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Halk Sağlığı Genel Müdürlüğü adına

On behalf of General Directorate of Public Health

Doç. Dr. Muhammed Emin DEMİRKOL, Genel Müdür (General Director)

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TÜRK HİJYEN VE DENEYSEL BİYOLOJİ DERGİSİ YAYIN İLKELERİ VE YAZIM KURALLARI

I) AMAÇ ve KAPSAM

Türk Hijyen ve Deneysel Biyoloji Dergisi (THDBD), T.C. Sağlık Bakanlığı, Halk Sağlığı Genel Müdürlüğü'nün yayın organı olan bilimsel bir dergidir. Dergi üç (3) ayda bir (Mart, Haziran, Eylül, Aralık) yayımlanır ve dört (4) sayıda bir cilt tamamlanır. Talep olması durumunda Ek Sayı çıkartılır.

Dergimizin amacı tıp alanında aşağıdaki konularda yapılan, bilimsel açıdan nitelikli ve literatüre katkı sağlayacak klinik ve deneysel araştırma yazılarını yayımlamaktır.

Dergide biyoloji, mikrobiyoloji, enfeksiyon hastalıkları, farmakoloji, toksikoloji, immünoloji, parazitoloji, entomoloji, kimya, biyokimya, gıda, beslenme, çevre, halk sağlığı, epidemiyoloji, patoloji, fizyopatoloji, moleküler biyoloji, genetik ve biyoteknoloji ile ilgili alanlardaki özgün araştırma, olgu sunumu, derleme, editöre mektup ve teknik rapor türündeki yazılar yayımlanır.

II) YAYIN İLKELERİ

Dergiye, daha önce başka yerde yayımlanmamış ve yayımlanmak üzere başka bir dergide inceleme aşamasında olmayan yazılar kabul edilir.

Dergi Yayın Kurulu tarafından uygun görülen yazılar, konu ile ilgili en az iki Bilimsel Danışma Kurulu Üyesi'nden (Hakem'den) olumlu görüş alındığında yayımlanmaya hak kazanır. Hakemlerin ve yazarların isimleri gizli tutulur. Hakemler değerlendirme süreçlerini en geç üç ay içinde tamamlar. Bu kurulların, yazının içeriğini değiştirmeyen her türlü düzeltme ve kısaltmaları yapma yetkileri vardır.

Yazıların bilimsel ve hukuki sorumluluğu yazarlara aittir.

Yazarlar araştırma ve yayın etiğine tam olarak uyum göstermelidir.

III) TELİF ve LİSANS

Makalelerin tıbbi ve etik sorumluluğu yazarlara aittir. Makalelerin ve kaynakların içeriğinden, yayımlanan makalelerdeki veriler, fikirler ve ifadelerden yazarlar sorumludur; editörler, yayın kurulu ve T.C. Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü bu konularda herhangi bir sorumluluk kabul etmemektedir. Yazarlara telif ücreti ödenmez.

Yazarlar, makalenin yayına kabul edilmesi halinde telif haklarını Türk Hijyen ve Deneysel Biyoloji Dergisi'ne devretmeyi kabul ederler. Ancak yazarlar aşağıdaki haklara sahiptir:

-Telif haklarının dışında kalan patent vb. tescil edilmiş haklar,

-Dergi ve kitap yayını dışında tüm eğitim faaliyetlerinde ücret ödemeden kullanılabilme hakkı,

-Ticari olmamak koşulu ile makaleyi çoğaltabilme hakkı.

Yazarlar, Telif Hakkı Devir Sözleşmesini imzalayarak, makalenin Türk Hijyen ve Deneysel Biyoloji Dergisi tarafından yayımlanmak üzere kabul edilmesi durumunda Creative Commons Alıntı GayriTicari-Türetilemez 4.0 Uluslararası (CC BY-NC-ND 4.0) kapsamında lisanslanacağını kabul ederler.

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IV) AÇIK ERİŞİM POLİTİKASI

Türk Hijyen ve Deneysel Biyoloji Dergisi açık erişim politikasını benimsemiştir. Açık erişim politikası Budapeşte Açık Erişim Girişimi (BOAI) kuralları esas alınarak uygulanmaktadır. BOAI'ye göre Açık Erişim, "Hakem değerlendirmesinden geçmiş bilimsel makalelerin, internet aracılığıyla; finansal, yasal ve teknik engeller olmaksızın, serbestçe erişilebilir, okunabilir, indirilebilir, kopyalanabilir, dağıtılabilir, basılabilir, taranabilir, tam metinlere bağlantı verilebilir, dizinlenebilir, yazılıma veri olarak aktarılabilir ve her türlü yasal amaç için kullanılabilir olması"dır. **https://www.budapestopenaccessinitiative.org/boai-10-translations/turkish-translation**

Dergide yayınlanan bilimsel yazılara, **Creative Commons Alıntı-GayriTicari-Türetilemez 4.0 Uluslararası Lisansı** çerçevesinde ücretsiz erişilebilir. Dergimiz, hakem değerlendirmesinden geçmiş bilimsel literatürün, herkese serbestçe ulaşılabilir kılınması, daha geniş bir küresel bilgi alışverişini desteklemesi ilkesine dayanarak içeriğine anında açık erişim sağlar. Türk Hijyen ve Deneysel Biyoloji Dergisi'nde yayımlanan tüm makaleler Açık Erişim talimatlarına uygundur.

Türk Hijyen ve Deneysel Biyoloji Dergisi yayımladığı makaleleri tüm dünyada serbestçe çevrimiçi erişilebilir kılmak için makalelere anında açık erişim sağlamaktadır. Makalelere erişim için abone olunmasında gerek yoktur. Dergi kullanıcısı olmadan da sistemdeki tüm makaleler ulaşılıp okunabilmektedir. Makale gönderme, değerlendirme ve yayımlama ücreti alınmamaktadır.

V) ÜCRET POLİTİKASI

Makale gönderilmesi, değerlendirilmesi ve yayımlanması için **ücret alınmaz**.

VI) ETİK KURALLAR

Araştırma ve yayın etiği kurallarına uymak yazarların sorumluluğundadır. Yazarlar **Helsinki Bildirgesi'**nde ana hatları çizilen ilkeleri izlemelidir. Yazarlar, bu tür bir çalışma söz konusu olduğunda, uluslararası alanda kabul edilen kılavuzlara ve yürürlükte olan tüm mevzuatta belirtilen hükümlere uymalıdır.

Etik kurul izni gerektiren tüm araştırmalar için Etik Kurul Onayı alınmış olmalı, belgelendirilmeli; kurul adı, tarih ve sayısı "Gereç ve Yöntem" bölümünde belirtilmelidir.

Klinik araştırmalarda, çalışmaya katılanlardan bilgilendirilmiş olur alındığının gereç ve yöntem bölümünde belirtilmesi gerekmektedir. Gönüllü ya da hastalara uygulanacak prosedürlerin özelliği tümüyle anlatıldıktan sonra kendilerinin bilgilendirilip onaylarının alındığını gösterir beyan "Gereç ve Yöntem" kısmında bulunmalıdır. Olgu sunumlarında ve araştırma makalelerinde hasta kimliğini içeren herhangi bir doküman kullanılmamalıdır. Hasta kimliğini ortaya çıkaracak bilgiler (fotoğraf vs.) kullanıldığında hastanın yazılı onayı gönderilmelidir.

Hayvanlar üzerinde yapılan çalışmalar için de gereken izinler alınmalı; yazıda deneklere ağrı, acı ve rahatsızlık verilmemesi için neler yapıldığı açık bir şekilde belirtilmelidir. Hayvan deneylerinde, çalışma "Laboratuar Hayvanlarının Bakım ve Kullanımı Kılavuzunda" (www.nap.edu/catalog/5140.html) belirtilen etik düzenlemelere göre yapılmalıdır ve yazarlar etik kurul onayı alındığını ve etik kurul tarih ve sayısını "Gereç ve Yöntem" kısmında beyan etmelidirler. Deneysel ve klinik ilaç çalışmalarında Türkiye Cumhuriyeti Sağlık Bakanlığı düzenlemelerine uygun olarak yapıldığı ve etik kurul onayı alındığı metin içinde belirtilmelidir.

Makalenin formatı ICMJE (International Committee of Medical Journal Editors) ve COPE (Committee on Publication Ethics) rehberlerine uygun olmalıdır.

VII) YAZI DİLİ

Dergimizin yazı dilleri Türkçe ve İngilizcedir. Dili Türkçe olan yazılar İngilizce "abstract" ile, dili İngilizce olan yazılar da Türkçe özetleri ile yer alırlar. Özet ve "Abstract" bölümleri bire bir çevirileri şeklinde yer almalıdır. Yazının hazırlanması sırasında, Türkçe kelimeler için Türk Dil Kurumundan (www.tdk.gov.tr), teknik terimler için Türk Tıp Terminolojisinden (www.tipterimleri.com) yararlanılması önerilir. Dili İngilizce olan yazıların mutlaka yazım ve dilbilgisi açısından yeterliliklerinin kontrol edilmiş olması gereklidir. Dil açısından yetersiz görülen yazılar değerlendirmeye alınmazlar..

VIII) YAZIM KURALLARI

Dergide yayımlanmak üzere gönderilen yazılar, Türk Hijyen ve Deneysel Biyoloji Dergisi yazım kurallarına göre hazırlanmalıdır.

Başvurular **www.turkhijyen.org** adresinden **"Çevrimiçi Makale Gönder, Takip Et, Değerlendir"** programı aracılığıyla on line olarak yapılmaktadır.

Yayımlanmak üzere gönderilecek yazılar;

* Bilimsel düzeyi yüksek, orijinal ve kaynak gösterilebilecek özellikte olmalıdır.

* Bilgiler ve kaynaklar son 5 (beş) yıla ait güncel verileri içermelidir.

1. "Telif Hakkı Devir Formu" tüm yazarlarca imzalanarak onaylandıktan sonra dergimizin makale kabul sistemine yüklenmelidir.

2. Makale başlığı, İngilizce başlık, kısa başlık, yazar ad(ları), yazar(lar)ın çalıştığı kurum(lar) ve birim(ler), yazışma işini üstlenen yazarın açık adresi, telefon numaraları (sabit ve cep), elektronik posta adresi belirtilmelidir.

a. Yazının başlığı kısa olmalı ve küçük harfle yazılmalıdır.

b. Sayfa başlarına konan kısa başlık 40 karakteri geçmemelidir.

c. Çalışma bilimsel bir kuruluş ve/veya fon ile desteklenmişse dipnot veya teşekkür bölümünde mutlaka yazılmalıdır. Herhangi bir ticari ürün ve/veya

şirketle bir ilişki yoksa, başvuru yazısında belirtilmelidir.

d. Makale, kongre/sempozyumda sunulmuşsa sunum türü ile birlikte dipnot şeklinde mutlaka belirtilmelidir.

3. Yazılardaki terimler mümkün olduğunca Türkçe ve Latince olmalı, dilimize yerleşmiş kelimelere yer verilmeli ve **Türk Dil Kurumu**'nun güncel sözlüğü kullanılmalıdır. Yazıların dili açık ve anlaşılır olmalı, imlâ ve yazım hataları olmamasına özen gösterilmelidir.

4. Metin içinde geçen mikroorganizma isimleri ilk kullanıldığında tam ve açık yazılmalı, daha sonraki kullanımlarda kısaltılarak verilmelidir. Mikroorganizmaların orijinal Latince isimleri italik yazılmalıdır: Örneğin; *Pseudomonas aeruginosa, P. Aeruginosa* gibi. Yazıda sadece cins adı geçen cümlelerde stafilokok, streptokok gibi dilimize yerleşmiş cins adları Türkçe olarak yazılabilir. Antibiyotik isimleri dil bütünlüğü açısından okunduğu gibi yazılmalı; uluslararası standartlara uygun olarak kısaltılmalıdır.

5. Metin içerisinde bahsedilen birimlerin sembolleri **Uluslararası Birimler Sistemi** (SI)'ne göre verilmelidir.

6. Yazılar bir zorunluluk olmadıkça "geçmiş zaman edilgen" kip ile yazılmalıdır.

7. Metnin tamamı 12 punto Times New Roman karakteri ile çift aralıkla yazılmalı ve sayfa kenarlarından 2.5 cm boşluk bırakılmalıdır.

8. **Araştırma yazıları**;

Türkçe Özet, İngilizce Özet, Giriş, Gereç ve Yöntem, Bulgular, Tartışma, Teşekkür (varsa) ve Kaynaklar bölümlerinden oluşmalıdır. Bu bölüm başlıkları sola yaslanacak şekilde büyük harflerle kalın yazılmalıdır. İngilizce makalelerde de Türkçe başlık, kısa başlık ve özet bulunmalıdır.

Dergimizin ve makalenizin olabildiğince fazla atıf alabilmesi için özetler son derece kapsamlı hazırlanmalı; gramer, imlâ ve yazım hataları barındırmamalıdır.

a) **Türkçe Özet**: Amaç, Yöntem, Bulgular ve Sonuç, alt başlıklarından oluşmalıdır (yapılandırılmış özet) **ve en az 250, en fazla 400 sözcük içermelidir.**

b) **İngilizce Özet (Abstract):** Türkçe Özet bölümünde belirtilenleri birebir karşılayacak şekilde "Objective, Method, Results, Conclusion" olarak yapılandırılmalıdır.

c) **Anahtar Sözcükler:** 3-8 arasında olmalı ve **Index Medicus Medical Subject Headings - (MeSH)**'de yer alan sözcükler kullanılmalıdır. Türkçe anahtar sözcüklerinizi oluşturmak için **http://www.bilimterimleri.com/** adresini kullanınız.

d) **Giriş:** Araştırmanın amacı ve gerekçesi güncel literatür bilgisi ile desteklenerek iki sayfayı aşmayacak şekilde sunulmalıdır.

e) **Gereç ve Yöntem:** Araştırmanın gerçekleştirildiği kurum/kuruluş ve tarih belirtilmeli, araştırmada kullanılan araç, gereç ve yöntem sunulmalı; istatistiksel yöntemler açıkça belirtilmelidir.

f) **Bulgular:** Sadece araştırmada elde edilen bulgular belirtilmelidir.

g) **Tartışma:** Araştırmanın sonunda elde edilen bulgular, diğer araştırıcıların bulgularıyla karşılaştırılmalıdır. Araştırıcı, kendi yorumlarını bu bölümde aktarmalıdır.

h) **Teşekkür:** Ana metnin sonunda kaynaklardan hemen önce yer almalıdır. Teşekkür bölümünde çalışmaya destek veren kişi, kurum/kuruluşlar yer almalıdır.

i) **Kaynaklar:** Yazarlar kaynakların eksiksiz ve doğru yazılmasından sorumludur. Kaynaklar, metnin içinde geçiş sırasına göre numaralandırılmalıdır. Numaralar, parantez içinde cümle sonlarında verilmelidir. Kaynakların yazılımı ile ilgili aşağıda örnekler verilmiştir. Daha detaylı bilgi için "Uniform Requirements for Manuscripts submitted to Biomedical Journals" (J Am Med Assoc 1997; 277: 927-934) **(http://www.nejm.org/)** bakılmalıdır.

Makalenizin Kaynaklar bölümünde Türk Hijyen ve Deneysel Biyoloji Dergisinde yayımlanmış makalelere atıf yapılmasına özen gösterilmelidir.

- Süreli yayın: Yazar(lar)ın Soyadı Adının baş harf(ler)i (altı veya daha az yazar varsa hepsi yazılmalıdır; yazar sayısı yedi veya daha çoksa yalnız ilk altısını yazıp "et al." veya "ve ark." eklenmelidir). Makalenin başlığı, Derginin Index Medicus'a uygun kısaltılmış ismi, Yıl; Cilt (Sayı): İlk ve son sayfa numarası.

- Standart dergi makalesi için örnek: Demirci M, Ünlü M, Şahin Ü. A case of hydatid lung cyst diagnosed by kinyoun staining of bronco-alveolar fluid. Turkiye Parazitol Derg, 2001; 25 (3): 234-5.

- Yazarı verilmemiş makale için örnek: Anonymous. Coffee drinking and cancer of the panceras (Editorial). Br Med J, 1981; 283:628.

- Dergi eki için örnek: Frumin AM, Nussbaum J, Esposito M. Functinal asplenia: Demonstration of splenic activity by bone marrow scan (Abstract). Blood, 1979; 54 (Suppl 1): 26a.

- Kitap: Yazar(lar)ın soyadı adının baş harf(ler)i. Kitabın adı. Kaçıncı baskı olduğu. Basım yeri: Yayınevi, Basım yılı.

- Örnek: Eisen HN. Immunology: an Introduction to Molecular and Cellular Principles of the Immun Response. 5th ed. New York: Harper and Row, 1974.

- Kitap bölümü: Bölüm yazar(lar)ın soyadı adının başharf(ler)i. Bölüm başlığı. In: Editör(ler)in soyadı adının başharf(ler)i ed/eds. Kitabın adı. Kaçıncı baskı olduğu. Basım yeri: Yayınevi, Basım yılı: Bölümün ilk ve son sayfa numarası.

- Örnek: Weinstein L. Swarts MN. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, eds. Pathologic Physiol ogy: Mechanism of Disease. Phidelphia. WB Saunders, 1974:457-72.

- Web adresi: Eğer doğrudan "web" adresi referans olarak kullanılacaksa adres ile birlikte parantez içinde bilgiye ulaşılan tarih de belirtilmelidir. Web erişimli makalelerin referans olarak metin içinde verilmesi gerektiğinde DOI (Digital Object Identifier) numarası verilmesi şarttır.

- Kongre bildirisi: Entrala E, Mascaro C. New stuructural findings in Cryptosporidium parvum oocysts. Eighth International Congress of Parasitology (ICOPA VIII). October,10-14, Izmir-Turkey. 1994.

- Tez: Bilhan Ö. Labirent savakların hidrolik karakteristiklerinin deneysel olarak incelenmesi. Yüksek Lisans Tezi, Fırat Üniversitesi Fen Bilimleri Enstitüsü, 2005.

GenBank/DNA dizi analizi: Gen kalıtım numaraları ve DNA dizileri makale içinde kaynak olarak gösterilmelidir. Konuyla ilgili ayrıntılı bilgi için "National Library of Medicine" adresinde "National Center for Biotechnical Information (NCBI)" bölümüne bakınız.

- Şekil ve Tablolar: Her tablo veya şekil ayrı bir sayfaya basılmalı, alt ve üst çizgiler ve gerektiğinde ara sütun çizgileri içermelidir. Tablolar, "Tablo 1." şeklinde numaralandırılmalı ve tablo başlığı tablo üst çizgisinin üstüne yazılmalıdır. Açıklayıcı bilgiye başlıkta değil dipnotta yer verilmeli, uygun simgeler (*,+,++, v.b.) kullanılmalıdır. Fotoğraflar "jpeg" formatında ve en az 300 dpi olmalıdır. Baskı kalitesinin artırılması için gerekli olduğu durumlarda fotoğrafların orijinal halleri talep edilebilir.

9. **Araştırma Makalesi** türü yazılar için kaynak sayısı **en fazla 40** olmalıdır.

10. **Derleme** türü yazılarda tercihen yazar sayısı ikiden fazla olmamalıdır. Yazar(lar) daha önce bu konuda çalışma ve yayın yapmış olmalı; bu deneyimlerini derleme yazısında tartışmalı ve kaynak olarak göstermelidir. Derlemelerde Türkçe ve İngilizce olarak başlık, özet **(en az 250, en fazla 400 sözcük içermelidir)** ve anahtar sözcükler bulunmalıdır. Derleme türü yazılar için kaynak sayısı **en fazla 60** olmalıdır.

11. **Olgu sunumlarında** metin yedi sayfayı aşmamalıdır. Türkçe ve İngilizce olarak başlık, özet ve anahtar sözcükler ayrıca giriş, olgu ve tartışma bölümleri bulunmalıdır. Olgu sunumu türü yazılar için kaynak sayısı **en fazla 20** olmalıdır.

12. **Editöre Mektup:** Daha önce yayımlanmış yazılara eleştiri getirmek, katkıda bulunmak ya da bilim haberi niteliği taşıyacak bilgilerin iletilmesi amacıyla yazılan yazılar, Yayın Kurulu'nun inceleme ve değerlendirmesinin ardından yayınlanır. Editöre Mektup bir sayfayı aşmamalı ve kaynak sayısı **en fazla 10** olmalıdır.

13. **Teknik Rapor** türü yazılar ilgili alanda önemli katkısı olabilecek bilgileri içermelidir. Teknik raporlarda Türkçe ve İngilizce başlık, tek paragraf olacak şekilde Türkçe ve İngilizce özet, Türkçe ve İngilizce olmak üzere anahtar kelimeler yer almalıdır. Kaynak sayısı **en fazla 10** olmalıdır.

14. Bu kurallara uygun olmayan metinler kabul edilmez.

15. Yazarlar teslim ettikleri yazının bir kopyasını saklamalıdır.

TURKISH BULLETIN OF HYGIENE AND EXPERIMENTAL BIOLOGY PUBLISHING POLICIES AND WRITING RULES

I) AIM and SCOPE

The Turkish Bulletin of Hygiene and Experimental Biology (TBHEB) is a publication of the "Republic of Turkey, Ministry of Health, General Directorate of Public Health ". The Journal is published every three months (March, June, September, December) and one volume consists of four (4) issues.

Goal of the our journal is to publish clinical and experimental research articles which are scientifically qualified and will provide a new contribution to the literature.

The journal publishes biology, microbiology, infectious diseases, pharmacology, toxicology, immunology, parasitology, entomology, chemistry, biochemistry, food safety, environmental, health, public health, epidemiology, pathology, pathophysiology, molecular biology, genetics, biotechnology in the field of original research, case report, reviews, letters to the editor and technical reports.

II) PUBLISHING POLICY

Articles which are not previously published in another journal or not currently under evaluation elsewhere can be accepted for the journal.

Articles approved by the Scientific Committee and Editorial Board are eligible to be released after receiving at least two positive opinions from the Scientific Committee members. The names of the reviewers and authors are kept confidential. Reviewers complete the evaluation processes within three months at the latest.Those committees have the authority to make all corrections and abbreviations but not to change the content of the article.

The authors have the all the scientific and legal responsibilities of the articles.

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V) PRICE POLICY

Article submission, evaluation and publication are free.

VI) ETHICAL RULES

The Turkish Bulletin of Hygiene and Experimental Biology expects the authors to comply with the ethics of research and publication. In case the authors do not have a local ethics committee, the principles outlined in the "Declaration of Helsinki" should have been followed. Authors must comply with the internationally accepted guidelines and provisions set out in all applicable legislation when it comes to this type of work.

Ethics Committee Approval must be obtained and documented for all researches requiring ethics committee approval; The name, date and number of the committee should be stated in the method section of the article.

In human research, a statement of the informed consent of those who participated in the study is needed in the section of the "Materials and Methods". In case of procedures that will apply to volunteers or patients, it should be stated that the study objects have been informed and given their approval before the study started. In case reports, information about the signed informed patient consent form should be included in the article. In case patient information (photograph, etc.) is used which shows patient ID, a written informed consent of the patient must be submitted.

In case animal studies, approval also is needed; it should be stated clearly that the subjects will be prevented as much as possible from pain, suffering and inconvenience. In animal experiments, the study should be conducted in accordance with the ethical regulations specified in the "Guide to the Care and Use of Laboratory Animals" (www.nap.edu/catalog/5140.html) and the authors should declare that the ethics committee approval was obtained and the date and number of the ethics committee in the "Materials and Methods" section. Experimental and clinical drug studies performed in accordance with the Republic of Turkey Ministry of Health regulations and ethics committee approval must be stated in the article.

The format of the article should be in accordance with ICMJE (International Committee of Medical Journal Editors) and COPE (Committee on Publication Ethics) guidelines.

VII) LANGUAGE of the JOURNAL

The official languages of the our Journal are Turkish and English. The manuscripts written in Turkish have also abstracts in English, and the articles in English have also abstracts in Turkish. The Turkish and English abstracts should be literal translations of each other. When preparing manuscripts, the Turkish Language Institution (www.tdk.gov.tr) is advised for consulting Turkish words and Turkish Medical Terminology (www.tipterimleri.com) for technical terms. Manuscripts in English must absolutely be checked for spelling and grammar. Manuscripts considered insufficient in language will not be considered for evaluation.

VIII) WRITING RULES

Manuscripts submitted for publication in the journal should be prepared according to the writing rules of the Turkish Journal of Hygiene and Experimental Biology.

Applications are made online at www.turkhijyen.org via the "Online Manuscript Submit, Track, Evaluate Program".

Articles to be submitted for publication;

* Should have a high scientific level, be original and suitable for reference.

* Information and references should contain up-to-date data for the last 5 (five) years.

1. The "Copyright Transfer Form" (Copyright Release Form) after being signed by all authors should be uploaded using the article accepting system of the journal.

2. The title of article, Turkish title, short title, author name(s), names of institutions and the departments of the author(s), full address of the

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corresponding author, telephone numbers (landline and mobile), e-mail address should be given.

a. The title should be short and written in lower case.

b. The short title should not exceed 40 characters.

c. The study supported by a fund or scientific organisation must be mentioned in a footnote or in the acknowledgements.

d. The study presented in a conference/symposium must be mentioned with the type of presentation in footnotes or in the acknowledgements.

3. For Turkish studies; Terms used in articles should be in Turkish and Latin as much as possible, according to the latest dictionary of the "Turkish Language Institution". The language of the articles should be clear, and care should be taken to avoid spelling and writing mistakes.

4. Latin names of microorganisms used for the first time in the text have to be written in full. If these names are used later, they should be abbreviated in accordance to international rules. The original Latin names of microorganisms should be written in Italic: for example, Pseudomonas aeruginosa, P.aeruginosa. Names of antibiotics should be abbreviated in accordance with international standards.

5. Symbols of the units mentioned in the text should be according to "The Système International (SI).

6. Articles should be written in one of the "past perfect, present perfect and past" tenses and in the passive mode.

7.Only one side of A4 paper should be used and should have a 2.5 cm margin on each side. 12 pt, Times New Roman font and double line space should be used.

8. **Research Articles;**

Research papers should consist of Turkish abstract, English abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements (if any), and References sections. These sections should be written in bold capital letters and aligned left. English articles should have a Turkish abstract and title in Turkish. (If the all of the authors from abroad the manuscript and abstract can be write English language).

Abstracts should be prepared in an extremely comprehensive way; it should not contain grammatical, spelling and writing errors.

a) **Turkish Abstract** should consist of the subheadings of Objective, Methods, Results and Conclusion (Structured Abstract). It should be between 250 and 400 words.

b) **English Abstract:** The abstract should be structured like the Turkish abstract (Objective, Methods, Results, and Conclusion). It should be between 250 and 400 words.

c) **Key words** The number of keywords should be between 3-8 and the terminology of the Medical Subjects Headings (Index Medicus Medical Subject Headings-MeSH) should be used.

d) **Introduction:** The aim of the study, and references given to similar studies should be presented briefly and should not exceed more than two pages.

e) **Materials and Methods:** The date of the study, institution that performed the study, and materials and methods should be clearly presented. Statistical methods should be clearly stated.

f) **Results:** The results should be stated clearly and only include the current research.

g) **Conclusions:** In this section, the study findings should be compared with the findings of other researchers. Authors should mention their comments in this section.

h) **Acknowledgements** should be placed at the end of the main text and before the references. In this section, the institutions/departments which supported the research should be stated.

i) **References:** Authors are responsible for supply complete and correct references. References should be numbered according to the order used in the text. Numbers should be given in brackets and placed at the end of the sentence. Examples are given below on the use of references. Detailed information can be found in "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (J Am Med Assoc 1997 277: 927-934) and at **http://www.nejm.org/general/text/requirements/1.htm.**

- **Periodicals:** Author(s) Last Name initial(s) name of author(s) (if there are six or fewer authors, all authors should be written; if the number of authors are seven or more, only the first six of the authors should be written and the rest as "et al"). The title of the article, the abbreviated name of the journal according to the Index Medicus, Year; Volume (Issue): The first and last page numbers.

- **Example of standard journal article:** Demirci M, Unlü M, Sahin U. A case of hydatid cyst diagnosed by kinyoun staining of lung bronco-alveolar fluid. Türkiye Parazitol Derg, 2001; 25 (3): 234-5.

- **Example of an article with authors unknown:** Anonymous. Coffee drinking and cancer of the pancreas (Editorial). Br Med J, 1981; 283:628.

- **Example of journal supplement:** Frumin AM, Nussbaum J, Esposito M. Functional asplenia: Demonstration of splenic activity by bone marrow scan (Abstract). Blood, 1979; 54 (Suppl 1): 26a.

- **Books:** Surname of the author(s) initial name(s) of author(s). The name of the book. The edition number. Place of publication: Publisher, Publication year. - Example: Eisen HN. Immunology: an Introduction to the Principles of Molecular and Cellular Immune Response. 5th ed. New York: Harper and Row, 1974.

- **Book chapters**: The author(s) surname of the chapter initial(s) letter of the name. Section title. In: Surname of editor(s) initial (s) letter of first name(s) ed / eds. The name of the book. Edition number. Place of publication: Publisher, year of publication: The first and last page numbers of the chapter.

- **Example:** Weinstein L. Swarts MN. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, eds. Pathologic Physiology: Mechanism of Disease. Phidelphia. WB Saunders, 1974:457-72.

- **Web address:** If a "web" address is used as the reference address, the web address date should be given in brackets with the address. The DOI (Digital Object Identifier) number must be provided, when a web access article used in the text as a reference.

- **Congress papers:** Entrala E, Mascaro C. New structural findings in Cryptosporidium parvum oocysts. Eighth International Congress of Parasitology (ICOPA VIII). October, 10-14, Izmir-Turkey. 1994.

- **Thesis:** Bilhan Ö. Experimental investigation of the hydraulic characteristics of labyrinth weir. Master Thesis, Science Institute of Firat University, 2005.

- **GenBank / DNA sequence analysis:** DNA sequences of genes and heredity numbers should be given as references in the article. For more information, check "National Library of Medicine" and "National Center for Biotechnical Information (NCBI)".

- **Figure and Tables:** Each table or figure should be printed on a separate sheet, the top and bottom lines and if necessary column lines must be included. Tables should be numbered like "Table 1." and the table title should be written above the top line of the table. Explanatory information should be given in footnotes, not in the title and appropriate icons (*,+,++, etc.) should be used. Photos should be in "jpeg" format. In case the quality of the photos is not good for publication, the originals can be requested.

9. **Research articles** should have **up to 40** references.

10. **In reviews**, it is preferred to have not more than two authors. Author(s) must have done research and published articles previously on this subject; they should discuss their experience and use as reference in the review. Reviews should have Turkish and English titles, abstracts (**it should contain minimum 250, maximum 400 words**) and key words. Reference numbers for the review should be **maximum 60**.

11. **Case reports** should have a maximum of seven pages of text. Case report should have a Turkish and English title, abstract, keywords and also introduction, case description and discussion sections should be given. Number of references should be **maximum 20**.

12. **Letters to Editor**: Written to make criticisms, additions to previously published articles or scientific updates are published after review and assessment of the Editorial Board. Letters should not exceed one page of text and must be supported with **up to 10** references.

13. **Technical report** should contain information that may contribute significantly to the relevant field. Technical reports should include Turkish and English titles, Turkish and English abstracts in a single paragraph, keywords in Turkish and English. The number of references should be **maximum 10**.

14. The articles which do not comply with the journal rules are not accepted.

15. Authors should keep a copy of the article that they submit.

ETİK İLKELER VE YAYIN POLİTİKASI

ETİK İLKELER

Türk Hijyen ve Deneysel Biyoloji Dergisi Editör ve Hakemleri, Uluslararası Tıp Dergisi Editörleri Komitesi (ICMJE), Yayın Etiği Komitesi (COPE), Dünya Tıp Editörleri Birliği (WAME), Bilim Editörleri Konseyi (CSE), Avrupa Bilim Editörleri Birliği (EASE), ABD Ulusal Tıp Kütüphanesi (NLM), Dünya Tıp Birliği (WMA) ve Ulusal Bilgi Standartları Örgütü (NISO) rehber kurallarına uymaktadır.

