technique (an anastomosis technique in which the posterior vessel wall is sutured primarily). Because the posterior wall is sutured primarily, vessel manipulation is minimal, and vessel rotation is not required. Therefore, the narrowness of the field or the shortness of the vascular stump does not constitute a significant impediment. Consistent with previous studies, no difference was observed between the warm ischemia times of the flaps in this study (15).

There are some limitations to our study. One of the weaknesses of this study is that pain is a subjective concept, and objective measurement is difficult, as the response to pain varies from person to person. Consequently, the basis of pain measurement is to believe the pain intensity stated by the patient. In addition, the fact that it is not clear in which part of the patient's use of PCA pump for pain can be considered a limitation in the study. However, during the early postoperative period, especially during the first 4–6 hours, patients may not be able to clearly indicate which part of the pain pump they use for pain relief because they are not fully oriented and cooperative with the effect of anesthesia. Another weakness of the study is the limited number of cases in the study sample. Furthermore, patients who underwent delayed or bilateral autologous reconstruction were not included in the study.

In conclusion, costal sparing surgery significantly reduces pain during the early postoperative period, and we surmise that employing costal sparing surgery during recipient site preparation in autologous breast reconstruction has a positive effect on both patient morbidity and economic considerations. Our study will shed light on future studies.

Conflict of interest: *Author declares that there is no conflict of*

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

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REFERENCES

1. Pusic AL, Matros E, Fine N, et al. Patient-reported outcomes 1 year after immediate breast reconstruction: Results of the mastectomy reconstruction outcomes consortium study. J Clin Onco 2017;35(22):2499.
2. Parikh RP, Myckatyn TM. Paravertebral blocks and enhanced recovery after surgery protocols in breast reconstructive surgery: Patient selection and perspectives. J Pain Res 2018;11:1567.
3. Berg K, Kjellgren K, Unosson M, et al. Postoperative recovery and its association with health-related quality of life among day surgery patients. BMC nursing 2012;11(1):1-10.
4. Wu CL, Richman JM. Postoperative pain and quality of recovery. Current opinion in anesthesiology 2004;17(5):455-60.
5. Coley KC, Williams BA, DaPos SV, et al. Retrospective evaluation of unanticipated admissions and readmissions after same day surgery and associated costs. J Clin Anesth 2002;14(5):349-53.
6. Hickey OT, Nugent NF, Burke SM, et al. Persistent pain after mastectomy with reconstruction. J Clin Anesth 2011;23(6):482-8.
7. Marcusa DP, Mann RA, Cron DC, et al. Prescription opioid use among opioid-naive women undergoing immediate breast reconstruction. Plast Rec Surg 2017;140(6):1081-90.
8. Sacks JM, Chang DW. Rib-sparing internal mammary vessel harvest for microvascular breast reconstruction in 100 consecutive cases. Plast Rec Surg 2009;123(5):1403-7.
9. Dowell D, Noonan RK, Houry D. Underlying factors in drug overdose deaths. Jama 2017;318(23):2295-6.
10. Macdonald L, Bruce J, Scott NW, et al. Long-term follow-up of breast cancer survivors with post-mastectomy pain syndrome. Br J Cancer 2005;92(2):225-30.
11. Coderre TJ, Katz J. Peripheral and central hyperexcitability: Differential signs and symptoms in persistent pain. Behav&Brain Sci 1997;20(3):404-19.
12. Srivastava A, Tripathi DM, Zaman W, et al. Subcostal versus transcostal mini donor nephrectomy: Is rib resection responsible for pain related donor morbidity. The J Uro 2003;170(3):738-40.
13. Parrett BM, Caterson SA, Tobias AM, et al. The ribsparing technique for internal mammary vessel exposure in microsurgical breast reconstruction. Ann Plast Surg 2008;60(3):241-3.
14. Malata CM, Moses M, Mickute Z, et al. Tips for successful microvascular abdominal flap breast reconstruction utilizing the “total rib preservation” technique for internal mammary vessel exposure. Ann Plast Surg 2011;66(1):36-42.
15. DiCandia M, Moses M, Mickute Z. Internal mammary vessel exposure with total rib preservation during free flap breast reconstruction-technique and pitfalls. Br J Surg 2009;96(S5):67.

SELÇUK TIP DERGİSİ
SELCUK MEDICAL JOURNAL

Araştırma Makalesi / Research Article

Selcuk Med J 2023;39(1): 12-18
DOI: 10.30733/std.2023.01587

NEÜ YAYINEVİ

Investigation of the Anticancer Activity of DCZ0415, a Small Molecular Inhibitor of TRIP13, in U87 Human Glioblastoma Multiforme Cells

TRIP13'ün Küçük Bir Moleküler İnhibitörü Olan DCZ0415'in, U87 İnsan Glioblastoma Multiforme Hücrelerindeki Antikanser Etkinliğinin İncelenmesi

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Geliş Tarihi/Received: *12 January 2023*
Kabul Tarihi/Accepted: *25 February 2023*

Öz

Amaç: Tiroid Hormon Reseptörü Etkileşimli Protein 13 (TRIP13); mayotik rekombinasyonda rol oynayan, iğ-toplanma kontrol noktasında görevli bir proteindir. Son yıllarda yapılan çalışmalar TRIP13'ün glioblastoma multiforme (GBM) de dahil olmak üzere çok sayıda kanserde potansiyel bir tümör indükleyicisi olabileceğini ortaya koymuştur. Bu çalışmada TRIP13'ün küçük bir moleküler inhibitörü olan DCZ0415'in U87 insan GBM hücrelerindeki antikanser etkinliğinin araştırılması amaçlandı.
Gereçler ve Yöntem: DCZ0415'in U87 hücrelerindeki olası antikanser etkisi sitotoksisite analizi, koloni formasyon analizi ve apoptoz analizi ile belirlendi. Ayrıca qRT-PZR analizi ile DCZ0415'in apoptoz, invazyon ve Transforme Edici Büyüme Faktörü-Beta (TGF-β) sinyal yolağı ile ilişkili genlerin mRNA seviyeleri üzerine etkisi araştırıldı.
Bulgular: DCZ0415, U87 hücre proliferasyonunu doz ve zaman bağımlı şekilde inhibe etti. U87 hücrelerinde DCZ0415'in 48 saat için IC_{50} dozu 19,77 µM olarak belirlendi. Bu dozda DCZ0415 uygulaması U87 hücrelerinde apoptozu indükledi ve hücrelerin koloni oluşturma yeteneklerini baskıladı. Ayrıca DCZ0415 apoptoz, invazyon ve TGF-β sinyal yolağı ile ilişkili genlerin mRNA seviyelerini antikanser etkiye yol açabilecek şekilde değiştirdi.
Sonuç: Kanserde yeni bir onkogenik faktör olarak değerlendirilen TRIP13'ün bir inhibitörü olan DCZ0415, GBM hücrelerinde antikanser etkiye sahiptir. Bu açıdan, TRIP13'ün GBM için önemli bir terapötik hedef olabileceği ve DCZ0415'in GBM hücrelerinde antikanser etkiye yol açan etkili bir inhibitör olarak değerlendirilebileceği düşünülmektedir.

Anahtar Kelimeler: TRIP13, DCZ0415, Glioblastoma multiforme, TGF-β sinyal yolağı

Abstract

Aim: Thyroid Hormone Receptor Interacting Protein 13 (TRIP13) is a protein involved in spindle-aggregation checkpoint, which plays a role in meiotic recombination. Recent studies have revealed that TRIP13 may be a potential tumor inducer in many cancers, including glioblastoma multiforme (GBM). We aimed to investigate the anticancer activity of DCZ0415, a small molecule inhibitor of TRIP13, in U87 human GBM cells in this study.
Materials and Methods: The possible anticancer effect of DCZ0415 on U87 cells was determined by cytotoxicity, colony formation, and apoptosis assays. In addition, the effects of DCZ0415 on mRNA levels of genes which were involved in apoptosis, invasion and Transforming Growth Factor-Beta (TGF-β) signaling pathway were investigated by qRT-PCR analysis.
Results: DCZ0415 inhibited U87 cell proliferation in a dose and time dependent manner. The IC_{50} dose of DCZ0415 for 48 hours was determined as 19.77 µM in U87 cells. DCZ0415 treatment at this dose induced apoptosis and suppressed colony forming abilities of U87 cells. In addition, DCZ0415 altered mRNA levels of genes associated with apoptosis, invasion and TGF-β signaling pathway, which could lead to anticancer effects.
Conclusion: DCZ0415, an inhibitor of TRIP13 which has been evaluated as a new oncogenic factor in cancer, has an anticancer effect on GBM cells. In this respect, it is thought that TRIP13 may be an important therapeutic target for GBM and DCZ0415 may be considered as an effective inhibitor that causes anticancer effects in GBM cells.

Key words: TRIP13, DCZ0415, Glioblastoma multiforme, TGF-β signaling pathway

Cite this article as: Guclu E, Cinar Ayan I. Investigation of the Anticancer Activity of DCZ0415, a Small Molecular Inhibitor of TRIP13, in U87 Human Glioblastoma Multiforme Cells. Selcuk Med J 2023;39(1): 12-18

Disclosure: *None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this article. The research was not sponsored by an outside organization. All authors have agreed to allow full access to the primary data and to allow the journal to review the data if requested.*

INTRODUCTION

Glioblastoma multiforme (GBM), also called Grade IV glioma, is the most common malignant primary brain tumor (1). Treatment approaches for GBM include surgery, temozolomide-based chemotherapy, radiotherapy, and their combinations. However, GBM is a highly lethal type of cancer with a high recurrence rate. Accordingly, median survival of the disease is 15 months (2, 3). The identification and targeting of new molecules associated with GBM is crucial for the development of new therapeutic approaches.

TRIP13 (Thyroid Hormone Receptor Interaction Protein 13), first identified as a protein that interacts with human papillomavirus protein E1, is found in AAA+ (ATPases associated with various cellular activities) family. TRIP13, a protein consisting of 432 amino acids, is encoded by a gene on chromosome 5p15.33. This protein plays a role in meiotic recombination and is involved in the spindle-assembly checkpoint that maintains genomic stability and prevents aneuploidy (4, 5). Recent studies show that TRIP13 plays an oncogene role in cancer pathogenesis. It has been found that TRIP13 expression is increased in thyroid cancer (4), ovarian cancer (6), and prostate cancer (7) tissues compared to adjacent tissues.

In hepatocellular carcinoma (8), bladder cancer (9), esophageal cancer (10) and cervical cancer (11), increased TRIP13 expression was associated with lower survival and advanced tumor stage. It was stated that TRIP13 mRNA level is high in advanced gliomas and a negative correlation is found between TRIP13 and the overall survival rate. Therefore, TRIP13 is also considered as an oncogenic factor for GBM (12). And, targeting TRIP13 in tumor tissues with high TRIP13 expression may be a therapeutic approach as well as important for identifying TRIP13-related signaling pathways. DCZ0415 is a small molecule inhibitor that has been shown to bind to TRIP13 by nuclear magnetic resonance (NMR) spectroscopy and pull-down, and is used to target TRIP13 and has shown anticancer activity in several cancer cells (13-15).

Transforming growth factor (TGF)-β is another molecule associated with malignancy in gliomas, and the signaling pathway involving TGF-β is associated with processes such as angiogenesis, invasion and stem cell maintenance in gliomas (16). In this respect, TGF-β signaling pathway is also considered an important therapeutic target (17). In this study, we aimed to investigate possible anticancer activity of DCZ0415, an inhibitor of TRIP13, which is also evaluated as an oncogene for GBM, in U87 cells and to evaluate this effect in terms of genes associated with apoptosis, invasion, and TGF-β signaling pathway.

MATERIALS AND METHODS

Cell Culture and DCZ0415 Treatment

Human U87 GBM cell line was obtained from American Type Culture Collection. U87 cells were cultured in DMEM containing 2 mM L-glutamine in the presence of 1 % penicillin/streptomycin and 10% FBS and the cells were grown at 37°C, 5% CO_2 and 95% humidity. DCZ0415 was purchased commercially (Biosynth, SPD47043) and stock solution was prepared with 0.1% DMSO in medium.

Cytotoxicity Assay

U87 cells were seeded in 96-well plates and after 24 hours, cells were incubated with 5, 10, 15, 20, 25, 50, 75, 100, 150 and 200 μM DCZ0415 for 24, 48, and 72 hours. And, XTT assay was performed as described in our study (18). The half-maximal inhibitory concentration (IC_{50}) of DCZ0415 was determined by GraphPad Prism software using % cell inhibition values.

Colony Formation Assay

In order to evaluate the effect of DCZ0415 treatment at IC_{50} dose on the colony forming abilities, U87 cells were seeded in 6-well plates. Then, the cells were cultured by changing the medium every other day for approximately 10 days. Afterwards, cells were treated with cold methanol and stained with crystal violet dye and colonies were counted.

Apoptosis Analysis

The effect of DCZ0415 treatment at IC_{50} dose on apoptosis was determined with FITC Annexin V method (BioLegend, 640.922). For this, cell suspensions were prepared using Annexin V Binding Buffer from control and DCZ0415-treated cells. Cell suspensions (100 μl) were transferred to test tubes. Then, FITC Annexin V and 7-ADD dye were added. After 15 minutes, samples were placed on a flow cytometer and percentages of early apoptosis, late apoptosis, and necrosis were determined.

qRT-PCR Analysis

The changes observed in mRNA level of genes associated with apoptosis, invasion, and TGF-β signaling pathway after DCZ0415 treatment at IC_{50} dose were evaluated by qRT-PCR analysis. For this, firstly, total RNA was isolated (GeneAll, 301-001) and then cDNA was synthesized (Bio-Rad, 170-8891). Preparation of the reaction components and setting up the reaction were performed as previously described

Table 1. Primer sequences of target and reference genes

Gene	Forward primer (5'->3')	Reverse primer (5'->3')
CASP3	CTCTGGAATATCCCTGGACAAC	ACATCTGTACCAGACCGAGA
CASP7	GTCACCATGCGATCCATCAA	CGCCCATACCTGTCACTTTATC
CASP8	GATTCAGAGGAGCAACCCTATT	AGCAGAAAGTCAGCCTCATC
CASP9	CGACCTGACTGCCAAGAAA	GACAGCCGTGAGAGAGAATG
BAX	GTCACTGAAGCGACTGATGT	ACTCCCGCCACAAAGATG
BCL2	GGAGGATTGTGGCCTTCTTT	GTTCAGGTACTCAGTCATCCAC
CYCS	GGAGAGGATACACTGATGGAGTA	GTCTGCCCTTTCTTCCTTCTT
FAS	CTTTTCGTGAGCTCGTCTCTGA	CTCCCCAGAAGCGTCTTTGA
FADD	GACAGCATCGAGGACAGATAC	CTGTTGCGTTCTCCTTCTCT
TNFA	CCAGGGACCTCTCTCTAATCA	TCAGCTTGAGGGTTTGCTAC
TNFR1	CTCCTTCACCGCTTCAGAAA	GTCCACTGTGCAAGAAGAGAT
MMP2	GGCACCCATTTACACCTACA	CCAAGGTCAATGTCAGGAGAG
MMP9	GGGCTTAGATCATTCCTCAGTG	GCCATTCACGTCGTCCTTAT
TIMP1	GTCAACCAGACCACCTTATACC	TATCCGCAGACACTCTCCA
TIMP2	AAGGAAGTGGACTCTGGAAAC	CAGGCCCTTTGAACATCTTTATC
TGFβ1	CGTGGAGCTGTACCAGAAATAC	CTAAGGCGAAAGCCCTCAAT
TGFβR1	GTTCCGTGAGGCAGAGATTTAT	ACCAGAGCTGAGTCCAAGTA
TGFβR2	GTCGCTTTGCTGAGGTCTATAA	CTCTGTCTTCCAAGAGGCATAC
SMAD2	GGGACTGAGTACACCAAATACG	TACCTGGAGACGACCATCAA
SMAD3	CCTGAGTGAAGATGGAGAAACC	GGCTGCAGGTCCAAGTTATTA
SMAD4	TCCAGCATCCACCAAGTAATC	GCAGTGCTGGTAGCATTAGA
SMAD7	AGGCTGTGTTGCTGTGAA	TCCATCGGGTATCTGGAGTAA
ZEB1	GGCTCCTATAGCTCACACATAAG	TGCTGAAAGAGACGGTGAAG
ZEB2	CCCATTCTGGTTCCTACAGTTC	GGGAAGAACCCGTCTTGATATT
ACTB	GGACCTGACTGACTACCTCAT	CGTAGCACAGCTTCTCCTTAAT

(18). Primers designed for target and reference genes are presented in Table 1.

Statistical Analysis

Data from experiments performed in triplicate are presented as mean ± SD. qRT-PCR results were analyzed using $2^{-\Delta\Delta CT}$ method by normalizing with ACTB reference gene. Control and dose groups were compared using t-tests in GraphPad Prism software. And, $P < 0.05$ was considered significant.

RESULTS

DCZ0415 Suppresses Proliferation and Colony Forming Capacity of U87 Cells

The effect of DCZ0415 on proliferation of U87 cells was investigated by XTT method. Accordingly, DCZ0415 suppressed the proliferation of U87 cells (Fig 1). IC_{50} doses of DCZ0415 for 24, 48 and 72 hours in U87 cells were found as 82.56 µM, 19.77 µM, and 13.16 µM, respectively.

Since it would be more convenient to treat U87 cells with DCZ0415 at a low dose and in a short time, in subsequent experiments, U87 cells were treated with 19.77 µM DCZ0415 for 48 hours. According to colony analysis results, DCZ0415 significantly inhibited the colony forming capacity of U87 cells ($P = 0.0011$) Colony numbers in control and DCZ0415-treated cells were found as 125.33±10.05 and 66.33±4.03, respectively (Fig 2).