Yazarlara yönelik; dergi politikası gereğince, uluslararası anlaşmalara uygun bir etik kurul tarafından araştırma protokollerinin onaylanması gereklidir. [WMA Helsinki Deklarasyonu - İnsan Denekleri İçeren Tıbbi Araştırmalar için Etik İlkeler (son güncelleme: Ekim 2013, Fortaleza, Brezilya)", "Tüm araştırma çalışmaları için laboratuvar hayvanlarının bakım ve kullanımı kılavuzu (8. baskı, 2011) "ve / veya" Hayvanları içeren Biyomedikal Araştırmalara Yönelik Uluslararası Rehber İlkeler (2012)]. Gönderilen makale, etik kurul onayı içermemesi durumunda değerlendirme için işleme alınmaz.

ÇIKAR ÇATIŞMASI POLİTİKASI

Türk Hijyen ve Deneysel Biyoloji Dergisi'nin editör incelemesi, uluslararası editör organizasyonları (ICMJE, EASE, WAME, COPE, CSE,…) tarafından belirlenen İyi Editörlük Uygulamalarına uygundur. WAME, yayın sürecinde (makalenin gönderilmesi, hakem incelemesi, editör kararları ve yazarlar, hakemler ve editörler arasındaki iletişim) yazar, hakem veya editörlerin, süreçteki sorumluluklarını (akademik dürüstlük, çalışmanın yürütülmesinde ve raporlanmasında yanıltma olmaması, karar ve hükümlerin uygunluğunu) etkileyebilecek herhangi bir rekabetçi çatışma varsa çıkar çatışmasının mevcut olduğunu belirtmektedir.

Türk Hijyen ve Deneysel Biyoloji Dergisi, tüm yazarların, hakemlerin ve editörlerin, WAME tarafından yukarıda belirtilen herhangi bir rekabet çıkarının yanı sıra, ailevi, kişisel, finansal, politik veya dini konularla ilgili herhangi bir çıkar çatışmasını editöre bildirmesini istemektedir. Herhangi çıkar çatışması ve finansal desteğin olup olmadığı, makalelerin sonunda beyan edilmelidir.

Yazarlar inceleme sürecinde bölüm editörlerinden hiçbiri ile iletişime geçmemelidir. Makalelerin süreci ile ilgili tüm gerekli bilgiler dergi sekreterliğinden edinilebilir. Editör ve hakemlerin isimleri yazarlara verilmez. Türk Hijyen ve Deneysel Biyoloji Dergisi'nin çift kör gözden geçirme ilkeleri nedeniyle, yazarların ve hakemlerin isimleri diğer kişilerce bilinmemektedir.

YAYIN POLİTİKASI ve HAKEM DEĞERLENDİRME SÜRECİ

Türk Hijyen ve Deneysel Biyoloji Dergisi (THDBD), **çift kör hakem değerlendirmesi** ile Türkçe ve İngilizce dillerinde T.C. Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü tarafından yayımlanmaktadır. THDBD yayın ilkelerinde belirtilen konularda yazılan makaleleri kapsamaktadır. Sadece çevrimiçi (online) başvurular kabul edilmektedir. Dergiye kabul edilme sürecindeki değerlendirilmelerde aranan temel özellikler özgünlük ve bilim literatürüne katkıdır.

Makaleler gönderildikten sonra öncelikle editörler tarafından değerlendirilmektedir. Editörler hakem değerlendirme sürecinden önce makalelerin THDBD yazım kurallarına uygun yazılıp yazılmadığını değerlendirmektedirler. Bu kurallara göre yazılmayan makaleler değerlendirilmeye alınmamaktadır.

Sonrasında, makalenin değerlendirilmesi için iki hakem atanır. Hakemler aynı alanda yayınları olan uzmanlar arasından seçilir. Makaleler ihtiyaç halinde istatistik editörü tarafından da kontrol edilebilir. Tüm makaleler İngilizce dil editörü tarafından da incelenir. Hakemlere değerlendirme için 30 gün süre verilmektedir ve eğer herhangi bir düzeltme istenirse yazarların gerekli düzeltmeleri yapmak için 30 gün süresi vardır. Editör, editör yardımcıları, istatistik editörü ve İngilizce dil editörü kabul edilen makalenin asıl anlamını değiştirmeyen küçük düzeltmeler yapabilirler.

ETHICAL PRINCIPLES AND PUBLICATION POLICY

ETHICAL PRINCIPLES

The Editorial Board and Reviewers of the Turkish Bulletin of Hygiene and Experimental Biology adhere to the guidelines of the International Committee of Medical Journal Editors (ICMJE), Committee on Publication Ethics (COPE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), European Association of Science Editors (EASE), the US National Library of Medicine (NLM), the World Medical Association (WMA), and National Information Standards Organization (NISO).

For Authors; as journal's policy, an approval of research protocols by an ethics committee in accordance with international agreements "WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects (last updated: October 2013, Fortaleza, Brazil)" ,"Guide for the care and use of laboratory animals (8th edition, 2011)" and/or "International Guiding Principles for Biomedical Research Involving Animals (2012)" is required for all research studies. If the submitted manuscript does not include ethics committee approval, it will not be processed for evaluation.

CONFLICT OF INTEREST POLICY

Turkish Bulletin of Hygiene and Experimental Biology's editorial review process is in accordance with the Good Editorial Practice set by international editorial organizations (ICMJE, EASE, WAME, COPE, CSE, …). WAME indicates that "conflict of interest exists when an author, reviewer, or editor in the publication process (submission of manuscripts, peer review, editorial decisions, and communication between authors, reviewers and editors) has a competing interest that could unduly influence his or her responsibilities (academic honesty, unbiased conduct and reporting of research, and integrity of decisions or judgments) in the publication process".

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Authors should not contact any of the section editors during the review process. All necessary information regarding the process of a manuscript can be obtained from the editorial office. However, the names of the handling editor and the reviewers are not given to the author(s). Due to the Turkish Bulletin of Hygiene and Experimental Biology's double-blinded review principles, the names of authors and reviewers are not known to the other.

PUBLICATION POLICY and PEER REVIEW PROCESS

Turkish Bulletin of Hygiene and Experimental Biology (TBHEB) is published in Turkish and English by Republic of Turkey Ministry of Health General Directorate of Public Health which processing through **double-blind peer review**. The journal covers clinical and experimental research articles on subjects from all medical areas, review articles, case reports, letters to the editor, manuscripts on historical evolution of diseases and medicine and content on ethical problems. Articles are accepted only by online submission. The basic features supposed to be in an article during assessment processes for the acceptance to journal are the contribution to the scientific literature and the originality.

All manuscripts are underwent an initial process by the Editors after the submission. Editors firstly evaluates whether the manuscript is prepared according to the journal's manuscript submitting rules before peer review process. Manuscripts which are not appropriate for those rules will not be evaluated.

Then, two reviewers are assigned for manuscript evaluation. Reviewers are selected among specialists who also have publications in literature in the same medical field. Articles might be checked by the statistical editor in addition if needed. All manuscripts are also inspected for English grammatical and manuscript preparation rules. 30 days are given for reviewer and, if any corrections are required, the author(s) has 30 days to make the requested corrections. The Editor-in-Chief, Associate Editors, statistical editor and English language editor may make minor corrections to accepted manuscripts that do not change the main meaning of the manuscript.

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TÜRK HİJYEN VE DENEYSEL BİYOLOJİ DERGİSİ

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- Metin içerisinde bahsedilen birimlerin sembolleri the Système International (SI)'e göre verildi.
- Yazılar "miş'li geçmiş" zaman edilgen kip ile yazıldı.
- Metnin tamamı 12 punto Times New Roman karakteri ile çift aralıkla yazıldı.
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- The number of words in Turkish and English abstracts (between 300-500) is checked.
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- All abbreviations are reviewed and non-standard abbreviations are corrected
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- Photos are in JPEG format.
- References are given at the end of the sentence in brackets and are listed in order of use in the text.
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- References are reviewed, and the name of all authors, spelling and punctuation are controlled according the writing rules.

Furthermore, please check.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

The effect of nasal staphylococcal carriage on the incidence of prosthesis infection in orthopedic surgery patients: A cohort study

Ortopedik protez cerrahisi hastalarında nazal stafilokok taşıyıcılığının protez enfeksiyonları üzerine etkisi: Bir kohort çalışması

Elif AGÜLOĞLU BALİ[1] (ID), Halit ÖZSÜT[2] (ID), Ömer Naci ERGİN[3] (ID), Serap ŞİMŞEK YAVUZ[2] (ID)

ABSTRACT

Objective: Prosthetic infections are commonly caused by coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus* (*S. aureus*). Despite recommendations for preoperative *S. aureus* screening, data on screening for staphylococcal carriage, including CoNS, remains limited. Our study aimed to explore the relationship between nasal carriage of *S. aureus* and other staphylococci and prosthesis infections.

Methods: This prospective cohort study, conducted from January to September 2019, included 151 patients undergoing prosthetic surgery. Nasal cultures for staphylococcal carriage were collected 24 hours before surgery. Patients were monitored and blood tests were conducted at 1, 2, and 3 months postoperatively to assess prosthesis infection development. Infection rates were compared between patients with and without nasal carriage.

Results: In the postoperative three-month period, 5 patients (3.3%) developed prosthetic infections. Preoperative nasal swabs revealed methicillin-

ÖZET

Amaç: *Staphylococcus aureus* (*S.aureus*) ve koagülaz negatif stafilokoklar (KNS) protez enfeksiyonlarının en sık etkenleridir. Diz ya da kalça protezi ameliyatlarından önce nazal *S.aureus* taşıyıcılığının taranmasını öneren yayınlar olmasına karşın, başta KNS olmak üzere diğer stafilokok türlerinin taranmasına dair veriler sınırlıdır. Çalışmamız, *S.aureus* ve diğer stafilokokların nazal taşıyıcılığı ile protez enfeksiyonları arasındaki ilişkiyi araştırmayı amaçlamıştır.

Yöntem: Çalışmamız prospektif bir kohort çalışması olup, çalışmaya Ocak-Eylül 2019 tarihleri arasında protez cerrahisi geçiren 151 hasta dahil edilmiştir. Nazal stafilokok taşıyıcılığı taraması için preoperatif 24 saat önce burun sürüntü kültürleri alınmış ve değerlendirilmiştir. Hastalar yakın izlenerek ameliyat sonrası 1., 2. ve 3. aylarda polikliniğe çağrılmış ve kan tetkikleri istenmiştir. Nazal taşıyıcılık saptanan ve saptanmayan hastalar arasında protez enfeksiyonu oranları karşılaştırılmıştır.

Bulgular: Postoperatif 3 aylık dönemde 5 (%3.3) hastada protez infeksiyonu gelişti. Enfeksiyon gelişen

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resistant coagulase-negative staphylococci (MR-CoNS) in four patients and methicillin-sensitive coagulase-negative staphylococci (MS-CoNS) in one patient. None of the patients had preoperative Staphylococcus aureus. The increased risk of prosthetic infection was significantly associated with nasal CoNS carriage (p=0.025). The infected group exhibited elevated leukocyte, neutrophil, and lymphocyte counts, as well as increased C-reactive protein (CRP) levels at 24 hours post-surgery.

Conclusion: This study found no evidence of an association between *S. aureus* carriage and prosthesis infections, but an association was found for CoNS carriage. However, it is not possible to establish a clear relationship between carrying CoNS and infection. The results of our study suggest that nasal carriage screening before prosthetic surgery may be unnecessary. Further studies are needed to clarify the relationship between nasal carriage and prosthetic infection. Monitoring leukocyte and CRP levels in the early postoperative period may indicate prosthesis infection.

Key Words: Nasal staphylococcal carriage, prosthesis infection, osteomyelitis, *S.aureus*, coagulase-negative staphylococci, C-reaktive protein

hastalardan dördünün preoperatif sürüntü kültüründe metisiline dirençli koagülaz negatif stafilokok (MRKNS), birinde metisiline duyarlı koagülaz negatif stafilokok (MSKNS) üremesi oldu. Enfekte hastaların hiçbirinde nazal *S. aureus* taşıyıcılığı saptanmadı. Protez enfeksiyonu gelişme riski preoperatif nazal KNS taşıyıcılarında anlamlı olarak yüksek bulundu (p=0.025). Enfeksiyon gelişen hastalarda postoperatif 24. saat lökosit, nötrofil, lenfosit ve CRP düzeyleri yüksekti.

Sonuç: Bu çalışmada *S. aureus* taşıyıcılığı ile protez enfeksiyonları arasında bir ilişki olduğuna dair kanıt bulunamamıştır, ancak KNS taşıyıcılığı için bir ilişki bulunmuştur. Bununla birlikte, mevcut literatür verileriyle KNS taşıyıcılığı ile enfeksiyon arasında net bir ilişki kurmak mümkün değildir. Çalışmamızın sonuçları, protez cerrahisi öncesinde nazal taşıyıcılık taramasının gereksiz olabileceğini düşündürmektedir. Nazal taşıyıcılık ve protez enfeksiyonu arasındaki ilişkiyi netleştirmek için daha fazla çalışmaya ihtiyaç vardır. Ameliyat sonrası erken dönemde lökosit ve CRP düzeylerinin izlenmesi protez enfeksiyonuna işaret edebilir, hastaların yakın takibi önerilir.

Anahtar Kelimeler: Nazal stafilokok taşıyıcılığı, protez infeksiyonu, osteomyelit, *S.aureus*, koagülaz negatif stafilokok, C-reaktif protein, lökositoz

INTRODUCTION

Hip and knee arthroplasties are highly successful elective surgeries, with a 10-year survivorship rate exceeding 95%. In the United Kingdom (UK) and United State of America (USA) , around 800,000 such procedures are performed annually, with projections indicating this could rise to over 4 million by 2030. The rate of periprosthetic infection stands at approximately 1% for hip replacements and varies between 1% and 2% for knee replacements each year (1). Numerous bacteria have been linked to prosthetic joint infections (PJI), the microbiological profile can be influenced by several factors, including the timing of the surgical intervention, post-surgical soft tissue complications like poor wound healing, hematoma, and wound dehiscence, previous infections, known colonization with drug-resistant organisms like *Staphylococcus* spp, and the patient's underlying comorbidities (2).

Early prosthetic joint infections (PJIs) typically occur within the first three months following surgery, primarily involving bacteria introduced during the surgical procedure. These infections often involve

organisms with significant virulence or a large bacterial load, including *Staphylococcus aureus*, CoNS, as well as a higher proportion of anaerobic and aerobic gram-negative bacilli, and polymicrobial infections. Conversely, delayed infections usually manifest between three months and one year post-surgery, often involving less virulent bacteria that were also likely introduced during or shortly after the initial surgery. In these later cases, the prevalence of polymicrobial and aerobic gram-negative bacterial infections decreases as these are more typical of earlier presentations (2).

Various patient-specific comorbidities and demographic factors significantly elevate the risk of developing periprosthetic joint infections (PJI). Critical risk factors encompass previous joint infections, septicemia, active cutaneous or deep tissue infections, and blood transfusions. Furthermore, individual patient factors such as uncontrolled diabetes, malnutrition, morbid obesity, smoking habits, alcohol consumption, immunocompromising diseases, drug use, and nasal carriage of *S. aureus* also contribute to the increased vulnerability to PJIs (1). The community prevalence of MRSA has been increasing over the course of the last decade, and patient colonization with *S. aureus* has been shown to be an independent risk factor for the development of a surgical site infection after total hip or knee arthroplasty (3).

S. aureus colonisation and infection have a complex and multifactorial relationship, which is not yet fully understood. The mucosal surfaces that harbor *S. aureus* include the nose, throat, vaginal wall, and GI tract. Nasal carriage is probably most important because nose-picking could effectively disseminate the bacterium to other body surfaces and other hosts (4). However, various immunological mechanisms may also play a role in the pathogenesis of infection. There are studies indicating that *S. aureus* nasal colonisation may play a role in the pathogenesis of autoimmune disease due to its effects on the innate and adaptive immune response (5). Transient or persistent *S. aureus* colonization induces specific systemic immune responses. Humoral responses are the most studied of these and little is known of cellular responses induced by colonization. Nasal *S. aureus* carriage screening/decolonization procedures are heterogeneous and the evaluation of their clinical impact remains complex. On the other hand, there are studies that recommend screening and decolonisation of *S. aureus* carriage before cardiovascular surgeries, hip or knee replacement surgeries and in neonatal intensive care units (6).

CoNS are the most abundant micro-organisms colonizing human skin and mucous membranes. Among healthy individuals this commensal flora of CoNS remains relatively stable over time. However, hospital admissions, antibiotic treatment, and chemotherapy have all been shown to alter the flora, reduce the variety of strains, and result in accumulation of multi-drug-resistant CoNS. The role of CoNS as a significant contributor to bloodstream infections has been increasingly acknowledged, particularly in the context of immunocompromised individuals and those with indwelling medical devices. (7). There are studies indicating that CoNS carriage increases the risk of bacteraemia and sepsis in neonates and patients with malignancy (8,9).

The aim of this study was to screen for preoperative nasal staphylococcal carriage in patients who underwent knee or hip prosthesis surgery in the orthopedic clinic of our hospital and to compare the prosthetic infection rates in the first 3 months postoperatively in patients with and without staphylococcal carriage.

MATERIAL and METHOD

Study Population and Design

This study was designed as a prospective observational cohort study. A total of 151 patients older than 18 years of age who underwent knee or hip prosthesis implantation between January and September 2019 at the Istanbul University Medical

Faculty Orthopedics Clinic were included in the study. Preoperative nasal staphylococcal carriage was analyzed in these patients and the rates of prosthesis infection in the first 3 months postoperatively were evaluated.

A total of 151 patients older than 18 years of age who underwent knee or hip prosthesis implantation between January and September 2019 at the Istanbul University Medical Faculty Orthopedics Clinic were included in the study. Pregnant, pediatric patients and patients scheduled for revision surgery due to infected prostheses were not included in the study. Preoperative nasal staphylococcal carriage was analyzed in these patients and the rates of prosthesis infection in the first 3 months postoperatively were evaluated.

Data collection

Epidemiological and clinical information was recorded on a form. Demographic data, surgery indications, duration of surgery, preoperative, and postoperative hemogram data, sedimentation, CRP and procalcitonin levels were obtained from anamnesis and patient records. The patients and/or their families were informed that data from the research would be submitted for publication and gave their consent. Nasal swabs were taken from patients undergoing knee or hip replacement surgery 24 hours before surgery and cultured for the presence of bacteria. After incubation in 5% sheep blood agar at 37°C for 24 hours, the formation of round, smooth-surfaced yellow-white colonies represented growth. The colonies with positive catalase test were considered as staphylococci and DNAase test was performed. DNAase test positive strains were identified as *S. aureus* and negative strains were identified as CoNS. Antibiograms were performed on Mueller-Hinton medium and susceptibility to cefoxitin, cefazolin, penicillin, rifampicin, fusidic acid, trimethoprim-sulfomethoxazole, doxycycline, ciprofloxacin, erythromycin, clindamycin, gentamicin, and linezolid were determined using the disk diffusion method. Since our study was not an interventional study, no decolonisation treatment was given to patients with growth in nasal swab cultures, and routine prophylaxis schemes were applied peroperatively.

Definitions

Antibiotic susceptibilities were evaluated according to antibiotic zone diameters determined by European Committee on Antimicrobial Susceptibility Testing 2019 data. Patients were examined in the first, second and third post-operative months. Data such as swelling, redness, temperature rise, pain and movement restrictions, if any, were recorded in the patient form. Infection findings were evaluated according to the prosthetic joint infections guide published by the Infectious Diseases Society of America in 2013.

IBM SPSS Statistics for Windows Version 21.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA) was used for statistical analysis of the study data. Data are expressed as mean, standard deviation and percentage (%)". Student's t-test was used to analyze normally distributed numerically measured variables and the Mann-Whitney U test was used to analyze non-normally distributed numerically measured variables. x2 test or Fisher's exact test was used to analyze categorical variables. And $p < 0.05$ was considered statistically significant in all analyses

The study was approved by the Istanbul University Faculty of Medicine Clinical Research Ethics Committee (Date: 09.07.2018 and Number: 993).

RESULTS

Demographic Characteristics

Thirty-four (22.5%) patients were male and 117 (77.5%) were female; The mean age of the patients was 63.1 years; the mean height and weight were 161.1 cm and 78.3 kg, respectively, and the mean body mass index (BMI) was 30.3 kg/m^2. Thirty-four (22.5%) patients were smokers and 117 (77.5%) were non-smokers.

Comorbid Conditions

71 (47%) patients had hypertension, 40 (26.5%) had diabetes, 18 (11.9%) had hypothyroidism, and 7 (4.6%) had rheumatoid arthritis; 30 patients had no comorbidities. Sixteen patients overall and one patient who developed infection were immunosuppressed.

Surgical Characteristics

76 (50.4%) and 75 (49.6%) patients underwent hip and knee prosthesis surgery, respectively. The indication was gonarthrosis in 76 (100%) patients who underwent knee prosthesis surgery. Indications for hip prosthesis surgery were coxarthosis in 30 (19.8%) patients, congenital hip dislocation in 20 (13.2%) patients, avascular femoral head necrosis in 9 (5.9%) patients, and avascular femoral head necrosis in 15 (9.9%) patients. The mean operation time was 120.5 minutes overall and 132 minutes for those who developed infection.

Preoperative Nasal Swab Culture Results

Figure 1 shows the distribution of preoperative nasal swap culture results.

Characteristics of Patients Developing Prosthesis Infection

Prosthesis infection developed in 5 (3.3%) patients in the postoperative 3-month period. Only one in five patients who developed infection was a smoker. The characteristics of the patients are shown in Table 1.

Preoperative and Postoperative Hemogram, CRP, and Sedimentation Values

Preoperative and postoperative 24th hour hemogram, CRP and sedimentation results of infected and uninfected patients are shown in Table 2.

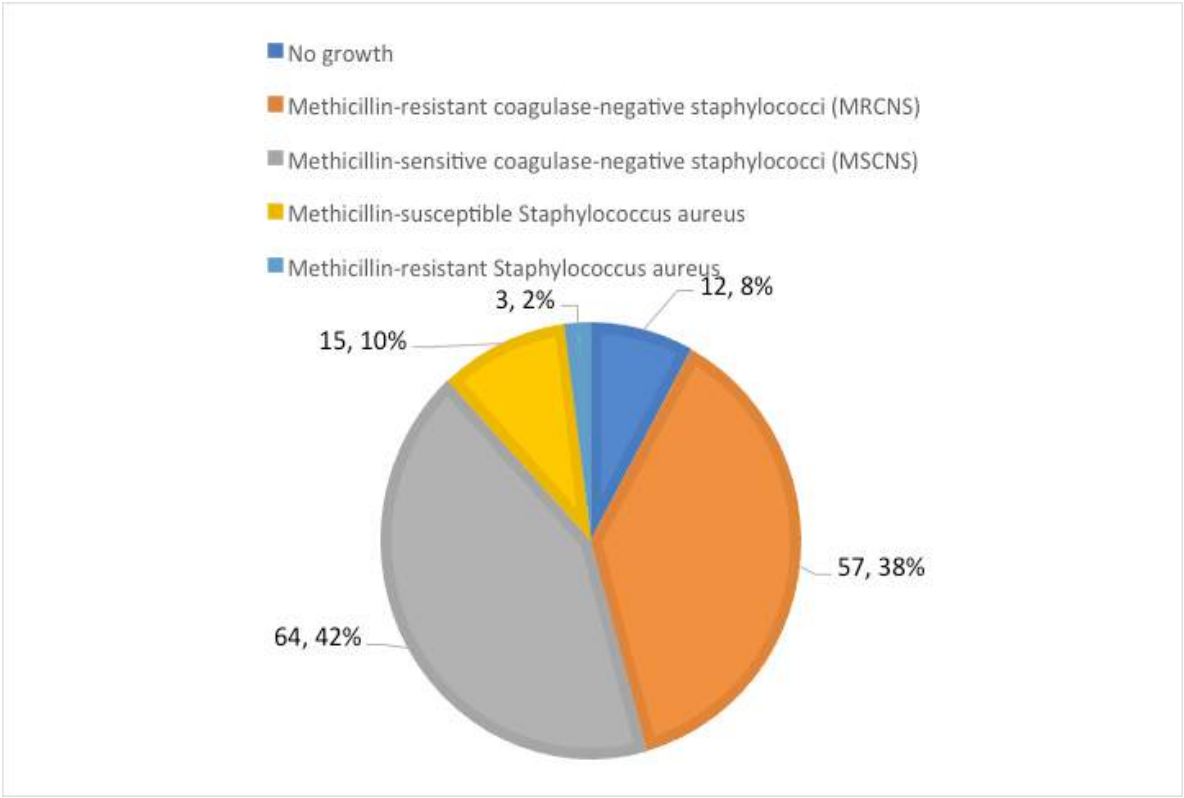

Figure 1. Distribution of preoperative nasal swab culture results according to factors

Table 1. Characteristics of Patients Developing Prosthesis Infection

Patient number	Age	Sex	Comorbidity	Surgery type	Surgery indication	Nasal swab culture	Infection development time (post operative/day)	Tissue culture
Patient 1	73	Female	Rheumatoid arthritis	Hip replacement surgery	Femoral neck fracture	MRCoNS	11 days	*Proteus mirabilis*
Patient 2	71	Female	Hypertension	Hip replacement surgery	Femoral neck fracture	MRCoNS	30 days	*Corynebacterium* spp.
Patient 3	71	Female	Hypertension	Knee replacemant surgery	Osteoarthritis	MSCoNS	45 days	No growth
Patient 4	49	Female	No comorbidity	Hip replacement surgery	Congenital hip dislocation	MRCoNS	49 days	C/G group streptococci
Patient 5	43	Male	Cerebrovascular disease	Hip replacement surgery	Heterotrophic ossification	MRCoNS	2 days	*Klebsiella pneumoniae*

MRCoNS: Methicillin-Resistant Coagulase Negative Staphylococci

MSCoNS: Methicillin-Sensitive Coagulase Negative Staphylococci

Table 2. Demographic characteristics of patients with and without postoperative prosthesis infection; preoperative and postoperative 24th hour laboratory values

Characteristics	**Patients without prosthesis infection (n = 146)**	**Patients with prosthesis infection (n = 5)**	***p***
Age, mean ± SD	63.17 ± 11.46	61.80 ± 14.09	0.793
Height (cm)	161.18 ± 8.90	158.60 ± 9.47	0.525
Weight (kg)	78.64 ± 14.55	68.80 ± 8.87	0.136
BMI (kg/m^2)	30.37 ± 0.48	27.69 ± 5.24	0.312
Operation time (min)	120.068 ± 25.723	132 ± 27.522	0.310
Preoperative leukocyte count (mm^3)	7495,83 ± 2376,86	8740 ± 4383.39	0.561
Preoperative neutrophil count (mm^3)	5002.08 ± 2315.39	6460 ± 4388.39	0.500
Preoperative lymphocyte count (mm^3)	1827.08 ± 594.16	1620 ± 216.79	0.439
Preoperative CRP level (mg/dL)	9.74 ± 17.65	9.60 ± 7.40	0.985
Preoperative sedimentation rate (mm/hour)	28.65 ±20.09	43.60 ±34.55	0.113
Postoperative leukocyte count (mm^3)	9413.47 ± 2619.41	14680 ± 3778.49	**< 0.01**
Postoperative neutrophil count (mm^3)	7308.65 ± 2403	11680 ± 3824.52	**< 0.01**
Postoperative lymphocyte count (mm^3)	1331.91 ± 545.14	1940 ± 733.48	**0.017**
Postoperative CRP level (mg/dL)	92.80 ± 82.97	139 ± 102.596	0.230
Postoperative sedimentation rate (mm/hour)	60.32 ±28.26	68.40 ±27.92	0.534

In the univariate analysis, leukocyte and neutrophil levels at the 24-hour postoperative mark were notably elevated in patients presenting with infections ($p < 0.01$). Additionally, lymphocyte counts measured 24 hours after surgery were significantly greater in these patients ($p = 0.017$).

There was no statistically significant difference in pre- and post-operative erythrocyte sedimentation rate and procalcitonin levels between patients with and without infection.

The study assessed the risk of developing an infection associated with preoperative nasal carriage of *S. aureus* using univariate analysis. The results showed no significant difference ($p = 0.256$). However, patients colonized with CoNS had a significantly increased likelihood of infection on univariate analysis ($p = 0.025$).

The mean BMI of patients with preoperative nasal *S. aureus* carriage was 32.71 compared to a mean BMI of 29.96 in non-carriers.

In our investigation, our findings indicate a lack of substantial relationship between smoking and immunosuppression in elevating the likelihood of post-operative prosthesis infection development ($p = 0.890$, $p = 0.528$). Furthermore, no notable variance was observed in the duration of surgery between patients who experienced infection and those who did not ($p=0.275$).

DISCUSSION

Our study suggests that carrying CoNS in the nasal passage increases the risk of prosthesis infection. No other studies in the literature have shown a link between nasal carriage of CoNS and an increased risk of prosthesis infection. Although our study adds value to the literature with this finding, further research will be needed to establish a clear association due to the small number of cases.

Furthermore, in five patients who developed an infection, the pathogens were different from CoNS found in nasal carriers. Although this finding is statistically significant, it is challenging to argue that there is an increased risk of prosthesis infection in CoNS carriers.

The prevalence of CoNS carriage in the community is high, but the intricacies of this phenomenon have been little studied. Nasal carriage of methicillin-resistant coagulase-negative staphylococci (MR-CoNS) is highly prevalent in community subjects, but its dynamic has been little investigated (10).

Studies have shown that CoNS weaken host defences and promote infection by damaging the natural barriers of the skin and mucous membranes (11). In addition, some factors, such as bacterial interactions in the human nose, may influence *S. aureus* colonisation and sometimes prevent colonisation. (12). Studies have demonstrated that nasal carriage of CoNS triggers various immunological mechanisms.Although CoNS have a much lower pathological potential than *S. aureus*, antigens of *S. haemolyticus* and *S. epidermidis* can induce dysfunctional immune responses. In vitro studies have shown that *S. epidermidis* may antagonise or cooperate with *S. aureus* in biofilm formation. These studies on the interactions of CoNS in mixed biofilms with *S. aureus* have primarily included healthy individuals (13). Indeed, it has been shown in various studies that there is a relationship between nasal CoNS carriage and the frequency and severity of atopic dermatitis in children (14). Considering the relationship of CoNS with other bacteria and their immunological effects, we hypothesise that nasal CNS carriage facilitates the development of infection by various mechanisms, although the infectious agents were non-CNS pathogens in 5 patients who developed infection. Our conclusion that the presence of CoNS in the nasal region increases the likelihood of prosthesis infection is supported by the results of studies investigating the relationship between CoNS and the development of infection.

If the results of our study are confirmed by further studies, it will be possible to clearly demonstrate the effect of nasal CoNS

on the development of prosthesis infections.

While the literature reports that nasal carriage of *S. aureus* is a risk factor for developing prosthetic infections, our study did not find such an association. The relationship between nasal *S. aureus* carriage and infection development has been evaluated in the literature and screening is recommended especially before cardiothoracic and orthopedic implant placement. In the current investigation, there was no development of prosthesis infection in any of the 18 patients with preoperative *S. aureus* carriage within the initial 3 months post-surgery (p = 0.256). In some studies, preoperative *S. aureus* carriage was found to be associated with infection, but there are also studies that did not find such a relationship. A landmark randomized-controlled trial evaluating the impact of S aureus decolonization reported a significant reduction in health care–associated S aureus infection, but could not find a significant reduction in PJIs in an underpowered subgroup analysis in orthopedic patients (15).

Prosthesis infection developed in five patients in our study. Of these, four patients were female and one was male. One study reported male sex as a risk factor for developing knee prosthesis infection (16). In another study investigating the risk factors for the development of hip prosthesis infection, female sex was reported as a protective factor against infection. (17) Since the number of female patients was significantly higher in the present study, the incidence of prosthesis infection was naturally higher in female patients.

In various publications, advanced age has been shown as a risk factor for the development of infections (18,19). In this study, the average age of women participating was 64.0 years, with those who developed an infection having an average age of 66.3 years.This finding is important in terms of demonstrating the increased risk of infection with age and is consistent with the literature.

A previous study reported that higher BMI was associated with a higher risk of infection and a BMI > 30 kg/m^2 is considered as a risk factor for the development of prosthesis infection (19).

In the present study, mean the BMI was 30.37 kg/m^2 among all patients compared to 27.69 kg/m^2 among patients who developed infection. There was no statistically significant difference between patients with and without infection in terms of BMI (p = 0.312). This may be attributed to the small number of patients who developed postoperative prosthesis infection.

The mean BMI of patients with preoperative nasal *S. aureus* carriage was 32.71 compared to a mean BMI of 29.96 in non-carriers. Although there was no statistically significant difference due to the small sample size (p = 0.059), BMI could be considered a risk factor for *S. aureus* carriage. In a study in which risk factors for staphylococcal carriage were evaluated, it was shown that every 2.5 kg/m^2 increase in BMI increased the risk of nasal staphylococcal carriage by 7%, which is consistent with our results (20).

One of the infected patients was diagnosed with rheumatoid arthritis, one with hypertension and one with diabetes. Diabetes is a known risk factor for the development of prosthesis infection.

In a cohort study conducted in patients with knee and hip prosthesis, it was found that the presence of diabetes increased the risk of infection 1.55 times (21). In the present study, no significant relationship was found between diabetes and infection risk (p = 0.187). This may also be attributed to the small number of patients. In the literature, rheumatoid arthritis is considered a risk factor for prosthesis infection. In a study comparing patients who underwent hip or knee replacement due to osteoarthrosis or rheumatoid arthritis, the risk of developing infection in the rheumatoid arthritis group was found to be 4.08 times higher than the osteoarthrosis group (22). However, no significant increase in infection risk was found in patients with rheumatoid arthritis in the present study (p = 0.204). Although a statistically significant increase could not be shown in our study due to the fact that

only six patients had rheumatoid arthritis, it is also interesting that one of the five patients who developed infection was diagnosed with rheumatoid arthritis.

There was no significant association between immunosuppression and development of prosthesis infection ($p = 0.528$). However, immunosuppression is considered a risk factor for the development of prosthesis infection (23). In a study evaluating the development of bone joint infection in patients who underwent hematopoietic stem cell transplantation, it was reported that immunosuppression constituted a serious risk factor and treatment response and prognosis were worse in immunosuppressed patients (23). In a case-control study conducted to evaluate the development of prosthesis infection in solid organ transplant patients, immunosuppression was determined as a risk factor for the development of infection (24).

Similarly, no significant relationship was found between smoking and development of infection. However, smoking is considered a risk factor for the development of infection. In another study, it was reported that the risk of developing prosthesis infection increased 1.8-fold in patients who had been smoking for more than one year (25). In the literature also reported that smoking increased MRSA colonization (26).

The lack of such a significant relationship in the present study may be due to the low number of smokers and patients who developed infection.

Leukocyte, neutrophil and lymphocyte counts were significantly increased in the infection group 24 hours after surgery ($p < 0.01$). In the literature, the sensitivity (79%) and specificity (46%) of leukocytosis in predicting prosthesis infection were found to be low and it was stated that it should not be used as a predictor unless there are additional abnormal findings (27). When comparing the CRP and erythrocyte sedimentation rates at 24 hours after surgery, no significant difference was observed. Although not statistically significant, postoperative CRP was found to be higher in the infection group than in the non-infection group. Similarly, a study in the literature reported that a CRP value > 10 mg/dL on postoperative day 4 was predictive of infection development in patients undergoing lumbar instrumentation (28). Early postoperative CRP elevation may be indicate developing of infection.

This study has several strengths. Firstly, the results of the study are valuable as it is a prospective cohort study. Secondly, patients underwent monthly postoperative evaluations for three months, during which they received comprehensive examinations and close monitoring for signs of prosthesis infection through blood tests. Thirdly, this is a rare study to evaluate CoNS carriage in relation to prosthesis infection development and may provide an idea for further studies. Our study has also several limitations. Firstly, our sample size was small and the number of patients who developed infection was 5 patients. The multivariate analysis was not performed due to the low number of patients who developed infection. Secondly, patients could be followed up for the first three months postoperatively. Finally, subtyping of CoNS grown in preoperative nasal swab cultures could not be performed due to inadequate laboratory facilities.

In conclusion; our study found no association between nasal *S. aureus* carriage and prosthesis infection. While nasal carriage of *S. aureus* is considered a significant risk for prosthetic infections, it is also likely that CoNS contribute to the pathogenesis of prosthetic infections. As the pathogens in patients with prosthesis infection differ from those causing CoNS, and the number of patients who went on to develop infection was 5, it is impossible to establish a clear link between carrying CoNS and infection. Considering the results of our study and the available literature data, it is not possible to make a clear recommendation regarding nasal staphylococcal carriage screening. Further studies are needed to clearly demonstrate the relationship between carriage and the pathogenesis of infection development. An understanding

of the pathogenic mechanisms of CoNS would facilitate the development of therapeutic approaches aimed at preventing prosthetic infections and combating their complications.

Finally, monitoring of leukocyte and CRP levels in the early postoperative period can be an indicator of prosthesis infection.

ETHICS COMMITTEE APPROVAL

* The study was approved by the Istanbul University Faculty of Medicine Clinical Research Ethics Committee (Date: 09.07.2018 and Number: 993).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Araştırma Makalesi/Original Article
Makale Dili "İngilizce"/Article Language "English"

Türk Hijyen ve Deneysel Biyoloji Dergisi

Questioning: Is *Cocos nucifera* oil antifungal effective for dental and oral health or not?

Sorgulama: *Cocos nucifera* yağı diş ve ağız sağlığı için antifungal etkili midir, değil midir?

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ABSTRACT

Objective: Aim of this study was to investigate the antifungal properties of different acrylic resins coated with *Cocos nucifera* oil. The escalating threat to human health posed by serious *Candida* spp. infections and the growing challenge of antimicrobial resistance underscores the pressing need for novel antifungal compounds. In response to this, our study explored the antifungal properties arising from the application of *Cocos nucifera* oil coating on different acrylic resins. Since there are no previous studies in the literature investigating the relationship between *Candida albicans*, *Cocos nucifera* oil, and commonly used 3D-printed acrylic resins, this topic deserves further investigation.

Methods: In our study, six discs were fabricated using the heat-cured method, and the other six discs were fabricated using the 3D-printing method. The surfaces of the experimental acrylic discs were coated with *Cocos nucifera* oil, but the surfaces of the acrylic discs in the control group were not coated with *Cocos nucifera* oil. The antifungal activities of acrylic resins

ÖZET

Amaç: Bu çalışmanın amacı *Cocos nucifera* yağı ile kaplanmış farklı akrilik reçinelerin antifungal özelliklerinin araştırılmasıdır. Ciddi *Candida* spp. enfeksiyonlarının insan sağlığına yönelik tehditini arttırması ve antimikrobiyal direncin giderek artması, yeni antifungal bileşiklere olan acil ihtiyacın altını çizmektedir. Bu sorunlara cevap olarak, çalışmamızda *Cocos nucifera* yağ kaplamasının farklı akrilik reçineler üzerine uygulanmasından kaynaklanan antifungal özellikleri araştırılmıştır. Literatürde *Candida albicans*, *Cocos nucifera* yağı ve yaygın olarak kullanılan 3D baskılı akrilik reçineler arasındaki ilişkinin araştırılması konusunda daha önce yapılmış herhangi bir çalışma olmadığından dolayı bu konunun araştırılmasına ihtiyaç vardır.

Yöntem: Çalışmamızda, altı adet disk, ısıyla sertleştirme yöntemi kullanılarak, diğer altı adet disk ise 3D baskı yöntemi kullanılarak üretilmiştir. Deneysel akrilik disklerin yüzeyleri *Cocos nucifera* yağı ile kaplanmıştır. Kontrol grubundaki akrilik disklerin yüzeyleri ise *Cocos nucifera* yağı ile kaplanmamıştır. *Cocos nucifera* yağı ile kaplanmış akrilik reçinelerin *C.*

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coated with *Cocos nucifera* oil against *C. albicans* ATCC 90028 strains were determined using the plate counting method.

Results: Contrary to expectations, *Cocos nucifera* oil did not exhibit any antifungal effects; it was determined that *C. albicans* promoted growth increase on acrylic discs coated with *Cocos nucifera* oil than on the control acrylic discs. Thus *Cocos nucifera* oil was found to promote *C. albicans* growth.

Conclusion: This study demonstrated that both heat-cured and 3D-printed acrylic resins coated with *Cocos nucifera* oil did not have any antifungal effects against *C. albicans*, the contrary, the acrylic resins coated with *Cocos nucifera* oil promoted *C. albicans* growth.

Key Words: *Candida albicans*, antifungal, coconut oil, 3D-printed acrylic resins, heat-cured acrylic resin

albicans ATCC 90028 suşlarına karşı antifungal aktiviteleri plate counting yöntemi kullanılarak belirlenmiştir.

Bulgular: Beklenenin aksine, *Cocos nucifera* yağının antifungal etki göstermediği; aksine, *Cocos nucifera* ile kaplanmış akrilik diskler üzerinde, kontrol akrilik disklere göre daha fazla *C. albicans* üremesi olduğu görülmüştür. *Cocos nucifera* yağının *C. albicans* üremesini teşvik ettiği belirlenmiştir.

Sonuç: Bu çalışma ile *Cocos nucifera* yağı ile kaplanmış hem ısıyla sertleştirilmiş hem de 3D baskılı akrilik reçinelerin *C. albicans*'a karşı herhangi bir antifungal etkiye sahip olmadığı, aksine *Cocos nucifera* yağı ile kaplanmış akrilik reçinelerin *C. albicans* üremesini arttırdığı sonucuna varılmıştır.

Anahtar Kelimeler: *Candida albicans*, antifungal, hindistan cevizi yağı, 3D-baskılı akrilik reçine, ısıyla sertleştirilmiş akrilik reçine

INTRODUCTION

Yeasts of the genus *Candida* are inherent components of the typical microbiota found in the skin, oral cavity, gastrointestinal tract, and genitourinary tract (1). *Candida albicans* is the most prevalent strain in oral candidiasis and denture stomatitis (2), a common inflammatory condition of oral mucosa in individuals wearing dentures (3). Although other *Candida* species can cause infections, they occur less frequently (4). In addition to infections, several other factors affect the development of denture stomatitis, including the nocturnal use of oral cavity prostheses and poor oral hygiene (5). The irregularities and pores that develop on the surface of acrylic resin denture base materials can transform the inner surface of dentures into a reservoir of microorganism (6).

Complete and partial edentulism lead to an imbalance in all components of the stomatognathic system. Consequently, practitioners and researchers aim to restore all affected functions (7). In the past decade, digital technologies have emerged as alternatives to conventional (heat-cured) fabrication of acrylic removable dentures (8). Computer-aided design and computer-aided manufacturing (CAD-CAM), incorporating additive techniques such as 3D-printing, has facilitated the manufacture of removable dentures (9). The advantages of 3D-printing include the ability to build intricate geometries by adding materials layer-by-layer, thereby minimizing waste (10). Therefore, the use of 3D-printing to create completely removable denture bases has become increasingly popular in both research and clinical practice. Although it is equal to or surpasses the conventional method in terms of the overall accuracy of the produced dentures (11), this method still has some limitations concerning its mechanical and physical properties (10, 12). Research has indicated that 3D-printed acrylic resins exhibit higher surface roughness and inferior surface characteristics than heat-cured acrylic resins (7, 13, 14).

The widespread use of acrylic resin for edentulism has led to growing emphasis on maintaining optimal denture hygiene. Mechanical and chemical methods are commonly recommended to prevent the buildup of microorganisms and subsequent oral mucosal inflammation during denture hygiene (6). Efforts have been made to produce an antimicrobial acrylic resin. Nonetheless, the inclusion of such agents may alter the characteristics of acrylic resins, ultimately affecting their appearance and strength (15). Additionally, a variety of antifungal agents are readily available for treating oral *Candida* infections. Despite their proven efficacy, these drugs are associated with undesirable side effects such as bitter taste, allergic reactions, and potential drug interactions. Investigating natural products obtained from plants that are traditionally used in medicine can offer valuable substitutes for antifungal agents (5). Studies have indicated that natural substances, particularly plant essential oils, have the potential to combat various strains of *Candida* spp., including the highly prevalent *C. albicans* (16). Among these, coconut oil, scientifically known as *Cocos nucifera*, has garnered attention because of its efficacy against fungal infections. Given the accessibility, unique composition, and affordability of coconut oil, it is crucial to investigate its efficacy as an antifungal agent (17). Although they are widely used in clinical practice, the effects of natural products against fungal pathogens remains controversial (5, 18). Furthermore, there is a notable gap in studies investigating the effects of coconut oil on different types of denture resins against *C. albicans*. Thus, this study aimed to assess the antifungal activity of *Cocos nucifera* oil on both heat-cured and 3D-printed acrylic resin surfaces and compare their effectiveness.

The hypotheses tested were as follows:

1) The application of *Cocos nucifera* oil would have antifungal activity against *C. albicans* growth

2) 3D-printed resin discs would provide an equal surface topology against *C. albicans* compared to heat-cured acrylic resin.

This study has the potential to shed light on the role of alternative medicine in digital and conventional dentistry. Previous literature lacks evidence of the effects of *Cocos nucifera* oil on both conventional and 3D-printed denture resins against *C. albicans*. This study was prompted by the urgent need to control the growth of *C. albicans*, a major cause of denture stomatitis.

MATERIAL and METHOD

The sample designs

In this study, a total of 12 acrylic discs were fabricated: heat-cured and 3D-printed. Six discs were fabricated by heat-cured method and other six discs were fabricated by 3D-printing method. The fabricated rectangular samples had a width of 2 cm, length of 5 cm, and thickness of 1 cm.

Heat-cured acrylic resin

The heat-cured acrylic resin discs were fabricated according to the manufacturer's instructions, as described in a previous study (7). Wax patterns were cut using modelling wax (Hindustan Modelling Wax No. 2, The Hindustan Dental Products, Hyderabad, India) according to predetermined dimensions and invested in Type 3 dental stones (Moldano, Kulzer GmbH, Hanau, Germany) using a mixing ratio of 100 g powder to 30 mL water under vacuum for 30 min, following the manufacturer's instructions. Subsequently, the flask was immersed in water at 100°C for five minutes. Once the flask was separated, any remaining wax remnants were removed. A gypsum separator (Model Sep, Harvest Dental Inc., California, United States) was used to isolate the mold. The resin was mixed in a beaker at a ratio of 35 g powder to 14 mL liquid and stirred with a spatula for one minute.

The acrylic resin (Meliodent Heat Cure, Kulzer GmbH, Hanau, Germany) reached packable consistency within 10 min and was poured into the mold. The flask underwent a 15-minute immersion in boiling water, after which the heat source was deactivated. The polymerization process utilized a short cycle method

according to the manufacturer's guidelines. After a 20-minute boiling period, the flask was cooled gradually in a water bath. Following the completion of the polymerization phase, the specimens were removed from the mold. Subsequently, the printed specimens were trimmed, ground, and buffed using a polishing machine (Interlab, Hull, UK) with the application of pumice powder, emery paper, and tungsten carbide bur.

3D-printed acrylic resin

Wax patterns were digitized to obtain a high-resolution STL format using a chairside software (CEREC 5.0.0, Dentsply-Sirona, NY, USA). The resulting STL file was processed using a software (Blender for Dental 3.3.1, Blender, New York, USA). Subsequently, resin discs were produced through 3D printing using an LCD printer (FreeShape 120, Ackuretta, Taipei, Taiwan), and resin material (Optiprint Laviva, Dentona AG, Dortmund, Germany) in a light pink hue (LOT:48230) was formulated for disc fabrication. The printing was completed with a wavelength of 405 nm, maximum laser speed of 5000 mm/s, layer thickness of 100 µm, and build angle of 90°, as recommended to achieve optimum material properties (19, 20). After printing, the specimens were cleaned using a dual-tank cleaning bath treatment for 3-minute each, containing 99.8% isopropanol (CLEANI; Ackuretta, Taipei, Taiwan), followed by a 3-minute post-curing phase using an ultraviolet curing unit (Tray-LED; Ackuretta, Taipei, Taiwan) with a 405 nm LED wavelength and 39 W power at 60 °C, following the manufacturer's instructions. The printed specimens were ground using water lubricant, followed by polishing using silicon carbide papers with grain sizes of 400 (P600), 600 (P1200), and 1200 (P2500). Subsequently, they were polished with 0.05 µm MasterPrep Alumina discs.

Test microorganism

The test microorganism, *C. albicans* ATCC 90028, was obtained from the culture collection of the School of Pharmacy, Department of Pharmaceutical Microbiology Research Laboratory at Istanbul Medipol University.

Inoculum preparation

C. albicans suspension was prepared from 3 days culture and the yeast density was adjusted to $4.6x10^3$ CFU/mL by using a densitometer and diluting (21).

Determination of the *Candida* growth on acrylic resin discs

The antifungal activities of acrylic resins coated with *Cocos nucifera* oil against *C. albicans* ATCC 90028 strains were determined using the plate counting method described by Hoş et al. (21).

Each the acrylic disc surfaces (2 cm x 5 cm) were sterilized for 10 min using UV light (Philips TUV 30 W, Ultraviolet) (21). The test (experimental) group was flooded with 1.5 mL of warm (37 °C) *Cocos nucifera* oil (Bio Planete, Bram, France), and the control group was kept without the *Cocos nucifera* oil coating. A 100 µL of a 24-hour yeast culture (4.6×10^3 CFU/mL) was transferred onto the test and control groups, and the surfaces were covered with polyethylene (PE) film (1.5 cm × 4 cm). Three discs of 3D-printed acrylic resin were coated with *Cocos nucifera* oil and other three discs of 3D-printed acrylic resin were not coated with *Cocos nucifera* oil, as those were controls. Three discs of heat-cured acrylic resin were coated with *Cocos nucifera* oil and other three discs of heat-cured acrylic resin were not coated with *Cocos nucifera* oil, as those were controls. Hence, the tests were performed with three replicates. The discs were incubated for 24 h at 37 ±1°C with relative humidity (RH) exceeding 90%. Following incubation, the discs were washed with 20 mL of a 0.87% NaCl solution. A 100 µL of this solution was spread-plated onto Potato Dextrose Agar. After the 72-hour incubation at 37 ±1°C, the colonies were counted.

Statistical analysis

Statistical analyses were performed using SPSS version 26 (SPSS Inc., Chicago, IL, USA). The

distribution and homogeneity of the data were assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests. The findings revealed that the data did not display a homogenous distribution, and non-parametric tests were employed as a result of the limited sample size. Mann-Whitney U test was used for statistical analysis, and a significance level of $p < 0.05$ was used for the analysis.

The study was approved by the Non-Interventional Clinical Research Ethics Committee of Istanbul Medipol University (Date: 22.04.2022 and Number: 363).

RESULTS

Cocos nucifera oil

Table 1 presents the number of *C. albicans* colonies for control without *Cocos nucifera* oil and experimental resins coated with *Cocos nucifera* oil. The findings revealed that *C. albicans* demonstrated growth increase on *Cocos nucifera* oil-coated acrylic discs than on the control acrylic discs. Notably, there was a statistically significant difference between the control group and the *Cocos nucifera* oil-coated acrylic discs for the 3D-printed acrylic resins (p:0.022). In the control group of 3D-printed acrylic resin discs, the median value of *C. albicans* count was 266 (172-465) CFU/sample whereas the median value was 1817 (882-1907) CFU/sample for 3D-printed acrylic resin discs coated with *Cocos nucifera* oil.

Additionally, heat-cured acrylic resins revealed a statistically significant difference between the control group and the *Cocos nucifera* oil-coated discs (p =0.001). Although the control group did not show *C. albicans* growth, the *Cocos nucifera* oil-coated discs had a median value of 315 (302-324) CFU/sample. The findings are shown in Figure 1.

Table 1. Comparison of the numbers of *C. albicans colonies on acrylic resin surfaces (3D-printed / Heat-Cured) coated with/without Cocos nucifera oil*

	3D- Printed				Heat Cured				
	Mean	S.D.	Med.	Min-Max	Mean	S.D.	Med.	Min-Max	*p*
Control	301	149.6	266	172-465	0	0	0	0	0.037
Experimental	1535.3	567.6	1817	882-1907	313.7	11.1	315	302-324	0.050
p	0.022				0.001				

Mean= mean, S.D.= standard deviation, Med= Median, Min-Max= Minimum-Maximum, *p*= p-value

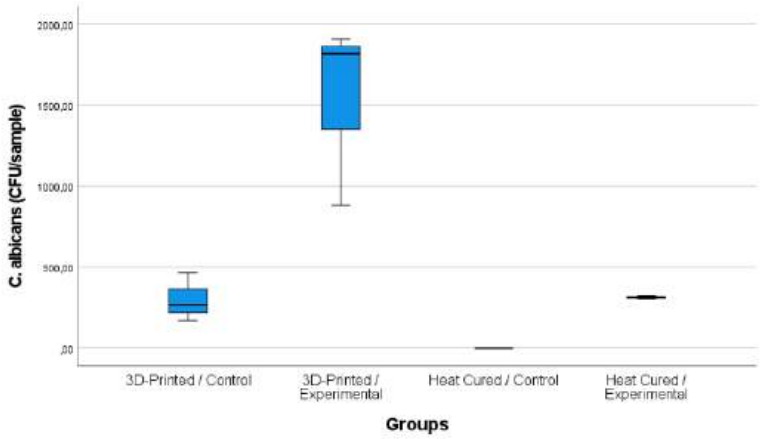

Figure 1. Effects of *Cocos nucifera* oil on *C. albicans* growth

Manufacturing techniques

The number of *C. albicans* colonies for 3D-printed acrylic resins and heat-cured acrylic resins coated with *Cocos nucifera* oil are shown in Table 2. It was found that *C. albicans* exhibited growth increase on 3D-printed acrylic resin discs than on heat-cured acrylic resins. The number of *C. albicans* colonies was statistically significant in control groups (p :0.037), with no growth observed in heat-cured acrylic resin discs, but a median value of 266 (172-465) CFU/sample was found in 3D-printed acrylic resin discs. The effects of different manufacturing techniques on *C. albicans* growth were highlighted by these findings. Statistically significant differences were found between the manufacturing techniques (p :0.050) in the groups coated with *Cocos nucifera* oil. The median values of *C. albicans* count in 3D-printed acrylic resin discs coated with *Cocos nucifera* oil was 1817 (882-1907) CFU/sample, while in heat-cured acrylic resin discs coated with *Cocos nucifera* oil, it was 315 (302-324) CFU/sample. The schematic representation is given in Figure 2.

Table 2. Comparison of the number of *C. albicans colonies in terms of the control and experimental groups*

		n	M	S.D.	p
3D -Printed	Control	3	301.0	149.6	0.022*
	Experimental	3	1535.3	567.6	
Heat-cured	Control	3	0.0	0.0	0.001*
	Experimental	3	313.7	11.1	

n= sample size, M= mean, S.D.= standard deviation, p= p-value

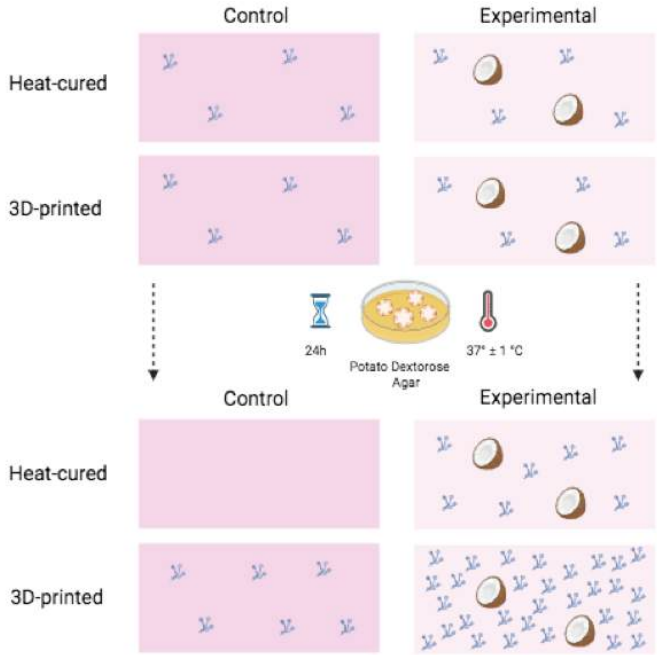

Figure 2. Graphical abstract
Schematic representation of the effects about differently manufactured acrylic resin discs coated with *Cocos nucifera* oil against to *C. albicans*.
: *Cocos nucifera* : *C. albicans*

DISCUSSION

In the present study, we investigated the antifungal activity of coconut oil on heat-cured and 3D-printed acrylic resin discs. As modern medicine comes with the risk of side effects, individuals are increasingly turning towards traditional practices as complementary therapies to improve their overall health. This quest for a solution for denture stomatitis has unveiled a potential natural remedy. According to Kumar et al. (17) and Krishnamoorthy et al. (16), in vitro studies have demonstrated that when *Cocos nucifera* oil is incorporated into tissue conditioner, it was showed a significant reduction in the colonization of *C. albicans*. However, our study yielded contrasting results, with *C. albicans* growth in the presence of *Cocos nucifera* oil. Shino et al. (22) conducted a study on the impact of *Cocos nucifera* oil on early childhood caries. They concluded that *Cocos nucifera* oil showed antifungal activity. Our findings challenge the literature on the potential benefits of *Cocos nucifera* oil for inhibiting *C. albicans* growth.

The initial step in the colonization and invasion of host tissues by *C. albicans* is its ability to adhere to acrylic resin surfaces. In addition, rough surfaces can increase the accumulation of organisms in prostheses even after cleaning. Yeast growth is aided by rough surfaces. The topology of improperly polished prostheses also favors yeast growth (15). Baciu et al. (7) revealed that among the tested denture base resins (heat-cured, CAD/CAM milled, and 3D-printed), 3D-printed acrylic resin exhibited the highest surface roughness. These results are supported by those of Helal et al. (13) and Zeidan et al. (14) In this study, *C. albicans* showed more growth increase on the 3D-printed acrylic resin surface coated with *Cocos nucifera* oil than on the heat-cured acrylic resin surface coated with *Cocos nucifera* oil.

A study conducted by da Silva et al. (19) showed that, despite the lower surface roughness compared to heat-cured acrylic resins, *C. albicans* exhibited growth increase on 3D-printed acrylic resins. They indicated that surface free energy of 3D-printed acrylic resins may be more likely associated with that colonization than their surface roughness. As previously described by Gad et al. (23), the surface roughness, the surface free energy, and the contact angle of the resin are also responsible for *C. albicans* growth. Moreover, the inferior surface characteristics of 3D-printed acrylic resin can be linked to the processing techniques employed, including light polymerization and printing methodologies, such as the layering technique, printing orientation, and associated parameters (7).

In conclusion; To sum up, the first hypothesis was rejected, as *Cocos nucifera* oil did not have antifungal activity against *C. albicans*. Furthermore, the second null hypothesis was also rejected, as the 3D-printed acrylic resin discs coated with *Cocos nucifera* oil showed more *C. albicans* growth increase compared to the heat-cured acrylic resin discs coated with *Cocos nucifera* oil.

In conclusion, this pioneer study proved that both heat-cured and 3D-printed acrylic resin discs coated with *Cocos nucifera* oil do not have any antifungal effects against *C. albicans*. This is the first report proving that both heat-cured and 3D-printed acrylic resins coated with *Cocos nucifera* oil do not have antifungal effects against *C. albicans*. Contrary to expectations, *Cocos nucifera* oil did not exhibit antifungal effects; instead, it unexpectedly promoted a favorable environment for *C. albicans* growth, particularly on 3D-printed acrylic resin discs. These findings challenge the initial hypotheses and underscore the interactions between natural products, material surfaces, and microbial behavior. Additionally, 3D-printed acrylic resin promoted more *C. albicans* growth increase compared to conventional acrylic resin. It is needed further investigation to determine its efficacy against other fungal species. To expand on these findings, it is imperative to thoroughly investigate relationship among *C. albicans*, *Cocos nucifera* oil and widely utilized 3D-printed resins. Future research should

focus on elucidating the underlying mechanisms that contribute to the observed discrepancies and investigating ways to reduce the enhanced growth of *C. albicans* in 3D-printed acrylic resin.

ETHICS COMMITTEE APPROVAL

* The study was approved by the Non-Interventional Clinical Research Ethics Committee of Istanbul Medipol University (Date: 22.04.2022 and Number: 363).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

Glukokortikoid ile oluşturulan insülin direncinde timokinonun karaciğer DNA hasarının tamiri ve moleküler yolaklar üzerindeki etkileri

Investigation of the effect of thymoquinone on liver tissue DNA damage and molecular pathways in glucocorticoid-induced insulin resistance

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ÖZET

Amaç: Bu çalışmada, insülin direncine bağlı olarak meydana gelen doku hasarının önlenmesinde veya tamir edilmesinde çok sayıda etken maddenin rolü moleküler düzeyde incelemektedir. Bu çalışma, *Nigella sativa*'nın başlıca etken maddesi olan ve pek çok yararlı etkisi bilinen timokinonun (TK) deneysel insülin direncinde karaciğer dokusundaki DNA hasarının tamiri ve bazı moleküler yolaklar üzerindeki etkilerinin değerlendirmesi amacıyla yapılmıştır.

Yöntem: Bu çalışmada, glukokortikoid ile indüklenen insülin direnci modeli oluşturulmuştur. Kontrol (K) 7 adet, İnsülin Direnci (İD) 7 adet, Timokinon (TK) 7 adet, timokinon ile korunma (TKI) 7 adet ve tedavi (ITK) 7 adet, tedavi kontrol için insülin direnci+metformin (İM) 5 adet olmak üzere toplam 40 adet rat kullanılmıştır. Çalışma sonucunda karaciğer dokusunda metabolik genlerin (antioksidan: Gpx, Sod, nekrotik: Rip1, Rip3, otofajik: Atg3 Atg5, apoptotik: Kaspaz 3 Kaspaz 8 Kaspaz 9, DNA hasarını onaran: KU70, KU80, TP53) ekspresyon seviyeleri, Real-time qPCR yöntemi ile belirlenmiştir. Gruplar arasındaki farklılık kontrol grubunun ekspresyonun

ABSTRACT

Objective: This study investigates the role of various compounds in the prevention or repair of tissue damage associated with insulin resistance at the molecular level. It aims to evaluate the effects of thymoquinone (TK), the principal active compound of *Nigella sativa* known for its numerous beneficial effects, on DNA damage and some molecular pathways in experimental insulin resistance.