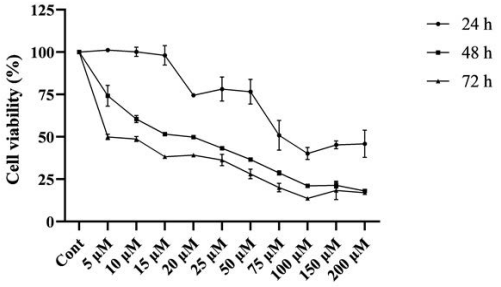

Figure 1. Effect of DCZ0415 treatment at different doses and times on U87 cell proliferation.

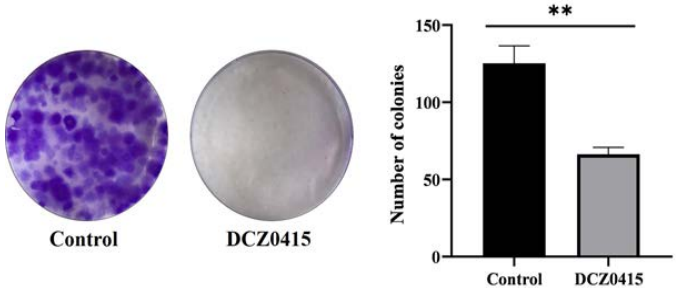

Figure 2. Effect of DCZ0415 treatment on colony forming capacity of U87 cells. **P < 0.01.

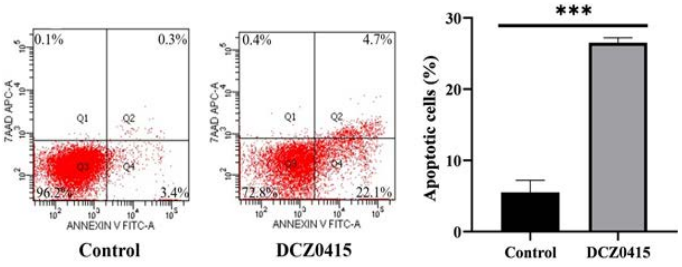

Figure 3. Effect of DCZ0415 treatment on apoptosis in U87 cells. ***P < 0.001.

DCZ0415 Induces Apoptosis in U87 Cells

Apoptotic effect of DCZ0415 was evaluated with flow cytometer-based FITC Annexin V method and the percentages of early apoptosis, late apoptosis, and necrosis were determined in the control and the DCZ0415-treated cells. Accordingly, % total apoptosis was determined as 5.5±1.52 in control group and 26.5±0.63 in DCZ0415-treated group. Accordingly, DCZ0415 treatment significantly increased the apoptosis rate in U87 cells (Fig 3; P < 0.001).

DCZ0415 Affects mRNA Level of Important Apoptosis Genes

The effect of DCZ0415 on mRNA levels of CASP3, CASP7, CASP8, CASP9, BAX, BCL2, CYCS, FAS, FADD, TNFA, and TNFR1 in U87 cells was evaluated by qRT-PCR analysis. Accordingly, a significant increase was observed in mRNA levels of CASP3, CASP9, BAX, and CYCS as 4.41 (P= 0.004672), 9.07 (P= 0.000238), 1.79 (P= 0.009565) and 9.7 (P= 0.000898) fold, respectively, in DCZ0415-treated cells compared to the control group (Fig 4).

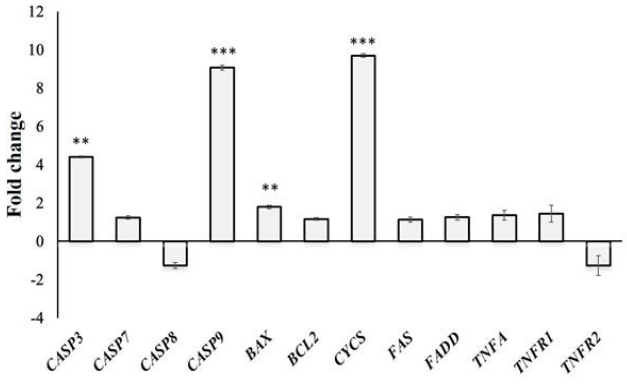

Figure 4. Effect of DCZ0415 treatment on apoptosis-related genes in U87 cells. **P < 0.01; ***P < 0.001.

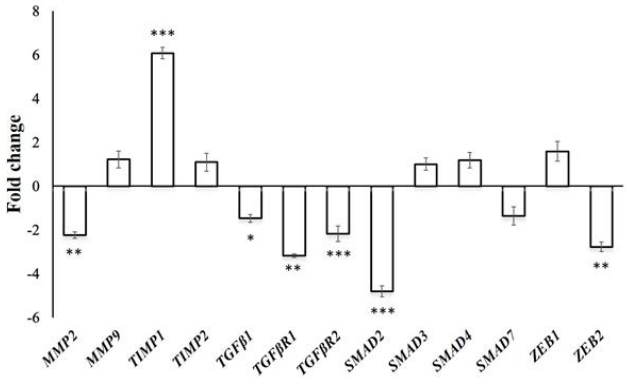

Figure 5. Effect of DCZ0415 treatment on invasion and TGF-β signaling-related genes in U87 cells. *P < 0.05; **P < 0.01; ***P < 0.001.

DCZ0415 Affects expression level of Invasion and TGF-β Signaling-Related Genes

The effect of DCZ0415 treatment at IC50 dose on mRNA levels of MMP2, MMP9, TIMP1, and TIMP2 genes which are associated with invasion, and, TGFβ1, TGFβR1, TGFβR2, SMAD2, SMAD3, SMAD4, SMAD7, ZEB1 and ZEB2 genes associated with TGF-β signaling were evaluated. Accordingly, after DCZ0415 treatment, MMP2 (2.23 fold; P=0.006202), TGFβ1 (1.47 fold; P= 0.018663), TGFβR1 (3.17 fold; P= 0.001715), TGFβR2 (2.17 fold; P= 0.000749), SMAD2 (4.81 fold; P= 0.000087) and ZEB2 (2.77 fold; P= 0.006447) gene expressions were significantly decreased while TIMP1 (6.08 fold; P= 0.000001) gene expression was significantly increased when compared to the control group (Fig 5).

DISCUSSION

Identification of new molecules that are effective in cancer development and determination of the relationship between these molecules and known signaling pathways are very important for improving the new treatment approaches. Recent studies have revealed that expression of TRIP13 gene is increased in many cancers, including GBM, and in this respect, TRIP13 is considered as a new oncogenic factor (12). In support of this, silencing of TRIP13 has been shown to have anticancer effects in various cancer types. It has been shown that TRIP13 expression is high in bladder cancer tissues, and increased TRIP13 expression is related to advanced tumor stage, metastasis and low survival. Silencing TRIP13

in bladder cancer cells resulted in suppression of proliferation, increase in apoptosis, and cell cycle arrest (9). Similarly, it has been shown that TRIP13 expression is high in ovarian cancer tissues and cells, and silencing of TRIP13 induces apoptosis and inhibits cell proliferation, invasion, and migration (6). Zhang et al. (5) also stated that TRIP13 plays an important role in tumorigenesis of GBM. And, silencing of TRIP13 in GBM cells has been shown to inhibit proliferation, migration, and invasion.

DCZ0415, whose anticancer activity was investigated in GBM cells in this study, is a small molecule inhibitor used to target TRIP13. In a study with multiple myeloma cells, DCZ0415 has been shown to inhibit cell growth and induce apoptosis. It has also been noted that the combination of DCZ0415 with melphalan or panbinostat has a synergistic effect (13). Similarly, it has been reported that DCZ0415 treatment reduces cell proliferation, arrests the cell cycle, and promotes apoptosis in colon cancer cells (14). Xu et al. (15) also stated that DCZ0415 suppresses proliferation, migration, and invasion of hepatocellular carcinoma cells. And, researchers have demonstrated that DCZ0415 has a synergistic effect with olaparib, a PARP1 inhibitor, in hepatocellular carcinoma cells.

In this study, we first performed cytotoxicity analysis to investigate the possible anticancer activity of DCZ0415 in U87 human GBM cells and treated the cells with different concentrations of DCZ0415 for 24, 48, and 72 hours. Accordingly, DCZ0415 suppressed U87 cell proliferation, and, IC_{50} dose of DCZ0415 for 48 hours was determined as 19.77 μM. We performed colony analysis after cytotoxicity analysis, and accordingly, treatment of DCZ0415 at IC_{50} dose for 48 hours also inhibited the colony forming capacity of U87 cells. These results demonstrate the suppressive effect of DCZ0415 on U87 cell proliferation.

One of the important indicators of anticancer activity is the induction of apoptosis. In other words, anticancer drugs are expected to induce apoptosis in cancer cells (19). In our study, the anticancer activity of DCZ0415 was evaluated in terms of apoptosis with the FITC Annexin V method, and accordingly, apoptosis was induced in GBM cells by DCZ0415 treatment at IC_{50} dose for 48 hours. In mammalian cells, apoptosis occurs in two ways as intrinsic and extrinsic pathways in which many proteins are involved, and these two pathways can be induced in anticancer activity (20). The intrinsic pathway, also called the mitochondrial pathway, is characterized by Cytochrome-c release from mitochondria to the cytoplasm, depending on the activity of Bcl-2 protein members. The release of Cytochrome-c leads to the formation of apoptosome, resulting in the activation of Caspase-9. And then, effector Caspases (Caspase-3, -6 and -7) are activated. Apoptotic cell death is triggered by the cleavage of substrates by effector Caspases (21). The extrinsic pathway is triggered by binding of extracellular ligands to tumor necrosis factor (TNF) receptor superfamily members, which are located on the cell surface and are called death receptors. Intracellular domains of death receptors, called the death domain, are effective in the transmission of the apoptotic signal (22, 23). In this study, the effect of DCZ0415 on apoptosis was also evaluated at the molecular level, and the expression levels of genes encoding important proteins that function in intrinsic and extrinsic pathways of apoptosis were evaluated by qRT-PCR analysis. Accordingly, DCZ0415 significantly increased mRNA levels of CASP3, CASP9, BAX, and CYCS genes, which encode Caspase-3, -9, Bax and Cytochrome-c proteins, are involved in intrinsic pathway of apoptosis, in GBM cells. In this respect, DCZ0415 is thought to be effective on the intrinsic pathway of apoptosis.

Distant organ metastases are rarely encountered in gliomas. However, the cells have property of infiltrative growth, which provides invasiveness to tumor. Tumor invasiveness is one of the most important causes of recurrence and GBM cells can invade as individually or collectively by remodeling the extracellular matrix (24, 25). Matrix metalloproteinases (MMPs) are enzymes that play a role in tumor invasion by degrading extracellular matrix components. MMPs are inhibited by tissue inhibitors of metalloproteinases (TIMPs) (26). To evaluate the effect of DCZ0415 on invasion, we analyzed mRNA levels of MMP2, MMP9, TIMP1, and TIMP2 genes. And, DCZ0415 decreased MMP2 gene expression while increased TIMP1 gene expression. This result suggests that DCZ0415 may exert an antiinvasive effect in U87 cells by regulating mRNA levels of invasion-related genes.

In this study, anticancer activity of DCZ0415 in GBM cells was also evaluated in terms of the TGF-β signaling. TGF-β family members bind to two receptors in the cell. As a result of this binding, Smad2 and -3 are activated by phosphorylation and form a complex with Smad4. This complex imports to the nucleus and regulates expression of TGF-β target genes such as ZEB1 and ZEB2. One of the TGF-β target genes is Smad7 which acts as a negative regulator in this

pathway (27, 28). It has been shown that activation of the pathway is increased in aggressive and highly proliferating gliomas and this increase is associated with poor prognosis (29). In this study, the effect of DCZ0415 on mRNA levels of TGF-β signaling-related genes was evaluated and it was determined that TGF-β pathway was suppressed at mRNA level. This result shows that the anticancer effect of DCZ0415 in GBM cells may be related to suppression of TGF-β pathway. In addition, considering that DCZ0415 is a TRIP13 inhibitor, it is thought that there may be a possible relationship between TRIP13 and TGF-β signaling pathway.

In this study, the anticancer effect of DCZ0415, a small molecule inhibitor of TRIP13, which has been shown to have an oncogenic role in many cancers in recent years, was investigated in GBM cells. Our findings revealed that DCZ0415 suppresses proliferation and induces apoptosis in GBM cells, and DCZ0415 regulates the expression of genes associated with apoptosis, invasion, and TGF-β signaling. And, it is thought that TRIP13 can be evaluated as a new target for GBM and DCZ0415 can be an effective inhibitor in this respect. However, this study, which presents the first data on the activity of DCZ0415 in GBM cells, has several limitations, such as the fact that it was performed with a single cell line representing GBM, and changes in expression level of genes were investigated only at mRNA level. In order to fully elucidate the molecular mechanism of DCZ0415 in GBM cells, further studies are needed. In this respect, it is also believed that this study will contribute to further studies that will investigate the efficacy of DCZ0415.

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

Financial conflict of interest: *Authors declare that they did not receive any financial support in this study.*

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REFERENCES

1. Caffery B, Lee JS, Alexander-Bryant AA. Vectors for glioblastoma gene therapy: Viral & non-viral delivery strategies. Nanomaterials (Basel) 2019;9(1):105.
2. Malacrida A, Rivara M, Di Domizio A, et al. 3D proteome-wide scale screening and activity evaluation of a new ALKBH5 inhibitor in U87 glioblastoma cell line. Bioorg Med Chem 2020;28(4):115300.
3. Yang K, Wu Z, Zhang H, et al. Glioma targeted therapy: Insight into future of molecular approaches. Mol Cancer 2022;21(1):39.
4. Yu L, Xiao Y, Zhou X, et al. TRIP13 interference inhibits the proliferation and metastasis of thyroid cancer cells through regulating TTC5/p53 pathway and epithelial-mesenchymal transition related genes expression. Biomed Pharmacother 2019;120:109508.
5. Zhang G, Zhu Q, Fu G, et al. TRIP13 promotes the cell proliferation, migration and invasion of glioblastoma through the FBXW7/c-MYC axis. Br J Cancer 2019;121(12):1069-78.
6. Zhou W, Yu X, Sun S, et al. Increased expression of MMP-2 and MMP-9 indicates poor prognosis in glioma recurrence. Biomed Pharmacother 2019;118:109369.
7. Dong L, Ding H, Li Y, et al. TRIP13 is a predictor for poor prognosis and regulates cell proliferation, migration and invasion in prostate cancer. Int J Biol Macromol 2019;121:200-6.
8. Yao J, Zhang X, Li J, et al. Silencing TRIP13 inhibits cell growth and metastasis of hepatocellular carcinoma by activating of TGF-β1/smad3. Cancer Cell Int 2018;18:208.
9. Gao Y, Liu S, Guo Q, et al. Increased expression of TRIP13 drives the tumorigenesis of bladder cancer in association with the EGFR signaling pathway. Int J Biol Sci 2019;15(7):1488-99.
10. Di S, Li M, Ma Z, et al. TRIP13 upregulation is correlated with poor prognosis and tumor progression in esophageal squamous cell carcinoma. Pathol Res Pract 2019;215(6):152415.
11. Liu X, Shen X, Zhang J. TRIP13 exerts a cancer-promoting role in cervical cancer by enhancing Wnt/β-catenin signaling via ACTN4. Environ Toxicol 2021;36(9):1829-40.
12. Chen SH, Lin HH, Li YF, et al. Clinical significance and systematic expression analysis of the thyroid receptor interacting protein 13 (TRIP13) as human gliomas biomarker. Cancers (Basel) 2021;13(10):2338.
13. Wang Y, Huang J, Li B, et al. A small-molecule inhibitor targeting TRIP13 suppresses multiple myeloma progression. Cancer Res 2020;80(3):536-48.
14. Agarwal S, Afaq F, Bajpai P, et al. DCZ0415, a small-molecule inhibitor targeting TRIP13, inhibits EMT and metastasis via inactivation of the FGFR4/STAT3 axis and the Wnt/β-catenin pathway in colorectal cancer. Mol Oncol 2022;16(8):1728-45.
15. Xu J, Lamouille S, Derynck R. TGF-beta-induced epithelial to mesenchymal transition. Cell Res 2009;19(2):156-72.
16. Frei K, Gramatzki D, Tritschler I, et al. Transforming growth factor-β pathway activity in glioblastoma. Oncotarget 2015;6(8):5963-77.
17. Birch JL, Coull BJ, Spender LC, et al. Multifaceted transforming growth factor-beta (TGFβ) signalling in glioblastoma. Cell Signal 2020;72:109638.
18. Güçlü E, Çınar Ayan İ, Dursun HG, et al. Tomentosin induces apoptosis in pancreatic cancer cells through increasing reactive oxygen species and decreasing mitochondrial membrane potential. Toxicol In Vitro 2022;84:105458.
19. Pfeffer CM, Singh ATK. Apoptosis: A target for anticancer therapy. Int J Mol Sci 2018;19(2):448.
20. Pistritto G, Trisciuoglio D, Ceci C, et al. Apoptosis as anticancer mechanism: Function and dysfunction of its modulators and targeted therapeutic strategies. Aging (Albany NY) 2016;8(4):603-19.

21. Fulda S. Targeting apoptosis for anticancer therapy. Semin Cancer Biol 2015;31:84-8.
22. Guicciardi ME, Gores GJ. Life and death by death receptors. FASEB J 2009;23(6):1625-37.
23. Cui J, Zhao S, Li Y, et al. Regulated cell death: Discovery, features and implications for neurodegenerative diseases. Cell Commun Signal 2021;19(1):120.
24. Zhou XY, Shu XM. TRIP13 promotes proliferation and invasion of epithelial ovarian cancer cells through Notch signaling pathway. Eur Rev Med Pharmacol Sci 2019;23(2):522-9.
25. Vollmann-Zwerenz A, Leidgens V, Feliciello G, et al. Tumor cell invasion in glioblastoma. Int J Mol Sci 2020;21(6):1932.
26. Han J, Jing Y, Han F, et al. Comprehensive analysis of expression, prognosis and immune infiltration for TIMPs in glioblastoma. BMC Neurol 2021;21(1):447.
27. Xu H, Ma Z, Mo X, et al. Inducing synergistic DNA damage by TRIP13 and PARP1 inhibitors provides a potential treatment for hepatocellular carcinoma. J Cancer 2022;13(7):2226-37.
28. Yan X, Liu Z, Chen Y. Regulation of TGF-beta signaling by Smad7. Acta Biochim Biophys Sin (Shanghai) 2009;41(4):263-72.
29. Bruna A, Darken RS, Rojo F, et al. High TGFbeta-Smad activity confers poor prognosis in glioma patients and promotes cell proliferation depending on the methylation of the PDGF-B gene. Cancer Cell 2007;11(2):147-60.

SELÇUK TIP DERGİSİ
SELCUK MEDICAL JOURNAL

Araştırma Makalesi / Research Article

Selcuk Med J 2023;39(1): 19-23
DOI: 10.30733/std.2023.01606

NEÜ YAYINEVİ

SARS-CoV-2 VOC 202012/01(B.1.1.7) Variant: Is it More Dangerous?

SARS-CoV-2 VOC 202012/01(B.1.1.7) Varyantı: Daha mı Tehlikeli?

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Geliş Tarihi/Received: *5 July 2022*
Kabul Tarihi/Accepted: *13 February 2023*

Öz

Amaç: *Bu çalışmada; SARS-CoV-2'nin İngiltere Varyantı (VOC 202012/01-B.1.1.7) ile enfekte olan hastaların demografik ve klinik özelliklerinin saptanması ve İngiltere Varyantı olmayan SARS-CoV-2 ile enfekte hastalarla karşılaştırılarak farklılıkların ortaya konması amaçlanmaktadır.*
Hastalar ve Yöntem: *Konya İli'nde 02-11 Şubat 2021 tarihleri arasında PCR testi pozitif olarak sonuçlanan ve varyant analiz sonucu VOC 202012/01 (B.1.1.7) olan 671 vaka ile aynı tarihler arasında PCR test sonucu pozitif olan ve varyant olmayan 2284 vakanın, Sağlık Bakanlığı Halk Sağlığı Yönetim Sistemindeki (HSYS) kayıtları 24.02.2021 tarihi baz alınarak taranmıştır. Yapılan taramada yaş, cinsiyet, temaslılık durumu, daha önce COVID-19 geçirme durumu, hastaneye ve yoğun bakım ünitesine yatma durumu, yatış süresi, entübasyon ve exitus durumları kaydedilmiştir.*
Bulgular: *Varyant varlığı/yokluğuna göre hastanede yatış durumu arasında istatistiksel olarak anlamlı fark yoktu (p= 0,234). Varyant pozitif olan hastaların %1.9'u, varyant pozitif olmayanların ise %3.9'u yoğun bakım ünitesine yatırıldı. Varyant pozitif olmayan hastalarda YBÜ'ye kabul oranı, pozitif olanlara göre anlamlı olarak daha yüksekti (p= 0.013).*
Sonuç: *Bu çalışmanın bulguları ışığında SARS-CoV-2 VOC 202012/01(B.1.1.7)'nin hastaneye yatış ve yoğun bakıma yatış açısından daha tehlikeli olmadığını söylemek mümkündür.*

Anahtar Kelimeler: Covid-19, B.1.1.7, varyant, VOC

Abstract

Aim: This study aimed to determine the demographic and clinical characteristics of patients infected with the VOC 202012/01-B.1.1.7 variant of SARS-CoV-2 and to compare these patients with those infected with other variants of SARS-CoV-2, in order to demonstrate the differences.
Patients and Methods: Records of 671 patients with VOC 202012/01 (B.1.1.7)(VOC+) who tested positive in the PCR (polymerase chain reaction) test, between February 2–11, 2021 in Konya Province, and were found to have the VOC 202012/01 (B.1.1.7), according to variant analysis and 2284 (VOC−) patients who also tested positive in the PCR test between the same dates but did not have the variant (VOC−) were screened in the Public Health Administration System of the Turkish Ministry of Health, on February 24, 2021. Age, gender, hospitalization status, and admission to the intensive care unit (ICU) were recorded from the screening results.
Results: There was no statistically significant difference between hospitalization status, according to the presence/absence of the variant (p= 0.234). Of the patients who were variant-positive and those who were not, 1.9% and 3.9% were admitted to the ICU, respectively. The rate of admission to the ICU was significantly higher for patients who were not positive for the variant as compared to those who were (p= 0.013).
Conclusıons: In the light of the findings of this study, it is possible to state that SARS-CoV-2 VOC 202012/01(B.1.1.7) is not more dangerous in terms of hospitalization and admission to the ICU.

Key words: Covid-19, B.1.1.7, variant, VOC

Cite this article as: Aslanlar DA, Aydemir O, Kunt M, Koc E, Koc M. SARS-CoV-2 VOC 202012/01(B.1.1.7) Variant: Is it More Dangerous? Selcuk Med J 2023;39(1): 19-23

Disclosure: *None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this article. The research was not sponsored by an outside organization. All authors have agreed to allow full access to the primary data and to allow the journal to review the data if requested.*

INTRODUCTION

As of March 30, 2021, the world has been greatly affected by the Coronavirus disease (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), with 128 million cases and 2.8 million deaths (1). A total of 2,492,977 cases and 31,385 deaths have been reported in Turkey so far (2).

Since the onset of the pandemic, multiple variants of SARS-CoV-2 have been observed throughout the world. Variants of concern (VOC) have been defined based on evidence of increased transmissibility, disease severity, and the ability to evade immunity provided by previous infections or vaccines. The VOC 202012/01 (B.1.1.7), which was first detected in the UK, has spread rapidly worldwide. The transmission rate of the virus was estimated to be 43%–90% in early February 2021, when the aforementioned variant became the predominant one in the UK and constituted 95% of the cases (3-5). This rate is evidently higher than the transmission rate of the SARS-CoV-2 strain that already existed in other countries, such as the United States (4).

The VOC 202012/01(B.1.1.7) was first detected on September 20, 2020 in the UK, after which it was identified in Turkey on January 1, 2021, approximately three months later (6, 7). This date onward, the number of cases increased from 15 to 128 on January 29, 2021. It was reported that the patients with this variant constituted 75% and 85% of all cases on March 30 and April 12, 2021, respectively (7).

At this stage in the pandemic, demonstrating whether this variant of SARS-CoV-2 would pose a danger in terms of exceeding hospital capacity, considering its virulence and the demographic characteristics of the patients affected, would contribute to the fight against the pandemic.

In this study, it was aimed to determine the demographic and clinical characteristics of patients infected with the VOC 202012/01-B.1.1.7 variant of SARS-CoV-2 and to compare these patients with those infected with other variants of SARS-CoV-2, in order to demonstrate the differences.

PATIENTS AND METHODS

Records of 671 patients with VOC 202012/01 (B.1.1.7)(VOC+) who tested positive in the PCR (polymerase chain reaction) test, between February 2–11, 2021 in Konya Province, and were found to have the VOC 202012/01 (B.1.1.7), according to variant analysis and 2284 (VOC−) patients who also tested positive in the PCR test between the same dates but did not have the variant (VOC−) were screened in the Public Health Administration System of the Turkish Ministry of Health, on February 24, 2021. Age, gender, hospitalization status, and admission to the intensive care unit (ICU) were recorded from the screening results. Patients who completed the ten-day isolation period according to the COVID-19 Guidelines published by the Turkish Ministry of Health, were included in the study.

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee (2021/019).

Statistical Analysis

The research data was analyzed using SPSS (Statistical Package for the Social Sciences) for Windows 22.0 (SPSS Inc, Chicago, IL). Descriptive statistics were expressed with median [interquartile range (Q1-Q3)], frequency distribution, and percentage values. Pearson's Chi-Square Test was used to evaluate categorical variables. Normality of distribution was analyzed using visual (histograms and probability graphs) and analytical (Kolmogorov–Smirnov Test) methods for all variables. For variables that were not normally distributed, the Mann–Whitney U Test was used as the statistical method to compare two independent groups and assess statistically significant differences. Independent predictors for predicting ICU admission were analyzed using Logistic Regression analysis. The Hosmer-Lemeshow test was used for model fit. Values of $p < 0.05$ were considered statistically significant.

RESULTS

A total of 2955 cases were examined, including 671 (22.7%) patients who were VOC (+). The median ages of patients who were VOC (+) and VOC (−) were 36 (23–52) and 43 (29–59), respectively, wherein the difference was statistically significant ($p < 0.001$). In addition, 55.7% patients who were VOC (+) and 55.6% patients who were VOC (−) were female, and the difference was not statistically significant ($p = 0.935$) (Table 1).

Of the patients who were VOC (+) and VOC (−), 8.6% and 10.2% were hospitalized, respectively. There was no statistically significant difference between hospitalization status, according to the presence/absence of the variant ($p = 0.234$) (Table 2). Of the patients who were variant-positive and those who were not, 1.9% and 3.9% were admitted to the ICU, respectively. The rate of admission to the ICU

Table 1. Distribution of Some Descriptive Characteristics by Variant Status

	VOC (+) (n = 671)	VOC (−) (n = 2284)	p
Age (year), median (Q1-Q3)	36 (23–52)	43 (29–59)	<0.001*
Gender, n (%)			
Male	297 (44.3)	1015 (44.4)	0.935
Female	374 (55.7)	1269 (55.6)	

n: Number of cases; %: Column percentage

Table 2. Distribution of Some Clinical Characteristics by Variant Status

	VOC (+) (n = 671)	VOC (−) (n = 2284)	p
Hospitalized patients, n (%)	58 (8.6)	233 (10.2)	0.234
Admission to the ICU, n (%)	13 (1.9)	90 (3.9)	0.013*

n: Number of cases; %: Column percentage

Table 3. Independent Effects of Age, Gender, and VOC Positivity In Predicting Admission to The ICU

	p	OR	95% CI
Age	<0.001	1.082	1.067–1.097
Gender			
Male	Reference		
Female	0.472	0.589	0.568–1.299
VOC Positivity			
Negative	Reference		
Positive	0.363	0.754	0.410–1.386

OR: Odds ratio; CI: Confidence interval

was significantly higher for patients who were not positive for the variant as compared to those who were (p = 0.013) (Table 2).

Of the 2955 patients included, the median age of 103 patients admitted to the ICU was 71 (60–76), whereas the median age of 2852 patients who were not admitted to the ICU was 41 (27–57). The ages of patients who were admitted to the ICU was found to be significantly higher than the ages of those who were not (p <0.001). In addition, of the patients who were admitted and not admitted to the ICU, 52.4% and 55.7% were female, respectively, wherein the rates were similar (p = 0.509).

Logistic regression analysis was used to evaluate the independent effects of some characteristics in predicting admission to the ICU. Accordingly, it was found that age had an independent effect (p <0.001), whereas gender and VOC positivity did not (p = 0.472 and p = 0.363, respectively) (Table 3).

DISCUSSION

In this study, hospitalization status and admission to the ICU as well as demographic characteristics were analyzed in patients who were confirmed to be VOC (+) and VOC (−), by laboratories in Konya province accredited by the Turkish Ministry of Health.

There was no difference between patients who were VOC (+) and VOC (−), in terms of hospitalization status. However, admission to the ICU was found to be more common among patients who were VOC (−) as compared to those who were VOC (+).

At the time of this research, the B.1.1.7 mutation was detected in 22.7% of the patients who tested positive for COVID-19 via PCR testing. This rate was found to be 26% in a study conducted by Yilmaz et al. in Istanbul (8). In another study conducted in the UK, the rate of VOC (+) cases was reported to be 93% between January 25 and 31 2021 (3). Within the same time period, the positivity rate of the B.1.1.7/SGTF variant reached 90% of all SARS-CoV-2 variants circulating in Madrid [4]. On the other hand, it was estimated that 13.3% of the confirmed COVID-19 cases in Portugal were caused by the VOC 202012/01 (B.1.1.7) in January 2021 (9). The B.1.1.7 variant became the dominant variant, approximately three months after it was first detected in the UK in September 2020. In Turkey, the B.1.1.7 variant, which was first reported in Istanbul, spread throughout the

country over time. The time when variant strains become dominant may vary by the date and region where they are reported. The B.1.1.7 variant became dominant in Turkey, approximately a month after this study was conducted.

In this study, of the patients who were VOC (+) and VOC (−), 8.6% and 10.2% were hospitalized, respectively, wherein the rates were similar. The hospitalization status of those who were positive for the variant and those who were not was also found to be similar to the results by Yilmaz et al. in İstanbul (8). Moreover, in another study conducted in the UK, Davies et al. found no clear evidence that VOC 202012/01 results in more or less severe disease as compared to the pre-existing variants (10). In Denmark, a study revealed that individuals infected with the B.1.1.7 strain had a 42% higher risk of hospitalization compared to individuals infected with other strains of SARS-CoV-2 (11). Nyberg et al. found that the risk of hospitalization within 14 days of a positive test was 1.52 times higher in patients diagnosed with COVID-19 and infected with the B.1.1.7 variant, in the UK (3). In another study conducted with PCR-positive cases in Norway between December 20, 2020 and May 2, 2021, it was shown that the B.1.1.7 variant led to a 1.6-fold increase in the risk of hospitalization (12). Different results were reported in terms of hospitalization status or risk of hospitalization associated with the B.1.1.7 variant in the studies listed. In the present study, there was no difference in terms of hospitalization status. This could be attributed to the fact that the patients were followed up for ten days, they were not evaluated in terms of comorbidities, and that the B.1.1.7 variant was not the dominant variant in Konya and Turkey at the time of this study.

In the present study, 1.9% and 3.9% of the patients who were positive for the variant and those who were not, respectively, were admitted to the ICU, the latter displaying a higher rate of admission to the ICU than the former. According to Yilmaz et al., there was no difference between patients who were VOC (+) and VOC (−), in terms of admission to the ICU (8). A study conducted in Spain has shown that patients infected with the B.1.1.7 variant had a two-fold higher risk of admission to the ICU than those not infected with the said variant (4). Stirrup et al. determined that the risk of admission to the ICU was not different between patients infected and not infected with the B.1.1.7 variant, whereas Whittaker et al. found no difference between patients infected with the B.1.1.7 variant and those who were VOC (−), in terms of the time from symptom onset to hospitalization and length of hospital or ICU stay (13, 14). Funk et al. reported that a larger proportion of patients who were VOC (+) were admitted to the ICU compared to those who were not (15). According to a UK-based study, patients infected with B.1.1.7 had a significantly higher risk of admission to the ICU compared to those who were not infected with B.1.1.7 (6). A study from Norway showed that patients infected with B.1.1.7 had a higher risk of admission to the ICU than those who were not (12). In the present study, the age of patients who were not infected with the variant was significantly higher than the age of those who were. In addition, evaluation of the factors affecting admission to the ICU with multivariate analysis revealed that age was a factor affecting admission to the ICU. In various studies, age was reported to be one of the important factors that lead to an increased risk of admission to the ICU (4, 6, 12, 13, 15, 16). With respect to hospitalization status, there may be other factors involved such as the fact that patients were followed up for ten days and that they were not evaluated in terms of comorbidities.

Our study includes some limitations. Positive patients were followed up for ten days through the system. This time period could have been 28 days or longer, in order to obtain a better picture in terms of hospitalization and admission to intensive care. This study included patients who visited outpatient clinics and emergency departments of hospitals and tested positive in a PCR test. Our study may not entirely represent society, since there are other people who may not have undergone PCR testing, those who may be positive for COVID-19 but at home, or those who are moving within the society with or without symptoms. These can be considered the limitations of the present study.

CONCLUSION

In the light of the findings of this study, it is possible to state that SARS-CoV-2 VOC 202012/01(B.1.1.7) is not more dangerous in terms of hospitalization and admission to the ICU. Further clinical trials evaluating disease severity should be conducted. The B.1.1.7 variant is highly infectious, which may lead to a more rapid increase in the number of cases in Konya Province and in Turkey. This can further increase the pre-existing hospital burden. The number of wards and intensive care beds allocated to patients with COVID-19 should be reconsidered in the hospitals of Turkey. In addition, ongoing efforts toward vaccination should be accelerated, to minimize the effects of the

COVID-19 pandemic.