Methods: The insulin resistance model was induced using glucocotiocoids. A total of 40 Wistar-Albino rats were utilized, comprising 7 in the control (C) group, 7 in the insulin resistance (IR) group, 7 receiving TK, 7 protection with TK (TKIR), and undergoing treatment with TK (IRTK) and 5 for tretament control with insulin resistance plus metformin (IM) groups. Expression levels of metabolic genes (antioxidant: Gpx, Sod; necrotic: Rip1, Rip3; autophagic: Atg3, Atg5; apoptotic: Caspase 3, Caspase 8, Caspase 9; DNA repair: KU70, KU80, TP53) in liver tissue were determined using Real-time qPCR. Differences between groups were assessed by comparing fold changes in expression against the

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artış-azalış kat (fold changes) sayısı ile karşılaştırılıp değerlendirilmiştir. İstatistiksel analizler SPSS programı ile gerçekleştirilmiştir.

Bulgular: DNA hasarını onaran KU70 ve KU80 gen ekspresyonları, TK verilen gruplarda artış göstermiştir. TP53 geni I grubunda belirgin bir artış gösterirken, tedavi için TK verilen grupta azalmıştır.Apoptotik Kaspaz8 geni ekpresyonu ID grubunda artmış, TK verilen gruplarda azalmış, Kaspaz 3 ve Kaspaz 9 gen ekspresyonlarında ise önemli bir farklılık saptanmamıştır. Antioksidan (Gpx1,Sod1) gen ekspresyonu bakımından, gruplar arasında anlamlı bir fark görülmemiştir. OtofajikAtg5, nekrotikRip1 ve Rip3 genlerinin ekspresyonu I grubunda artarken, TK verilen gruplarda azalmıştır.

Sonuç: Sonuç olarak TK'nun, insülin direncini azaltma, glukoz toleransını iyileştirme ve pankreatik ß-hücre fonksiyonlarını destekleme gibi etkilerinin; antioksidan özelliklerinin yanı sıra, KU70 ve KU80 genleri aracılığı ile DNA onarımını attırması, antiapoptotik genleri upregüle etmesi ve otofajik ve nekrotik yolakları inhibe etmesi gibi mekanizmalardan kaynaklanabileceği sonucuna varılmıştır. Bu bulguların, insülin direncine bağlı komplikasyonların tedavisi ve önlenmesinde TK'nun karaciğer koruyucu etkisinin biyokimyasal temellerini anlamada yararlı bilgiler sağlayabileceği düşünülmektedir.

Anahtar Kelimeler: İnsülin direnci, timokinon, karaciğer, DNA hasarı, DNA tamiri, moleküler yolaklar

control group. Statistical analysis was performed using SPSS.

Results: The expression levels of DNA repair and damage genes KU70 and KU80 increased in the groups receiving TK. The TP53 gene exhibited a significant increase in the IR group but decreased in the TK treatment group. Apoptotic Caspase 8 gene expression was elevated in the IR group and reduced in the TK groups, while no significant differences were observed for Caspase 3 and Caspase 9 expression. No significant differences were noted in antioxidant gene expression (Gpx1, Sod1) among the groups. Autophagic Atg5, and necrotic Rip1 and Rip3 gene expressions increased in the IR group but decreased in the TK groups.

Conclusion: The results suggest that TK may reduce insulin resistance, improve glucose tolerance, and support pancreatic ß-cell functions through mechanism including enhancing DNA repair via KU70 and KU80, upregulating antiapoptotic genes, and inhibiting autophagic and necrotic pathways. These results could provide valuable insights into the biochemical basis for the hepatoprotective effects of TK in the treatment and prevention of insulin resistance-related complications.

Key Words: Insulin resistance, thymoquinone, liver, DNA damage, DNA repair, molecular pathways

GİRİŞ

İnsülin direnci (İD), hücrelerin insüline normalden daha az duyarlı olması nedeniyle kan glukozunun yükselmesi ve pankreasın fazla miktarda insülin üretmesine rağmen hücrelerin bu insüline yeterli yanıt vermemesi durumunu ifade etmektedir. İnsülin direnci, metabolik sendrom olarak bilinen çeşitli metabolik, hücresel ve klinik sorunlara (hipertansiyon, yüksek plazma trigliserid, düşük HDL, hiperinsülinemi, Tip 2 diyabet, koroner kalp hastalığı ve damar anormallikleri vs) neden olur. Hücrelere zarar veren reaktif oksijen türlerinin fazlalığı, insülin direncinin patogenezinde önemli bir etiyolojik faktör olup, kas, karaciğer ve yağ dokusunda insülin direncinin gelişmesini kolaylaştıran maddelerinin birikimiyle oluşan bir tür hücresel strese yol açar (1-4).

TK, çörek otu (*Nigella sativa*) tohumunda bulunan ve geleneksel tıbbi değere sahip aktif bir ana bileşiktir. İyi bilinen antioksidan, antiinflamautuvar,

antiobezite, antihipertansif, antihiperlipidemik ve antidiyabetik otofajik özelliklerinin yanında, diyabette hiperglisemi, hiperinsülinemi, hiperlipidemi, insülin direncini önemli ölçüde önlemesi sayesinde insülin direncinin yönetiminde alternatif bir doğal ilaç olarak kullanılabileceğine dair görüşler vardır (5-9).

TK'un antidiyabetik etkilerini, pankreatik adacıkların bütünlüğünün korunması, glukoz-insülin homeostazisi, lipid metabolizması, hepato-renal fonksiyonları ve histomorfolojisini iyileştirerek gösterirken (10), hepatoprotektif etkilerinin, güçlü antioksidan, antiinflamatuar, antifibrotik, antiapoptotik/proapoptotik ve antikanserojenik özelliklerinden kaynaklandığı (11) düşünülmektedir.

Bu çalışma; deneysel insülin direncinde karaciğer dokularındaki DNA hasarının tamiri ve moleküler yolaklar (apoptotik, otofajik, nekrotik, oksidatif yolak) üzerine, tedavi ve korunma için TK uygulanmasının, etkisinin araştırılması amacıyla planlandı.

GEREÇ ve YÖNTEM

Bu çalışmada, "Glukokortikoid ile indüklenen insülin direncinde timokinonun vitamin D metabolizması üzerine etkisi" isimli yüksek lisans tezinden sağlanan karaciğer dokuları kullanıldı. İnsülin direnci tanısı konulmasında da bu tezden alınan değerler kullanıldı (12).

Deney hayvanları

Çalışma için, Van Yüzüncü Yıl Üniversitesi Deneysel Uygulama ve Araştırma Merkezi'nden ağırlıkları 150 ile 200 gr arasında değişen 40 adet Wistar-Albino erkek sıçan sağlandı. Ratlar deneme süresince 12 saat karanlık/aydınlık, sıcaklığı 22 ± 2 °C olarak ayarlanmış odalarda sürekli olarak yem ve taze su bulunan kafeslerde barındırıldı.

Deneme gruplarının hazırlanması(12)

1. Grup (Kontrol-K): Rastgele seçilen yedi adet rat kontrol grubu olarak ayrıldı. Bu grubun ilk kan glukoz düzeyi kuyruktan alınan kanda tespit edildi. İntraperitoneal (i.p) yoldan 7 gün boyunca serum fizyolojik enjekte edilip son 7 gün gavaj ile ayçiçek yağı verildi.

2. Grup (İnsülin Direnci-İ): Yedi adet rata 7 gün boyunca deksametazon 1mg/kg/gün (i.p.)uygulandı.

3. Grup (Timokinon-TK): Yedi adet rattan oluşan gruba 7 gün boyunca ağız gavajı ile TK 10mg/kg/gün olarak uygulandı.

4. Grup (Timokinon+ İnsülin direnci-TKİ): Yedi adet rata 7 gün süresince ağız gavajı ile TK10 mg/kg/gün (i.p.) uygulandı. Daha sonra 7 gün boyunca intraperitoneal olarak enjeksiyonla deksametazon 1mg / kg / gün uygulandı.

5. Grup (İnsülin direnci+ Timokinon-İTK): Yedi adet rattan oluşan gruba 7 gün süresince deksametazon 1mg/kg/gün (i.p.) uygulandı. Daha sonra 7 gün boyunca ağız gavajı ile TK 10mg/kg/gün verildi.

6. Grup (İnsülin direnci+metformin-İM): Beş adet rattan oluşan gruba 7 gün boyunca deksametazon 1mg/kg/gün (i.p.) uygulandı. Toz haldeki metformin (40 mg/kg/gün) şeklinde suda eritilerek, ağız gavajı ile 6 gün verildi ve deneme 10. güne tamamlandı.

Bütün gruplarda, son uygulamanın yapılmasından 2 saat sonra kuyruk veninden alınan kan örneklerinde glukoz düzeyleri saptandı (Biyosensor glukoz ölçüm cihazı ve stripleri, Lifechek, Türkiye).

Karaciğer dokularının toplanması

Steril ortamda, sakrifiye edilen ratlardan steril bisturi ve pens yardımıyla alınan yaklaşık 200 mg karaciğer dokusu 2 ml steril RNAaz free tüplere konulduktan sonra, üzerini kaplayacak kadar RNA stabilizatör konuldu.

Doku homojenatının hazırlanması

Dokulardan 80 mg kadarı steril tüplere alındı. Dokuların üzerine 0,2 ml steril fosfat tamponu eklenerek homojenize edildi. Homojenize edilen dokular 1500 rpm'de 4 °C derecede sıcaklıkta santrifüj edildi. Tüpün üstünde kalan sıvı kısım atıldı ve hemen total RNA izolasyon aşamasına geçildi.

İnsülin direncinin hesaplanması (13)

Kan şekeri (FUJI DRI-CHEM NX500, Japonya), serum insülin düzeyleri (Rat INS ELISA Kiti- USBN Kod No: CEA448Ra) ölçülerek elde edilen sonuçlara göre insülin direnci hesaplandı.İnsülin direncinin hesaplanması, aşağıdaki formüle göre yapıldı.

HOMA IR= Plazma glukoz (mg/dL) × açlık plazma insülin (mu/L)/405.

RNA eldesi ve cDNA izolasyonu

Karaciğer örneklerinden total RNA izolasyon protokolüne göre (geneAll, Güney Kore) RNA eldesi yapıldı. Uygun ölçüm sonucunda Total mRNA'dan High-Capacity cDNA ReverseTranscription izolasyon kiti (WizScriptTM cDNA Synthesis) kullanılarak cDNA sentezi gerçekleştirildi.

Komplementer DNA (cDNA) sentezi

WizScriptTM cDNA Synthesis Kit protokolüne göre, cDNA karışımı 200 µl'lik PCR tüpünde hazırlandı (Her bir tüpte 10 µl olacak şekilde karışım hazırlandı). gDNA eliminasyonu yapılmış RNA'lar 10'ar µl alınarak diğer tüplere eklendi. Qiagen Rotor-Gene Q Software-Run cihazı ile reverse transkripsiyon aşağıdaki programa göre gerçekleştirildi.

cDNA sentezinde kullanılan uygulamanın sıcaklıkları ve süreleri; 25°C'de 10 dk, 37°C'de 120 dk, 85°C'de 5 dk, 25°C'de 1 dk, olarak ayarlandı.

RT-PCR analizleri

Tüm gruplarda rat karaciğer dokusundan elde edilen RNA izolasyon ürünlerinde genlerin ekspresyonu real time-PCR ile belirlendi. Elde edilen cDNA'lar kullanılarak tablo 3'de primer sekans dizilimleri verilen hedef genlerin mRNA transkripsiyon düzeyleri belirlendi. Her bir gene ait primerlerin uygun optimizasyon şartları belirlendi. RT-qPCR reaksiyonu ROTOR-GENE Q (Qiagen, Germany) cihazında gerçekleştirildi. Nekrotik hücre ölümüyle bağlantılı olan Rip1 ve Rip3 geni; otofajik hücre ölümüyle bağlantılı olan Atg3 ve Atg5 genleri; apoptotik hücre ölümüyle bağlantılı olan Kaspaz -8, Kaspaz -9, Kaspaz -3 genleri ve DNA hasarını onaran KU70, KU80 ile tümör önleyici TP3 genlerinin transkripsiyon düzeyleri belirlendi. Ekspresyon analizlerinde kontrol geni olarak Aktin Beta (ACTB) kullanıldı. Çalışmada amplikasyon tespiti için SYBR Green master mix (WizpureTM qPCR Master) kullanıldı. Her bir örnek 3 bağımsız tekrar ile tekrarlandı, bir ct (cycletreshold) amplifikasyonların logaritmik fazının başlangıcı itibariyle belirlendi. Ekspresyon analizlerinde genlerin ürünleri 2-ΔΔCt değerleri ile belirlendi. Gruplar arasındaki farklılık kontrol grubunun ekspresyonun artış-azalış kat (fold changes) sayısı ile karşılaştırılıp değerlendirildi.

Qiagen Rotor Gen model gerçek zamanlı-qPCR cihazı kullanılarak çoğaltıldı. WizpureTM qPCR Master (SYBR) protokolüne göre, her birörnek için aşağıdaki karışım hazırlandı. Real-time PCR'da kullanılan karışımın bileşenleri ve miktarları; Mastermix 10µl, Forward Primer 2µl, Reverse Primer 2µl, Su (Nükleaz-Free) 5µl, cDNA 1µl olarak yapıldı.

Aşağıda RT-PCR analizinde kullanılan hedef genlerin forward ve reverse primerlerine ait baz dizilimleri verildi (Tablo 1).

Hazırlanan 200 µl PCR tüpleri Real-time PCR cihazında (Qiagen, Rotorgene, USA) ilgili cDNA'lar çoğaltıldı.

İstatistik Analiz

Elde edilen veriler; Ortalama, Standart Sapma, Minimum ve Maksimum değer olarak ifade edilmiştir. Bu özellikler için grupları karşılaştırmada Tek Yönlü Varyans analizi yapılmıştır. Varyans analizini takiben farklı grupları belirlemede, Tukey çoklu karşılaştırma testi kullanılmıştır. Bu özellikler arasındaki ilişkiyi belirlemede, genel ve gruplarda ayrı ayrı olmak üzere Pearson korelasyon katsayıları hesaplanmıştır. Hesaplamalarda istatistik anlamlılık düzeyi %5 olarak alınmış ve hesaplamalar için SPSS (ver: 22) istatistik paket programı kullanılmıştır.

Bu çalışma, Van Yüzüncü Yıl Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nun onayı ile gerçekleştirilmiştir (Tarih:25.04.2024, Karar no: 2024/04-07).

Tablo 1. Ekspresyon analizinde kullanılan primerler ve dizilimleri

Genler	Forward (5'-3')	Reverse (5'-3')
Kaspaz P3	CGAAACTCTTCATCATTCAGG	GAGCATTGACACAATACACGG
Kaspaz P8	GATGTCCTGGTGCTATTTCAGAG	CCTCCTTGTCCATGTCTTCTG
Kaspaz P9	TCTCACACCAGAAACACCCA	GTCGTTCTTCACCTCCACCA
ATG3	GGGAAGAATTGAAAGTGAAGG	TGAACTGAACACATAGGAGG
Atg5	CTATTTGACGCTGGTAACTGAC	CTGATGTGAAGGAAGTTGTCTGG
Rip1	CTTCAGCACCTAGCTCCGTC	TGTCCAGGTCTTTCCTCTCCA
Rip3	CCATCCAGACCCAACAAGAG	GACGAAGATTCACCATAGCC
Gpx1	TCCACCGGTATGCCTTCTC	TCTCTTCATTCTTGCCATTCTCC
Sod1	GCTTCTGTCGTCTCCTTGCT	CATGCTCGCCTTCAGTTAATCC
TP53	CTGGACGACAGGCAGACTTT	GTCCCGTCCCAGAAGATTCC
KU70	GCCCACCCTCTTCTGTCC	GGGCTCTGCTCCTCCTCT
KU80	ACAGAAGACCACGACTCCAG	AGTTGCTGAAGACTCTCGCT
GAPDH	AACCCATCACCATCTTCCAG	GCCATCCACAGTCTTCTGAG

BULGULAR

Çalışma sonunda belirlenen HOMA-IR verileri aşağıdaki tabloda verildi (Tablo 2).

Çalışma sonucunda elde edilen veriler aşağıda verilmiştir.İnsülin direnci değerleri de Göktuğ (2022)'un yüksek lisans tezinden alınmıştır (Tablo 5). HOMA-IR düzeyleri I grubunda en yüksek ve kontrole göre önemli ($p \leq 0.05$) bulunurken, diğer gruplar arasında birbirileri ve kontrole göre önemli bir fark bulunmadı (13).

KU70 gen ekspresyonu korunma için TK(TKI) verilen grupta 5.9 kat, tedavide (ITK) ise 23 kat up regule olurken, TK ve ID gruplarında ise önemli oranda down regule oldu. Tedavi kontrol (IM) grubunda ise kontrolden farklı değildi.KU80 gen ekspresyonu, tedavi için TK verilen grupta (ITK) 5 kata yakın up regüle olurken, TK ve ID grubunda kontrole göre down regule oldu. Tedavi kontrol (IM) grubunda ise kontrolden farklı değildi.TP53; ID grubunda 6 kat up regüle oldu. Tedavi için TK (ITK) verilen grupta metformin verilen gruba yakındı. TK ve TKI grupları kontrole yakın bulundu (Tablo 3).

Tablo 2. Çalışma gruplarından elde edilen HOMA-IR sonuçları (13).

	K	TK	I	TKI	ITK	IM
HOMA-IR	544±61.4b	420±122.6b	1080±293.8a	682±125.6ab	443±90.75b	787±120.09ab

a, b, c...: Farklı küçük harfi alan gruplar arasındaki fark istatistik olarak anlamlıdır.

Tablo 3. Çalışma gruplarından elde edilen DNA hasarını tamir genleri ekspresyon sonuçları

	K	TK	I	TKI	ITK	IM
KU70	1	$0,24 \pm 0,02^{a}$	$0,42 \pm 0,11^{a}$	$5,90 \pm 0,56^{b}$	$23,03 \pm 0,81^{c}$	$1,74 \pm 0,07^{a}$
KU80	1	$0,05 \pm 0,01^{a}$	$0,59 \pm 0,02^{ab}$	$1,26 \pm 0,09^{bc}$	$4,99 \pm 0,40^{d}$	$1,80 \pm 0,44^{c}$
TP53	1	$1,05 \pm 0,01^{a}$	$6,7 \pm 0,15^{c}$	$0,89 \pm 0,001^{a}$	$2,15 \pm 0,09^{b}$	$2,73 \pm 0,43^{b}$

a, b, c...: Farklı küçük harfi alan gruplar arasındaki fark istatistik olarak anlamlıdır.

Kaspaz 3 ve Kaspaz 9 genlerinin ekspresyonu bakımından gruplar arasında ortalama farklar açısından önemli bir farklılık gözlemlenmemiştir. Ama Kaspaz 3 geninin bütün çalışma gruplarında kontrole göre 2 kattan fazla up regule olduğu görüldü. Kaspaz 8; ID grubunda 2,9 kat up regüle olurken, TK ve metformin verilince yarıya yakın down regule olduğu belirlendi (Tablo 4).

Gpx geni; bütün çalışma grupları arasında ortalama değerlerde anlamlı bir fark bulunmamakla birlikte, tümünün kontrole göre 1.5 kattan fazla up regule olduğu görüldü. Sod geni;bütün çalışma gruplar arasında ortalama değerlerde anlamlı bir fark bulunmamakla birlikte gen ekspresyonu, kontrole göre 2 kat civarında up regule olduğu saptandı (Tablo 5).

Atg3 gen ekspresyonu bakımından, bütün çalışma grupları ve kontrol grubu arasındaki ortalamaların istatistiksel olarak anlamlı olmadığı görüldü.Atg5 geni; ID grubunda kontrole göre 6 kat up regüle olurken, korunma ve tedavi için TK verilen (TKI ve ITK) gruplarda kontrole göre ve tedavi kontrole göre (IM) azaldı (Tablo 6).

Rip1 gen ekspresyonu; ID grubunda kontrol grubuna göre 2.4 kat arttı, tedavi kontrol (IM) ve korunma ve tedavi için TK verilen (TKI ve ITK) gruplarında yarı yarıya azaldı. Aynı şekilde Rip3 geni de ID de 7,5 kat artarken, tedavi kontrol (IM) ve korunma için TK verilen (TKI ve ITK) gruplarında çok azaldı. Ancak tedavi için TK verilen (ITK) grupta da azalmakla birlikte, yine de kontrolden 5 kata yakın daha fazla up regüle olduğu görüldü (Tablo 7).

Tablo 4. Çalışma gruplarından elde edilen apoptozis genleri ekspresyon sonuçları

Gruplar	K	TK	I	TKI	ITK	IM
Kaspaz 3	1	2,56 ± 0,43	2,56 ± 0,43	2,66 ± 0,46	2,76 ± 0,48	2,87 ± 0,51
Kaspaz 8	1	1,48 ± 0,03^{b}	2,99 ± 0,01^{a}	0,52 ± 0,03^{d}	0,59 ± 0,09^{cd}	0,72 ± 0,02^{c}
Kaspaz 9	1	0,22 ± 0,02	1,10 ± 0,28	1,04 ± 0,15	0,44 ± 0,11	1,00 ± 0,47

a, b, c...: Farklı küçük harfi alan gruplar arasındaki fark istatistik olarak anlamlıdır.

Tablo 5. Çalışma gruplarından elde edilen antioksidan enzim genleri ekspresyon sonuçları

Gruplar	K	TK	I	TKI	ITK	IM
Gpx	1	1,52 ± 0,07	1,52 ± 0,07	1,54 ± 0,07	1,57 ± 0,08	1,60 ± 0,09
Sod	1	1,87 ± 0,02	1,87 ± 0,02	1,92 ± 0,02	1,98 ± 0,03	2,03 ± 0,03

a, b, c...: Farklı küçük harfi alan gruplar arasındaki fark istatistik olarak anlamlıdır.

Tablo 6. Çalışma gruplarından elde edilen otofajik genleri ekspresyon sonuçları

	K	TK	I	TKI	ITK	IM
Atg3	1	1,31 ± 0,02	1,31 ± 0,02	1,33 ± 0,02	1,34 ± 0,02	1,36 ± 0,02
Atg5	1	2,04± 0,03^{ab}	6,20 ± 0,93^{c}	0,67 ± 0,07^{a}	0,54 ± 0,02^{a}	3,73 ± 0,20^{b}

a, b, c...: Farklı küçük harfi alan gruplar arasındaki fark istatistik olarak anlamlıdır.

Tablo 7. Çalışma gruplarından elde edilen nekrotik genleri ekspresyon sonuçları

	K	TK	I	TKI	ITK	IM
Rip1	1	0,05 ± 0,01^{a}	2,39 ± 0,02^{c}	0,56 ± 0,04^{b}	0,40± 0,03^{ab}	0,76 ± 0,22^{b}
Rip3	1	0,05 ± 0,01^{a}	7,45 ± 0,49^{c}	0,62 ± 0,12^{a}	4,99 ± 0,04^{b}	0,39 ± 0,05^{a}

a, b, c...: Farklı küçük harfi alan gruplar arasındaki fark istatistik olarak anlamlıdır.

TARTIŞMA

Geleneksel tıpta uzun süredir kullanılan *Nigella sativa* (NS; Ranunculaceae), diyabetes mellitusta (DM) glukoz toleransını iyileştirerek (arttırarak), hepatik, hepatik glukoneogenezi azaltarak, kan glukozunu ve lipit dengesizliğini normalleştirerek ve pankreastan insülin salgılanmasını uyararak DM tedavisinde adjuvan tedavi olarak önemli etkinlik göstermiştir. NS, insülin direncini azaltır, insülin sinyalini artırır (13). TK, çoğunlukla antioksidan etkisinin yanı sıra pankreatik ß-katenin ekspresyonunun yukarı regülasyonu yoluyla vücuttaki insülin seviyelerinin dengede tutulmasını sağlar (14). Biyokimyasal ve ultrastrüktürel bulgular, *N. sativa* ekstraktının ve timokinonun terapötik olduğunu ve oksidatif stresi azaltarak STZ diyabetine karşı koruduğunu, dolayısıyla pankreatik ß hücre bütünlüğünü koruduğunu göstermektedir (15).

TK'un apoptozisi önleme ve çeşitli bağışıklık ve hücresel sinyal yollarını düzenleme, interferonlar, interlökinler, TNF-α gibi bağışıklık faktörlerini, oksidatif stres seviyelerini, düzenleyici T hücrelerini ve NF-κß, JAK-STAT, MAPK gibi hücresel sinyal yollarını etkileyerek moleküler düzeyde işlev gösterdiği bilinmektedir (16-19).

TK, antioksidan enzimlerin (Gpx, Sod, GR, CAT) ve indirgenmiş glutatyonun (GSH) üzerindeki olumlu etkileri ile oksidatif stresin olumsuz etkilerini iyileştirir. Antioksidan özellikleri sayesinde oksidatif stresi azaltır, hepatik fonksiyon bozukluklarına karşı koruma sağlar, hepatik steatozu ve oksidatif stresi iyileştirir. TK ve nanopartikül formu da, antioksidan özellikleri sayesinde apoptotik belirteçlerin ve inflamatuar sitokinlerin aşağı regülasyonu ile karaciğer hasarını azaltır (20,21). TK, bozulmuş glukoz toleransını ve insülin direncini önler, HFD (high-fructose diet) kaynaklı hepatik TBARS artışını ve Sod, katalaz ve GSH tükenmesini iyileştirir (22). Gpx ve Sod gen ekspresyonlarındaki artışlar, oksidatif stresin yönetiminde olumlu bir etki olduğunu gösterir. Ancak bu çalışmada, tedavi grupları arasında karaciğer dokusundaki gen ekspresyonunu düzeylerinde belirgin bir farklılık gözlemlenmemiştir. Bu durum,TK'un antioksidan enzimlerin aktivitesini etkilemektense, ilişkili sinyal yollarını düzenleyerek etkili olabileceğini düşündürmektedir.Ayrıca, insülin direncine bağlı etkilerin yalnızca oksidatif stresle değil, inflamasyon, hücresel stres ve metabolik yolların bozulması ile ilişkili olabileceği ya da antioksidan enzimlerin aktivitesinin gen ekspresyonundan sonra post-translasyonel modifikasyonları ile düzenlenebileceği olasılıkları da göz önünde bulundurulmalıdır.

TK, oksidatif stres ve DNA hasarını azaltarak, karaciğer hasarını önlemeye yardımcı olur. Aynı zamanda, DNA onarım süreçlerini destekleyici etkiler gösterir (23-26). Böylece DNA hasarını ve apoptozisi azaltarak karaciğer hasarını da engeller (21,22). Bu çalışmada da TK'un DNA onarımında olumlu etkiler sağladığı görülmektedir. TK, hem korunma hem de tedavi amaçlı uygulamalarda KU70 ve KU80 gen ekspresyonunu artırmış ve bu genlerdeki artışlar, özellikle tedavi grubunda belirgin olup, DNA hasarını önlemede önemli bir rol oynar. TK'un DNA onarım mekanizmalarını düzenlemesi, DNA hasarını azaltarak hem hücre koruma hem de tedavi amaçlı kullanımlarda etkili bir DNA koruyucu olarak işlev görmesini sağlar.

TK, oksidatif stres ve DNA hasarını azaltarak,

karaciğer hasarını önlemeye yardımcı olur. Aynı zamanda, DNA onarım süreçlerini destekleyici etkiler gösterir. TK, hem korunma hem de tedavi amaçlı kullanıldığında KU70 ve KU80 gen ekspresyonunu artırarak DNA onarımını teşvik eder. Özellikle tedavi grubunda KU70 ve KU80 genlerinde belirgin artışlar gözlenmiştir. Bu sonuçlar, TK'un DNA onarım genlerini düzenleyerek hasarları azalttığını ve hem korunma hem de tedavi süreçlerinde fayda sağladığını göstermektedir.Aynı şekilde, TK'un TP53 gen ekspresyonunu azaltıcı etkisi, DNA hasar yanıtının düzenlenmesinde olumlu bir katkıda bulunarak, insülin direncinin (IR) neden olduğu aşırı TP53 aktivitesini dengeleyebileceğini göstermektedir. Tedavi gruplarında TP53 ekspresyon seviyeleri kontrol gruplarına yakın olup, metformin verilen gruba benzer etki gösterdiği ve bu gen için potansiyel koruyucu etkisinin bir kanıtı olabilir.

TK'un apoptotik sürece katkıları; insülin üreten β hücrelerinin apoptozisini baskılayarak (10), apoptotik hücre ölümünü sağlayarak, NF-κB ve MEK sinyalini inhibe ederek (27), hücre döngüsünü durdurarak (22), p53 aracılı apoptozis ve kaspaz aktivasyonunu etkileyerek (23, 28) gerçekleşmektedir.Apoptozis, fazla veya anormal hücreleri ortadan kaldırarak doku dengesini korumada önemli bir işlevi olan düzenlenmiş bir hücresel kendini yok etme mekanizmasıdır (19).TK'un yüksek glukoz verilen PC12 hücre hattında oksidatif DNA hasarı ve bazı apoptotik parametreleri üzerinde yararlı etkileri ortaya konulmuştur (29-31). Bu çalışmada Kaspaz 3, Kaspaz 9'da gruplar arasında fark olmamakla birlikte Kaspaz 8'de bütün gruplarda kontrole göre 2 kattan fazla artış gözlenmiştir. İnsülin direnci (İR) grubunda Kaspaz 8 gen ekspresyonundaki bu artış, hücre ölümüne katkıda bulunabilirken, TK ve metformin ile tedavinin ekspresyonu belirgin bir şekilde azaltması,apoptozis sürecini sınırlamış olabilir. Bu sonuçlar, TK'un apoptozis mekanizmasını düzenleyici etkisini ve DNA hasarına yanıt olarak apoptozis azaltabileceğini göstermektedir. TK'un, apoptotik yanıtı hafifleterek hücresel hasarın kontrol altına alınmasına katkı sağladığı söylenebilir.

Son yıllarda yapılan araştırmalar, TK'un otofaji, nekrozis ve PI3K/Akt/mTOR gibi kritik hücresel yollar üzerinde önemli düzenleyici etkileri olduğunu göstermiştir. Nekroz ve otofaji ile ilgili etkiler dolaylı olarak, oksidatif stres, apoptozis ve DNA hasarı gibi konularla ilişkilendirilebilir (16,32,33).Böylece TK'un özellikle metabolik hastalıklar, kanser ve diğer kronik hastalıkların tedavisinde potansiyel bir terapötik ajan olarak değerlendirilmesi olanaklarını düşündürmektedir.

Otofaji, hücresel atıkların temizlenmesi ve hücresel homeostazın korunması için kritik bir süreçtir. Otofaji süreçlerindeki düzensizliklerin diyabet, obezite ve diğer kronik hastalıkların gelişimine katkıda bulunabileceğini vurgulamışlardır. TK'un otofaji süreçleri üzerindeki yararlı etkilerine dair bilgiler, metabolik bozuklukların tedavisinde bir seçenek olarak kullanılabileceğini göstermektedir (9,32).Bu çalışmada karaciğer dokusunda Atg3 gen ekspresyonundaki anlamlı değişikliklerin olmaması, TK'un bu gen üzerindeki etkisinin sınırlı olduğunu gösterebilir. Ancak, Atg5 genindeki belirgin değişiklikler, TK'un otofaji sürecini etkileyebileceğini ve özellikle ID durumunda otofajik yanıtı modüle edebileceğini ortaya koyabilir.

Nekrozis, patolojik hücre ölümünün bir formu olarak, genellikle inflamatuar yanıtlarla ilişkilidir ve bu durum, çeşitli hastalıkların ilerlemesinde rol oynayabilir. TK'un nekrozis üzerindeki yararlı etkileri, onun potansiyel terapötik kullanımını desteklemektedir (33,34).Bu çalışmada her iki nekrozis genindeki artış, IR'nin hücresel nekrozisi artırdığını ve tedavi seçeneklerinin bu süreci etkileyebileceğini gösterir. Metformin ve TK'un Rip1 ve Rip3 gen ekspresyonlarını azaltma etkisi, bu tedavilerin nekrozisi sınırlama potansiyelini ortaya koyabilir. TK'nun nekrozis süreçlerini azaltarak modüle etme yeteneğine sahip olduğuna işaret sayılabilir.