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

Financial conflict of interest: *Authors declare that they did not receive any financial support in this study.*

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REFERENCES

1. World Health Organization (WHO). WHO Coronavirus (COVID-19) Dashboard. Available from: https://covid19.who.int Accessed March 31, 2021.
2. T.C. Sağlık Bakanlığı. COVID-19 Bilgilendirme Platformu. Available from: https://covid19.saglik.gov.tr/genelkoronavirüs-tablosu Accessed April 1, 2021.
3. Nyberg T, Twohig KA, Harris RJ, et al. Risk of hospital admission for patients with SARS-CoV-2 variant B.1.1.7: Cohort analysis. BMJ 2021;373: n1412.
4. Martínez-García L, Espinel MA, Abreu M, et al. Emergence and spread of B.1.1.7 lineage in primary care and clinical impact in the morbi-mortality among hospitalized patients in Madrid, Spain. Microorganisms 2021;9(7):1517.
5. World Health Organization (WHO). COVID-19 Weekly Epidemiological Uptade. Available from: https://www.who.int/publications/m/item/weekly-epidemiological-update-oncovid 19-18-may-2021.
6. Patone M, Thomas K, Hatch R, et al. Mortality and critical care unit admission associated with the SARSCoV-2 lineage B.1.1.7 in England: An observational cohort study. Lancet Infect Dis 2021;21(11):1518-28.
7. T.C. Sağlık Bakanlığı. COVID-19 Bilgilendirme Platformu Available from: https://covid19.saglik.gov.tr/ Accessed April 15, 2021.
8. Yilmaz H, Cakir M, Komurcu SZM, et al. Evaluation of patients with COVID-19 and the United Kingdom mutations in a training and research hospital in Istanbul. North Clin Istanb 2021;8(4):317-20.
9. Borges V, Sousa C, Menezes L, et al. Tracking SARS-CoV-2 lineage B.1.1.7 dissemination: Insights from nationwide spike gene target failure (SGTF) and spike gene late detection (SGTL) data, Portugal, week 49 2020 to week 3 2021. Euro Surveill 2021;26(10):2100131.
10. Davies NG, Jarvis CI; CMMID COVID-19 Working group, et al. Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. Nature 2021;593(7858):270-4.
11. Bager P, Wohlfahrt J, Fonager J, et al. Risk of hospitalisation associated with infection with SARS-CoV-2 lineage B.1.1.7 in Denmark: An observational cohort study [published correction appears in Lancet Infect Dis 2021;21(11): e341]. Lancet Infect Dis 2021;21(11):1507-17.
12. Veneti L, Seppälä E, Larsdatter Storm M, et al. Increased risk of hospitalisation and intensive care admission associated with reported cases of SARS-CoV-2 variants B.1.1.7 and B.1.351 in Norway, December 2020 -May 2021. PLoS One 2021;16(10): e0258513.
13. Stirrup O, Boshier F, Venturini C, et al. SARS-CoV-2 lineage B.1.1.7 is associated with greater disease severity among hospitalised women but not men: Multicentre cohort study. BMJ Open Respir Res 2021;8(1): e001029.
14. Whittaker R, Kristofferson AB, Seppälä E, et al. Trajectories of hospitalisation for patients infected with SARS-CoV-2 variant B.1.1.7 in Norway, December 2020- April 2021. J Infect 2021;83(4):e14-7.
15. Funk T, Pharris A, Spiteri G, et al. Characteristics of SARS-CoV-2 variants of concern B.1.1.7, B.1.351 or P.1: Data from seven EU/EEA countries, weeks 38/2020 to 10/2021. Euro Surveill 2021;26(16):2100348.
16. Davies NG, Abbott S, Barnard RC, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science 2021;372(6538):eabg3055.

SELÇUK TIP DERGİSİ
SELCUK MEDICAL JOURNAL

Araştırma Makalesi / Research Article

Selcuk Med J 2023;39(1): 24-28
DOI: 10.30733/std.2023.01605

NEÜ YAYINEVİ

Identification of the Mutation in *DCLRE1C* Gene by PCR-RFLP

DCLRE1C Genindeki Mutasyonun PCR-RFLP ile Tanımlanması

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Geliş Tarihi/Received: *5 August 2022*
Kabul Tarihi/Accepted: *15 January 2023*

Öz

Amaç: *DCLRE1C* genindeki mutasyonlar Artemis proteininin fonksiyonel olarak bozulmasına neden olur ve T/B hücre gelişimi olumsuz etkilenir. Bu mutasyonun bir sonucu olarak, genellikle ağır kombine ve kombine immün yetmezlik (CID) kliniği ortaya çıkar. Akraba evliliğinin yaygın olduğu bölgemizde bu mutasyona bağlı CID vakalarına sıklıkla rastlanmaktadır. Bu nedenle şüpheli hastalar ilgili gen mutasyonu açısından vakit kaybetmeden değerlendirilmelidir. Mutasyonların tespitinde daha karmaşık ve maliyetli yöntemlerin kullanılmakla birlikte daha ucuz ve hızlı yöntemlere ihtiyaç olduğu açıktır. Bundan dolayı çalışmada *DCLRE1C* geni ekzon 3 (c.194C>T; p.T65I) ve ekzon 14 (c.1669_1670insA; p.T577Nfs*21) mutasyonlarının Polimeraz Zincir Reaksiyonu-Restriksiyon Parça Uzunluk Polimorfizmi (PZR-RFLP) yöntemi kullanılarak belirlenmesi amaçlandı.
Hastalar ve Yöntem: Çalışma 2017-2020 yılları arasında kliniğimizde DCLRE1C mutasyonu ile takip edilen 14 hasta, 2 ebeveyn ve 10 sağlıklı kontrol dahil edildi. Mutasyon bölgeleri ve uygun restriksiyon enzimleri içeren primerler ile PZR-RFLP analizi gerçekleştirildi.
Bulgular: Analiz sonucunda 12 hasta *DCLRE1C* geni ekzon 3 açısından homozigot mutant, 2 ebeveyn ekzon 3 açısından heterozigot, 2 hasta ekzon 3 ve ekzon 14 açısından compound heterozigote genotipde olduğu bulundu. Mutasyonlar, Sanger DNA dizilimi ile doğrulandı. PZR-RFLP yöntemi ile ilgili bölgedeki mutasyonlar hızlı ve güvenilir bir şekilde belirlendi.
Sonuç: Çalışma, PZR-RFLP yönteminin primer immün yetmezliklerde özellikle bilinen mutasyonların tespiti ve aile taraması gibi durumlarda kullanılabilecek ucuz, güvenli ve hızlı bir yöntem olduğunu göstermiştir.

Anahtar Kelimeler: Artemis, *DCLRE1C*, PZR-RFLP

Abstract

Aim: Mutations in the *DCLRE1C* gene result in functional impairment of the Artemis protein and T/B cell development is adversely affected. As a result of this mutation, a clinic of severe combined and combined immunodeficiency (CID) generally occurs. In our region where consanguineous marriage is common, CID cases due to this mutation are frequently encountered. Therefore, suspected patients should be evaluated promptly for the relevant gene mutation. It is clear that more complicated and costly methods are used in the detection of mutations and there is a need for cheaper and faster methods. Therefore, in this study, it was aimed to determine the mutations of *DCLRE1C* gene exon 3 (c.194C>T; p.T65I) and exon 14 (c.1669_1670insA; p.T577Nfs*21) by using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method.
Patients and Methods: The study was carried out between 2017 and 2020 and study included 14 patients followed up with *DCLRE1C* mutation in our clinic, 2 parents and 10 healthy controls. PCR-RFLP analysis was performed with primers containing mutation sites and appropriate restriction enzymes.
Results: As a result of the analysis, 12 patients were homozygous mutant for *DCLRE1C* gene exon 3, 2 parents were heterozygous for exon 3, and 2 patients were heterozygous for exon 3 and exon 14 and were found to be compound heterozygous genotype. Mutations were confirmed by Sanger DNA sequencing. Mutations in the relevant region were determined quickly and reliably by the PCR-RFLP method.
Conclusion: The study showed that the PCR-RFLP method is a cheap, safe and fast method that can be used in cases such as family screening, especially for the detection of known mutations in primary immunodeficiencies.

Key words: Artemis, *DCLRE1C*, PCR-RFLP

Cite this article as: Karaselek MA, Kuccukturk S, Kapakli H, Hazar E, Guner SN, Keles S, Kurar E, Reisli I. Identification of the Mutation in *DCLRE1C* Gene by PCR-RFLP. Selcuk Med J 2023;39(1): 24-28

Disclosure: *None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this article. The research was not sponsored by an outside organization. All authors have agreed to allow full access to the primary data and to allow the journal to review the data if requested.*

INTRODUCTION

The *DCLRE1C* (DNA Cross-Link Repair 1C) gene is a gene located on the short arm of the tenth chromosome, 47.2 kb in length and consists of 14 exons. This gene encodes a protein called Artemis, which is a nuclease with 5'-3' exonuclease activity on single-stranded DNA (1,2). Artemis also plays an important role in repairing double strand breaks through non-homologous end joining and in V(D) J recombination (3,4). Mutation(s) in the DCLRE1C gene result in functional impairment of Artemis and T/B cell development is adversely affected, cause severe combined immunodeficiency (SCID) resulting in increased sensitivity to ionizing radiation (5,6). Although mutations in this gene result in the SCID phenotype, some hypomorphic mutations in Artemis have been shown to cause combined immunodeficiency (CID) with autoimmunity, granulomatous inflammation, lymphoproliferative disease and malignancy (6,7).

Consanguineous marriages are common in our region. The incidence of primary immunodeficiency (PID), many of which are autosomal recessive, is increasing accordingly. Therefore, rapid screening of mutations in individuals with a family history of consanguineous marriage is of great importance. DNA sequencing methods are widely used in the identification of relevant mutations in these patients. However, considering the cost and duration of these methods, it is not considered appropriate to use them for screening purposes. For this purpose, based on our previous experience, it was thought that the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method might be suitable for detecting *DCLRE1C* mutation (8). From this point of view, we aimed to use a fast, reliable and low-cost method of mutation in exon 3 (c.194C>T; p.T65I) and exon 14 (c.1669_1670insA; p.T577Nfs*21) previously detected in our patients. It was aimed to determine by the PCR-RFLP method. In addition, the obtained results were confirmed by Sanger DNA sequence analysis.

PATIENS AND METHODS

Patients

The prospective study was carried out between 2017 and 2020. A total of 14 patients with mutations in exon 3 and exon 14 of the DCLRE1C gene, 2 parents and 10 healthy controls were included in the study. Patient recruitment and the studies reported herein were approved by Institutional Review Board (2017/803). Written informed consent was obtained from participating patients' guardians and healthy controls. The methods applied to patients and controls are shown in Figure 1.

PCR and PCR-RFLP

PCR primers were designed to cover mutation sites in exon 3 (forward primer, 5'-GTTAGTCACCAAGATGGCTCATT-3' and reverse primer, 5'-GGCTCGTTAACAACAACTCCT-3') and exon 14 (forward primer, 5'-GGCTGGGACAGCCAATCAGATA-3' and reverse primer, 5'- AGAGTAAGTATCCTTTGGG-3).

A PCR protocol for exon 3 was used and cycling conditions were initial denaturation of 94°C for 6 minutes followed by 30 cycles of 94°C for 30 seconds, annealing beginning at 66°C for 30 seconds and 72°C 30 seconds. A final extension of 72°C for 10 minutes was applied. Resulting PCR products were visualized by 2% agarose gel. PCR-RFLP method was applied to PCR products. The PCR products were incubated for 1 hour at 37°C with DdeI (New England Biolabs®Inc.).

A PCR protocol for exon 14 was used and cycling conditions were initial denaturation of 94°C for 6 minutes followed by 30 cycles of 94°C for 10 seconds, annealing beginning at 59,6°C for 100 seconds and 72°C 10 seconds. A final extension of 72°C for 10 minutes was applied. Resulting PCR products were visualized by 2% agarose gel. PCR-RFLP method was applied to PCR products. The PCR products were incubated for 1 hour at 37°C with PstNI (New England Biolabs®Inc.).

Sanger DNA Sequence Analysis

The 3 genotypes determined by the RFLP method were confirmed by DNA sequencing. The mutation

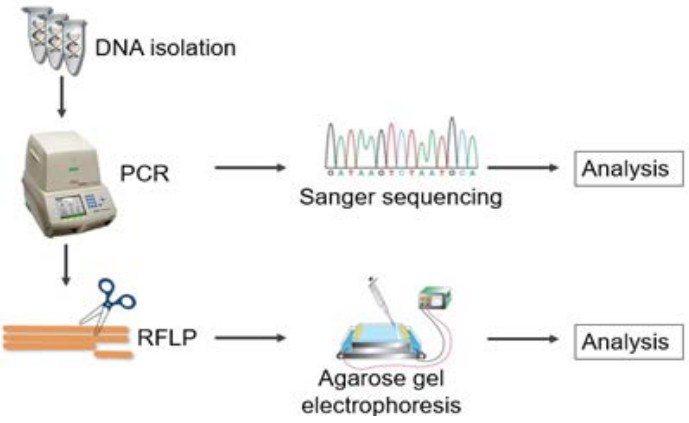

Figure 1. The methods applied to patients and controls

Table 1. Laboratory findings of patients with mutations (*: Patients with exon 14 mutations)

Patients	Age of onset	ALC (cell/µl)	B cell (cell/µl)	T cell (cell/µl)	CD4 T cell (%)	CD8 T cell (%)	Ig (mg/dl)
P1	3 years	1000	40	680	34	26	IgG:892; IgM:944; IgA:23
P2	4 years	6000	480	2940	6,2	1,2	IgG:1710; IgM:159; IgA:6.6
P3	2 years	2000	16	540	4,9	6,7	IgG:135; IgM:15; IgA:25
P4	4 years	200	0	13	6	52	IgG:240; IgM:127;IgA:6
P5	5 years	1240	13	603	6,3	6,2	IgG:1040;IgM:20.9;IgA:24
P6	5 years	900	36	558	23	27	IgG:560;IgM:54;IgA:19
P7	4 years	1500	85	820	19	18	IgG:450;IgM:52;IgA:20
P8	9 years	2440	24	2025	22	36	IgG:1440;IgM:86.5;IgA:6.6
P10	2 years	800	22	547	1,4	1,9	IgG:240;IgM:35;IgA:6.6
P16*	6 years	791	31	490	4,3	5,6	IgG:489;IgM:132;IgA:64.3
P17*	3 years	2170	238	1388	12,4	11,9	IgG:1340;IgM:157;IgA:6.6
P28	2 years	1370	123	643	20	38	IgG:65;IgM:113;IgA:6
P45	6 mounts	2000	18	1200	52	19	IgG:202;IgM:46;IgA:25
P46	6 years	400	76	304	57	21	IgG:456;IgM:179;IgA:6

region of the DCLRE1C gene in exon 3 and exon 14 was amplified with the primers used in the RFLP method, and sequence analysis was performed with the Sanger DNA sequence analysis method.

RESULTS

The M/F ratio of the patients included in the study was 5/9. The mean age of 14 patients was 4±2.17 years. Ten individuals were included in the study as healthy controls, and the M/F ratio was 5/5 and the mean age was 5±2.01 years. The laboratory and clinical findings of the patients are shown in Table 1 and Table 2.

PCR-RFLP

As a result of PCR-RFLP analysis, 12 patients were homozygous mutant for exon 3, 2 parents were heterozygous for exon 3, and 2 patients were heterozygous for exon 3 and exon 14 and were found to be compound heterozygous genotype.

DNA amplicons of 117 bp were obtained after PCR amplification of exon 3 mutation region. DdeI enzyme recognition region 5'….C^TNAG….3'; 3'….GANT^C….5' is selected according to the wild type allele. In the absence of mutation, enzyme digestion was performed in both alleles 94 and 23 bp DNA fragment was obtained after PCR-RFLP and these results were interpreted as normal homozygous genotype (C/C). If the patient is homozygous mutant genotype (T/T) 117 bp DNA fragment was obtained. In the case of heterozygote (C/T), three DNA fragments will be obtained: 117 bp, 94 bp and 23 bp. Accordingly, 12 patients were found to be homozygous mutant, 2 parents heterozygous, and 10 healthy controls homozygous wild genotype (Figure 2).

Table 2. Clinical features at admission of patients with mutations (*: Patients with exon 14 mutations)

Patients	Age of onset	Clinical features at admission
P1	3 years	Recurrent infections, anal wart
P2	4 years	Recurrent infections
P3	2 years	Recurrent infections, unidentified skin lesions, aphthous stomatitis
P4	4 years	Recurrent infections, unidentified skin lesions
P5	5 years	Recurrent infections
P6	5 years	Verruca vulgaris infection
P7	4 years	Recurrent infections
P8	9 years	Recurrent infections, severe varicella infection, Verruca vulgaris infection
P10	2 years	Verruca vulgaris, mycobacterial skin infection
P16*	6 years	Recurrent infections, granulomatous skin lesions, vitiligo
P17*	3 years	Recurrent infections, disseminated varicella infection, vitiligo
P28	2 years	Recurrent infections
P45	5 years	Recurrent infections
P46	6 years	Recurrent infections

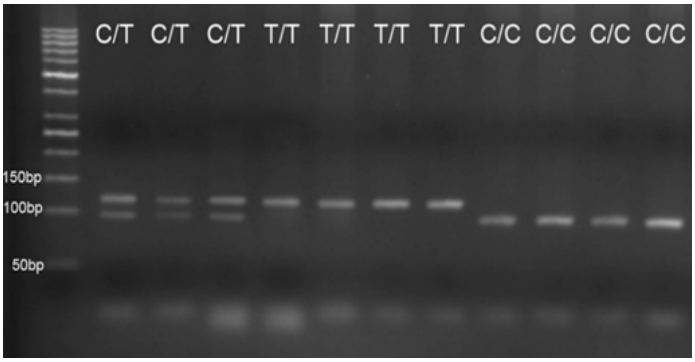

Figure 2. RFLP result of 3 different genotypes for DCLRE1C gene exon 3. (C/T): Heterozygous, (T/T): Homozygous mutant, (C/C): Homozygous normal.

DNA amplicons of 157 bp were obtained after PCR amplification of exon 14 mutation region. PstNI enzyme recognition region 5'….CAGNNN^CTG….3'; 3'….CTG^NNNGAC….5' is selected according to the mutant type allele. In the absence of mutation, enzyme digestion was not performed in both 157 bp DNA fragment was obtained after PCR-RFLP and these results were interpreted as homozygous wild genotype. If the patient is homozygous mutant genotype 136 bp and 21 bp DNA fragment was obtained. In the case of heterozygote, three DNA fragments will be obtained: 157 bp, 136 bp and 21 bp. According to these results, 2 patients were heterozygous (-/A) for exon 14 and the other patients were found to be homozygous wild genotype (-/-) (Figure 3).

DNA Sequence Analysis

Mutations identified by PCR-RFLP were confirmed by DNA sequence analysis and the presence of mutations was confirmed.

DISCUSSION

This study showed that RFLP was a fast and safe method for detecting the homozygous and heterozygous mutations in exon 3 (c.194C>T; p.T65I) and exon 14 (c.1669_1670insA; p.T577Nfs*21) previously detected in our patients with DCLRE1C gene defect and was also low-cost method.

Artemis protein, encoded by the DCLRE1C gene, is an endonuclease that plays a critical role in the unfolding of hairpins during V(D)J recombination in T and B cell development (9). In addition to this critical role, Artemis also plays a key role in repairing double-stranded DNA breaks, resulting in increased radiosensitivity as a result of Artemis gene mutation (1,6,10). Although hypomorphic mutations arising

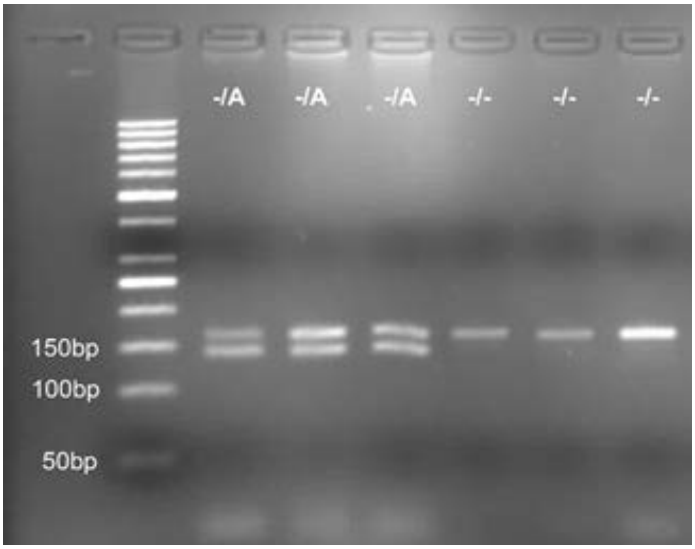

Figure 3. RFLP result of 2 different genotypes for DCLRE1C gene exon 14. (-/A): Heterozygous, (-/-), Homozygous normal.

from the DCLRE1C gene result in decreased V(D)J recombination, mutations that completely abolish Artemis expression or function also seriously affect T and B cell development. Many mutations have been detected in the DCLRE1C gene so far, and SCID is generally seen in patients with this mutation. On the other hand, in some mutations in this gene, patients appear in the CID table (6).

Since 2010, 17 patients with CID due to DCLRE1C mutation have been identified in our center (3,7,8,11). It is noteworthy that this number is higher than other regions in our country. This is due to the prevalence of consanguineous marriages, the geographical characteristics and the ethnic origin of the population in our region. Therefore, it is very important to identify these mutations as soon as possible. Although there are many methods such as DNA sequence analysis, exome analysis, array analysis, RT-RCR and RFLP for the identification of mutations at the DNA level, DNA sequence analysis method is among the most preferred methods. However, the RFLP method, which is less costly, requires less laboratory equipment, and is easier to apply, can be used to identify known mutations. CD19 deficiency, which is a PIY deficiency due to CD19 gene mutation, was defined by us in 2010 (12). Thereupon, a family scan was made with the PCR-RFLP method for this mutation, and the relevant mutation in the CD19 gene was detected (13). Therefore, in our study, the RFLP method based

on the RE cutting logic was preferred. Although the PCR-RFLP method is a reliable method, it is currently used for the identification of some bacterial species (14-16). By using PCR-RFLP method, homozygous mutant in 12 patients, heterozygous in 2 parents and compound heterozygous genotype in 2 patients were detected in DCLRE1C gene exon 3.

As a result, molecular identification of mutations in patients who are thought to be diagnosed with primary immunodeficiency in line with clinical and laboratory findings is of great importance for patients' lives. Because hematopoietic stem cell transplantation is among the life-saving treatments for these patients. Therefore, we believe that the RFLP method can be used as a rapid screening method in such patients, especially in patients with a family history in known mutations.

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

Financial conflict of interest: *Authors declare that they did not receive any financial support in this study.*

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REFERENCES

1. Pannicke U, Hönig M, Schulze I, et al. The most frequent DCLRE1C (ARTEMIS) mutations are based on homologous recombination events. Human Mutat 2010;31(2):197-207.
2. Gerodimos CA, Chang HHY, Watanabe G, et al. Effects of DNA end configuration on XRCC4-DNA ligase IV and its stimulation of Artemis activity. J Biol Chem 2017;292(34):13914-24.
3. Felgentreff K, Lee YN, Frugoni F, et al. Functional analysis of naturally occurring DCLRE1C mutations and correlation with the clinical phenotype of ARTEMIS deficiency. J Allergy Clin Immunol 2015;136(1):140-50.
4. Löbrich M, Jeggo P. A process of resection-dependent nonhomologous end joining involving the goddess artemis. Trends Biochem Sci 2017;42(9):690-701.
5. Darroudi F, Wiegant W, Meijers M, et al. Role of artemis in DSB repair and guarding chromosomal stability following exposure to ionizing radiation at different stages of cell cycle. Mutat Res 2007;615(1):111-24.
6. Lee PP, Woodbine L, Gilmour KC, et al. The many faces of Artemis-deficient combined immunodeficiency-two patients with *DCLRE1C* mutations and a systematic literature review of genotype–phenotype correlation. Clin Immunol 2013;149(3, Part B):464-74.
7. Volk T, Pannicke U, Reisli I, et al. *DCLRE1C* (ARTEMIS) mutations causing phenotypes ranging from atypical severe combined immunodeficiency to mere antibody deficiency. Hum Mol Genet 2015;24(25):7361-72.
8. Karaselek MA, Kapaklı H, Keleş S, et al. Intrauterine detection of *DCLRE1C* (Artemis) mutation by restriction fragment length polymorphism. Pediatr Allergy Immunol 2019;30(6):668-71.
9. Ma Y, Pannicke U, Schwarz K, et al. Hairpin opening and overhang processing by an artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. Cell 2002;108(6):781-94.
10. Ijspeert H, Lankester AC, van den Berg JM, et al. Artemis splice defects cause atypical SCID and can be restored in vitro by an antisense oligonucleotide. Genes Immun 2011;12(6):434-44.
11. Gul E, Sayar EH, Gungor B, et al. Type I IFN–related NETosis in ataxia telangiectasia and artemis deficiency. J Allergy Clin Immunol 2018;142(1):246-57.
12. Van Zelm MC, Reisli I, Van Der Burg M, et al. An antibody-deficiency syndrome due to mutations in the CD19 gene. N Engl J Med 2006;354(18):1901-12.
13. Karaselek MA, Kapaklı H, Güner ŞN, et al. A family screening of CD19 gene mutation by PCR-RFLP. Eur J Clin Exp Med 2022(2):141-5.
14. Dib C, Fauré S, Fizames C, et al. A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 1996;380(6570):152-4.
15. Ota M, Fukushima H, Kulski JK, et al. Single nucleotide polymorphism detection by polymerase chain reaction-restriction fragment length polymorphism. Nat Protoc 2007;2(11):2857-64.
16. Tarhan G, Kogagöz T, Cesur S, et al. The place and importance of Pcr-Rflp Method in determination of Mycobacteria species in routine laboratory practice. J Adv Biotech Micro 2017;3:77-61.

SELÇUK TIP DERGİSİ
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Araştırma Makalesi / Research Article

Selcuk Med J 2023;39(1): 29-34
DOI: 10.30733/std.2023.01594

NEÜ YAYINEVİ

Clinical and Demographic Features of Twenty-Nine Patients with Psoriatic Arthritis "Sine Psoriasis"

Cilt Bulgusu Olmayan 29 Psöriyatik Artrit Hastasının Klinik ve Demografik Özellikleri

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Geliş Tarihi/Received: *21 January 2023*
Kabul Tarihi/Accepted: *22 February 2023*

Öz

Amaç: Psoriatik Artrit (PsA), sedef hastalığı (PsO) olan hastaların %10-30'unu etkileyen kronik ilerleyici inflamatuar bir hastalıktır. "PsA Sine sedef hastalığı" terimi, "cilt belirtileri olmadan PsA teşhisi konan hastaları" tanımlamak için kullanılır. Bu çalışmada "PsA" "sine psoriasis"in demografik ve klinik özelliklerinin CASPAR kriterlerine göre tanımlanması amaçlandı.
Hastalar ve Yöntem: 2016-2022 yılları arasında CASPAR kriterlerine göre PsA sine psoriasis tanısı alan 29 hasta çalışmaya dahil edildi. Romatizmal hastalığı ve herhangi bir cilt tutulumu olan hastalar çalışma dışı bırakıldı.
Bulgular: Çalışmaya dahil edilen 29 hastanın tamamına CASPAR kriterlerine göre PsA tanısı konuldu. Hastaların 16'sı kadındı. Hastaların ortalama (±SS) yaşı 45±11 idi. Hastaların ortalama (±SD) PsA süresi 6,2±3,0 yıldı. PsA'lı hastaların birinci derece akrabalarında psoriazis öyküsü %54,1; ikinci derece akrabalarında psöriazis öyküsü ise %45,9 olarak saptandı. Hastaların %39,4'ünde poliartiküler, %35,7'sinde oligoartiküler, %24,9'unda aksiyal tutulum vardı. Tüm hastaların 19'unda (%65,5) DİP tutulumu mevcuttu. Hastaların %88.9'unda tırnak bulguları mevcuttu. Ayrıca hastaların 17'sinde (%58,6) entezit, 18'inde (%62) daktilit saptandı.
Sonuç: PsA'yı düşündüren klinik semptom ve bulguları olan ve ailede psöriazis öyküsü olan hastalar, PsA sine psoriasisi olarak sınıflandırılabilir. Daktilit ve DIP artritli hastalar, ailesel sedef hastalığı PsA'nın bir alt grubunu temsil edebilir.

Anahtar Kelimeler: Sedef hastalığı, psoriatik artrit, distal interfalangeal eklem

Abstract

Aim: Psoriatic Arthritis (PsA) is a chronic progressive inflammatory disease that affects 10-30% of patients with psoriasis (PsO). The term "PsA sine psoriasis" is used to describe "patients diagnosed with PsA without skin manifestations". In this study, it was aimed to define the demographic and clinical features of "PsA" "sine psoriasis" according to CASPAR criteria.
Patients and Methods: Twenty-nine patients diagnosed with PsA sine psoriasis according to CASPAR criteria between 2016-2022 were included in the study. Patients with rheumatic diseases and any skin involvement were excluded from the study.
Results: All twenty-nine patients included in the study were diagnosed with PsA according to the CASPAR criteria. 16 of the patients were female. The mean (±SD) age of the patients was 45±11 years. The mean (±SD) PsA duration of the patients was 6.2±3.0 years. A history of psoriasis in the first-degree relatives of patients with PsA was 54.1%; A history of psoriasis in second-degree relatives was found in 45.9%. 39.4% patients had polyarticular, 35.7% had oligoarticular, 24.9% had axial involvement. DIP involvement was present in 19 (65.5%) of all patients. Nail findings were present in 88.9% of the patients. Besides, enthesitis was detected in 17 (58.6%) and dactylitis was in 18 (62%) of patients.
Conclusion: Patients with clinical symptoms and findings suggestive of PsA and a family history of psoriasis can be classified as PsA sine psoriasis. Patients with dactylitis and DIP arthritis, familial psoriasis may represent a subgroup of PsA.

Key words: Sine psoriasis, psoriatic arthritis, psoriasis, distal interphalangeal joint

Cite this article as: Karabulut Y, Kurtulus D, Saritas F, Gucenmez S, Yilmaz Z, Simsek S, Esen I. Clinical and Demographic Features of Twenty-Nine Patients with Psoriatic Arthritis "Sine Psoriasis" Selcuk Med J 2023;39(1): 29-34

Disclosure: *None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this article. The research was not sponsored by an outside organization. All authors have agreed to allow full access to the primary data and to allow the journal to review the data if requested.*

INTRODUCTION

Psoriatic Arthritis (PsA) is a chronic progressive inflammatory disease that affects 10-30% of patients with psoriasis (PsO) (1). Since joint damage occurs in more than half of untreated cases, the importance of diagnosing and treating the disease is increasing (1). In 60-70% of psoriatic arthritis (PsA) cases, psoriasis precedes arthritis. Since there is no specific test for the diagnosis of the disease, the presence of skin lesions is of great importance in the diagnosis (1,2). In approximately 20% of PsA patients, joint involvement may precede skin involvement. There may be delays and difficulties in the diagnosing PsA when there is no psoriatic skin lesions and the patient is not aware of skin lesion history. The term "PsA sine psoriasis" is used to describe "patients diagnosed with PsA without skin manifestations" (3). Does the definition of “PsA sine psoriasis” refer to a patient with typical PsA who has not yet developed skin psoriasis but will eventually develop if followed long enough or a patient who may have latent psoriasis that cannot be detected during clinical evaluation or only a patient with psoriasis in his family? These questions remain unanswered. A positive family history of psoriasis may be a helpful clue when diagnosing ‘’PsA sine psoriasis". It is important to identify patients who could benefit from approved but expensive new treatments for PsA. In particular, the questions remain unanswered whether the two SpA subtypes, namely peripheral SpA and PsA sine psoriasis, should be grouped together and ultimately treated in the same way or considered as two separate diseases. On the other hand, without psoriatic skin lesions, PsA can be diagnosed according to Classification Criteria For Psoriatic Arthritis (CASPAR) criteria (4). The diagnosis of PsA can be made using imaging methods, familial history of psoriasis, joint involvement features, enthesitis, dactylitis, and nail findings, and laboratory tests performed to exclude similar diseases (4).

This study aimed to retrospectively define the demographic and clinical features of “PsA sine psoriasis” patients diagnosed with PsA according to CASPAR criteria.

PATIENTS AND METHODS

Twenty-nine patients diagnosed with PsA sine psoriasis according to CASPAR criteria between 2016-2022 were included in the study. Patients older than 18 years of age, no signs of psoriatic skin disease, diagnosed as PsA according to CASPAR criteria, had a history of psoriasis in a first or second-degree relative, and without other rheumatic diseases were included in the analysis.

Sociodemographic characteristics, PsA duration, PsA domain findings, family history of rheumatic disease or PsO, radiological and laboratory findings, treatment history and comorbidities were recorded retrospectively. CASPAR criteria were used to for diagnosing PsA. Ethics committee approval was obtained from Ankara Medical Park Hospital Ethics Committee (dated: 07.12.2022, decision number: E2-22-2987).

Statistical Analysis

Statistical analysis was performed using SPSS Statistics for Windows, Version 23.0 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). The conformity of numerical variables for normal distribution was examined by visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). Descriptive statistics were expressed by mean ±standard deviation or median and interquartile range (IQR) according to distribution of the numeric variables and percentage [n(%)] for categorical variables. As the type of this study was a descriptive study, we did not make any statistical inference.

RESULTS

All twenty-nine patients included in the study were diagnosed with PsA according to the CASPAR criteria. 16 (55.2%) of the patients were female. The mean age was 45±11 years. The mean PsA duration was 6.2±3.0 years. All patients had either first or second-degree family history of psoriasis. 8 (27.6%) of the patients were consulted to the rheumatology clinic from other clinics (orthopedics, physiotherapy, internal medicine, etc.). The percentage of patients who applied directly to the rheumatology outpatient clinic was 21 (72.4%). None of the patients had any signs of psoriasis. No patients had a personal history of psoriasis. (Table 1)

Considering the articular involvement distribution: 11(39.4%) polyarticular, 10 (35.7%) oligoarticular, 7 (24.9%) axial involvement patterns and 19 (65.5%) DIP involvement. Human leukocyte antigen (HLA-B27) was studied in 14 of 29 patients and was positive in only three patients. Erythrocyte Sedimentation Rate (ESR, mm/h) and C-reactive protein (CRP, mg/dl) were high in all patients. Nails were affected in 24/27 (88.9%) of the patients (64% toe nails, 36% fingernails). The most common nail sign was pitting. The association of DIP-nail involvement was remarkable, nail involvement

Table 1. Demographic and Clinical Features Patients with Psoriatic Arthritis “Sine Psoriasis” (n= 29)

Age (mean±SD) (years)		45±11
Female, n(%)		16 (55.2)
Family History of psoriasis (%)		100
PsA duration (mean±SD) (years)		6.2±3.0
Articular Involvement Joint Pattern n(%)		(n=28)
	Polyarticular	11 (39.4)
	Oligoarticular	10 (35.7)
Axial		7 (24.9)
Distal Interphalangeal Involvement n(%)		19 (65.5)
Dactylitis n(%)		18 (62)
Enthesitis n(%)		17 (58.6)
Uveitis n(%)		1 (3.4)
Nail Involvement n(%)		24/27 (88.9)
Inflammatory Bowel Disease n(%)		-
Comorbidity number >2 n(%)		14 (48.3)
Erythrocyte Sedimentation Rate (ESR) (mean±SD) mm/h		47±11
C-reactive protein (CRP) (mean±SD) mg/dl		12±3
HLA B27a n(%) (+)		3/14 (21)
RFb n(%) (+)		-
Anti-CCPc n(%) (+)		-
ANAd n(%) (+)		-

a Human leukocyte antigen (HLA) B27
b Rheumatoid factor
c Anti-Cyclic Citrullinated Peptide Antibody
d Antinuclear Antibodies

was observed in all cases with DIP involvement.