TK'un oksidatif stresi ve DNA hasarını azaltma etkileri, nekrozu dolaylı yoldan etkileyebilir,

çünkü oksidatif stres genellikle hücresel nekroza yol açabilir (35,36). TK'un hücre koruyucu etkileri nedeniyle otofaji ve nekrozis süreçlerini düzenleme kapasitesine sahip olduğunu göstermektedir. TK'nun nekrozisi engelleyici etkisi, Rip-1, ve Rip-3 gibi nekrozis belirteçlerinin azaltılmasıyla gösterilmiştir. Otofaji, DNA hasarının onarımında rol oynayabilir ve DNA onarımdan sorumlu mekanizmaların etkilenmesi otofajiyi etkileyebilir.

Sonuç olarak elde edilen bulgular, TK'un antioksidan özellikleriyle oksidatif stresi azaltarak ve DNA onarım süreçlerini destekleyerek insülin direncine karşı korunma ve tedavide yararlı olabileceğini, ayrıca, apoptozis, otofaji ve nekrozis süreçlerini modüle ederek hücresel hasarı yönetebilme potansiyeline sahip olduğunu, dolayısı ile insülin direncine bağlı komplikasyonlarından olan karaciğer hasarının tedavisinde hepatoprotektif bir mekanizma sunabileceğini göstermektedir. Bu çalışmada elde edilen gen ekspresyon sonuçlarını doğrulamak amacıyla daha ileri protein düzeyinde analizlerin (Western blot, ELISA gibi) yapılması ve TK'nun uzun vadeli etkilerinin incelenmesinin, TK'un insülin direncinde metabolik yolaklar üzerindeki yararlı etkilerinin mekanizmalarını daha kapsamlı şekilde aydınlatılmasını sağlayabileceği düşünülmektedir.

ETİK KURUL ONAYI

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Yazarlar bu makale ile ilgili herhangi bir çıkar çatışması bildirmemişlerdir.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

The dual role of cold atmospheric plasma as a promising modulator in melanoma

Melanomada umut verici bir düzenleyici olarak soğuk atmosferik plazmanın ikili rolü

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ABSTRACT

Objective: Cold Atmospheric Plasma (CAP),known for its antibacterial properties and potential therapeutic benefits, is emerging as a promising alternativeto traditional chemical disinfectants. This study aims to investigate the dual role of CAP in skin disinfection and its potential effects on melanoma. Specifically, the study examines how CAP affects gene expression and the potential implications of these effects on both skin health and cancer progression.

Methods: In this study, gene expression analysis was performed on human skin epithelial keratinocytes (HaCaT) post-treatment with Cold Atmospheric Plasma (CAP) and melanoma cell lines. Raw gene expression data were obtained from Array Express (E-GEOD-46343) and Gene Expression Omnibus (GSE22301) databases. The data were normalized using the Robust Multichip Average (RMA) method. For the melanoma cell lines, normal human melanocytes were used as the control group to ensure a comparative analysis of gene expression changes between cancerous and non-

ÖZET

Amaç: Antibakteriyel özellikleri ve potansiyel terapötik faydalarıyla bilinen Soğuk Atmosferik Plazma (SAP), geleneksel kimyasal dezenfektanlara umut verici bir alternatif olarak ortaya çıkmaktadır. Bu çalışma, SAP'nın cilt dezenfeksiyonundaki çift rolünü ve melanom üzerindeki potansiyel etkilerini araştırmayı amaçlamaktadır. Özellikle, SAP'nın gen ekspresyonunu nasıl etkilediği ve bu etkilerin hem cilt sağlığı hem de kanser progresyonu üzerindeki potansiyel sonuçları incelenmiştir.

Yöntem: Bu çalışmada, insan deri epitelyal keratinositlerinin (HaCaT) Soğuk Atmosferik Plazma (SAP) tedavisi sonrası ve melanom hücre hatlarının gen ekspresyonu analiz edilmiştir. Ham gen ekspresyon verileri Array Express (E-GEOD-46343) ve Gene Expression Omnibus (GSE22301) veritabanlarından alınmıştır. Veriler Robust Multichip Average (RMA) yöntemiyle normalize edilmiştir. Melanom hücre hatlarında, kanserli ve kanser olmayan hücreler arasındaki gen ekspresyonu değişimlerinin karşılaştırmalı analizini sağlamak amacıyla kontrol grubu olarak normal insan melanositleri kullanılmıştır.

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cancerous cells. Differentially expressed genes (DEGs) were identified using R packages Limma, affy, and edgeR. Common genes were identified with Venny 2.1.0, and hierarchical clustering analysis was performed using Gene Cluster v3.0. Pathway analysis was conducted using DAVID and KEGG, DAVID provides functional annotation clustering, while KEGG offers pathway mapping to identify significant biological processes. In addition, network analysis was performed using GeneMANIA and Cytoscape software.

Results: This study revealed significant differential gene expressions highlighting CAP's impact on both antimicrobial activity and cancer-related gene regulation. Key genes involved in oxidative stress responses, including ARID1A, NIPBL, BPTF, and OTUB1, showed notable changes. Downregulation of ARID1A, NIPBL, and BPTF is linked to impaired DNA repair and increased genomic instability, critical in melanoma progression. In contrast, upregulation of OTUB1 promotes cell survival and resistance to apoptosis. These findings suggest CAP's dual role in enhancing antimicrobial defenses and influencing cancer-related pathways, particularly through the modulation of oxidative stress responses.

Conclusion: Our findings provide a foundation for understanding the molecular effectsof CAP and its potential in developing novel therapeutic strategies for melanoma.These studies clarify CAP's beneficial impacts on skin health and potential carcinogenic risks, providing robust scientific data for future applications.

Key words: CAP, gene expression, disinfection, melanoma, oxidative stress, DEG

Diferansiyel olarak eksprese edilen genler (DEG) R paketleri Limma, affy ve edgeR kullanılarak belirlenmiştir. Ortak genler Venny 2.1.0 ile tanımlanmış, hiyerarşik kümeleme analizi Gene Cluster v3.0 ile yapılmıştır. Yolak analizi DAVID ve KEGG kullanılarak yapılmıştır, DAVID fonksiyonel anotasyon kümelemesi sağlarken, KEGG önemli biyolojik süreçleri tanımlamak için yolak haritalaması sunmaktadır. Ek olarak, ağ analizi GeneMANIA ve Cytoscape yazılımları kullanılarak gerçekleştirilmiştir.

Bulgular: Bu çalışma, SAP'nın hem antimikrobiyal aktivite hem de kanserle ilişkili gen düzenlemesi üzerindeki etkisini vurgulayan önemli farklı gen ifadelerini ortaya koymaktadır. ARID1A, NIPBL, BPTF ve OTUB1 dahil olmak üzere oksidatif stres tepkilerinde yer alan anahtar genler, dikkate değer değişiklikler göstermiştir. ARID1A, NIPBL ve BPTF'nin aşağı regülasyonu, melanom ilerlemesinde kritik olan bozulmuş DNA onarımı ve artan genomik instabilite ile bağlantılıdır. Buna karşılık, OTUB1'in yukarı regülasyonu hücrenin hayatta kalmasını ve apoptoza direncini desteklemektedir. Bu bulgular, SAP'nın antimikrobiyal savunmaları geliştirmede ve özellikle oksidatif stres tepkilerinin modülasyonu yoluyla kanserle ilişkili yolakları etkilemede ikili bir rol oynadığını göstermektedir.

Sonuç: Bulgularımız, SAP'nın moleküler etkilerinin anlaşılması ve melanom için yeni tedavi stratejilerinin geliştirilmesindeki potansiyeli için bir temel oluşturmaktadır. Bu çalışmalar, CAP'nin cilt sağlığı üzerindeki olumlu etkilerini ve olası kanserojen risklerini netleştirerek, gelecekteki uygulamalar için sağlam bilimsel veriler sunmaktadır.

Anahtar Kelimeler: SAP, gen ekspresyonu, dezenfeksiyon, melanoma, oksidatif stres, DEG

INTRODUCTION

Disinfectants are effective in reducing and preventing disease transmission, but their potential adverse effects on humans and the environment, such as aquatic ecosystem pollution and resistant populations, are increasing. Disinfectants must be strictly controlled to protect human and environmental health. Because chemical disinfectants can irritate the skin, trigger allergies and disrupt the microbiota (1). Given these challenges, CAP offers a safer alternative due to its non-thermal properties

and minimal environmental impact.

Gaseous partially ionized Cold Atmosphere Plasma (CAP), also known as Non-Thermal atmospheric pressure plasma (NTAPP), has reactive species that do not require high temperatures and has antibacterial abilities (2). Comprehensive antibacterial activities prevent diseases associated with multidrug-resistant bacteria (3). Cell growth and tissue regeneration can improve wound healing using cold atmospheric plasma (CAP). CAP helps manage permanent wounds and surgeries that require infection control and good healing (4, 5).

Melanoma is spreading worldwide, and people with fair skin are often more exposed to the harmful effects of the sun. Additionally, mutations and family histories may also increase the risk of disease. The risk of melanoma increases significantly due to genetic changes. For example, mutations in the melanocortin 1 receptor (MC1R) and other genes increase the risk of melanoma (6, 7).

Melanoma has also been associated with disinfectant exposure, especially frequent and persistent exposure. These elements damage skin cells and structure (8). Genetics, UV light, and disinfectants contribute to melanoma (9).

In this study, we used advanced computational methods and comprehensive analyzes to identify genes that show significant changes after exposure to CAP in human skin epithelial keratinocytes. By discovering how CAP exposure affects molecular pathways in keratinocytes, we have gained valuable insight into cancer biology and believe it will shed light on future studies that may reveal potential biomarkers or treatment targets for melanoma.

MATERIAL and METHOD

Obtaining and Normalizing Whole-Genome Expression Data

Whole genome expression data of human skin epithelial keratinocytes were obtained from Array Express (E-GEOD-46343) (10). Gene expression data of Melanoma cell lines and melanocyte controls were obtained from the Gene Expression Omnibus (GSE22301). Affymetrix Human Genome U133A 2.0 Array is used for the analysis (11).

In order to address technical differences across the arrays and eliminate background noise, the raw sample data undergo normalization using the Robust Multichip Average (RMA) approach, implemented using the R packages Limma and affy. (12, 13). Additionally, to account for potential batch effects between the datasets (E-GEOD-46343 and GSE22301), the “ComBat” function from the sva package was applied. This method adjusts for systematic biases arising from batch effects, ensuring comparability across datasets. However, we acknowledge that while batch correction minimizes technical variance, some residual effects may persist, which could potentially influence the interpretation of subtle gene expression differences.

Identification of Differentially Expressed Genes and Commonly Altered Genes

Differentially Expressed Genes (DEGs) analysis is a molecular biology approach that compares the levels of gene expression across distinct sample groups, such as healthy and diseased tissues or cells treated with different substances (14, 15).

The main goal of DEGs analysis is to identify genes that are expressed differently in the circumstances.

By doing DEG analysis using the R package edgeR, we identified statistically significant genes (p-value < 0.05) with a high expression change ratio in human skin epithelial keratinocytes (HaCaT) after 60 seconds of cold plasma treatment. We compared these results to an untreated control group and also compared them to a melanoma cell line in comparison to normal human melanocytes.

A Venn diagram was produced using the web application Venny 2.1.0 to identify the common genes that exhibit substantial changes in expression in both cold plasma-treated epithelial cells and melanoma cells, as compared to their respective control groups

(16, 17).

Hierarchical Cluster Analysis

To demonstrate the capacity of common genes with the highest variation between groups to distinguish between the compared groups, hierarchical cluster analysis was conducted.

Genes identified were grouped hierarchically based on their mean standardized expression values (Euclidean Gene Cluster 3.0). After clustering, the data was standardized and visualized with Treeview. The hierarchical clustering enabled the visualization of distinct gene expression patterns, thereby highlighting the differential expression profiles and helped in the identification of key genes involved in the response to CAP exposure as well as melanoma (18).

Pathway Analysis

The pathways associated with our genes were identified by Database for Annotation, Visualization and Integrated Discovery (DAVID) which is an online tool to elucidate the biological pathways associated with the common differentially expressed genes. Kyoto Encyclopedia of Genes and Genomes (KEGG) database within DAVID is used to map the genes by using known pathways that may be implicated in the response to exposure to CAP in human skin epithelial keratinocytes (19, 20).

Network Analysis

GeneMANIA software was used and genes in similar pathways were identified with Cytoscape version 3.10.2 to explore the network and pathway relationships between the selected differentially expressed genes and other genes. The corresponding network provided valuable insights into the functional associations and putative regulatory mechanisms of the discovered genes. The identification of important genes and crucial pathways that may have major roles in the response to NTAPP exposure and melanoma development was achieved via the visualization of the network (21, 22).

Statistical analysis

The statistical analysis was performed using the R programming environment with packages such as Limma, affy, and edgeR. Data were expressed as mean ± standard deviation (SD). The Shapiro-Wilk test was applied to determine the data's suitability for normal distribution (Data not shown). For normally distributed data, comparisons between groups were made using a t-test. Non-parametric data were analyzed using the Mann-Whitney U test. Statistical significance was set at a p-value < 0.05. To show the clustering of selected genes, the pathways they are involved in, and their network relations, Euclidean Gene Cluster, DAVID, and GeneMANIA software were used.

This study does not require Ethics Committee Approval.

RESULTS

Differential Expression Analysis and Volcano Plot Visualization

The volcano plot shown in Figure 1A shows the comparative study of gene expression between human skin epithelial keratinocytes treated with CAP and the control group. Every data point corresponds to a specific gene, where the x-axis represents the log2 fold change (LFC) and the y-axis indicates the negative logarithm (base 10) of the adjusted p-value. Genes that exhibit a substantial increase in expression ($p<0.01$) in response to plasma therapy are visually emphasized in red, while genes that show a significant decrease in expression ($p<0.01$) are visually represented in green. The gray dots represent genes that do not show statistically significant changes in expression. The horizontal blue dashed line depicts the significance threshold, which denotes the critical level at which p-values are deemed significant ($p<0.01$). This image clearly illustrates a noticeable difference between the treated and control groups, with a large number of genes displaying substantial

differences in expression. This emphasizes the influence of plasma therapy on the regulation of genes in human skin epithelial keratinocytes. A total of 407 genes exhibited substantial up-regulation after NTAPP therapy, while 10868 genes showed down-regulation.

Heat Map Visualization of Differentially Expressed Genes

The heat map in Figure 1B provides a complete display of the genes that are expressed differently between the control group and human skin epithelial keratinocytes treated with CAP. Each row represents a gene, and each column represents samples from either the control or treatment groups. The color red represents an increase in gene expression, whereas the color green represents a decrease. The intensity of the color reflects the degree of gene expression. The heat map clearly displays distinct clustering patterns that demonstrate substantial variations in gene expression profiles between the treated and non-treated groups. Differentiation emphasizes the substantial impact of plasma treatment on the gene expression pattern of human skin epithelial keratinocytes, indicating how this intervention might regulate certain biological processes.

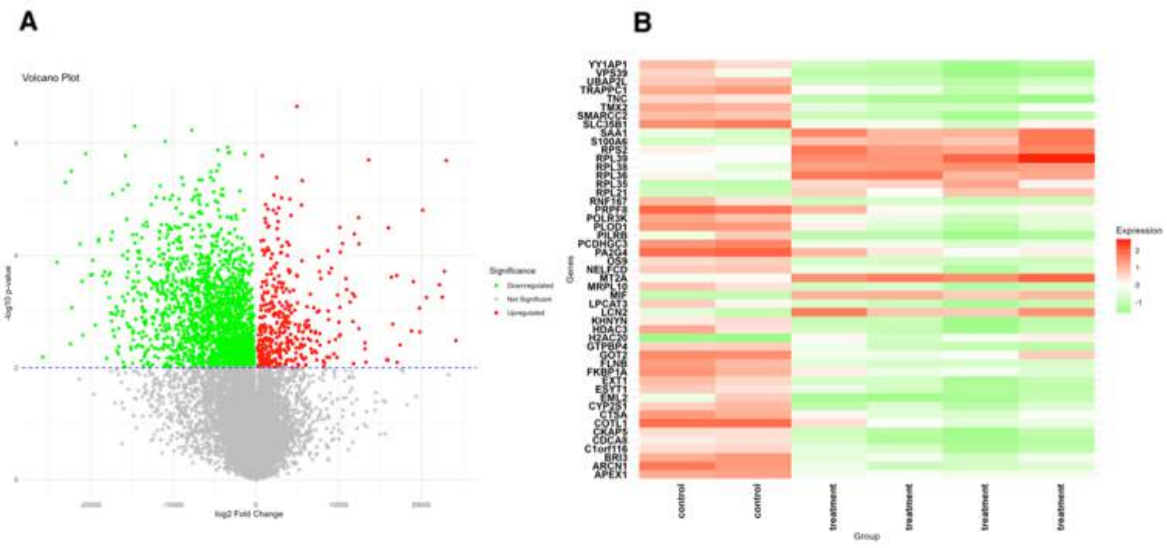

Figure 1. (A) Differential gene expression analysis showing genes that are statistically significantly up- or down-expressed in Non-Thermal Atmospheric Pressure Plasma (NTAPP)-treated human skin epithelial keratinocytes compared to the control group. (B) Heat map analysis demonstrating that the differentially expressed genes can significantly distinguish between treated and non-treated groups.

Identifying Molecular Distinctions and Therapeutic Target Genes

Figure 2A illustrates the comparison of gene expression between melanoma cell lines and normal human melanocyte cell lines. The x-axis of the graph indicates the log2 fold change (LFC) of each gene, while the y-axis represents the -log10 adjusted p-value. Each dot on the graph represents an individual gene. The red dots indicate genes that are considerably elevated in melanoma cell lines

($p<0.01$), whereas the green dots reflect genes that are strongly downregulated ($p<0.01$). The gray dots represent genes that have not shown any substantial change in their expression levels. The horizontal dashed lines represent the levels used to determine statistical significance. Disparities in gene expression patterns between the two cell line groupings, emphasizing significant changes in gene regulation linked to melanoma. This investigation highlights the molecular distinctions between malignant melanoma cells and normal melanocytes, offering valuable insights into possible routes and targets for therapeutic intervention. A total of 83 genes exhibited substantial up-regulation, whilst 154 genes showed down-regulation in melanoma cell lines as compared to normal human melanocyte cell lines. Furthermore, the use of hierarchical cluster analysis revealed that these genes had the ability to differentiate between melanoma and healthy groups, as seen in Figure 2B.

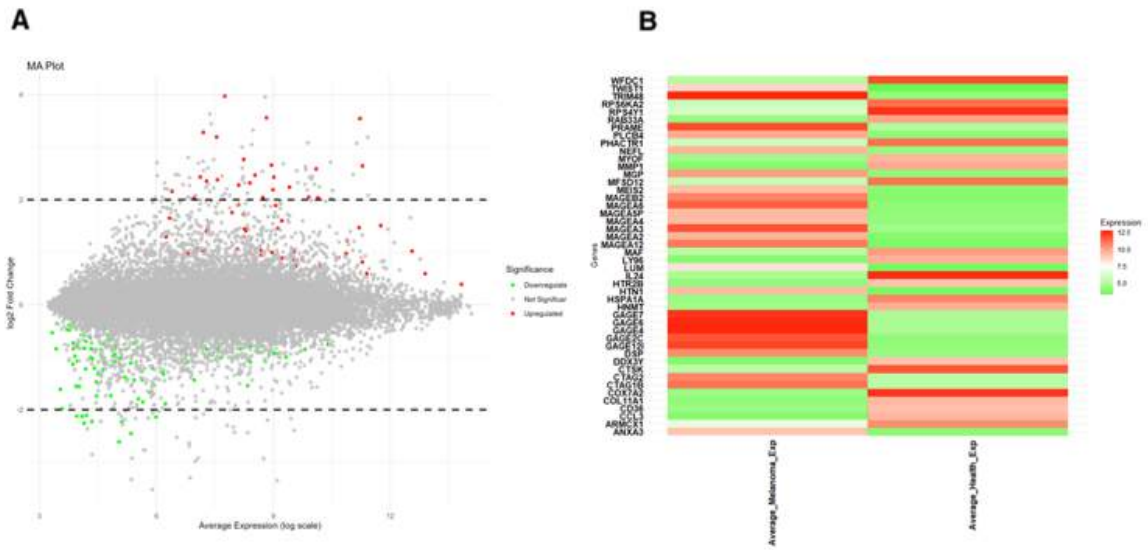

Figure 2. (A) A scatter plot illustrating the significantly up- and down-regulated genes between melanoma cells and healthy skin cells. The data reveal distinct gene expression patterns, indicating substantial alterations in gene regulation associated with melanoma. (B) A heat map of gene expression profiles differentiating between melanoma and healthy skin cells. The expression data highlights clear distinctions, underscoring the potential of these genes to serve as biomarkers for melanoma detection and characterization.

The Venn diagram (Figure 3) shows the intersection of genes that are highly regulated in both CAP-treated human skin epithelial keratinocytes and melanoma cell lines. More precisely, the data reveals that there are 12 genes that are noticeably reduced in expression in both scenarios, as seen in Figure 3A. Additionally, there are 3 genes that exhibit increased expression, as shown in Figure 3B. A comprehensive inventory of these genes that have been downregulated and elevated is shown in Table 1.

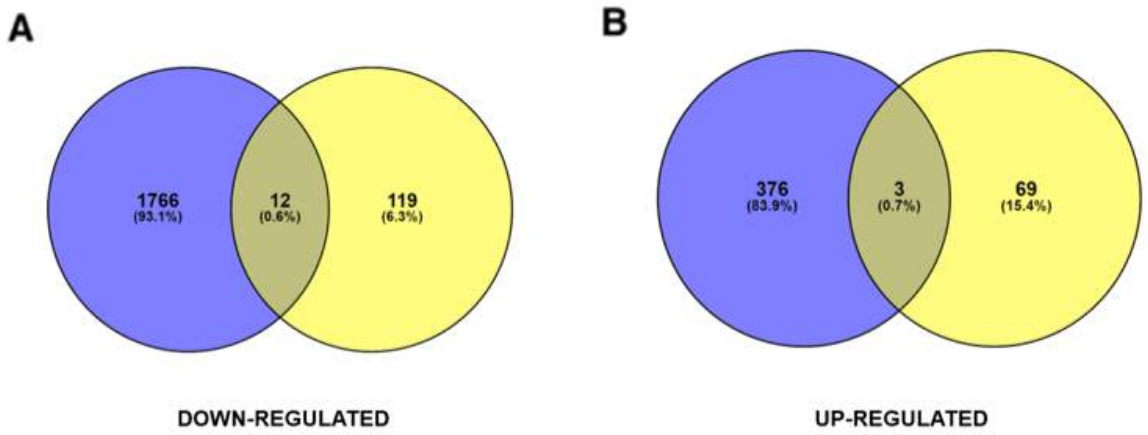

Figure 3. The Venn diagrams illustrate the overlap of significantly regulated genes between NTAPP-treated human skin epithelial keratinocytes and melanoma cell lines. (A) The left diagram shows 12 genes that are significantly downregulated in both NTAPP-treated keratinocytes and melanoma cell lines compared to their respective controls. (B) The right diagram shows 3 genes that are significantly upregulated in both NTAPP-treated keratinocytes and melanoma cell lines. These overlaps highlight potential common pathways and molecular mechanisms affected by NTAPP treatment and associated with melanoma progression.

Table 1. Differentially expressed genes common to NTAPP-treated human skin epithelial keratinocytes and melanoma cell lines. The table categorizes the genes into up-regulated and down-regulated groups

up-regulated genes	down-regulated genes
CARMIL1	SLC7A1
OTUB1	SMG7
GUK1	EXOC7
	NUP54
	POU2F1
	DST
	NIPBL
	ARID1A
	MRPL57
	DCK
	ZBTB39
	BPTF

Biological Processes and Molecular Functions

The functional annotation clustering study yielded five separate groups, as shown in Table 2. Melanoma cell lines and CAP-treated human epithelial keratinocytes share a set of 15 genes with differential expression. Clusters of genes have been shown to be more abundant in relation to certain biological processes, cellular components, or molecular activities. An example instance is Cluster 1, which comprises genes associated with the nucleoplasm,

nucleus, and phosphoproteins, and has an enrichment value of 1.42. Cluster 2 consists of genes involved in chromatin remodeling, transcription control, and RNA polymerase II activity. This cluster has an enrichment value of 0.84. The genes related to cytoplasmic components and coiled coil domains are emphasized in Cluster 3, with an enrichment value of 0.58. Cluster 4, which has an enrichment value of 0.2, consists of genes associated with the cell membrane and plasma membrane. Cluster 5, which has an enrichment value of 0.17, specifically targets genes related to isopeptide bonds, ubiquitin-like conjugation, and metal-binding. for their regulation in response to CAP therapy and the advancement of melanoma.

Table 2. Functional annotation clustering of differentially expressed genes common to NTAPP-treated human skin epithelial keratinocytes and melanoma cell lines

Annotation Cluster 1	**Enrichment Score: 1.42**	**Count**	**P-Value**	**Benjamini**
GOTERM_CC_DIRECT	Nucleoplasm	9	1.90E-03	1.30E-01
GOTERM_CC_DIRECT	Nucleus	8	8.70E-02	1.00E+00
UP_KW_PTM	Phosphoprotein	10	3.30E-01	1.00E+00
Annotation cluster 2	**Enrichment Score: 0.84**	**Count**	**P-Value**	**Benjamini**
GOTERM_BP_DIRECT	Chromatin remodeling	3	2.40E-02	1.00E+00
GOTERM_CC_DIRECT	Chromatin	4	3.90E-02	8.10E-01
UP_KW_CELLULAR_COMPONENT	Nucleus	5	5.10E-02	6.70E-01
UP_KW_BIOLOGICAL_PROCESS	Transcription regulation	5	1.40E-01	1.00E+00
GOTERM_BP_DIRECT	Negative regulation of transcription by RNA polymerase II	3	1.50E-01	1.00E+00
UP_KW_BIOLOGICAL_PROCESS	Transcription	3	1.50E-01	1.00E+00
UP_KW_DISEASE	Intellectual disability	3	1.80E-01	1.00E+00
GOTERM_BP_DIRECT	Positive regulation of transcription by RNA polymerase II	3	2.20E-01	1.00E+00
GOTERM_MF_DIRECT	RNA polymerase II cis-regulatory region sequence-specific DNA binding	3	2.40E-01	1.00E+00
GOTERM_BP_DIRECT	Regulation of transcription by RNA polymerase II	3	3.20E-01	1.00E+00
UP_SEQ_FEATURE	COMPBIAS:Pro residues	3	3.80E-01	1.00E+00
UP_KW_MOLECULAR_FUNCTION	DNA-binding	3	5.50E-01	1.00E+00
Annotation cluster 3	**Enrichment Score: 0.58**	**Count**	**P-Value**	**Benjamini**
UP_KW_DOMAIN	Coiled coil	4	2.30E-01	1.00E+00
UP_SEQ_FEATURE	COMPBIAS:Basic and acidic residues	6	2.70E-01	1.00E+00
UP_KW_CELLULAR_COMPONENT	Cytoplasm	7	2.80E-01	1.00E+00
Annotation Cluster 4	**Enrichment Score:0.2**	**Count**	**P-Value**	**Benjamini**
UP_KW_DOMAIN	Coiled coil	4	2.30E-01	1.00E+00
UP_SEQ_FEATURE	COMPBIAS:Basic and acidic residues	4	6.40E-01	1.00E+00
UP_KW_CELLULAR_COMPONENT	Cytoplasma	4	7.70E-01	1.00E+00
Annotation Cluster 5	**Enrichment Score: 0.17**	**Count**	**P-Value**	**Benjamini**
UP_KW_DOMAIN	Coiled coil	3	8.90E-01	1.00E+00
UP_KW_CELLULAR_COMPONENT	Cell membrane	5	9.50E-01	1.00E+00
GOTERM_CC_DIRECT	Plasma membrane	3	5.30E-01	1.00E+00
GOTERM_CC_DIRECT	Membrane	3	7.40E-01	1.00E+00
UP_KW_CELLULAR_COMPONENT	mMembrane	3	8.10E-01	1.00E+00

Elucidating Key Interactions and Regulatory Mechanisms

In Figure 4, a network analysis of 15 genes that showed differential expression in human skin epithelial keratinocytes and melanoma cell lines that were constructed using Cytoscape is shown. In this network, each node stands for a gene, and the connections between them show several kinds of interactions, such physical interactions, common pathways, or co-expression. Larger nodes signify genes with higher degrees of connectivity, highlighting their potential central roles in the network. Key genes, such as NUP54, CAD, and DST, show extensive interactions, suggesting their importance in the regulatory processes affected by CAP treatment and melanoma.

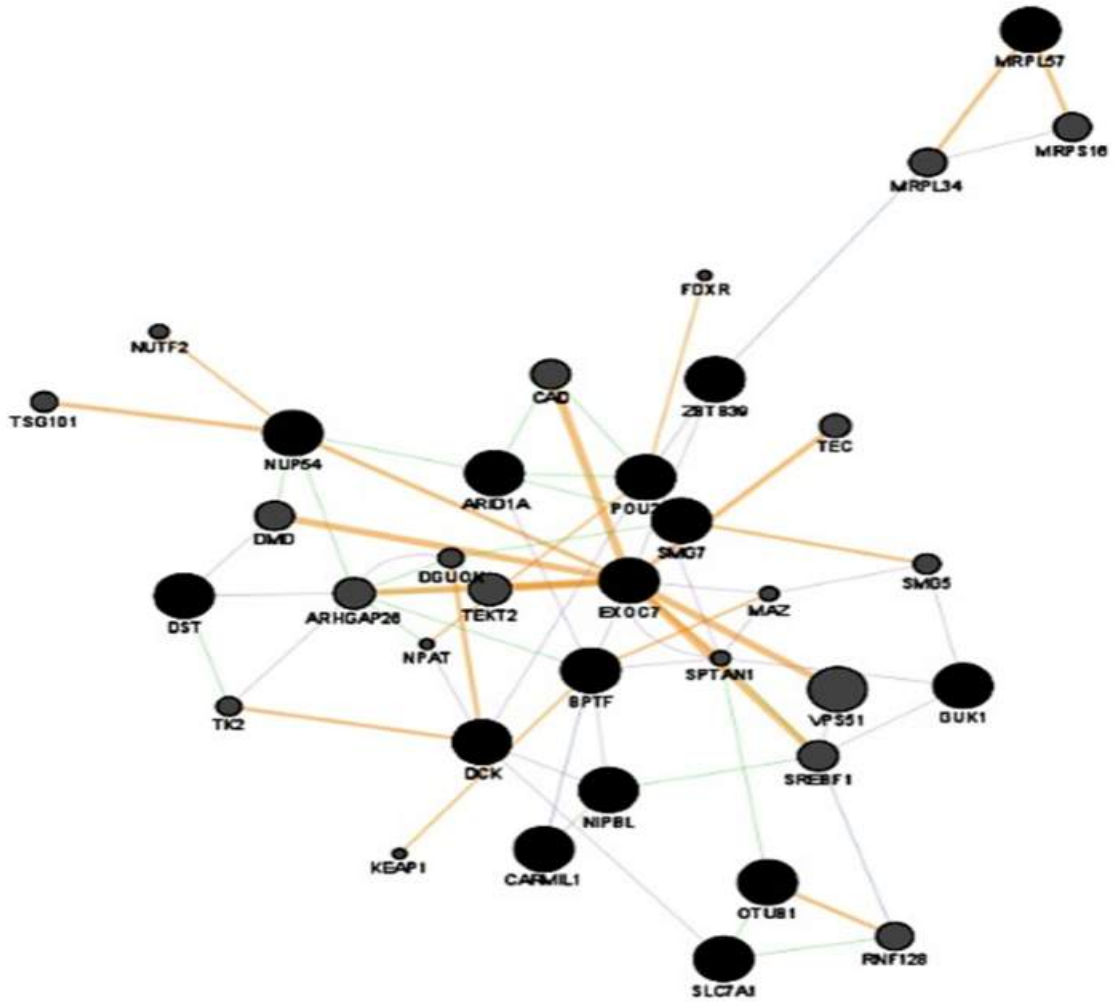

Figure 4. Network analysis of 15 differentially expressed genes common to NTAPP-treated human skin epithelial keratinocytes and melanoma cell lines. Each node represents a gene, with larger nodes indicating higher degrees of connectivity. The edges between nodes represent various types of interactions, such as co-expression, physical interactions, or shared pathways. Key hub genes, such as NUP54, CAD, and DST, exhibit extensive interactions, suggesting their central roles in the regulatory processes affected by NTAPP treatment and melanoma progression.