Enthesitis was detected in 17/29 (58.6%) cases. Achilles the plantar region was the most commonly affected enthesis regions. Enthesitis was diagnosed with physical examination in 13 of 17 patients, 3 with USG and 1 with MRI. 12 of 17 patients had enthesitis in multiple regions (such as patellar ligament and iliac crest). Dactylitis was detected in 18 (62%) of patients' history and current examination findings. Nail findings were observed in 16 (88.8%) of patients with dactylitis. In addition, these patients have DIP joint involvement. Obesity, hyperlipidemia, hypertension, and diabetes mellitus were the most common comorbidities when considering the frequency of co-morbidities in the patients. 14/29 (48.3%) of the patients had two or more comorbidities. Antinuclear Antibodies (ANA), Anti-Cyclic Citrullinated Peptide Antibody (Anti-CCP), Rheumatoid factor (RF), were all negative in all patients.

Table 2. Drug Use in Patients with Psoriatic Arthritis “Sine Psoriasis” (n= 29)

NSAID1 n(%) (%)	29 (100)
PRD2 n(%) (%)	16 (55.1)
csDMARD3 n(%) (%)	15 (51.7)
Anti-TNFs 4 n(%) (%)	14 (48.3)
Non-TNF Biologics n(%) (%)	5 (17.2)

1 Non-Steroidal Anti-Inflammatory Drugs
2 Prednisolone below 7.5 mg/day
3 Conventional synthetic disease modifying antirheumatic drugs
4 Tumor Necrosis Factor (TNF) Inhibitors

Intra-articular steroid injection, and systemic steroids below 7.5 mg/day were used in appropriate cases. Conventional DMARDs in 15 (51.7)%, tumor necrosis factor (TNF) inhibitors (anti-TNFs) in 14 (48.3%), and non-TNF biologics in 5 (17.2%) of patients were used in the management of PsA.

DISCUSSION

The relationship between the onset of skin and joint complaints in patients with psoriatic arthritis (PsA) has always been a matter of interest (5). Considering that joint involvement may precede the onset of rash in 10-20% of cases, ‘’PsA sine psoriasis” should not be a rare diagnosis. Using data from the COMOSPA and PerSpA studies, the prevalence of PsA sine psoriasis was calculated to range from 3.1% to 5.5% of all SpA (3). From the perspective of rheumatologists, some findings suggest psoriatic arthritis (PsA) even if the patient does not have psoriasis. According to the CASPAR criteria, the presence of psoriasis is not mandatory for the diagnosis of PsA (3,4).

Few studies have evaluated the demographic characteristics of patients with PsA sine psoriasis. In a study comparing peripheral SpA (pSpA) and PsA sine psoriasis, the mean age of patients with pSpA was 32.8 - 42.2 (6). In studies of PsA sine psoriasis involving 20, 57, and 100 patients, respectively, the

age of onset was similar (7). In our study, the patients' mean age was also similar to the studies mentioned. Looking at the distribution of males and females using the same studies, the prevalence of male sex in PsA sine psoriasis varied between 20-55% (7). In our study, the female-male ratio was also similar.

HLA-B27 is the shared genetic factor for SpA subtypes. The prevalence of HLA-B27 in pSpA ranges from 27 to 62.3% (6). In PsA, data from the PerSpA study confirmed the lower prevalence of HLA-B27 at 18.2%, similar to our study. In other studies, conducted in patients with psoriasis, HLA-B27 was associated with early psoriasis and PsA onset; HLA-Cw6 was found to be associated with delayed onset of PsA (8). In addition, HLA-B27 was associated with axial involvement and symmetrical sacroiliitis in PsA, while it was negatively associated with a family history of psoriasis (9). Scarpa et al. studied 57 patients (31 female, 26 male) with undifferentiated spondyloarthritis in their study on the clinical and genetic aspects of PsA sine psoriasis (10). The outcome of the study was that the subset of PsA sine psoriasis was defined by dactylitis and/or DIP arthritis, HLA Cw6, and a family history of psoriasis (10). Although the HLA Cw6 antigen could not be examined in our study, family history of DIP arthritis, lower extremity dactylitis, and PsA sine psoriasis was observed as a clinical pattern frequently found in cases (11). In addition, HLA-B27 was studied in 14 of 29 patients in the study group, and it was found to be positive in only 3 of them, and 3 of the cases were cases with axial involvement. In the literature, while HLA-B27 in patients with peripheral involvement did not show a significant increase, HLA-B27 positivity was detected in 45% of patients with axial involvement (3).

In current literature on PsA sine psoriasis, 85.7% of the cases have arthritis, 62-75% dactylitis, and 35-55% enthesitis (12). In PsA sine psoriasis, the small joints of the hands and feet are dominantly affected which is different from pSpA which mainly involves the large joints of the lower extremities. In our study, 35.7% patients had oligoarticular involvement and 39.4% had polyarthritis; small joints were affected more than large joints. The association of asymmetric oligoarticular involvement and dactylitis was also remarkable in our patients.

24.9% of our patients had axial involvement. Although it may be difficult to distinguish patients with PsA axial involvement from ankylosing spondylitis, the presence of cervical involvement without lumbar involvement, the presence of asymmetric sacroiliitis and the presence of non-marginal coarse syndesmophytes in the vertebrae may help in the differentiation (3). Peripheral involvement may or may not be present in PsA with axial involvement. Three of the patients in our study group had axial and peripheral involvement together with entheseal involvement.

In the literature, DIP arthritis predominates in approximately 15% of all PsA cases (12). Although it is stated that DIP involvement is common in PsA sine psoriasis cases, the rate is not stated (13). In our study, about 65.5% of patients had DIP involvement accompanying the oligoarticular and polyarticular joint involvement. Besides, nail involvement is very common and predictive of PsA (6,14,15). In the literature, nail changes were observed in 88% of PsA sine psoriasis, similar to our study (16,17). Unlike skin lesions, there is a close relationship between nail changes and DIP joint involvement (6,12,16). Nail finding was one of the important findings that contributed to the diagnosis since there were no skin findings in our PsA sine psoriasis patients. The association of DIP-nail involvement was remarkable in our group, and nail involvement was observed in all cases with DIP involvement in our series. According to current classification criteria, patients with PsA “sine psoriasis” are included in the broad spectrum of the undifferentiated SpA or peripheral SpA subset (3,6,18). It can be thought that the diagnosis of PsA "sine psoriasis" may be easier if dactylitis and DIP involvement, which are two features of classical PsA, are found in patients with a first and/or second-degree family history in the patient group diagnosed with undifferentiated Spa or pSpA (3,12). In their study, Oliveri et al. (7) reported that fifteen patients with PsA sine psoriasis met the Amor criteria for spondyloarthritis and the European Spondyloarthropathy Study Group (ESSG) criteria (11). Patients with peripheral musculoskeletal disease (arthritis or enthesitis, or dactylitis) with and without skin psoriasis may meet both the CASPAR and ASAS pSpA classification criteria. The family history of psoriasis should be absolutely questioned in patients diagnosed with pSpA or undifferentiated SpA according to AMOR and European Spondylarthropathy Study Group (ESSG). When CASPAR criteria are applied to cases with a family history of psoriasis, some patients may be diagnosed with SpA sine psoriasis. Study results of Oliveri et al. suggested that the clinical spectrum of PsA sine psoriasis is as broad as that of PsA.

Enthesitis has a key pathogenic role in PsA (3,19-

21). In the literature, the percentage of enthesitis in PsA sine psoriasis has been reported as 35-55% (3,20-21). Enthesitis was detected in 58.6% of patients in this study most commonly in lower extremities. The rate of enthesitis reported in PsA sine psoriasis cases may be higher than in the literature with the use of MRI, USG and if the enthesitis examination is performed regularly and carefully.

Dactylitis is seen in about 30% of PsA cases. (22-24). Generally, the involvement of the toes is more than the hand (25). The relationship between dactylitis and involvement of DIP joints in PsA patients is remarkable (3,23). In the literature, dactylitis has been reported in 62-75% of PsA sine psoriasis cases. Dactylitis was seen at a rate of 18/29 in the patients included in our study. It can be considered as one of the strong clues leading to the diagnosis of PsA in cases with PsA sine psoriasis, especially in patients with a family history of psoriasis. In our study, it was more common in the lower extremities. Nail findings were observed in 16/18 of the patients with dactylitis. In addition, almost all these patients have DIP joint involvement.

Ocular involvement is seen in 7-33% of PsA patients (3,23). In contrast to AS and enteropathic arthritis; bilateral, posterior, insidious, and chronic uveitis can be seen in psoriatic arthritis. The percentage of uveitis in PsA sine psoriasis has been reported to be 2-25% (3,25). Chronic anterior uveitis was observed in 1 of 29 patients in our study group. The patient also had psoriasis in his first-degree relatives, and DIP involvement, HLA-B27 negative, and nail findings were remarkable. Inflammatory Bowel Disease (IBD), which is well known to be associated with SpA, was not detected in our series. There is no IBD rate reported in the literature in patients with PsA sine psoriasis.

It has been reported that comorbidities such as hypertension, diabetes mellitus, cardiovascular disease, metabolic syndrome, and obesity were all frequent in patients with psoriasis and psoriatic arthritis (3,25-27). In 48.3% of the patients included in our study, two or more comorbidities accompanied the diagnosis of PsA. The most common comorbidities were hypertension, diabetes mellitus, obesity, and cardiovascular disease.

There are no diagnostic laboratory tests for PsA. Although RF and anti-CCP negativity seem to be distinctive laboratory features, low titer RF positivity was found in 5-16% of patients in recent studies (3,28). In all patients in our study, ANA, anti-CCP, RF were negative.

The aim of treatment in patients with PsA is to improve the quality of life, provide symptom control, prevent structural damage, and minimal disease activity or remission (28). Biologic drugs can be considered in case of unresponsiveness to conventional DMARD treatment during PsA treatment (29-31). In our study treatment choices were parallel to current guidelines.

The limited number of patients and the short follow-up period can be said to be the main limitations of our study. We think that studies with longer follow-up period examining the characteristics of PsA sine psoriasis patients in different ethnic and geographical regions are needed.

CONCLUSION

This study provides one of the largest Sine Psoriasis patients data with recent classification criteria. Findings of present study proves the importance of anamnesis. The set of PsA classification criteria proposed in 2006, with better specificity and sensitivity than those previously published, allows the classification of the disease in the absence of psoriasis if typical PsA findings are present. A first- or second-degree relative of psoriasis patients without skin lesions will help the diagnosis. Patients with clinical symptoms and findings suggestive of PsA and a family history of psoriasis can be classified as PsA sine psoriasis. In this spectrum, patients with dactylitis and DIP arthritis, familial psoriasis may represent a subgroup of the disease.

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

Financial conflict of interest: *Authors declare that they did not receive any financial support in this study.*

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REFERENCES

1. Veale D, Rogers S, Fitzgerald O. Classification of clinical subsets in psoriatic arthritis. Br J Rheumatol 1994;33:133-8.
2. Gladman DD. Clinical features and diagnostic considerations in psoriatic arthritis. Rheum Dis Clin North Am 2015;41:569-79.
3. Ziade N, Bou Absi M, Baraliakos X. Peripheral spondyloarthritis and psoriatic arthritis sine psoriase: Are we dealing with semantics or clinically meaningful differences? RMD Open 2022;8(2):e002592.
4. Chimenti MS, Triggianese P, Salandri G, et al. A multimodal

eye assessment in psoriatic arthritis patients sine-Psoriasis: Evidence for a potential association with systemic inflammation. J Clin Med 2020;9(3):719.
5. Ogdie A, Langan S, Love T, et al. Prevelance and treatment patterns of psoriatic arthritis in the UK. Rheumatology 2013;52:568-75.
6. del Río-Martínez P, Navarro-Compán V, Díaz-Miguel C, et al. Similarities and differences between patients fulfilling axial and peripheral ASAS criteria for spondyloarthritis: Results from the Esperanza cohort. Semin Arthritis Rheum 2016;45:400-3.
7. Olivieri I, Padula A, D'Angelo S, et al. Psoriatic arthritis sine psoriasis. J Rheumatol Suppl 2009;83:28-9.
8. Scarpa R, Cosentini E, Manguso F, et al. Clinical and genetic aspects of psoriatic arthritis "sine psoriasis". J Rheumatol 2003;30:2638-40.
9. Eder L, Haddad A, Rosen CF, et al. The incidence and risk factors for psoriatic arthritis in patients with psoriasis: A prospective cohort study. Arthritis Rheumatol 2016;68:915-23.
10. Queiro R, Sarasqueta C, Belzunegui J, et al. Psoriatic spondyloarthropathy: A comparative study between HLA-B27 positive and HLA-B27 negative disease. Semin Arthritis Rheum 2002;31:413-8.
11. FitzGerald O, Haroon M, Giles JT, et al. Concepts of pathogenesis in psoriatic arthritis: Genotype determines clinical phenotype. Arthritis Res Ther 2015;17:1-11.
12. López-Medina C, Molto A, Sieper J, et al. Prevalence and distribution of peripheral musculoskeletal manifestations in spondyloarthritis including psoriatic arthritis: Results of the worldwide, cross-sectional ASAS-PerSpA study. RMD Open 2021;7.
13. Ritchlin CT, Kavanaugh A, Gladman DD, et al. Treatment recommendations for psoriatic arthritis. Ann Rheum Dis 2009;68:1387-94.
14. Tillett W, Helliwell P. To lump or split when assessing psoriatic arthritis - not mutually exclusive? J Rheumatol 2020;47:307-9.
15. Stolwijk C, van Onna M, Boonen A, et al. Global prevalence of spondyloarthritis: A systematic review and meta-regression analysis. Arthritis Care Res 2016;68:1320-31.
16. Errichetti E, Zabotti A, Stinco G, et al. Dermoscopy of nail fold and elbow in the differential diagnosis of early psoriatic arthritis sine psoriasis and early rheumatoid arthritis. J Dermatol 2016;43(10):1217-20.
17. Scarpa R, Oriente P, Pucino A, et al. Psoriatic arthritis in psoriatic patients. Br J Rheumatol 1984;23:246-50.
18. Rudwaleit M, van der Heijde D, Landewé R, et al. The assessment of spondyloarthritis international society classification criteria for peripheral spondyloarthritis and for spondyloarthritis in general. Ann Rheum Dis 2011;70:25-31.
19. Cardoneanu A, Burlui MA, Macovei LA, et al. Psoriatic arthritis “sine psoriasis” – A rare form of disease that raises positive and differential diagnosis problems. Ro J Rheumatol 2019;28:151-5.
20. Alinaghi F, Calov M, Kristensen LE, et al. Prevalence of psoriatic arthritis in patients with psoriasis: A systematic review and metaanalysis of observational and clinical studies. J Am Acad Dermatol 2019;80:e19:251-65.
21. Kurd SK, Gelfand JM. The prevalence of previously diagnosed and undiagnosed psoriasis in US adults: Results from NHANES 2003- 2004. J Am Acad Dermatol 2009;60:218-24.
22. Pavlica L, Perić-Hajzler Z, Jovelić A, et al. Psoriatic arthritis: A retrospective study of 162 patients. Vojnosanit Pregl 2005;62:613-20.
23. López-Medina C, Molto A, Claudepierre P, et al. Clinical manifestations, disease activity and disease burden of radiographic versus non-radiographic axial spondyloarthritis over 5 years of follow-up in the DESIR cohort. Ann Rheum Dis 2020;79:209-16.
24. Tillett W, Charlton R, Nightingale A, et al. Interval between onset of psoriasis and psoriatic arthritis comparing the UK clinical practice research Datalink with a hospital-based cohort. Rheumatology 2017;56:2109-13.
25. Gladman DD, Shuckett R, Russell ML, et al. Psoriatic arthritis (PSA)-- an analysis of 220 patients. Q J Med 1987;62:127-41.
26. Moltó A, Etcheto A, van der Heijde D, et al. Prevalence of comorbidities and evaluation of their screening in spondyloarthritis: Results of the International cross-sectional ASAS-COMOSPA study. Ann Rheum Dis 2016;75:1016-23.
27. Haroon M, FitzGerald O. Psoriatic arthritis: Complexities, comorbidities and implications for the clinic. Expert Rev Clin Immunol 2016;12:405-16.
28. Mahmood F, Coates LC, Helliwell PS. Current concepts and unmet needs in psoriatic arthritis. Clin. Rheumatol 2018;37:297-305.
29. Congi L, Roussou E. Clinical application of the CASPAR criteria for psoriatic arthritis compared to other existing criteria. Clin Exp Rheumatol 2010;28:304-10.
30. Sepriano A, Rubio R, Ramiro S, et al. Performance of the ASAS classification criteria for axial and peripheral spondyloarthritis: A systematic literature review and meta-analysis. Ann Rheum Dis 2017;76:886-90.
31. Coates LC, Corp N, van der Windt DA, et al. Grappa treatment recommendations: 2021 update. J Rheumatol 2022;49:52-4.