DISCUSSION

Cold atmospheric plasma has important roles in the medical field in treatments such as wound healing, bacterial sterilization, cleaning and tissue coagulation. However, the molecular mechanisms underlying these roles are complex as they involve many physical and chemical activities. Although studies reveal that CAP produces ROS and RNS, it is predicted that the presence of these reagents may cause oxidative stress, which may cause genetic abnormalities or disruptions in signaling systems, and this may facilitate the development of cancer. High ROS production of melanoma cells has the ability to damage DNA, accelerate tumor growth, and increase the possibility of metastasis (23, 24). Elevated amounts of ROS in melanoma cells may trigger the activation of oncogenic signaling pathways, such as the often mutant MAPK pathway. Consequently, blocking these pathways is a fundamental approach for treatment. ROS and RNS also regulate the tumor microenvironment, stimulate angiogenesis, and suppress immune responses, therefore facilitating melanoma proliferation. Melanoma cells are susceptible to ROS, so therapeutic strategies are focusing on targeting oxidative stress pathways.

We identified 15 genes that were expressed differently in CAP-treated melanoma cells than in healthy skin cells. 12 of these genes were downregulated in both CAP-treated cells and melanoma cells (SLC7A1, SMG7, EXOC7, NUP54, POU2F1, DST, NIPBL, ARID1A, MRPL57, DCK, ZBTB39 and BPTF). Three of them were upregulated in both CAP-treated cells and melanoma cells (CARMIL1, OTUB1 and GUK1). This result suggests a potential link between differential expression of these genes in response to CAP treatment and their role in melanoma progression.

ARID1A, NIPBL, and BPTF has a significant impact on ROS and RNS production in various cancer cells, including melanoma.

Downregulation of ARID1A facilitates cancer progression by causing impairments in the cell's response to DNA damage (25). Additionally, a decrease in NIPBL expression has been associated with an increase in oxidative stress and genomic instability. This encourages the growth of melanoma cells (26). Similarly, downregulation of BPTF increases ROS levels and causes DNA damage. The results highlight the important functions of these genes in controlling oxidative stress and influencing the behavior of cancer cells (27). Conversely, increased expression of OTUB1 has been associated with elevated levels of ROS in cancer cells, which may cause oxidative stress and affect the proliferation and survival of cancer cells (28).

Research suggests that the development of melanoma cancer is strongly dependent on the growth, movement, and creation of blood vessels in cells. Melanoma cells exhibit increased rates of proliferation and migration, which promote the growth and spread of tumors. Additionally, they display accelerated growth, resulting in the development of larger tumors. The process is influenced by several molecular processes and factors, one of which is the overexpression of astrocyte increased gene-1 (AEG-1). Studies suggest that AEG-1 promotes the growth and movement of melanoma cells, hence accelerating the development of tumors (29). Tissue inhibitor of metalloproteinases-3 (TIMP3) influences tumor growth and blood vessel formation by regulating the movement of endothelial cells, which is crucial for the spread of melanoma (30).

Furthermore, suppression of ARID1A, NIPBL, BPTF and ZBTB39 genes may greatly contribute to the aggressive behavior of malignancies. Downregulation or mutation of ARID1A, a component of the SWI/SNF chromatin remodeling complex, has been associated with increased cell proliferation and migration. ARID1A dysfunction may lead to structural disruption of chromatin and changes in gene expression, contributing to the development of aggressive cancer behavior (31). NIPBL is essential for regulating gene expression and maintaining the

connection between sister chromatids. Therefore, its downregulation may lead to increased susceptibility to genetic instability and accelerated tumor development (32). Also, BPTF, which stands for bromodomain PHD finger transcription factor, is a constituent of the NURF chromatin remodeling complex. The decrease in BPTF expression has been associated with enhanced movement and infiltration of melanoma cells, hence promoting metastasis (33). While there is a lack of extensive research on ZBTB39 in melanoma, its decrease in activity is linked to changes in gene expression that might potentially promote the advancement of cancer via several signaling pathways (34).

Other studies and investigations have shown that the up-regulation of OTUB1 has a major impact on cell proliferation, migration, and angiogenesis in melanoma cells. OTUB1, also known as OTU deubiquitinase and ubiquitin aldehyde binding 1, has a role in controlling cellular responses to oxidative stress and repairing DNA damage. The upregulation of this gene has been associated with increased cell proliferation and migration.

According to the functional annotation clustering findings of our work, out of the 12 genes that were downregulated, a number of them have previously been linked to important biological functions.

Accordingly, ARID1A is required to control the activation or deactivation of genes. When ARID1A is suppressed, it is often associated with increased cell growth and progression of tumors, leading to the aggressive nature of malignancies such as melanoma (35). If NIPBL is reduced, it can cause genomic instability, which is a hallmark of cancer progression. When downregulated, BPTF, an essential component of NURF, inhibits cell cycle control and processes involved in DNA repair (36). If ZBTB39 is downregulated, it can lead to uncontrolled cell proliferation and survival, which promotes cancer development (37). Additional downregulated genes, such as *SLC7A1*, *SMG7*, *EXOC7*, *NUP54*, *POU2F1*, *DST*, *MRPL57*, and *DCK*, have diverse functions in cellular processes including transport, RNA surveillance, vesicle traffic, nuclear pore complex function, transcriptional regulation, structural integrity, and other factors. These genes have not been extensively investigated in the context of cancer. The simultaneous decrease in the expression of these genes disrupts the balance in the cells, leading to the development of cancer features seen in melanoma. Conversely, increased activity of *CARMIL1*, *OTUB1* and *GUK1* genes also has important consequences. CARMIL1 increases the ability of melanoma cells to migrate and invade (38). OTUB1 affects protein degradation and signaling cascades. Its increased expression has been associated with improved cell survival and resistance to apoptosis, thus promoting cancer cell survival (39). GUK1 facilitates rapid growth of cancer cells by providing a consistent source of nucleotides for DNA replication (40).

Overall, the differential expression of these genes underscores the complex molecular interaction affected by CAP treatment. Downregulation of genes associated with cell cycle regulation, chromatin restructuring, and genomic stability, combined with upregulation of genes that promote cell motility and survival, appears to drive the aggressive behavior of melanoma.

Further functional studies are needed to confirm these findings and explore the therapeutic potential of targeting these genes in melanoma treatment.

In conclusion, despite its promising applications, CAP therapy leads to changes in gene expression that may have implications for melanoma development. Although CAP is effective in antimicrobial action and enhancing wound healing, long-term effects on gene expression and its potential to promote oxidative stress and genetic mutations require further investigation.

Our findings suggest that CAP could be a double-edged sword in clinical settings, offering therapeutic benefits in wound healing and antimicrobial defense, while also potentially modulating gene pathways that influence cancer progression. This highlights the need for careful clinical application, particularly in patients

with a predisposition to melanoma or other cancers.

These insights into CAP-induced gene expression changes may guide future experimental and clinical studies aimed at optimizing CAP use for melanoma treatment. For example, understanding which genes are differentially regulated by CAP can inform targeted therapeutic strategies that either mitigate cancer-promoting effects or enhance CAP's beneficial actions. Additional research, particularly clinical trials and in vitro and animal studies, is essential to confirm the safety and effectiveness of CAP in humans and to explore its full potential as an adjunctive therapy in melanoma treatment.

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

Tetanus immunisation status in the adult population: Investigation of IgG levels

Erişkin popülasyonda tetanoz bağışıklık durumu: IgG düzeylerinin incelenmesi

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ABSTRACT

Objective: Tetanus is a vaccine-preventable disease; however, approximately one million new cases of tetanus are reported worldwide each year. As a result of successful vaccination policies in developed countries, tetanus cases are observed in individuals who are not vaccinated or who have not completed the vaccination schedule. The aim of this study is to determine the quantitative tetanus antibody levels in patients admitted to our hospital and to analyse the correlation between age, sex and antibody levels.

Methods: The study included the serum of 408 adult patients over 18 years of age who were admitted to the outpatient clinics and services of our hospital between 01/01/2022 and 06/08/2024. Tetanus IgG antibody levels were quantitatively determined using the VIRCLIA ELISA device (Vircell, Spain) using the Tetanus VIRCLIA® IgG ELISA kit (Vircell, Spain) in the serum of patients arriving at our medical microbiology laboratory. The data analyzed with the SPSS (Statistical Package for Social Sciences) 20.0 package program. The conformity of the numerical data to the normal distribution was

ÖZET

Amaç: Tetanoz aşı ile önlenebilir bir hastalık olmasına rağmen dünya genelinde her sene yaklaşık bir milyon yeni tetanoz vakası olduğu bildirilmektedir. Gelişmiş ülkelerde uygulanan başarılı aşılama politikaları neticesinde tetanoz vakaları sadece aşılanmamış veya yetersiz aşılanmış bireylerde gözlenirken; gelişmemiş ülkelerdeki tetanoz vakaları yenidoğan mortalitelerine oldukça sık neden olmaktadır. Bu çalışmanın amacı; hastanemize başvuran hastalardaki tetanoz antikor düzeylerini kantitatif olarak saptayarak, yaş ve cinsiyete göre antikor düzeylerinin ilişkisini incelemektir.

Yöntem: Çalışmaya 01.01.2022-06.08.2024 tarihleri arasında hastanemizde çeşitli poliklinik ve servislere başvuran 18 yaş üstü 408 yetişkin hastanın serumları dahil edildi. Tıbbi Mikrobiyoloji laboratuvarımıza ulaşan hasta serumlarında Tetanus VIRCLIA IgG ELISA kiti (Vircell, İspanya) kullanılarak, VIRCLIA® ELISA cihazı (Vircell, İspanya) aracılığıyla kantitatif olarak tetanoz IgG antikor düzeyleri ölçüldü. Veriler SPSS (Statistical Package for Social Sciences) 20.0 paket programı ile analiz edildi. Sayısal verilerin normal dağılıma uygunluğu

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examined by visual (histogram) and analytical methods (Kolmogorov-Smirnov test). Mann Whitney U test was performed for pairwise comparisons in groups with significant differences between them. The statistical significance level for all tests was accepted as p<0,05.

Results: The mean ± standard deviation values of the ages of the adults included in the study were determined as 40.07±15.10. 233 (57.1%) of patients were female. In our study, non-protective tetanus antibody levels of <0.1 IU/mL were detected in 25 adults (6.1%) and protective tetanus antibody levels of ≥0.1 IU/mL were detected in 383 adults (93.9%). The median tetanus antibody level was 1.12 IU/mL in men and 0.99 IU/mL in women. However, there was no statistically significant difference between the sexes (p>0,447). A negative correlation was found between age and antibody level above 40 years of age (r=-0,334; p=0,001).

Conclusion: In our study, lower tetanus antibody levels were found in older ages, and antibody monitoring should be performed at older ages and a tetanus booster dose should be administered if necessary.

Key words: Tetanus, IgG, vaccine, antibody level, immunisation

görsel (histogram) ve analitik yöntemlerle (Kolmogorov-Smirnov testi) incelendi. Aralarında anlamlı fark bulunan gruplarda ikili karşılaştırmalar için Mann Whitney U testi uygulandı. Tüm testler için istatistiksel anlamlılık düzeyi p<0,05 olarak kabul edildi.

Bulgular: Çalışmaya dahil edilen yetişkinlerin yaşlarının ortalama ± standart sapma değerleri 40,07±15,10 olarak tespit edildi. Hastaların 233 (%57,1)'ünü kadın hasta oluşturmaktadır. Çalışmada 25 (%6,1) kişide <0.1 IU/ml düzeyinde korumasız tetanoz antikoru, 383 (%93,9) kişide ise ≥0.1 IU/ml seviyesinde koruyucu tetanoz antikoru tespit edildi. Erkeklerde ortanca antikor düzeyi 1.12 IU/ml, kadınlarda ise 0.99 IU/ml olarak saptandı. Cinsiyetler arası istatistiksel olarak fark saptanmadı (p=0,447). 40 yaş üstünde yaş ile antikor düzeyi arasında negatif korelasyon saptandı (r=-0,334; p=0,001).

Sonuç: Çalışmamızda ileri yaşlarda daha düşük tetanoz antikor düzeyi saptanmış olup, ilerleyen yaşlarda antikor takibi yapılarak gerekliyse tetanoz hatırlatma dozu uygulanmalıdır.

Anahtar Kelimeler: Tetanoz, IgG, aşı, antikor düzeyi, bağışıklama

INTRODUCTION

Tetanus is an acute disease caused by exotoxins produced by *Clostridium tetani*, an anaerobic bacillus, with a high mortality, usually involving the musculoskeletal system. There is a vaccine developed from the bacteria's toxin. Although it is a vaccine-preventable disease; it is an important public health problem that mostly affects newborns and young adults in developing and the elderly in developed countries (1). Tetanus is a notifiable disease and its incidence is estimated to be 18 per hundred thousand. Mortality rates due to tetanus vary between 30-50% worldwide. It is reported that at least half of the mortality cases belong to the neonatal period (2-4). Since 1948, diphtheria, tetanus and pertussis vaccines have been used as combined toxoid vaccines (5). Turkey's National Immunisation Program states that DaBT-IPA-Hib (Diphtheria, Acellular Pertussis, Tetanus, Inactivated Polio, *Haemophilus influenza* type B) is the first vaccination given in the second month of life.

The administration of three doses of vaccine continues at intervals of eight weeks. A booster dose is administered at 18 months. DaBT-IPA is administered as a single booster dose in the first grade of primary school and Td (Tetanus, Diphtheria) in the eighth year. Moreover, men receive a single dose of the

tetanus vaccine while serving in the military, while women receive two doses at least two weeks prior to delivery (3).The risk of developing the disease increases in older ages due to the decreasing tetanus antibodies with age. According to a study done in the US, the vaccination rate for individuals six years of age and older was 72.3%, while the rate for those over 70 was 31% (4).

In this study, tetanus immunoglobulin G (IgG) levels of patients over 18 years of age admitted to various outpatient clinics and wards in our hospital is aimed to be investigated quantitatively and the relationship of antibody titres with age and gender is analysed.

MATERIAL and METHOD

The tetanus antibody levels of the patients included in our study were obtained retrospectively from the hospital information system. All patients who were requested tetanus antibodies from various outpatient clinics and services between 01.01.2022 and 06.08.2024 were included in our study.

The serum of 408 adult patients over the age of 18 who applied to various outpatient clinics and services in Necmettin Erbakan University Medicine Faculty between 01.01.2022 and 06.08.2024 were included in the study. Tetanus IgG antibody levels were quantitatively detected by the VIRCLIA ELISA device (Vircell, Spain) using the Tetanus VIRCLIA® IgG ELISA kit (Vircell, Spain) in the serum of patients accepted in our Medical Microbiology laboratory.

Results were stated in International Units per mililiter (IU/mL). The detected antibody levels were grouped according to the investigations in the literature and kit recommendations. Antibody titers of <0.1 IU/mL were considered to be non-protective against tetanus, antibody levels between 0.1-1 IU/mL were considered protective, and antibody levels above 1 IU/mL were considered to be long-term protective against tetanus. Protection status and recommendations according to tetanus antibody levels are presented in Table-1 (6-8).

The data were analyzed with the SPSS (Statistical Package for Social Sciences) 20.0 package program. In the descriptive analysis, frequency data were shown as numbers (n) and percentages (%), while numerical data were given using minimum and maximum values. The conformity of the numerical data to the normal distribution was examined by visual (histogram) and analytical methods (Kolmogorov-Smirnov test). Mann Whitney U test was performed for pairwise comparisons in groups with significant differences. Chi-square test and Fisher's exact chi-square test were used to compare categorical data. The correlation of parameters that did not fit the normal distribution was analized by Spearman test. The statistical significance level for all tests was accepted as $p<0,05$.

Table 1. Protection, vaccination and antibody monitoring according to tetanus antibody levels

Tetanus IgG Levels	Protective Status	Vaccination and antibody monitoring
<0.1 IU/mL	No protection	A booster dose should be given, and the antibody level should be measured after 4-6 weeks.
0.1-0.5 IU/mL	There is protectiveness	A booster dose should be given, and the antibody level should be measured after 4-6 weeks.
0.5-1.1 IU/mL	There is protectiveness	Antibody level control is recommended after 2 years
1.1-5.0 IU/mL	There is protectiveness	Antibody level control is recommended after 5-10 years
>5.0 IU/mL	There is protectiveness	Antibody level control is recommended for 10 years

The study was approved by the Necmettin Erbakan University Non-Drug and Non-Medical Device Research Ethics Committee Ethics Committee (Date: 20.09.2024 and Number: 2024/5181).

RESULTS

The mean ± standard deviation values of the ages of the adults included in the study were determined as 40.07±15.10. Considering the average age of the patients in the study, two groups under the age of 40 and over the age of 40 were formed in terms of age. 233 (57.1%) of patients in the study were female.

In the study, the median tetanus IgG level of women was 0.99 (0.44-1.67) IU/mL and of men 1.12 (0.62-1.77) IU/mL (p=0,447). The median tetanus IgG level was found to be 1.28 (0.66-2.33) IU/mL in the group aged 40 and under, and 0.71 (0.23-1.36) IU/mL in those aged 40 and over.

The antibody level of 6.1% of the patients was below <0.1 IU/mL that is not protective against tetanus. The rate of having a protective antibody level against tetanus for 2-5 years was 25.2%. The rate of having a protective antibody level for 5-10 years was 42.4%. The rate of having a protective antibody level for more than 10 years was 7.1%. (Figure-1).

There was no statistically significant difference between the antibody levels in males and females (Figure-2).

Considering the relationship between antibody levels and patient ages, the high results obtained for tetanus IgG antibody levels in patients under 40 years of age was found to be statistically significant (Table-2).

There was moderate negative correlation between age and tetanus antibodies (r=-0,334; p=0,001).

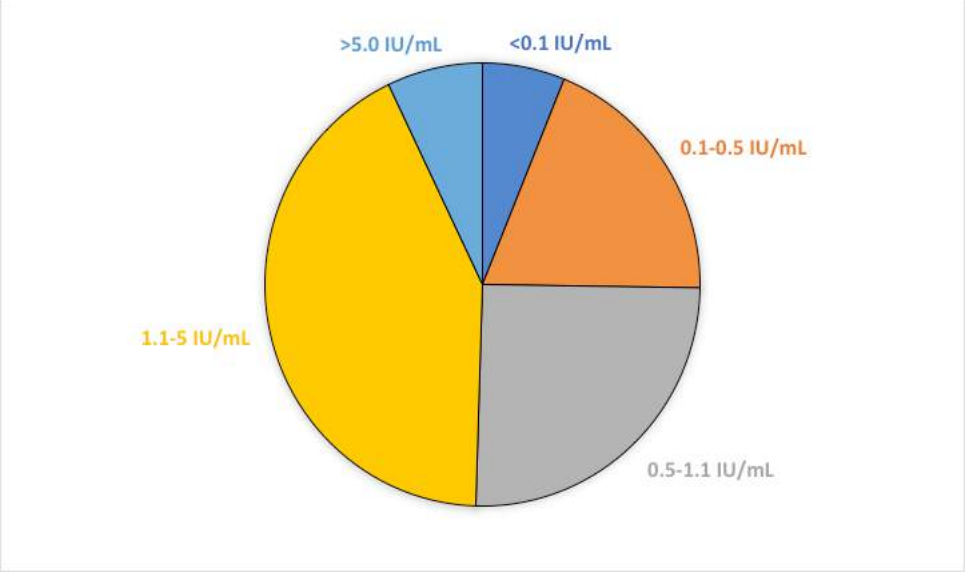

Figure 1. Antibody distribution of patients in the study.

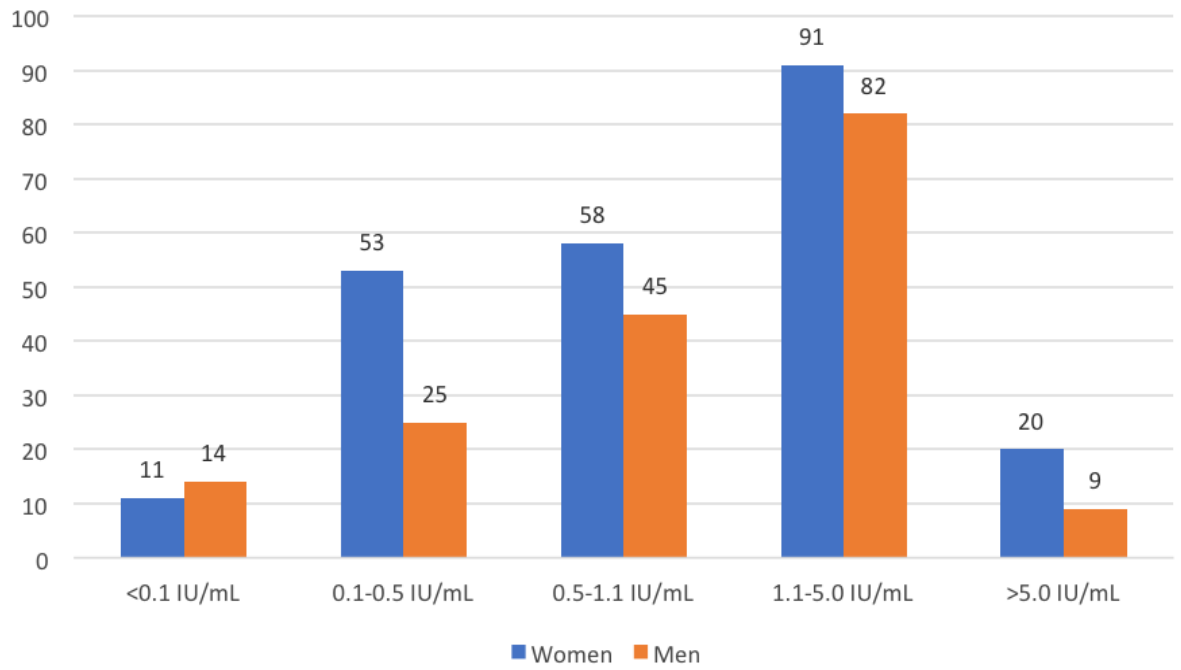

Figure 2. The relationship and distribution of antibody groups with gender

Table 2. The relationship and distribution of antibody groups with age

Tetanus IgG Level	Age group		p-value
	40 years and younger n (%)	Over 40 years old n (%)	
<0.1 IU/mL	2 (0.8)	23 (13.4)	0,001
0.1-0.5 IU/mL	32 (13.6)	46 (26.7)	
0.5-1.1 IU/mL	65 (27.5)	38 (22.1)	
1.1-5.0 IU/mL	117 (49.6)	56 (32.6)	
>5.0 IU/mL	20 (8.5)	9 (5.2)	

DISCUSSION

In our study, no statistically significant difference was found between genders in terms of tetanus antibodies, while a moderate negative correlation was found between age and antibody levels.

Tetanus is a vaccine-preventable disease with a high mortality and morbidity. For this reason, it is important to carry out vaccination programs in adulthood as well as childhood (9,10). Tetanus antibody levels vary depending on the development level and vaccination program of the country. A study

of 255 adults in India reported that 47% had antibody levels <0.1 IU/mL and were nonprotected against tetanus (11). In a study in Spain, tetanus antibodies were evaluated in 1296 people and 68.3% of people were found to have protective antibody levels (12). In a study conducted in healthcare workers in China, it was determined that 29.82% had an antibody level of <0.1 IU/ml (13). In a study conducted in 295 people in Edirne, protection against tetanus was found to be 98.6%, and in a study conducted in our hospital in 2021, protection against tetanus was found to be 82.5% (6,14). In our study, protection against tetanus was found to be 93.9% which is higher than the literature. We think that the reason for this may be due to the vaccination programs and the immune response of the patients included in the study.

In some studies in the literature, it is reported that higher tetanus antibody levels are seen in the male gender. It is reported that the reason for this situation may be the booster doses administered during military service and injury (12,15). In our study, although higher antibody titers were detected in the male gender, no statistically significant difference was found ($p>0.05$). The immune response to tetanus vaccination decreases over the years. Studies have shown that higher levels of antibodies are produced in young adults after vaccination than in the elderly. In the literature, it is reported that after 10 years or more, a booster dose should be given, especially in the elderly population (16). In many studies, antibody titers that are unprotected against tetanus (<0.1 IU/mL) have been detected, especially in the elderly population (17-19).

In our study, it was determined that 92% of the group with antibody titers lower than 0.1 IU/mL were adults over 40 years of age. In our study, similar to the literature, the decrease in tetanus antibodies as age increased was found to be statistically significant. ($r=-0.334$; $p<0.001$). The lower tetanus antibody levels in adults over 40 years of age were found to be statistically significant compared to adults aged 40 and under ($p<0.001$). Immigrants leaving countries where vaccination programs are not effectively implemented pose a significant risk of tetanus in the countries where they settle. In a multicenter study involving six European countries, it was found that 22.3% of migrants did not have a protective tetanus antibody titer (<0.1 IU/mL) (20).

Since our study is a retrospective study, the vaccination status of the individuals included in the study is unknown. We believe that more than 10 years have had passed since the vaccination of the adults included in our study whose tetanus antibody levels were not at a protective level.

Although tetanus is a vaccine-preventable disease with a high mortality and morbidity, it can still be seen worldwide. Preventive health services constitute the most important part of health policies in developed countries. After routine vaccination in our country since 1968, a single booster dose is recommended for men during military service and two doses during pregnancy for women.

It is essential to administer a booster dose to increase the levels of antibodies against tetanus that decrease in older ages. Especially for individuals over the age of 20, antibody levels should be followed every 10 years and a booster dose should be administered if necessary. The public should be informed about the risk factors, transmission routes, clinical findings and mortality of tetanus, and it should be ensured that the vaccinations of unvaccinated or of incompletely vaccinated people should be performed.

Since our study was planned retrospectively, we believe that the limitations of our study were the inability to obtain vaccination status, risk factors, and sociodemographic data of the individuals included in the study.

ETHICS COMMITTEE APPROVAL

* The study was approved by the Necmettin Erbakan University Non-Drug and Non-Medical Device Research Ethics Committee Ethics Committee (Date: 20.09.2024 and Number: 2024/5181).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

Evaluation of extracorporeal treatment methods in poisoned patients admitted to a university hospital

Bir üniversite hastanesine başvuran zehirlenme olgularına uygulanan ekstrakorporeal tedavi yöntemlerinin değerlendirilmesi

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ABSTRACT

Objective: This study aims to describe characteristics of poisonings in patients who were treated with extracorporeal methods between January 01, 2011 and December 31, 2017 in Dokuz Eylul University Drug and Poison Information Center (DEUDPIC).

Methods: This cross-sectional and descriptive study included patients who received extracorporeal treatment for poisoning. The data were obtained from the hospital automation system, extracorporeal treatment unit forms, and DEUDPIC forms. The severity was classified using the Poisoning Severity Score, and Glasgow Coma Scores (GCS) were recorded. Statistical analyses involved Kolmogorov-Smirnov tests for normality, Student's t-test for parametric comparisons, and Mann-Whitney U test for non-parametric data. Risk factors for mortality in specific common drug toxicities are determined using logistic regression analysis. Analyses were conducted using SPSS-24.

Results: A total of 75 cases participated in the study. The mean age was 51.5±19.7 years, with 60% of

ÖZET

Amaç: Bu çalışmanın amacı, 01 Ocak 2011-31 Aralık 2017 tarihleri arasında Dokuz Eylül Üniversitesi Tıp Fakültesi İlaç ve Zehir Danışma Merkezine (DEÜTF-ZDM) danışılan ve ekstrakorporeal tedavi yöntemleri uygulanan zehirlenme olgularının değerlendirilmesidir.

Yöntem: Bu kesitsel ve tanımlayıcı çalışma, zehirlenme nedeniyle ekstrakorporeal tedavi yöntemi uygulanan hastaları kapsamaktadır. Veriler, hastane otomasyon sistemi, ekstrakorporeal tedavi ünitesi formları ve DEÜTF-ZDM kayıt formlarından elde edilmiştir. Zehirlenme şiddeti, Zehirlenme Şiddet Skoru kullanılarak sınıflandırılmış, hastaların Glasgow Koma Skoru (GKS) kaydedilmiştir. Normalite analizi için Kolmogorov-Smirnov testi, parametrik veriler için Student-t testi ve parametrik olmayan veriler için Mann-Whitney U testi uygulanarak gerçekleştirilmiştir. En sık görülen zehirlenmelerde mortaliteyi etkileyen risk faktörlerini belirlemek için lojistik regresyon analizi uygulanmıştır. Verilerin analizi, SPSS-24 yazılımı kullanılarak yapılmıştır.

Bulgular: Çalışmaya 75 hasta dahil edildi. Ortalama yaş 51.5±19.7 yıl olup, %60'ı erkekti. En

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cases being male. Methanol (37.3%), lithium (18.7%), carbamazepine (10.7%), and digoxin (6.7%) caused the most poisonings, respectively. Extracorporeal treatment was used for "increasing the elimination of the substance exposed" in 74.7% of the cases for "renal support" in 25.3%, and the method was hemodialysis in all. Specific antidote treatment was applied to 29.3% of all cases and 67.9% of methanol poisonings. The most common complication of hemodialysis was hypotension (28%). A majority of the patients (73.3%) necessitated intensive care unit support. Three patients recovered with sequelae (4%), and 21 (28%) died. Among patients who suffered from methanol poisoning, pH<7.00, serum creatinine (SCr)>1.2 mg/dL, and lower GCS were associated with mortality in univariate analysis. In the multivariate regression analysis, the sole independent predictor of mortality was found to be the GCS (OR 0.62, 95% CI 0.39-0.99, p=0.05).

Conclusion: Despite adequate hemodialysis, mortality rate is high in the setting of common drug poisonings. A low GCS is the only independent predictor of mortality in the setting of methanol poisoning.

Key Words: Poisonings, methanol, hemodialysis, toxicity

fazla zehirlenmelere neden olan maddeler sırasıyla metanol (% 37,3), lityum (% 18,7), karbamazepin (% 10,7) ve digoksin (% 6,7) olarak tespit edildi. Ekstrakorporeal tedavi, vakaların %74,7'sinde "maruz kalınan maddenin eliminasyonunu artırmak" için, %25,3'ünde "renal destek" için kullanıldı ve tüm hastalara uygulanan yöntem hemodiyalizdi. Spesifik antidot tedavisi, tüm olguların %29,3'üne ve metanol zehirlenmelerinin %67,9'una uygulandı. Hemodiyalizin en sık komplikasyonu hipotansiyondu (%28). Hastaların çoğunluğunda (%73,3) yoğun bakım ünitesi ihtiyacı vardı. Üç hasta (%4) sekelle iyileşirken, 21 hasta (%28) hayatını kaybetti. Metanol ile zehirlenen hastalarda, pH<7.00, serum kreatinin (SCr)>1.2 mg/dL ve düşük GCS tek değişkenli analizde mortalite ile ilişkilendirildi. Çok değişkenli regresyon analizinde, mortalitenin tek bağımsız belirleyicisi GCS olarak bulundu (OR 0.62, %95 CI 0.39-0.99, p=0.05).