SELÇUK TIP DERGİSİ
SELCUK MEDICAL JOURNAL

Araştırma Makalesi / Research Article

Selcuk Med J 2023;39(1): 35-40
DOI: 10.30733/std.2023.01575

NEÜ YAYINEVİ

Relationship between Juvenile Myoclonic Epilepsy and Melatonin

Juvenil Miyoklonik Epilepsi ve Melatonin İlişkisi

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Geliş Tarihi/Received: *10 December 2022*
Kabul Tarihi/Accepted: *27 February 2023*

Öz

Amaç: Melatoninin sirkadiyen ritmi düzenleyerek antikonvülsan ve nöroprotektif özelliklere sahip olduğu bulunmuştur. Bu etkisinden yola çıkarak Juvenil myoklonik epilepsili (JME) hastalar ve sağlıklı bireylerde melatonin serum düzeylerinin belirlenmesi amaçlanmıştır. JME'li hastalar ile sağlıklı bireylerdeki serum melatonin seviyelerini karşılaştıran ilk çalışma olma özelliğini de taşımaktadır.
Hastalar ve Yöntem: Bu çapraz-kesitsel çalışmaya JME'li 30 hasta ve benzer cinsiyet ve yaş dağılımına sahip 30 sağlıklı kontrol dahil edildi. JME'li hastalar ve sağlıklı bireylerden, gece meydana gelen serum melatonin pik düzeyini ve sabahki en düşük serum melatonin düzeyini belirlemek üzere venöz kan örnekleri alınarak ELISA yöntemiyle çalışıldı.
Bulgular: Sağlıklı kontrollere kıyasla JME'li hastaların serum MELn (p = 0,002) ve MELm (p = 0,001) düzeyi daha düşük tespit edildi. Ayrıca JME'li hastalar ve kontrol grubu arasında MELn/MELm oranı ve MELn-MELm farkı açısından istatistiksel olarak anlamlı farklılıklar vardı (sırasıyla p = 0,005 ve 0,014).
Sonuç: Elde edilen sonuçlar JME'li hastalar ve kontrol grubunda melatoninin sirkadien ritminin korunduğunu fakat hastalarda serum melatonin düzeylerinin kontrollere göre düşük seyrettiğini göstermektedir. Bundan dolayı JME'li hastalarda melatonin düzeyinin tespiti hem hastalık etyolojisinin belirlenmesine hem de gerektiğinde melatonin takviyesi yapılması ile gereksiz ve yüksek doz antiepileptik ilaç kullanımının engellenmesine katkı sağlayabilir.

Anahtar Kelimeler: Juvenil miyoklonik epilepsi, melatonin, sirkadiyen ritim

Abstract

Aim: Melatonin has been found to have anticonvulsant and neuroprotective properties by regulating the circadian rhythm. Based on this effect, it was aimed to determine the serum levels of melatonin in patients with juvenile myoclonic epilepsy (JME) and healthy individuals. It is also the first study to compare serum melatonin levels in patients with JME and healthy individuals.
Patients and Methods: Thirty patients with JME and 30 healthy controls with similar gender and age distribution were included in this cross-sectional study. Venous blood samples were taken from patients with JME and healthy individuals to determine the peak serum melatonin level at night and the lowest serum melatonin level in the morning, and studied with the ELISA method.
Results: Compared to healthy controls, serum MELn (p = 0.002) and MELm (p = 0.001) levels of JME patients were determined to be lower than healthy controls. Moreover, there were statistically significant differences between MELn/MELm ratio and MELn-MELm difference between patients with JME and the control group (p = 0.005 and 0.014, respectively).
Conclusion: The results show that the circadian rhythm of melatonin is preserved in the patients with JME and the control group, but the serum melatonin levels in the patients are lower than in the controls. Therefore, the determination of melatonin level in patients with JME may contribute to both the determination of the etiology of the disease and the prevention of unnecessary and high-dose antiepileptic drug use by supplementing with melatonin when necessary.

Key words: Juvenile myoclonic epilepsy, melatonin, circadian rhythm

Cite this article as: Altas M, Genc BO, Kilinc I, Akyol Gurses A. Relationship between Juvenile Myoclonic Epilepsy and Melatonin. Selcuk Med J 2023;39(1): 35-40

Disclosure: *None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this article. The research was not sponsored by an outside organization. All authors have agreed to allow full access to the primary data and to allow the journal to review the data if requested.*

INTRODUCTION

Juvenile myoclonic epilepsy (JME) is an epileptic syndrome that occurs frequently during puberty and is classified separately within idiopathic generalized epilepsies by ILAE, which includes myoclonic jerks on awakening, generalized tonic-clonic seizures, and less frequently absence seizures (1,2). Although the pathophysiological mechanisms are unknown, there is some evidence of mild functional and structural disorders in the frontal lobes, and to a lesser extent extrafrontal (3). Lack of sleep increases the tendency to seizures in most patients (4). Other factors that cause seizures are alcohol intake, menstruation, fatigue, stress, tension, watching television, playing chess-like games, and irregular use of antiepileptic drugs (5). While clinical photosensitivity, that is, seizures triggered by flickering light, is seen approximately in 5%, photo-paroxysmal response is present at a rate of 30-40% in EEG (6).

The relationship between sleep and melatonin is well known. Melatonin, also known as the 'dark hormone', is a chronobiotic hormone synthesized in many organs such as the skin, lymphocytes and gastrointestinal tract, especially the pineal gland, and regulates major physiological processes such as sleep-wake cycle, pubertal development and seasonal adaptation in the body (7). Beyond being the major determinant of circadian rhythm, it is noteworthy that melatonin and analogous compounds using melatonin receptors are increasingly used in the management of depression, insomnia, Alzheimer's disease, diabetes, alopecia, obesity, migraine, cancer, immune and cardiac diseases (8,9). However, there are different data in the literature regarding the pathophysiology of epilepsy and the role of melatonin in its complementary treatment. In studies on epilepsy and melatonin, the relationship between different forms of epilepsy and circadian rhythm have been studied, and melatonin has generally been used as a determinant of the circadian rhythm (10,11).

Determining basal melatonin levels in JME patients can contribute to the understanding of JME pathophysiology and contribute to additional treatment modalities, however, no study has reported comparison of serum melatonin levels in patients with JME and healthy individuals in the literature.

In this study, it was aimed to determine melatonin levels in JME.

PATIENTS AND METHODS

The necessary ethics approval of the present study was obtained from the ‘’Institutional Ethics Committee of ’Institutional Ethics Committee of Meram Medical Faculty, Necmettin Erbakan University’’ enumerated as 2015/347 and dated as December 04th, 2015. A written consent form was then filled out by all participants, who were given in-depth information about the study that was to be conducted. The present study was conducted in accordance with Helsinki good clinical practice guidelines. (GCPG)

Participants

Between January 2016 and December 2016, JME patients who were admitted for control purposes were considered for this prospective study. Out of this epilepsy patient population, 30 consecutive JME patients were selected in accordance with the inclusion criteria. Healthy controls consisted of healthy individuals, mostly university hospital employees, without a history of epilepsy matching positively with JME patients age and sex criteria, and meeting all inclusion criteria. Neurological and physical examinations of patients diagnosed with JME were performed by the same neurologist in order to avoid evaluation bias. Demographic and clinical characteristics of the patients were recorded.

All patients in the JME group were receiving anti-epileptic drugs (AEDs) on daily basis administered orally. Out of these were valproic acid (VPA) (18 patients); levetiracetam (LEV) (7 patients); lamotrigine (LTG) (4 patients), and zonisamide (ZNS) intake (1 patient). The distribution of AED use of JME patients is presented in Table 1.

The inclusion criteria for the JME patient group were as follows; voluntary enrolment; age between 18 and 45 years; JME diagnosis as described in the 1989 revision to the ILAE criteria for clinical information and in EEG tests, lack of any chronic (mental retardation, insomnia, ie) or acute medical condition (status epilepticus, head injury, ie) other than JME as confirmed through previous medical reports and clinic examinations; no use of medications including antiaggregants, anticoagulants, beta blockers, non-steroidal anti-inflammatory drugs, products containing melatonin, corticosteroids, selective serotonin re-uptake inhibitors and antipsychotics; and no previous report of illicit drug or substance abuse or addiction. In addition, the participants were warned that sleep deprivation, excessive exercise, caffeine and alcohol use should be avoided for 72 hours before sampling, as it may affect melatonin levels and cause deviations from the basal value.

Table 1. The demographic, clinical and laboratory characteristics of patients and controls

	JME Patients (n=30)	Controls (n=30)	p value
Age, years, median [25th, 75th]	24.0 [20.0, 28.7]	24.0 [21.5, 29.0]	0.672*
Age of onset, years, median [25th, 75th]	16.5 [13.0, 20.2]		
Disease duration, years, median [25th, 75th]	7.0 [2.7, 10.2]		
Gender			
Male, n, %	16, 53.3%	16, 53.3%	1.000**
Female, n, %	14, 46.7%	14, 46.7%	
MELn (pg/mL), median [25th, 75th]	74.5 [53.7, 441.2]	590.0 [107.2, 760.5]	0.002*
MELm (pg/mL), median [25th, 75th]	35.5 [25.7, 226.2]	324.5 [78.2, 324.5]	0.001*
MELn / MELm, median [25th, 75th]	2.0 [1.8, 2.1]	1.7 [1.6, 1.9]	0.005*
MELn – MELm, median [25th, 75th]	39.5 [28.0, 205.7]	267.0 [38.5, 406.0]	0.014*
AED use			
VPA, n, %	18,60.0%		
LEV, n, %	7,23.3%		
LTG, n, %	4,13.3%		
ZNS, n, %	1,3.3%		

* Tested using Mann-Whitney Test
** Tested using Pearson's Chi – squared test
Bold: statistically significant results

Measurement of melatonin

Venous blood samples were taken from JME patients and healthy individuals at 03:00 under dim light to determine the peak serum melatonin level at night, and at 10:00 to determine the lowest serum melatonin level in the morning. The blood samples were centrifuged at 4°C and 1000 g speed for 10 minutes in a Hettich Rotina 46R (Hettich Zentrifugen, Tuttlingen, Germany) brand refrigerated centrifuge device, and the serums were separated. Serum samples were stored in New Brunswick U570 (New Brunswick Scientific, New Jersey, USA) refrigerator at -80°C until the parameters were studied. Melatonin (YH Biological Technology Company, Shanghai, China) levels in serum samples were studied by ELISA (Enzyme-linked immunosorbent assay) method. Serum melatonin levels were calculated according to the absorbance concentration calibration charts using the Biotek ELX 50 microplate washer (BioTek Instruments, Vermont, USA) and the Biorad Microplate absorbance reader xMark (Bio-Rad Laboratories, California, USA) system.

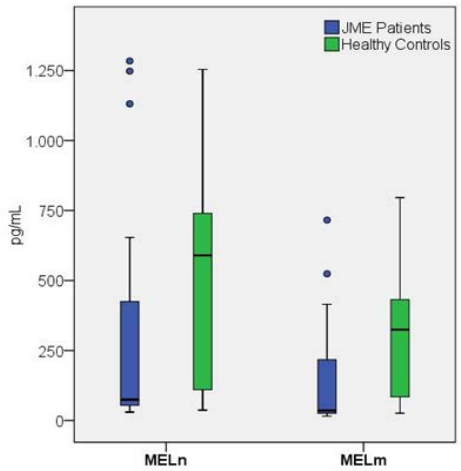

Figure 1. Median levels of MELn and MELm in patient and control groups.

Statistical analysis

The data obtained in the present study were analyzed using IBM SPSS statistics software version 20.0 (IBM Corp., Armonk, NY, USA). Using the Kolmogorov-Smirnov Test, normality was checked for each continuous variable. Non-normally distributed continuous variables were expressed as median [25th, 75th percentiles]. Categorical variables were expressed as numbers and percentages. The Mann–Whitney Test was used to compare non-normally distributed continuous variables between two independent groups. Categorical data between two groups were compared using Pearson's Chi-Square Test. A 2-tailed $p<0.05$ threshold value was considered for statistical significance.

RESULTS

The median [25th, 75th percentile] age of the 30 JME patients was 24.0 [20.0, 28.7] years; 14 (46.7%) of them were female and 16 (53.3%) were male. The median [25th, 75th percentile] age of the 30 healthy controls was 24.0 [21.5, 29.0] years; 14 (46.7%) were female and 16 (53.3%) were male. There were no statistically significant differences between the JME patients and those in the control group in terms of age and gender ($p = 0.672$ and 1.000, respectively).

Demographic and clinical characteristics of the participants are presented in Table 1.

The median [25th, 75th percentile] MELn level was 74.5 [53.7, 441.2] (pg/mL) in the JME patients, and 590.0 [107.2, 760.5] (pg/mL) in the control group. The median [25th, 75th percentile] MELm level was 35.5 [25.7, 226.2] (pg/mL) in JME patients and 324.5 [78.2, 324.5] (pg/mL) in the control group. There were statistically significant differences in MELn and MELm levels between the JME patients and control group (p = 0.002 and 0.001, respectively) (Figure 1). MELn and MELm levels for JME patients and control participants are presented in Table 1.

The median [25th, 75th percentile] MELn/MELm ratio was 2.0 [1.8, 2.1] in JME patients, and 1.7 [1.6, 1.9] in the control group. The median [25th, 75th percentile] MELn-MELm difference was 39.5 [28.0, 205.7] in JME patients and 267.0 [38.5, 406.0] in the control group. There were statistically significant differences in terms of MELn/MELm ratio and MELn-MELm difference between JME patients and those in the controls (p = 0.005 and 0.014, respectively) (Table 1).

DISCUSSION

We found statistically significant lower serum melatonin levels measured at night and in the morning in JME patients compared to healthy controls. The data obtained shows that the circadian rhythm of melatonin is preserved in the patient and control groups, but melatonin levels are lower in the patient group. Low melatonin levels in JME patients may play a triggering role by impairing sleep quality, or it may be a result of the existing disease. This is the first study to compare serum melatonin levels in patients with JME and healthy individuals. It is thought that the results of the study may reveal new information about the pathophysiology of JME and the role of melatonin in complementary therapy.

It is clearly known that the hormone melatonin, whose serum levels start to rise with the onset of darkness in mammals and rapidly decrease with the dawn, is closely related to the onset, maintenance, quality of sleep and the determination of the circadian rhythm (12). Decreased melatonin production and disruption of nocturnal melatonin secretion have been associated with various central nervous system diseases such as stroke, obsessive-compulsive disorder, and mood disorders (13). Due to its antioxidant effects, melatonin is used in the treatment of many neurological diseases such as epilepsy, amyotrophic lateral sclerosis, Alzheimer's disease, ischemic injury and head trauma due to its neuroprotective effects (14). Mitochondrial dysfunction and glutamate over activity, in which free radicals are involved in neuronal loss, are associated with disease progression in most of these diseases (8).

Studies of melatonin levels in humans are generally concerned with melatonin hormone physiology, metabolism and its role in the circadian rhythm. In studies related to melatonin epilepsy, the anticonvulsant and/or pro-convulsant role of melatonin and the effects of exogenous melatonin supplementation on the frequency and severity of seizures have been researched (15-18). Only very few studies have compared baseline melatonin levels between epilepsy patients and healthy individuals. In addition, there are great methodological differences between studies. Whereas some of these studies have investigated plasma or serum melatonin levels, others have investigated salivary melatonin levels. In some, the melatonin metabolite excreted in the urine was additionally measured. The biochemical methods used in the measurements of melatonin in the studies also do show a wide variety (such as RIA, ELISA, Atomic absorption, HPLC), and the commercial kits for each different method used also show differences among themselves. For this reason, the information related to basal melatonin levels, which are currently very few in the literature, show great differences among each other.

Low levels of melatonin have been reported in some types of epilepsy, and data obtained from human support that melatonin exerts an anticonvulsant effect by both reducing seizure frequency and improving EEG abnormality. Hence, its combination with other antiepileptic drugs may be beneficial (19). In a case report of a severe myoclonic epilepsy case; it has been reported that seizures that cannot be controlled with combined antiepileptic therapy could be controlled after melatonin is administered combined with phenobarbital (20). Similarly, Fauteck et al. (17) claimed that a single dose of 5-10 mg melatonin given in the evening can reduce the incidence of epileptic attacks in children and that melatonin can be used as an antiepileptic drug. There are also different data on the pathophysiology of epilepsy and the place of melatonin in its complementary treatment. Mahyar A. et al. (15) performed serum melatonin measurements in 37 children with simple febrile seizures, 37 children with complex febrile seizures, 37 children with epilepsy, and 37 children with fever only as controls,

they determined that there was no difference between the children with seizures and those in the control group in terms of serum melatonin levels. In addition, no significant difference was found between the groups in the seizure population (15). On the other hand, Guo et al., (21) reported that serum melatonin levels were low in children with epilepsy or complex febrile seizures, and they suggested that external melatonin supplementation may be beneficial in the treatment of epilepsy and febrile seizures in these children. In the study conducted by Yalin et al., (16) plasma melatonin levels at four different times of the day were compared in 10 participants with diurnal complex partial epilepsy, 10 with nocturnal complex partial epilepsy, and 10 healthy participants, and it was determined that melatonin levels in patients with both nocturnal complex partial epilepsy and diurnal complex partial epilepsy were found to be between 10:00 a.m. and 10:00 p.m. and between 01:00-05:00 a.m. always lower than those in the control groups, and the lowness at 10:00 a.m. was found to be statistically significant (16). In the present study, statistically significant lower serum melatonin levels were measured both at night and in the morning in the JME patients compared to those in the control group. Although melatonin levels were lower in the patient group, it was observed that the circadian rhythm of melatonin was preserved in both our patients and the control groups.

Bazil et al. (22) reported that basal melatonin levels in the saliva of patients with refractory temporal lobe epilepsy were lower than that of the controls' and increased threefold after seizure. They interpreted this finding as the anticonvulsant effects of melatonin (22). In another study in 54 children with convulsive seizures (febrile and epileptic), it was found that serum melatonin levels increased during seizure attacks and returned to normal level after 1 hour (23). Researchers have commented that the increase in melatonin produced by a convulsive seizure may express the response to the seizure in the body and aim to maintain homeostasis (23). However, Schapel et al. (24) reported that urinary 6-sulphatoxymelatonin excretion was increased in 30 untreated epilepsy patients with active epilepsy compared to healthy controls (24). They interpreted this situation as an increase in melatonin production in patients with untreated active epilepsy and phase differences compared to controls (24). On the contrary, Rao et al. (25) determined that serum melatonin levels did not change during and 2 hours after seizures and remained within normal limits as in healthy individuals. The fact that seizures are under control in almost all of our patients with JME and the lack of sleep deprivation in all of them within the last 72 hours eliminates the possibility of postictal melatonin elevation.