Sonuç: Yeterli hemodiyalize rağmen, ilaç zehirlenmelerinde ölüm oranı yüksektir. Düşük GCS, metanol zehirlenmesi durumunda ölüm oranının tek bağımsız belirleyicisidir.

Anahtar Kelimeler: Zehirlenme, metanol, hemodiyaliz, toksisite

INTRODUCTION

Poisonings are common health problems that may cause morbidity and mortality. According to the annual reports of the American Association of Poison Control Centers (AAPCC), the number of poisoning cases in 2022 was more than two million on average (1). In poisoning, extracorporeal therapy methods can be applied to increase the excretion of the substance from the body in cases when the level of the ingested agent in the blood is too high and/or there is insufficiency in the elimination organs. Extracorporeal therapy methods can be classified as intermittent hemodialysis, peritoneal dialysis, intermittent hemofiltration/hemodiafiltration, hemoperfusion, exchange transfusion, therapeutic plasma exchange, continuous renal replacement therapy, and extracorporeal membrane oxygenation (2-6). These methods can be used for both the removal of toxic agents and renal support. Lithium, ethylene glycol, methanol, valproic acid, salicylate, carbamazepine, theophylline, and tricyclic antidepressants are the most common poisonings in which extracorporeal therapy methods are used (7-9). AAPCC 2022 report shows that extracorporeal therapy methods are used in 0.13% of the poisoning

cases (1). However, in our country, there is limited data on the type and frequency of extracorporeal decontamination methods and for which poisoning agents they are applied, and the outcomes of poisoning cases.

This study aimed to find out the characteristics of hospital admission, demographic data, poisoning characteristics, clinical signs and symptoms, extracorporeal therapy and non- extracorporeal therapy methods applied, clinical follow-up and outcomes in poisoning cases who were consulted to Dokuz Eylul University Drug and Poison Information Center (DEUDPIC) between January 01, 2011 and December 31, 2017 and in whom extracorporeal therapy methods were applied. Determination of factors related to mortality in the common subgroup of toxicities is provided.

MATERIAL and METHOD

The study included cases admitted to Dokuz Eylul Hospital between January 01, 2011, and December 31, 2017, for poisoning, who were 18 years old and older and who had undergone extracorporeal therapy for treatment. Patients younger than 18 years of age and patients whose medical records were not accessed were excluded from the study.

DEUDPIC is a center working for the diagnosis and treatment of poisoning cases admitted to Dokuz Eylul University Hospital for detection, evaluation, prevention, and risk analysis. DEUDPIC records each case on a registration form. Data from registration forms, Probel Hospital Information Management System, and Extracorporeal Treatment Unit patient medical records were used in this investigation.

A nephrologist guided DEUDPIC to treat poisoning patients with hemodialysis. Drug blood levels were measured at the hospital before and after hemodialysis. The "Poisoning Severity Score" (PSS) was utilized to grade all DEUDPIC patients who had extracorporeal therapy as asymptomatic (0), mild (1), moderate (2), severe (3), or death (4) (10). In addition, Glasgow Coma Scores of the patients at the time of admission were evaluated (11).

Kolmogorov-Smirnov tests were performed for normality analysis. Categorical variables are presented as percentages, numeric variables are given as mean±standard deviation or median with the range, as appropriate. In the comparison of means, Student's t-test was used in parametric measurements, while the Mann-Whitney U test was used in non-parametric measurements. Chi-square test and Fisher's exact test were used to compare the numerical variables. Predictors of mortality were determined by using logistic regression analysis. Age, sex, and factors that were significantly associated with mortality in univariate analysis were included in the multivariate model. Results are shown as odds ratios (OR) with a 95% confidence interval. The data were analysed with SPSS-24 (SPSS INC., Chicago, IL, USA) software.

The study was approved by the Non-Interventional Research Ethics Committee of Dokuz Eylul University (Date: 04.10.2018 and Number: 2018/24-19).

RESULTS

Between 2011 and 2017, extracorporeal therapy was applied to 75 of the 831 (9.03%) poisoning cases consulted to DEUDPIC. Median age of the patients was 50 (18-88) years, and 60.0% were male. Acute poisoning (58.7%) was statistically significantly higher when compared with other types of poisoning (25.3% were chronic and 16.0% were acute on chronic) ($p<0.001$). It was found that 58.7% of the poisonings were unintentional, while 41.3% were intentional. It was determined that 40.0% of the female cases and 42.2% of the male cases had taken the agent intentionally ($p=0.848$). The rate of intentional exposure in the 18-49 age group was found to be statistically significantly high when compared with those aged 50 years and older (62.2% and 21.1%, respectively, $p<0.001$). The most common route was oral (97.3%) in all poisonings.

The most common agents causing poisoning in

cases treated with extracorporeal therapy were methanol (37.3%), lithium (18.7%), carbamazepine (10.7%), and digoxin (6.7%), respectively. It was found that 74.7% (56 cases) of all cases had been exposed to a single agent, while 22.7% (17 cases) had been exposed to more than one agent. Two (2.7%) cases were exposed to unknown agents (Table 1).

Table 1. Demographic and clinical characteristics of all patients

Characteristics	Female n=30 (%40.0)	Male n=45 (%60.0)	Total n=75 (100.0)
Age (year) (median, min-max)	50 (18-88)	49 (18-82)	50 (18-88)
Time of admission (hour)			
0-12	8	10	18
13-24	1	6	7
24<	3	6	9
Unknown	18	23	41
Type of poisoning			
Acute	9 (20.5)	35 (79.5)	44 (100.0)
Chronic	13 (68.4)	6 (31.6)	19 (100.0)
Acute on chronic	8 (66.7)	4 (31.6)	12 (100.0)
Route of poisoning			
Peroral	30 (41.1)	43 (58.9)	73 (100.0)
Inhalation	-	1 (100.0)	1 (100.0)
Unkown	-	1 (100.0)	1 (100.0)
Reason of poisoning			
Intentional	12 (38.7)	19 (61.3)	31 (100.0)
Unintentional	18 (40.9)	26 (59.1)	44 (100.0)
Unknown	-	-	-
GCS (median, min-max)	15 (3-15)	15 (3-15)	15 (3-15)
Toxin			
Single	21 (37.5)	35 (62.5)	56 (100.0)
Multiple	8 (47.1)	9 (52.9)	17 (100.0)
Methanol	2 (7.2)	26 (92.8)	28 (100.0)
Lithium	10 (66.7)	5 (33.3)	15 (100.0)
Carbamazepine	5 (62.5)	3 (37.5)	8 (100.0)
Digoxin	5 (100.0)	-	5 (100.0)
Metformin	3 (75.0)	1(25.0)	4 (100.0)
Others	4 (30.8)	9 (69.2)	13 (100.0)
Unknown	1 (50.0)	1 (50.0)	2 (100.0)
PSS			
Asymptomatic	1 (50.0)	1 (50.0)	2 (100.0)
Mild	10 (55.6)	8 (44.4)	18 (100.0)
Moderate	8 (40.0)	12 (60.0)	20 (100.0)
Severe	11 (31.4)	24 (68.6)	35 (100.0)

Table 1 (cont.). Demographic and clinical characteristics of all patients

Characteristics	Female n=30 (%40.0)	Male n=45 (%60.0)	Total n=75 (100.0)
Age (year) (median, min-max)	50 (18-88)	49 (18-82)	50 (18-88)
Clinical sign and symptoms			
Central nervous system	22 (39.3)	34 (60.7)	56 (100.0)
Acid-base disorders	13 (29.5)	31 (70.5)	44 (100.0)
Gastrointestinal system	12 (54.5)	10 (45.5)	22 (100.0)
Urinary system	12 (57.1)	9 (42.9)	21 (100.0)
Visual system	2 (14.3)	12 (85.7)	14 (100.0)
Cardiovascular system	3 (42.9)	4 (57.1)	7 (100.0)
Respiratory system	1 (16.7)	5 (83.3)	6 (100.0)
Reason of haemodialysis			
Remove the toxin or metabolite	19 (33.9)	37 (66.1)	56 (100.0)
Renal support	11 (57.9)	8 (42.1)	19 (100.0)
Complications associated with haemodialysis			
Hypotension	4 (19.0)	17 (81.0)	21 (100.0)
Clot formation in the catheter	4 (22.2)	14 (77.8)	18 (100.0)
Catheter-associated problems before dialysis	2 (66.7)	1 (33.3)	3 (100.0)
Nausea-vomiting	1 (50.0)	1 (50.0)	2 (100.0)
Catheter-associated problems after dialysis	1 (100.0)	-	1 (100.0)
Fever	-	1 (100.0)	1 (100.0)
Specific antidote therapy	2 (9.5)	19 (90.5)	21 (100.0)
Mechanical ventilation need	2 (10.5)	17 (89.5)	19 (100.0)
Admission			
Intensive care unit	19 (34.5)	36 (65.5)	55 (100.0)
Medical ward	11 (55.0)	9 (45.0)	20 (100.0)
Duration of hospital stay (median, min-max)	3 days (3 hours-84 days)	3 days (15 hours-53 days)	3 days (3 hours-84 days)
Outcome			
Alive	23 (42.6)	31 (57.4)	54 (100.0)
Dead	7 (33.3)	14 (66.7)	21 (100.0)

GCS: Glasgow Coma Scale; PSS: Poisoning Severity Score Percentages are calculated by row.

Median time elapsed from exposure to hospital admission was 23.4±33.5 hours, and 24.0% of admissions occurred within the first 12 hours. Clinical signs and symptoms of the patients at admission were classified according to PSS. While 2.7% (2 cases) of patients were asymptomatic, 24.0% (18 cases) had mild, 26.7% (20 cases) had moderate, and 46.7% (35 cases) had severe clinical signs and symptoms. The mean GCS at admission was 11.4±4.5.

Overall, the most common symptoms and findings were related to central nervous system complaints (74.7%), acid-base disorders (58.7%), and gastrointestinal system (29.4%) complaints, respectively. While the most common central system symptom was orientation-cooperation disorder (26 cases, 34.7%), it was followed by unconsciousness (14 cases, 18.7%), speech disorder (5 cases, 6.7%), tendency to sleep (5 cases, 6.7%) and other (6 cases, 8.0%) signs and symptoms. Acidosis (pH<7.35) was found in 58.7% (44 cases) of all cases.

The extracorporeal therapy method applied to all patients was "hemodialysis" (100.0%). Indications for hemodialysis were to increase the elimination of the exposed agent (74.7%, 56 cases) and renal support (25.3%, 19 cases).

Serum lithium, carbamazepine, digoxin, theophylline, phenytoin, ethanol, and blood carboxyhaemoglobin levels for carbon monoxide could be measured. Drug levels were monitored in 34 patients (45.3%) before hemodialysis and in 32 patients (42.7%) after hemodialysis. While all drug levels measured before hemodialysis treatment were toxic, 84.4% were found to decrease to non-toxic levels after hemodialysis.

No complications developed during hemodialysis in 36 patients (48.0%), and at least one complication was found to develop in 34 patients (45.3%). It was not known whether complications developed in five patients (6.7%). The most common complications related with hemodialysis were hypotension (28.0%), clot formation in the catheter (24.0%), and catheter-associated problems before hemodialysis (4.0%).

All patients received supportive care in addition to hemodialysis. Twenty-two (29.3%) patients received specific antidote treatment. Nineteen of these were suffering from methanol intoxication. Twenty (26.7%) of the patients were hospitalized in the medical ward, 55 (73.3%) needed intensive care support, and 19 (34.5%) of these who needed intensive care received respiratory support with mechanical ventilation. The number of male patients who required mechanical ventilation was found to be statistically higher when compared with female patients (p<0.002). Median duration of hospital stay was 3 days (3 hours - 84 days). Forty-one (54.7%) patients were discharged with complete recovery, 8 (10.7%) refused treatment and were lost to follow-up, and 2 (2.7%) were transferred to another hospital. Three (4.0%) of the cases recovered with sequelae, and 21 (28.0%) died (Figure 1). Except for the cases whose deaths were reported, the others were stated as "alive" (Table 1). It was found that the most common poisoning agent in the patients who died was methanol (52.4%), followed by digoxin (23.9%), lithium (9.5%), phenytoin (4.8%), tramadol (4.8%), and verapamil (4.8%), respectively.

Patients with methanol poisoning

The mean age of methanol poisonings was 51.1±12.9 (18-71) years. 60.7% of the cases were aged 50 years and older. The number of male cases was higher than the number of female cases (92.9% and 7.1%, respectively). It was found that 25.0% of methanol poisonings were intentional, while 75.0% were unintentional. The relationship between the reason of poisoning and gender was analysed, and it was determined that none of the female patients were exposed to methanol intentionally, while this rate was 26.9% in male patients. The time of admission was 32.5±24.5 hours in methanol poisoning. According to hospital records, discharge with wellness, leaving the hospital or voluntary discharge, discharge with sequelae, and transfer to another hospital were grouped as "survival group" (n=17, 60.7%). The cases whose deaths were reported in hospital records were considered the "non-survival group" (n=11, 39.3%). Clinical follow-up and results of the cases poisoned by methanol are shown in Tables 2 and 3.

The mean of GCS at admission was 10.95±4.72, and it was found that all the patients with <8 GCS at the time of admission died. The most common clinical sign and symptom after acidosis (pH<7.35) (96.0%) was related to the central nervous system (71.4%). The most common central nervous system symptom

was orientation-cooperation disorder (10 cases, 35.7%), followed by unconsciousness (5 cases, 17.9%), seizure (3 cases, 10.7%), syncope (1 case, 3.6%), agitation (1 case, 3.6%) and others (2 cases, 7.1%).

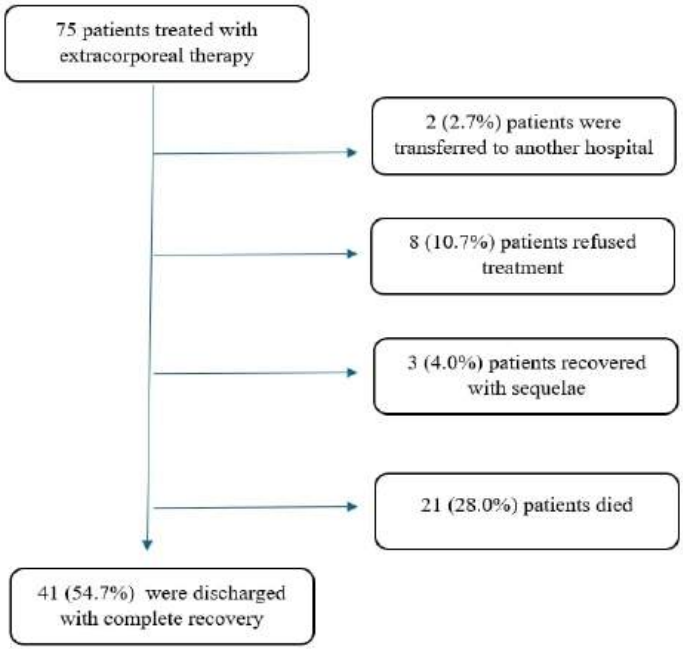

Figure 1. Follow-up and outcome characteristics of poisoning cases.

Table 2. Demographic and clinical characteristics of methanol poisonings

Characteristics		Survival group n (%)	Non-survival group n (%)	x2	*p**
Age (year)	<50 (n=11) ≥50 (n=17)	10 (90.9) 7 (41.2)	1 (9.1) 10 (58.8)	6.925	**0.016**
Gender	Female (n=2) Male (n=26)	2 (100.0) 15 (57.7)	0 (0.0) 11 (42.3)	1.394	0.505
Time of admission (hour)	≤24 (n=8) >24 (n=6)	5 (62.5) 6 (100.0)	3 (37.5) 0 (0.0)	2.864	0.209
Reason of poisoning	Intentional (n=7) Unintentional (n=21)	6 (85.7) 11 (52.4)	1 (14.3) 10 (47.6)	2.446	0.191
GCS	<8 (n=7) ≥8 (n=15)	0 (0.0) 12 (80.0)	7 (100.0) 3 (20.0)	12.320	**0.001**
PSS	0-1-2 (n=9) 3-4 (n=19)	7 (77.8) 10 (52.6)	2 (22.2) 9 (47.4)	0.249	1.619
pH	<7.00 (n=12) ≥7.00 (n=16)	4 (33.3) 13 (81.3)	8 (66.7) 3 (18.8)	6.601	**0.019**

Table 2 (cont.). Demographic and clinical characteristics of methanol poisonings

Characteristics		Survival group n (%)	Non-survival group n (%)	x2	p*
Mechanical ventilation need	Yes (n=12)	4 (33.3)	8 (66.7)	4.812	**0.028**
	No (n=13)	10 (76.9)	3 (23.1)		
Comorbidity	Yes (n=19)	11 (57.9)	8 (42.1)	0.197	1.000
	No (n=9)	6 (66.7	3 (33.3)		
Complications associated with haemodialysis	Yes (n=19)	7 (77.8)	2 (22.2)	1.619	0.249
	No (n=9)	10 (52.6)	9 (47.4)		
Specific antidote therapy	Yes (n=19)	14 (73.7)	5 (26.3)	4.169	0.095
	No (n=9)	3 (33.3)	6 (66.7)		
Admission	Intensive care unit (n=25)	14 (56.0)	11 (39.3)	2.174	0.258
	Medical ward (n=3)	3 (100.0)	0 (0.0)		

GCS: Glasgow Coma Scale; PSS: Poisoning Severity Score
**: Chi-square test and Fisher's exact test.*
Survival group (n=11): discharged with wellness + discharged voluntarily + discharged with sequelae + leaved the hospital + transferred to another hospital
Non-survival group (n=18): Cases whose deaths were reported in hospital records

Table 3. Characteristics of methanol poisonings

Characteristics	Survival group Median (min-max)	Non-survival group Median (min-max)	p¥
Age (year)	46 (18-66)	59 (48-71)	**0.007**
pH	7.08(6.77-7.30)	6.82 (6.75-7.21)	**0.008**
HCO_3 (mmol/L)	8.1(5.3-23.4)	5.8(4.7-9.5)	**0.003**
SCr (mg/dL)	0.89 (0.57-17.87)	1.41 (0.82-1.95)	**0.005**
GCS	15 (8-15)	7 (3-15)	**0.001**

SCr: Serum Creatinine
GCS: Glasgow Coma Scale
¥: Mann-Whitney U test.
Survival group (n=11): discharged with wellness + discharged voluntarily + discharged with sequelae + leaved the hospital + transferred to another hospital
Non-survival group (n=18): Cases whose deaths were reported in hospital records

Patients who survived had more optimal values of pH, HCO3, and better levels of GCS compared to those who died ($p<0.05$). Patients who experienced mortality were older and had higher levels of serum creatinine ($p<0.05$). Predictors of mortality among patients who had methanol intoxication are shown in Table 4. In univariate analysis, pH<7.00, SCr level >1.2 mg/dl, and lower GCS were associated with mortality among patients who suffered from methanol intoxication. In the multivariate regression analysis, the sole independent predictor of mortality was found to be the GCS (OR 0.62, 95% CI 0.39-0.99, p=0.05).

Table 4. The results of the univariate and multivariate analysis on the factors associated with mortality in methanol poisoning

Variable	Univariate analysis			Multivariate analysis		
	OR	**95% CI**	***p***	**OR**	**95% CI**	***p***
Age	1.14	1.03-1.26	0.014	1.09	0.94-1.27	0.268
Female sex	N/A	-	-	-	-	-
HCO_3	0.54	0.29-1.02	0.058	-	-	-
pH<7.00	8.67	1.53-49.2	0.015	0.94	0.04-24.9	0.971
pH<7.10	4	0.66-24.3	0.132	-	-	-
SCr>1.2	5.69	1.08-30	0.040	3.29	0.1-108	0.504
GCS	0.65	048-0.89	0.006	**0.62**	**0.39-0.99**	**0.050**

SCr: Serum Creatinine
GCS: Glasgow Coma Scale

DISCUSSION

In this study, 75 patients who were treated with extracorporeal therapy for poisoning in a 7-year period were evaluated. The number of patients was lower than reported studies in other countries (12-14), but it was higher than reported studies in Turkey (2,15-19) (Table 5). These other countries in the literature reflect the data of the national databases of each country, while the present study is the evaluation of only the poisoning cases admitted to Dokuz Eylul University Faculty of Medicine hospital in Izmir.

In a large number of studies in the literature, it has been reported that the number of women is higher than that of men in poisoning cases (1,2,15,16,20,21). In the present study, it was found that 60% of the cases were male. This difference from the literature can be due to the fact that only poisoning cases with severe clinical signs and symptoms that required the administration of the extracorporeal therapy method were included in the present study. In studies conducted by Jon B. Cole et al. and Ghannoum et al. in which poisoning cases that applied the extracorporeal therapy method, the number of male cases was found to be higher (52.5% and 53.9%, respectively) (14,22), similar to the present study. Similarly, in a large number of studies in the literature, it has been reported that most of the cases poisoned with methanol are men (76-92%) (23-25). Emma Kuusela et al. found that 82.1% of the methanol and ethylene glycol poisoning cases that were treated with hemodialysis were male (19,26). In line with the literature, it was found that 60% of all poisoning cases that applied extracorporeal therapy and 92.9% of the methanol poisoning cases were male in the present study.

There are conflicting results regarding the reasons of poisoning in various studies. In a study conducted in Iran, it was reported that more than half of the poisonings were intentional (suicide attempt, misuse) (27). Similarly, the 2014 Drug and Poison Information Center report also showed that intentional poisonings were frequent (approximately 67%) (2). However, the 2022 AAPCC report and 2017 annual report of the Israel Poison Information Center showed that unintentional poisonings were more frequent (76% and 74%, respectively) (1,20). Jon B. Cole et al. reported that 61% of the poisoning cases that applied extracorporeal therapy were unintentional (22). Similarly, it was found in the present study that approximately 60% of the cases had been exposed to the poisoning agent unintentionally.

Table 5. Studies reporting poisoning cases treated with extracorporeal therapy

Authors	Year	City/Region	Period	Number of patients treated with extracorporeal therapy
National				
Aydin B. *et al.*[2]	2017	İzmir/Turkey	1 year	2
Aydin K. *et al.*[15]	2020	Kayseri/Turkey	1 year	9
Baydin A. *et al.*[18]	2005	Samsun/Turkey	3 years	21
Canakci SE. *et al.*[16]	2018	Aydin/Turkey	3 years	14
Mutlu M. *et al.*[17]	2010	Black Sea Region/Turkey	5 years	2
Orhan U. *et al.*[19]	2022	Adana/Turkey	5 years	55
International				
Campion GH. *et al.* [12]	2019	New York City/USA	16 years	502
Darracq MA. *et al.* [13]	2012	California/USA	10 years	90
Ghannoum M. *et al.* [14]	2015	United States	29 years	40.258
		United Kingdom	2 years	343
		Denmark	9 years	481
		Canada	24 years	2709

In a study conducted by Holubek et al. in a 21-year-long period, it was stated that hemodialysis had an increasing trend in the choice of extracorporeal therapy method in poisoning cases, whereas the haemoperfusion method was preferred with a decreasing frequency (28). In an 11-year-long period study by Darracq et al., the most commonly preferred extracorporeal therapy method was hemodialysis; neither hemodialysis nor hemoperfusion and hemofiltration showed any temporal increase (13). Data from Turkey showed that the most preferred extracorporeal therapy method in poisoning patients was hemodialysis (42.85%), followed by hemoperfusion (35.72%), plasmapheresis (14.28%), and hemofiltration (7.15%) (16). In the present study, it was found that the extracorporeal therapy method chosen for poisoning cases in Dokuz Eylul University hospital was hemodialysis for all patients, while methods such as hemofiltration, hemoperfusion, hemodiafiltration, or plasmapheresis were not preferred as an extracorporeal therapy method in any of the patients. The reason why hemodialysis was preferred over other extracorporeal therapy methods can be explained by the increase in the efficacy of hemodialysis treatment with the developments in technology, higher molecular weights becoming a suitable indication for hemodialysis, and the increase in the tolerability of hemodialysis by patients (28). Our results are consistent with the literature regarding the reason for hemodialysis treatment (13). The reason of applied hemodialysis treatment was “to increase the elimination of the agents exposed to” in 74.7% of the patients, and it was “renal support” in 25.3%. This result shows that hemodialysis may be preferred in cases of poisoning not only to increase the elimination of the active agent, but also for renal support in cases such as the development of renal failure related to the poisoning agent.

Among the non-extracorporeal therapy methods administered to the patients, it was determined that all cases received supportive treatment, and

approximately one-third (29.3%) were treated with specific antidote treatment. It was found that there were 34 cases poisoned with agents for which a specific antidote can be used (methanol, digoxin, and tramadol), and specific antidote treatment was administered to 19 (55.8%) of these cases. All of the cases that were treated with a specific antidote were methanol poisoning cases. Zakharov et al. reported that specific antidote (ethanol and/or fomepizole) was used in 90% of the methanol poisonings (23), while this rate was reported as 80% by Thanacoody et al (29). Paasma et al. reported the rate of fomepizole use as 16% and ethanol use as 84.0% (30). In the present study, it was found that 19 (67.9%) of the cases poisoned with methanol were administered specific antidote treatment (ethanol and/or fomepizole). The reason for the lower rate of antidote treatment can be explained by the difficulty in accessing specific antidotes in our country.

According to the data in Turkey, the rate of need for follow-up and treatment in the intensive care unit varies between 2.4% and 47% in poisoning cases (16,31-34). This rate is 1.5% in the United States of America (1), 0.9% in Israel (20), 15.3% in Iceland (35), 0.7% in Paris (36), and 2.23% in Iran (37). In none of these studies were cases included that would be severe enough to require extracorporeal therapy. In the study conducted by Ghannoum et al., poisoning cases in which extracorporeal therapy was applied were evaluated, and the need for follow-up, monitoring, and treatment in the intensive care unit was reported as 78.5% in the USA and 84.4% in Canada (14). Similarly, in the study conducted in Aydin, this rate was reported as 85.7% (16). In the present study, it was found that most of the cases (73.3%) developed a need for follow-up and treatment in the intensive care unit.

In research conducted, the rate of death varies between 4.6% and 35.0% in patients who applied extracorporeal therapy (14,16,38,39). The reason for this wide range may be that the clinical findings and treatment-related complications vary depending on the exposure agents in poisoning cases treated with extracorporeal therapy. In this study, which analysed poisoning cases in which the extracorporeal therapy method was applied, the mortality rate was found to be 28.0% (21 cases), in parallel with the literature.

Emma Kuusela et al. found that increased anion gap and low pH, bicarbonate levels, and low GCS and need for mechanical ventilation at admission to hospital in cases poisoned with methanol were correlated with 90-day mortality (6). Similarly, low pH, low GCS, insufficient hyperventilation, respiratory arrest, duration of hospital admission longer than 24 hours, presence of seizures, and coma at admission have been associated with poor prognosis or mortality (24,25,30,40). In this study, in line with the literature, age, GCS, presence of need for mechanical ventilation, serum levels of pH, HCO3, and SCr at hospital admission were associated with mortality in methanol poisoning. Low GCS, pH<7.00, and SCr >1.2 mg/dL were found to be a predictor factors of mortality in methanol poisoning.

In addition to being retrospective, cross-sectional, and descriptive, this study had several limitations. Standard record forms and hospital automation systems from previous dates may have caused data loss. A university hospital planned this study, thus, its results cannot be extrapolated nationwide. Multi-center studies are needed to gather data on extracorporeal treatment in poisonings in our nation.

In conclusion; DEUDPIC was consulted regarding 75 poisoning cases that applied extracorporeal therapy. The extracorporeal therapy method administered to all of the cases was hemodialysis, and the most frequent poisoning agent was found as methanol. It was found that approximately three-quarters of the patients were monitored in the intensive care unit, the majority of these patients were methanol poisoning cases, and 28% of all cases died. It is noteworthy that the age, GCS, presence of need for mechanical ventilation, serum levels of pH, HCO3, and SCr at hospital admission are factors associated with mortality in cases of methanol poisoning. Low GCS, pH<7.00, and SCr>1.2 mg/dL are factors predicting mortality in methanol poisoning. Monitoring these parameters may be helpful for clinicians in the management of methanol poisonings.

ETHICS COMMITTEE APPROVAL

* The study was approved by the Non-Interventional Research Ethics Committee of Dokuz Eylul University (Date: 04.10.2018 and Number: 2018/24-19).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

Models of reimbursement to healthcare service providers and a practice based on diagnosis related groups

Sağlık hizmeti sunucularına geri ödeme modelleri ve teşhis ilişkili gruplara dayalı bir uygulama

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ABSTRACT

Objective: Reimbursement methods are important for the provision and continuity of health services. Reimbursement methods significantly affect elements such as quality and efficiency in the provision of health services. In addition, the size and diversity of the services provided by health institutions are also affected by reimbursement systems.Although there are many different reimbursement mechanisms for health service providers, case-based payment-DRG (Diagnosis Related Groups) is among the reimbursement methods used in terms of efficient use of resources and reimbursement according to costs. In this respect, reimbursement methods are explained in general terms in the study and the case-based-DRG method is discussed in detail.

Methods: In the study, case mix indexes (CMI) were calculated with the DRG data of 27 hospitals located in Ankara. The hospitals studied were evaluated according to their role groups and the severity levels and cost requirements of the cases were compared. In addition, the actual allocations sent according to the global budget in the studied hospitals were compared with the

ÖZET

Amaç: Sağlık hizmetlerinin sunulması ve devamlılığı açısından geri ödeme yöntemleri önem arz etmektedir. Geri ödeme yöntemleri sağlık hizmetinin sunumunda kalite ve verimlilik gibi unsurları önemli ölçüde etkilemektedir. Ayrıca sağlık kuruluşlarının sunduğu hizmetin büyüklüğü ve çeşitliliği de geri ödeme sistemlerinden etkilenmektedir. Sağlık hizmeti sunucularına yapılan geri ödeme mekanizmaları çok çeşitli olmakla beraber kaynakların verimli kullanılması ve maliyetlere göre geri ödeme yapılması açısından vaka başı ödeme-TİG (Teşhis İlişkili Gruplar) kullanılan geri ödeme yöntemleri arasında yer almaktadır. Bu itibarla çalışmada geri ödeme yöntemleri genel anlamda anlatılmış vaka başı-TİG yöntemi detaylı olarak ele alınmıştır.

Yöntem: Çalışmada Ankara ilinde yer alan 27 hastanenin TİG verileriyle vaka karma indeksleri (VKİ) hesaplanmıştır. Çalışılan hastaneler rol gruplarına göre değerlendirilerek vakaların ağırlık dereceleri ve maliyet gereksinimleri kıyaslanmıştır. Ayrıca çalışılan hastanelerde global bütçeye göre gönderilen fiili

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budget allocations formed according to the DRG-CMI and the differences between them were emphasized. The findings obtained as a result of the study indicate that reimbursement based on case-by-case basis is important for the financial sustainability of healthcare providers.

Results: BMI values of Group A hospitals were higher than Group B and C hospitals and A role group hospitals among the hospitals located in Ankara were affected negatively as role group by failure to perform planning according to DRG-CMI in 2023. C role group hospitals are the hospitals most positively affected. If planning were performed according to DRG-CMI the allowance distribution table of the hospitals would be formed very differently. Since the allowance planning performed according to DRG-CMI takes into consideration the complexity and costs of the cases, it shall lead to a more correct allowance distribution for A group hospitals compared to B and C group hospitals.