The studies mentioned above clarify that the melatonin levels may differ according to the type of epilepsy and it is still not clear whether there is a relationship between these differences in melatonin levels and antiepileptic treatment. Among all epilepsy syndromes, photosensitivity and sensitivity to sleep deprivation are well known in JME patients. Melatonin measurements in JME patients have been mostly conducted within the context of seizure and circadian rhythm relationship and as a determinant of the circadian rhythm (26). In this regard, the present study is the first to directly compare serum melatonin levels in patients with JME and healthy individuals.

The cross-sectional nature of the study and the small patient cohort are an important limitation and may affect the reliability of the present study's findings. There are also some inherent limitations in the present study, such as the fact that the contribution of additional melatonin to reduce the dose of antiepileptic therapy was not evaluated. Melatonin replacement in JME patients, reduction in seizure frequency and the effect of using lower doses in the use of AEDs and its contribution to the transition from combined therapy to mono-therapy may be the subject of further research. The present study excluded the evaluation of melatonin metabolites excreted in the urine. Despite all limitations of the present study, determination of statistically significant lower serum melatonin levels measured at night and in the morning in patients with JME will contribute to the literature both in illuminating the pathophysiology of the disease and in planning treatment management.

CONCLUSION

In this prospective study, it was determined that the melatonin levels of JME patients were lower compared to healthy controls. We suggest that melatonin levels should be studied in patients presenting JME for both determining disease etiology and preventing unnecessary use of high-dose antiepileptic drugs via melatonin supplementation.

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

Financial conflict of interest: *Authors declare that they did not receive any financial support in this study.*

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REFERENCES

1. Fisher RS, Cross JH, French JA, et al. Operational classification of seizure types by the International league against epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017;58:522-30.
2. Nordli DR Jr. Idiopathic generalized epilepsies recognized by the International League Against Epilepsy. Epilepsia 2005;46 Suppl 9:48-56.
3. Kim KY, Moon JU, Lee JY, et al. Distributed source localization of epileptiform discharges in juvenile myoclonic epilepsy: Standardized low-resolution brain electromagnetic tomography (sLORETA) Study. Medicine (Baltimore) 2022;101:e29625.
4. Lin CY, Wu T, Chang CW, et al. Reduced sleep quality is related to poor quality of life in patients with juvenile myoclonic epilepsy, a case-control study. Life (Basel) 2022;12:434.
5. Kleveland G, Engelsen BA. Juvenile myoclonic epilepsy: Clinical characteristics, treatment and prognosis in a Norwegian population of patients. Seizure 1998;7:31-8.
6. Syvertsen MR , Markhus R, Selmer KK, et al. Juvenile myoclonic epilepsy. Tidsskr Nor Laegeforen 2012;132:1610-3.
7. Samanta S. Physiological and pharmacological perspectives of melatonin. Arch Physiol Biochem 2020:1-22.
8. Singh M, Jadhav HR. Melatonin: Functions and ligands Drug Discov Today 2014;19:1410-8.
9. Pandi-Perumal SR, Trakht I, Srinivasan V, et al. Physiological effects of melatonin: Role of melatonin receptors and signal transduction pathways. Prog Neurobiol 2008;85:335-53.
10. Vasileva Z. Melatonin and Epilepsy. Folia Med (Plovdiv) 2021;63:827-33.
11. Quigg M. Circadian rhythms: Interactions with seizures and epilepsy. Epilepsy Res 2000;42:43-55.
12. Lynch HJ, Wurtman RJ, Moskowitz MA, et al. Daily rhythm in human urinary melatonin. Science 1975;187:169-71.
13. Anderson G, Maes M. Interactions of tryptophan and its catabolites with melatonin and the alpha 7 nicotinic receptor in central nervous system and psychiatric disorders: Role of the aryl hydrocarbon receptor and direct mitochondria regulation. Int J Tryptophan Res 2017;10:1178646917691738.
14. Lu J, Luo Y, Mei S, et al. The effect of melatonin modulation of mon-coding RNAs on central nervous system disorders: An updated review. Curr Neuropharmacol 2021;19:3-23.
15. Mahyar A, Ayazi P, Dalirani R, et al. Melatonin's effect in febrile seizures and epilepsy. Iran J Child Neurol 2014;8:24-9.
16. Yalin O, Arman F, Erdogan F, et al. A comparison of the circadian rhythms and the levels of melatonin in patients with diurnal and nocturnal complex partial seizures. Epilepsy Behav 2006;8:542-6.
17. Fauteck J, Schmidt H, Lerchl A, et al. Melatonin in epilepsy: First results of replacement therapy and first clinical results. Biol Signals Recept 1999;8:105-10.
18. Ross C, Davies P, Whitehouse W. Melatonin treatment for sleep disorders in children with neurodevelopmental disorders: An observational study. Dev Med Child Neurol 2002;44:339-44.
19. Petkova Z, Tchekalarova J, Pechlivanova D, et al. Treatment with melatonin after status epilepticus attenuates seizure activity and neuronal damage but does not prevent the disturbance in diurnal rhythms and behavioral alterations in spontaneously hypertensive rats in kainate model of temporal lobe epilepsy. Epilepsy Behav 2014;31:198-208.
20. Molina-Carballo A, Muñoz-Hoyos A, Reiter RJ, et al. Utility of high doses of melatonin as adjunctive anticonvulsant therapy in a child with severe myoclonic epilepsy: Two years' experience. J Pineal Res 1997 ;23:97-105.
21. Guo JF, Yao BZ. Serum melatonin levels in children with epilepsy or febrile seizures. Zhongguo Dang Dai Er Ke Za Zhi 2009;11:288-90.
22. Bazil CW, Short D, Crispin D, et al. Patients with intractable epilepsy have low melatonin, which increases following seizures. Neurology 2000;55:1746-8.
23. Molina-Carballo A, Muñoz-Hoyos A, Sánchez-Forte M, et al. Melatonin increases following convulsive seizures may be related to its anticonvulsant properties at physiological concentrations. Neuropediatrics 2007;38:122-5.
24. Schapel GJ, Beran RG, Kennaway DL, et al. Melatonin response in active epilepsy. Epilepsia 1995;36:75-8.
25. Rao ML, Stefan H, Bauer J. Epileptic but not psychogenic seizures are accompanied by simultaneous elevation of serum pituitary hormones and cortisol levels. Neuroendocrinology 1989;49:33-9.
26. Kasteleijn-Nolst Trenité DG, de Weerd A, Beniczky S. Chronodependency and provocative factors in juvenile myoclonic epilepsy. Epilepsy Behav 2013;28 Suppl 1:S25-9.

SELÇUK TIP DERGİSİ
SELCUK MEDICAL JOURNAL

Olgu Sunumu / Case Report

Selcuk Med J 2023;39(1): 41-43
DOI: 10.30733/std.2023.01604

NEÜ YAYINEVİ

A Rare Case of Porencephaly with Seizure Disorder, Organic Personality Change and Agitated Depression

Nöbet Bozukluğu, Organik Kişilik Değişikliği ve Ajite Depresyon ile Nadir Bir Porensefali Vakası

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Geliş Tarihi/Received: *1 June 2022*
Kabul Tarihi/Accepted: *8 September 2022*

Öz

Porensefali, serebral yarım küredeki kistler/boşluklar ile karakterize bir merkezi sinir sistemi bozukluğudur. Nöbetler, parezi, öğrenme güçlükleri, zeka geriliği ve nadiren psikiyatrik belirtiler gibi çeşitli klinik belirtilere sahiptir. Psikiyatrik belirtiler arasında psikoz en sık görülen belirtidir. Psikiyatrik sendromlarla başvuran porensefali ile ilgili sadece birkaç vaka raporu vardır. Bu vaka sunumunda nöbet bozukluğu, organik kişilik değişikliği ve ajite depresyon ile benzersiz bir porensefali vakasının tartışılması amaçlandı.

Anahtar Kelimeler: Porensefali, organik beyin sendromu, depresyon, olgu sunumları

Abstract

Porencephaly is a disorder of Central nervous system characterized by cysts/ cavities in the cerebral hemisphere. It has varied clinical manifestations like seizures, paresis, learning disabilities, mental retardation and rarely psychiatric manifestations. Among psychiatric symptoms, psychosis is the most commonly seen manifestation. There have been only few case reports of porencephaly presenting with psychiatric syndromes. Hereby it was aimed to discuss a unique case of porencephaly with seizure disorder, organic personality change and agitated depression.

Key words: Porencephaly, organic brain syndrome, depression, case reports

Cite this article as: Rayaji SG, Manju A, Pandit LV, Kshamaa HG. A Rare Case of Porencephaly with Seizure Disorder, Organic Personality Change and Agitated Depression. Selcuk Med J 2023;39(1): 41-43

Disclosure: *None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this article. The research was not sponsored by an outside organization. All authors have agreed to allow full access to the primary data and to allow the journal to review the data if requested.*

INTRODUCTION

Porencephaly is a disorder of central nervous system characterised by cysts/cavities in cerebral hemisphere. It is either congenital or acquired, incidence being 3.5 in 1,00,000 live births (1). The most common clinical manifestations based on localisation are seizures, mental retardation, paresis, learning disabilities and rarely psychiatric symptoms (2). Among psychiatric manifestations, psychosis is most commonly seen and involvement of prefrontal cortex and temporal lobe has been reported.

In this case report, it was aimed to discuss a unique case of porencephaly with seizure disorder, organic personality change and agitated depression.

CASE

Mr. X, a 55 year old male, pre-morbidly well adjusted with no significant family history, presented to psychiatry OPD with 15 years history of multiple episodes of loss of consciousness, lasting for 10-15 minutes followed by confusion and headaches for the next 3-4 hours, last such episode being 2 years ago. Following the onset of these episodes, family members have noticed changes in his behaviour, in the form of decreased interaction with family members, staying aloof and decreased interest in work. He was also not as active as he used to be. The patient, who would handle all finances by himself previously, with respect to calculations, counting and profits was observed taking help of family members for the same since past few years. He also would get irritated with trivial issues in the house like any change in the taste of the food or delay in work done at home which was not his usual self. He was sleeping and eating well during this time. Overall family members felt that he had become dull and restless. Even though he had these behavioural changes, the family members did not feel the need for consultation as his functioning was not impaired, hence treatment was not sought.

Since the past 20 days patient was feeling sad with frequent crying spells, had marked disturbance in sleep and had reduced appetite. Onset of these symptoms was following a stressful situation. Patient had supported his son in contesting for the Gram Panchayat elections and had spent 10 lakh rupees for the campaigning and election work. However, son had lost the elections 20 days back. Following this patient was irritable, fidgeting and was often seen crying alone. He would also talk to himself about how he lost the money and the way people had betrayed him by not voting for his son. He would constantly ruminate about the event and would feel that he should have not asked his son to contest the elections. He was not interested in taking food and would hardly eat anything, and when forced to eat he would get angry and would dismiss the family members. He would not sleep at night, would pace around the house and kept worrying about the elections and the loss. His duration of sleep reduced to around 2-3 hours per day. Patient started getting agitated if family members spoke to him or would ask him to eat food and sleep. This behavior worsened over 10 days for which he was brought for consultation.

On Mental Status Examination, patient was unkempt, restless and cried during the interview; had increased volume, decreased tone of speech and was ruminating about the stressful situation. He appeared agitated and depressed throughout the interview although subjectively he reported that he was fine. He was alert and the concentration was sustained with no memory dysfunction. He was evaluated as an in-patient, where in relevant blood investigations were done, which were within normal limits. In view of his age, onset of the symptoms and previous episodes of loss of consciousness, MRI Brain and EEG was done. MRI Brain revealed existence of a Porencephalic cyst in left temporal occipital region of size 3.6x2.7x2.7. EEG did not show any abnormality. Neurology opinion was sought for the above findings, where in no active

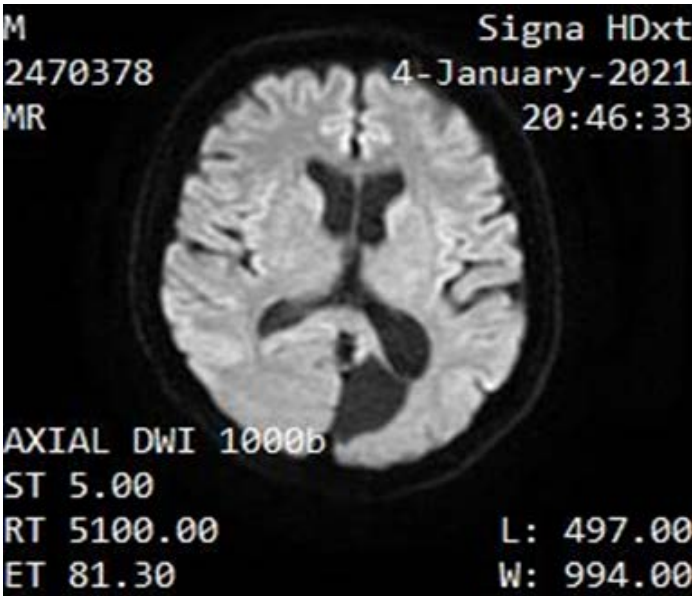

Figure 1. The appearance of a Porencephalic cyst in left temporal occipital region of size 3.6x2.7x2.7 in MR imaging

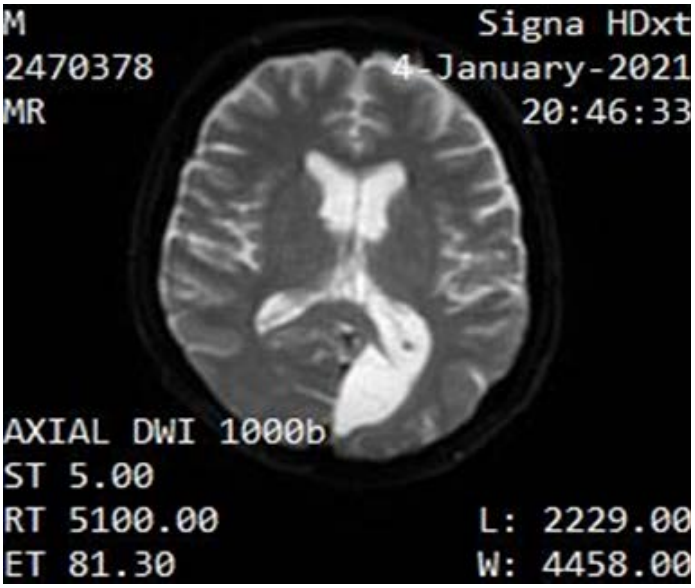

Figure 2. The appearance of a Porencephalic cyst in MR imaging of the patient

intervention was advised. No anti epileptics were started as the last episode of loss of consciousness was 2 years ago. Differential diagnosis of Vascular Dementia was considered as he was a case of Coronary Artery Disease since 2 years on medical management. However MMSE was 28. A diagnosis of adjustment disorder was also considered, but the symptoms were very severe affecting day to day functioning. Hence the current diagnosis of seizure disorder, Organic personality change with Severe depression without psychotic symptoms (ICD-10) i.e., Agitated depression was made. Patient was started on anti-depressants (Sertraline) and anti-anxiety drug (Clonazepam) and was discharged after 1 week. Since then patient was seen on regular follow up with gradual improvement in symptoms.

DISCUSSION

Porencephalic cyst is a malformation of cerebral cortex. It is usually Congenital and can be acquired following stroke or infection. Affected individuals present with mental retardation, seizures, paresis, learning disabilities and psychiatric symptoms (3). One case of late onset psychosis following stress has been reported in right medial frontal lobe porencephalic cyst (4). Another case of left frontal and temporal lobe involvement presented with psychosis in a 25 year old female with no past or family history of psychiatric illness (5). A rare case with epilepsy, infantile hemiplegia and antisocial personality showed the presence of a left-sided congenital cyst (6). A case of anger outbursts was also reported in a case of left fronto-temporal porencephalic cyst (7). However we have here discussed a patient presenting with seizures, organic personality change and agitated depression with presence of porencephalic cyst in the left temporal occipital region which has not been reported so far. It is still unclear if these manifestations concur by chance or if Porencephaly is the causal factor. The area of Porencephalic cyst seems to be of relevance. This case highlights the need for further research on Porencephaly and its presentations in Psychiatry.

Consent: written and informed consent was obtained from patient for publcation of this case report.

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

Financial conflict of interest: *Authors declare that they did not receive any financial support in this study.*

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REFERENCES

1. Wynne D, Jalil MF, Dhillon R. Endoscopic fenestration of a symptomatic porencephalic cyst in an adult. World Neurosurgery 2020;141:245-6.
2. Oommen AT, Sethy G, Minz NT, et al. Unusual presentation of porencephalic cyst in an adult. J Clin of Diagn Res 2017;11(2):OD12.
3. Ho SS, Kuzniecky RI, Gilliam F, et al. Congenital porencephaly: MR features and relationship to hippocampal sclerosis. American J Neuroradiology 1998;19(1):135-41.
4. Noyan OC Salcini, Talu BS, Eryilmaz G. Porencephalic cyst and late onset brief psychotic disorder. Case Reports 2016;2016:bcr2016215098.
5. Douzenis A, Rizos EN, Papadopoulou A, et al. Porencephaly and psychosis: A case report and review of the literature. BMC Psychiatry 2010;10(1):1-4.
6. Varma SL, Chadrasekaran S, Mohamad M, et al. Antisocial personality disorder with porencephalic cyst. European Psychiatry 1992;7(1):45.
7. Singh RK, Chaudhury S, Diwan C, et al. Porencephaly presenting with anger outbursts: A case report. Panacea J Med Sci 2018;8(3):126-7.