Conclusion: The Ministry of Health hospitals in the province of Ankara were analyzed by being divided according to the DRG data and role groups and CMI calculations were performed in this study. As expected, CMI values of A role group hospitals were found higher compared to the B and C group hospitals. In the case that reimbursement is made according to DRG-CMI, it is observed that A role group hospitals dealt with more severe and complex cases compared to the other role groups and needed higher allowance in terms of source requirement.

Key words: Reimbursement, case mix index, diagnosis related groups

ödenekleri TİG-VKİ'ye göre oluşan bütçe ödenekleri ile karşılaştırılmış ve arada oluşan farklar vurgulanmıştır. Çalışma sonucunda elde edilen bulgular vaka başı-TİG'e göre geri ödeme yapılmasının sağlık hizmeti sunucularının finansal sürdürülebilirlikleri açısından önem arz etmektedir.

Bulgular: A rol grubu hastanelerin B ve C grubu hastanelere göre VKİ değerleri daha yüksek çıkmıştır ve A rol grubu hastaneler TİG-VKİ'ye göre ödenek planlaması yapılmamasından negatif etkilenmiştir. C rol grubu hastaneler ise en fazla pozitif yönde etkilenen hastanelerdir. Eğer TİG-VKİ'ye göre bir planlama yapılmış olsaydı hastanelerin ödenek dağılım tablosu çok farklı şekilde oluşacaktı. TİG-VKİ'ye göre yapılan ödenek planlama vakaların kompleksliliği ve maliyetleri dikkat almasından dolayı A grubu hastanelerin B ve C grubu hastanelere göre daha doğru bir ödenek dağılımına neden olacaktır.

Sonuç: Ankara iline bağlı Sağlık Bakanlığı hastaneleri TİG verileri ile rol gruplarına göre ayrılarak analiz edilmiş ve VKİ hesaplamaları yapılmıştır. Beklenildiği gibi A rol grubu hastanelerin B ve C grubu hastanelere göre VKİ değerleri daha yüksek çıkmıştır. TİG-VKİ'ye göre geri ödeme yapılması durumunda A rol grubu hastanelerinin diğer rol gruplarına göre daha fazla ağır ve kompleks vakalara baktığı, kaynak gereksinimi açışından daha fazla ödenek ihtiyacı olduğu görülmektedir.

Anahtar Kelimeler: Geri ödeme, vaka karma indeksi, teşhis ilişkili gruplar

INTRODUCTION

Access to healthcare services is the leading fundamental rights for human beings. One of the main duties of states is providing its citizens with the access to healthcare services. It is becoming more expensive to provide healthcare services due the developed technology and increasing costs in the field of health. Therefore reimbursement after provision of healthcare service gets important (1).

Since sources are scarce, the concept of cost gets important for enabling individuals to live healthily using those scarce sources. The method of earning the incomes to be earned against the costs borne for sustaining the healthcare services and using the existing sources efficiently is important (2). The incomes that the healthcare service providers will earn for providing continuity of the service basically consist of the payments made by the state, insurance institutions or the individuals purchasing healthcare service directly. The methods of reimbursement are significant for provision of such incomes (3).

The fundamental purpose of development of models of reimbursement in healthcare services is enabling the diseased individuals to benefit from the healthcare service and protecting the patients against the financial risks that may arise (4). On the other hand, it is significant to create a reimbursement system according to costs for the service providers, a significant element of the health system to sustain their existence. In this context, the methods of reimbursement assume a duty of balance both for the service purchasers and for the service providers (5).

This paper explains the methods of reimbursement in general terms and discusses in detail the Diagnosis Related Groups (DRG) included in payment per case among the methods of reimbursement. The case mix indices (CMI) of hospitals located in the province of Ankara was calculated and the severity of hospitals' focus on complex cases and their resource requirements has been determined in this paper. Furthermore the global budget allowances which hospitals receive actually and the potential allowance distribution if they received allowance for DRG-CMI were calculated and both situations were compared.

METHODS OF REIMBURSEMENT

The payments for the service provided in health institutions are either paid personally by the individuals purchasing the service or paid by third party institutions (6). The financing of the services offered in Turkey are paid by third parties in general. Examining the distribution of financing of the health expenses, the share of governmental health expenses within the total health expenses realized as 76.4% in 2022. The rate of payment of health expenses out of pocket was 18.5% within the total health expenses (7).

The methods of reimbursement made to health institutions are divided into two as forward and backward payments on time basis. Forward payments are the payments made before the healthcare service is offered. These are daily payments, global budget payments, payment per capita-DRG (Diagnosis Related Groups) and budget payments. Backward payments on the other hand cover the payment made after the healthcare service is offered. The payments made per service could be held as an example for backward payments (8).

Fixed and variable payments are revealed in the methods of reimbursement depending on the quantity of the service offered. Fixed payments are payment of fixed amount regardless of the quantity of the service offered. Variable payments on the other hand cover the payments increasing/decreasing in line with the quantity of the service offered (8).

The methods of reimbursement could generally be listed as methods of payment per capita, payment per service, payment per day, global budget, budget with expense item, value based payment and payment per case (9).

Method of Payment per Capita

It is the method of payment made on the basis of the total number of individuals offered healthcare service. Payment is generally made in financial periods according to the number of individuals at the beginning of the period. The quantity and type of the service which individuals purchase from the service provider are not taken into consideration in this payment method. The method of payment per capita is more commonly used in the healthcare services where family practice is performed (8).

Method of Payment per Service

It covers the payments made for the service unit produced by the healthcare service providers. The transactions of reimbursement are performed over the amounts predetermined per service for the healthcare service offered. The health institutions send the invoices on service basis to the reimbursing institution in specific periods after the service offered and the payment is made to the health institutions after the necessary review is performed (10).

Method of Payment per Day

It consists of the systems of payment made for the healthcare services offered in the health institutions on the basis of duration. The payments made per day are generally used for intensive care at hospitals (1).

Different pricings are in question in the reimbursement made depending on the nature of the healthcare service provider, in the method of payment per day. There is difference between the unit prices per day to be received by an education and research hospital at the third grade and a small size hospital located in a county (11).

Global Budget Method of Payment

It is provision of a fixed budget to Health Institutions for the expenses they incurred. The reimbursement is realized in this payment method in specific periods by the reimbursing institution after reaching an agreement over a total amount independently from the quantity of the service given. A total expense top limit is defined in this payment method and it is targeted at controlling the health expenses for the healthcare service providers (12).

The global budget practice which started to be implemented in Turkey as of 2006 was enforced for the first time by concluding a lump sum price agreement among the Ministry of Health, the Ministry of Labor and Social Security and Ministry of Finance. The transaction of sending invoice to the Social Security Institution (SGK) by the hospitals within the scope of agreement ended in line with the global budget practice and the examinations are performed using the MEDULA system, the information network of SGK. Effort was paid to access the hospital invoice records electronically, decrease the paper costs, perform the transactions faster and prevent any potential abuses by preventing any potential mistakes, thanks to the MEDULA system. (5, 13).

Method of Budget with Expense Item

The method of budget payment with expense item is transferrin a fixed amount to the budget payment items for a specific period of time. Calculation is performed by taking the realizations of particularly the expenses of the previous year into consideration for each budget item in this method and considering the inflation and growth data of countries. Although it resembles the global budget payment system, the difference of this method from the global budget method is that the relevant payments are calculated at the level of budget items and payments are sent on the basis of those items (14).

Method of Value Based Payment

Another payment method which has started to become important today among the payment methods is the value based payment. It is expressed in the 2025-2027 medium term program published in the official gazette dated 05.09.2024 and numbered 32653 that value based reimbursement method will start to be used and an effective reimbursement shall be made by performing analyses regularly. The hospitals are assessed within the framework of infections, recovery process, patient safety, repetition of the disease as well as the concepts of effectiveness and cost in the value based payment. This payment method focuses on improvement of the quality of patient care. The value judgment in treatment of patients comes to the forefront at this point and it is quite difficult to measure this value (14).

Transformation into healthy individual and high quality life is assessed as output in the value based

payment system. On the other hand, when costs are calculated on patient bases rather than clinic basis, value increase shall be ensured since separate cost and output measurement shall be performed for each patient (Seyfioğlu, 2019).

Method of Payment per Case

The method of payment per case consists of the payments made by the health institutions for each case treated. This method deals with the amounts determined for each case or diseases and transactions of reimbursement are realized over those amounts. The similar treatments applied are divided into specific groups and payments are made according to similar case categories in the method of payment per case. In this manner, the categorized diseases have similar diagnoses and treatments, hospitalization periods and source consumptions within their own groups. Since the sources consumed by assessing the severity of the diseases within each group in the transactions of treatment shall be shaped according to these severity levels, reimbursements shall increase or decrease depending on these severity levels (2). Diagnosis related groups (DRG) are one of the most significant payment models used in the method of payment per case.

DIAGNOSIS RELATED GROUPS (DRG)

Diagnosis related groups (DRG) are a reimbursement model used in the method of payment per case. It is a classification method covering grouping of clinic and cost data in diagnoses and treatments and inclusion of similar diseases in specific groups. Furthermore DRG system is a system where the inpatients are subjected classification. It is a coding system where both the costs are controlled and clinic transactions are taken into consideration. The reimbursement method according to DRG-CMI adopts an approach of classifying the sources on the basis of severity of the cases and centering the concepts of cost and clinic (16). The purposes of reimbursement based on DRG-CMI include distributing fairly the scarce resources according to case severities and types, providing efficient and effective operation of the hospitals, collecting systematically the meaningful clinic data (17).

Doctors may tend to such diagnoses and treatments more expensive and more complex to perform among the case groups in DRG. None the less, they do not want to perform the cheaper transactions and may want to direct to other hospitals. Furthermore, method of payment case-DRG is very important for controlling costs. In certain cases, it may lead to too much service provision (18).

Cost control is kept at the forefront in DRG and DRG becomes important on which cases the hospitals shall head for. The DRG-CMI based payment system commonly used in the world was applied in Turkey in 2015 as 5% of the global budget and this rate was increased up to 10% in 2016. The transaction of sending payment on the basis of DRG was annulled in the Ministry of Health in August 2016 and merely used in statistical studies (14).

DRG Formation Process

Diseases are grouped according to the main diagnosis and disease transactions respectively in DRG. At the later stage, diseases are determined as relative value rather than treatment expenses. The DRG formation process is realized under 4 titles. These are the processes of pre-assessment, MDC (Major Diagnosis Classification), field assignment and detection of DRG respectively (17).

Pre-assessment covers the stages of general collection of data and detailed examination of the same. At this stage the information related to the disease obtained from the patient's file is examined in detail. Another issue to be assessed is assessment of demographic data covering information such as the patient's age, sex, period of hospitalization etc. Furthermore, the diagnoses containing clinic data, supplementary diagnoses and clinic transactions performed are realized in the pre-assessment stage (19).

MDC assignment consists of the processes where the processes of the data obtained after the pre-assessment are entered in the system. The reason for treatment of the patient is coded as the main diagnosis. MDC assignments conforming to the main diagnosis are performed as a consequence of coding the diseases supplementary to the main diagnosis and other factors. MDCs consist of 25 classes. MDC disease classes and abbreviations are given as example in the Table 1 (17).

Table 1. Example MDC Classification

MDC CLASSIFICATION	NUMBER OF DRG	MDC ABBREVIATION
MDC - 99 False DRGs	6	
MDC - 00 Preliminary - Major Diagnosis Classes	12	A
MDC - 01 Nervous System Diseases	53	B
MDC - 02 Eye Diseases	20	C
MDC - 03 ENT & Mouth Diseases	28	D
MDC - 04 Respiratory System Diseases	42	E
MDC - 05 Circulatory System Diseases	67	F
MDC - 06 Digestive System Diseases	52	G
MDC - 07 Hepatobiliary System Diseases	29	H
MDC- 08 Musculoskeletal and Collagen Tissue Diseases	79	I
MDC - 09 Skin and Breast Diseases	29	J
MDC - 10 Endocrinal and Metabolic Diseases	19	K
MDC - 11 Kidney and Urinary Tract Diseases	37	L
MDC - 12 Male Reproductive Organs Diseases	19	M
MDC -13 Female Reproductive Organs Diseases	20	N
MDC - 14 Pregnancy, Birth and Puerperium	17	O
MDC - 15 Newborn (and Other Neonates)	25	P
MDC- 16 Blood and Blood Producing Organs Diseases	10	Q
MDC - 17 Neoplastic Diseases	18	R
MDC - 18 Infectious and Parasitic Diseases	17	T
MDC - 19 Mental Health Disorders	13	U
MDC - 20 Alcohol/Drug Use/Organic Mental Health Disorders depending thereon	8	V
MDC - 21 Injury, Intoxication and Toxic Drug Effects	24	W,X
MDC - 22 Burnts	8	Y
MDC - 23 Factors Affecting Health and Other Type of Contacts Established through Healthcare Services	13	Z

Field assignment is performed according to the type of transaction performed for the patients in the stage of field assignment. These occur in three main classes and surgical field codes are included within the range of 01-39, other field codes are included within the range of 40-59 and medical field codes are included within the range of 60-99 (20).

DRG formation is realized in the final stage, the stages of disease diagnoses and treatments, field assignment and DRG formation grouped in the first three stages are formed automatically by the program (Figure 1) (21). DRG code structure consists of three sections. There is one letter in the first section. This indicates the MDC group. The field where it is located is determined in the second section. Here, it represents the field within the range of 01-99. A, B, C, D and Z letters are used in the third section. Those letters indicate that the source used vary from the higher to the lower. The letter Z represents the situations where the source is unique and distinction cannot be made (17).

The example on how the DRG code assignment occurs has been shown in Table 2.

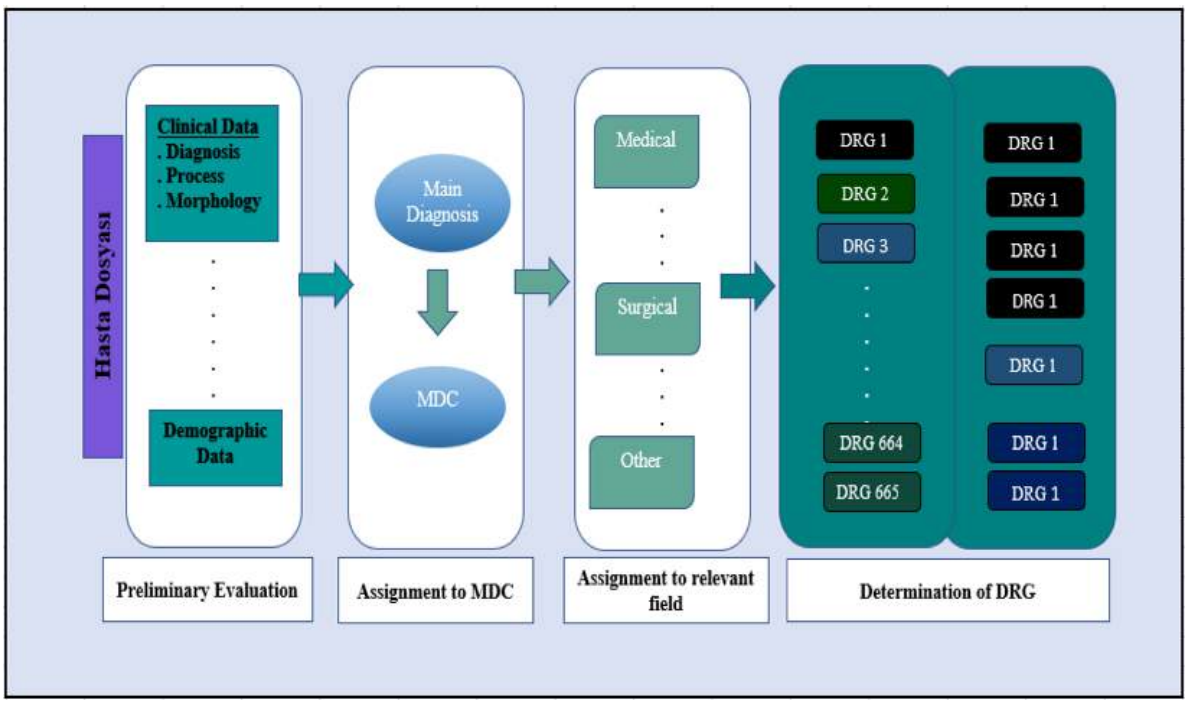

Figure 1. DRG Formation Process (14)

Table 2. Example DRG Code

DRG Code	MDC Abbreviation	Disease Diagnosis Code	Other Explanation
F60A	F Circulatory System Disorders	60 With Acute Myocardia Infarct, Without Invasive Cardiac Examination Transactions, Catastrophic	A With Severe KK
F60B	F Circulatory System Disorders	60 With Acute Myocardia Infarct, Without Invasive Cardiac Examination Transactions, Catastrophic	B Without Severe KK

DRG IN REIMBURSEMENT SYSTEMS

The stages of costing in the financing processes are started at hospitals after performance of the coding transactions according to DRG. Since classification is performed according to severity of the cases in the diagnoses and treatments performed at hospitals, costs are observed to have increased in line with the severity degree of the cases. DRG-CMI based payment system targets at making much payment for severe cases and making payment according to the degrees for cases not very severe. A fair payment system is realized in this manner (3).

DRG-CMI based payment system requires relative value and CMI (case mix index) for performing severity degree of the cases and source distribution. The relative value is a value indicating how much or few sources are used in the treatment of any disease compared to the sources used in the treatment of other diseases (Aral, 2014). In other words, the relative value is the severity of the cases compared to one another. It is found by dividing the average cost of a DRG into the average cost of all DRGs (23).

Calculation of the relative value is performed by dividing the average cost of a DRG into the average cost of all DRGs. Calculation of the relative value is realized with the following formula;

Relative Value=(Average Cost of a DRG)/(Average Cost of all DRGs)

CMI enables us to make comparison among hospitals. CMI is an index used for measuring how severe cases are the treatments performed at hospitals in medical terms (3).

Calculation of the CMI is realized with the following formula;

CCI=(ToTotal Relative Value)/(Total Number of Cases)

For example, assume that hospital A dealt with 100 cases and assuming that the total relative value of these cases compared to their severity in the DRG is 120; CMI is calculated as follows:

CMI= 120/100, CMI= 1,2

Finding the CMI calculated lower than one reveals the result that treatments simpler in terms of severity are performed at the hospital and finding CMI higher than one reveals the result that more complex cases are treated compared to the average (24).

There are two methods in the payment planning toward DRG-CMI. The first one of these is planning payment over the base price. The institution performing reimbursement determines a base price in this method. The payment is made by multiplying this base price with DRG relative value. The second is performing payment planning according to the budgeting method. In this method, the amount obtained as a consequence of multiplying the base price, total number of cases in the previous year and CMI values of the previous year constitutes the reimbursement size (14).

MATERIAL and METHOD

The research covers the 2023 DRG data of the hospitals in the province of Ankara affiliated with the Ministry of Health. The data of 30 hospitals were used but 3 hospitals were not included in the calculations due to merger with another hospital, in the paper. The remaining 27 hospitals were assessed according to the role group classes determined by the Ministry of Health. Research was performed in 5 hospitals in the AI role group, 3 in AI branch role group, 1 in AII role group, 1 in AII branch role group, 7 in B role group and 10 in C role group. Furthermore, the data used were obtained with reference to the permits of the Ministry of Health General Directorate of Public Hospitals dated 22.07.2024 and numbered E-96773357. The data obtained from the Ministry of Health were analyzed by means of Microsoft Excel program and CMIs were calculated. The purpose of CMIs calculated is calculating the source requirement degrees of the hospitals against the services they offer. Furthermore, the allowances which the hospitals covered in the research in 2023 from the global budget were compared to the situation if

allowance distribution is made according to DRG-CMI and the differences between were calculated. The restriction of the research is that it was performed only in the province of Ankara and does not cover the hospitals located in other provinces. The names of the hospitals covered in the research were coded since they did not want their names to be shared. The originality of the research is that the data of global budget actually realized were compared to the budget arising according to DRG/CMI.

The study was approved by the Ankara University, Presidency of Ethics Board (Date: 13.05.2024 and Number: 83).

RESULTS

The hospitals in the province of Ankara were firstly classified on role group basis. They were divided into totally 6 role groups being AI, AI Branch, AII, AII Branch, B and C role groups. The case mix indices were calculated using the DRG data of 2023. The CMIs found allowed to make an assessment of the hospitals in the province of Ankara in terms of both the role groups and the source requirements of province-wide hospitals. The province-wide average CMI value in Ankara realized as 1,39. The total and role group based DRG data of 27 hospitals located in the province-wide Ankara are given in the following Table 3.

AI role group hospitals consist of education and research hospitals and the advanced level operations are general performed in these hospitals. Examining Table-4, we observe that the average CMI of 6 hospitals of AI role group located in Ankara is 1,51. The AI group 3rd hospital is at the top of this group with CMI value of 1,72. As may be observed herein the AI group 3rd hospital is a hospital where top level treatments in terms of difficulty are performed. Depending on this, the source requirement becomes higher than other hospitals within the same role group. On the other hand, the AI group 4th hospital got the lowest value within the role group with its CMI of 1,08. It could be found out that this hospital actually serves as a second grade hospital.

Table 3. DRG Data according to Role Groups in Province-Wide Ankara

HOSPITALS ACCORDING TO ROLE GROUPS	NUMBER OF CASES	TOTAL RELATIVE VALUE	CMI
AI Role Group Hospitals	320.095,00	484.448,54	1,51
AI Branch Role Group Hospitals	56.026,00	80.239,35	1,43
AII Role Group Hospitals	13.459,00	14.852,15	1,10
AII Branch Role Group Hospitals	5.695,00	5.564,51	0,98
B Role Group Hospitals	58.773,00	58.964,04	1,00
C Role Group Hospitals	22.961,00	19.949,37	0,87
TOTAL	**477.009,00**	**664.017,96**	**1,39**

Table 4. AI Role Group Hospitals DRG Data

HOSPITALS ACCORDING TO ROLE GROUPS	NUMBER OF CASES	TOTAL RELATIVE VALUE	CMI
AI Group 1st Hospital	34.298,00	42.782,91	1,25
AI Group 2nd Hospital	101.014,00	142.303,38	1,41
AI Group 3rd Hospital	146.139,00	250.964,17	1,72
AI Group 4th Hospital	14.775,00	15.925,10	1,08
AI Group 5th Hospital	23.869,00	32.472,98	1,36
TOTAL	**320.095,00**	**484.448,54**	**1,51**

AI branch group role group hospitals are education and research hospital and they serve in a specific field in healthcare service. Examining the AI branch group hospitals, it could be observed that totally 3 hospitals in the province of Ankara belong to this role group (Table 5). The role group CMI average is 1,43. The highest CMI value is the AI branch group 2nd hospital with 2,45. This hospital is one which serves all over Turkey for advanced level and special group of patients and where treatments requiring advanced specialization on the case level are performed. The AI branch group 3rd hospital ranked as the second with CMI value of 1,55 is a hospital where treatments with high case severity level are performed. The high level of DRG values of these hospitals indicates that the source requirement is high.

On the other hand, the AI branch group 1st hospital received a value close to the role group average with CMI of 1,31. Since AI branch group hospitals generally perform treatments for a specific disease group, their patient groups are patients who need long term treatment and the treatment transactions performed require particular specialization. Furthermore the patients in this role group receive treatment for diseases which could be treated on outpatient basis and which require monitoring. For this reason it has received a value lower than the role group average.

There are two hospitals in AII and AII branch role groups (Table 6). Among those hospitals, the AII group 1st hospital has CMI of 1,10 and AII branch group 1st hospital has CMI of 0,98. Both hospitals have a CMI value lower than the general average of the province of Ankara. Furthermore AII branch group 1st hospital offers treatments for a special group in terms of service provided and performs transactions with daily added value not very high. For this reason the CMI of the hospital is found to be lower than the other AII group hospital.

Table 5. AI Branch Role Group Hospitals DRG Data

HOSPITALS ACCORDING TO ROLE GROUPS	NUMBER OF CASES	TOTAL RELATIVE VALUE	CMI
AI Branch Group 1st Hospital	34.190,00	44.693,43	1,31
AI Branch Group 2nd Hospital	1.845,00	4.519,89	2,45
AI Branch Group 3rd Hospital	19.991,00	31.026,03	1,55
TOTAL	**56.026,00**	**80.239,35**	**1,43**

Table 6. AII Role Group Hospitals DRG Data

HOSPITALS ACCORDING TO ROLE GROUPS	NUMBER OF CASES	TOTAL RELATIVE VALUE	CMI
AII Group 1st Hospital	13.459,00	14.852,15	1,10
TOTAL	**13.459,00**	**14.852,15**	**1,10**
AII Branch Group 1st Hospital	5.695,00	5.564,51	0,98
TOTAL	**5.695,00**	**5.564,51**	**0,98**

The hospitals in B role group are hospitals of smaller size compared to A role group and they lack education and research activities (Table 7). The average CMI of this role group was calculated as 1,00. It realized below CMI of 1,39 which is the average of Ankara. The hospital with the highest value of 1,43 within the group is the group B 7th hospital. And the hospital with the lowest CMI is the group B 2nd hospital. One may explain the high CMI value of the group B 7th hospital with being close to the center of the province and high county population. On the other hand the reason for low CMI of the group B 2nd hospital may be the fact that it serves in a small county and consequently has less branch doctors.

High CMIs could result from diversity of branch doctors, availability of advanced level medical devices, availability of advanced level intensive care services etc. in general terms. For this reason it could be expected that B role group hospitals have lower CMIs compared to the A role group hospitals. Furthermore CMIs of B role group hospitals could not be expected to be found high since they are hospitals located in big county centers and advanced level treatments cannot be performed in those hospitals.

The hospitals in C role group are county state hospitals which are smaller in size compared to the group B hospitals (Table 8). The average CMI of the role group realized as 0,87. The hospital with the highest value was group C 2nd hospital. It has CMI of 1,30 which is approximately fifty percent higher than the average of the role group. CMI may have resulted from preference of this hospital by the patients who cannot take appointment in the province since it is close to the center of the province. On the other hand, the hospital with the lowest CMI was group C 10th hospital with the value of 0,61. This hospital is the hospital of county with the lowest population density within the role group. Lowness of this value may have resulted from low population.

The allowances received by 27 hospitals located in Ankara from 2023 Ministry of Health global budget and the relative value as well as the allowances to be received in the case allowance is planned according to DRG/CMI are given in Table 9.

Table 7. B Role Group Hospitals DRG Data

HOSPITALS ACCORDING TO ROLE GROUPS	NUMBER OF CASES	TOTAL RELATIVE VALUE	CMI
Group B 1st Hospital	10.433,00	12.441,50	1,19
Group B 2nd Hospital	6.555,00	4.860,06	0,74
Group B 3rd Hospital	7.841,00	7.837,28	1,00
Group B 4th Hospital	6.411,00	6.807,35	1,06
Group B 5th Hospital	10.405,00	7.632,55	0,73
Group B 6th Hospital	12.565,00	12.847,95	1,02
Group B 7th Hospital	4.563,00	6.537,35	1,43
TOTAL	**58.773,00**	**58.964,04**	**1,00**

Table 8. C Role Group Hospitals DRG Data

HOSPITALS ACCORDING TO ROLE GROUPS	NUMBER OF CASES	TOTAL RELATIVE VALUE	CMI
Group C 1st Hospital	506,00	483,48	0,96
Group C 2nd Hospital	1.315,00	1.711,98	1,30
Group C 3rd Hospital	3.989,00	3.205,67	0,80
Group C 4th Hospital	2.105,00	1.792,37	0,85
Group C 5th Hospital	3.123,00	2.753,48	0,88
Group C 6th Hospital	730,00	712,16	0,98
Group C 7th Hospital	5.205,00	4.488,48	0,86
Group C 8th Hospital	2.362,00	2.105,26	0,89
Group C 9th Hospital	2.182,00	1.816,35	0,83
Group C 10th Hospital	1.444,00	880,14	0,61
TOTAL	**22.961,00**	**19.949,37**	**0,87**

Table 9. Allowance Distribution Table

HOSPITALS ACCORDING TO ROLE GROUPS	NUMBER OF CASES	TOTAL RELATIVE VALUE	CMI	TRANSFORMED CMI	GLOBAL BUDGET ALLOWANCE SENT FOR INPATIENT	ALLOWANCE TO BE SENT ACCORDING TO DRG	ALLOWANCE TO BE SENT ACCORDING TO DRG/CMI	DIFFERENCE ACCORDING TO DRG (TRY)	DIFFERENCE ACCORDING TO DRG (%)	DIFFERENCE ACCORDING TO DRG/CMI (TRY)	DIFFERENCE ACCORDING TO DRG/CMI (%)
AI-1	4.298	2.783	1,25	0,90	919.976.459	757.549.668	650.260.778	162.426.791	18%	269.715.681	36%
AI-2	101.014	142.303	1,41	1,01	2.140.574.789	2.519.741.603	2.442.668.747	-379.166.814	-18%	-302.093.958	-14%
AI-3	146.139	250.964	1,72	1,23	3.519.308.558	4.443.779.620	5.251.374.925	-924.471.063	-26%	-1.732.066.368	-49%
AI-4	14.775	15.925	1,08	0,77	278.994.735	281.983.021	209.147.443	-2.988.286	-1%	69.847.292	25%
AI-5	23.869	32.473	1,36	0,98	1.307.015.516	574.993.501	538.301.734	732.022.015	56%	768.713.781	59%
TOTAL	**320.095**	**484.449**	**1,51**	**1,09**	**8.165.870.056**	**8.578.047.413**	**9.091.753.628**	**-412.177.357**	**-5%**	**-925.883.572**	**-11%**
AI Br-1	34.190	44.693	1,31	0,94	859.337.611	791.378.918	711.875.398	67.958.693	8%	147.462.213	17%
AI Br-2	1.845	4.520	2,45	1,76	205.657.275	80.032.919	134.919.609	125.624.356	61%	70.737.666	34%
AI Br-3	19.991	31.026	1,55	1,11	779.453.025	549.372.605	586.723.672	230.080.420	30%	192.729.353	25%
TOTAL	**56.026**	**80.239**	**1,43**	**1,03**	**1.844.447.912**	**1.420.784.442**	**1.433.518.679**	**423.663.469**	**23%**	**410.929.233**	**22%**
AII-1	13.459	14.852	1,10	0,79	284.357.358	262.984.479	199.701.614	21.372.879	8%	84.655.745	30%
TOTAL	**13.459**	**14.852**	**1,10**	**0,79**	**284.357.358**	**262.984.479**	**199.701.614**	**21.372.879**	**8%**	**84.655.745**	**30%**
AII Br-1	5.695	5.565	0,98	0,70	99.663.797	98.529.827	66.248.468	1.133.970	1%	33.415.329	34%
TOTAL	**5.695**	**5.565**	**0,98**	**0,70**	**99.663.797**	**98.529.827**	**66.248.468**	**1.133.970**	**1%**	**33.415.329**	**34%**
B-1	10.433	12.442	1,19	0,86	124.149.754	220.299.512	180.780.671	-96.149.758	-77%	-56.630.917	-46%
B-2	6.555	4.860	0,74	0,53	77.366.854	86.056.251	43.906.243	-8.689.397	-11%	33.460.611	43%
B-3	7.841	7.837	1,00	0,72	159.725.728	138.773.376	95.449.792	20.952.352	13%	64.275.936	40%
B-4	6.411	6.807	1,06	0,76	107.850.867	120.536.582	88.073.671	-12.685.715	-12%	19.777.196	18%
B-5	10.405	7.633	0,73	0,53	74.890.382	135.148.257	68.220.193	-60.257.875	-80%	6.670.189	9%
B-6	12.565	12.848	1,02	0,73	210.754.426	227.496.452	160.074.036	-16.742.026	-8%	50.680.390	24%
B-7	4.563	6.537	1,43	1,03	173.113.913	115.755.738	114.121.828	57.358.175	33%	58.992.085	34%
TOTAL	**58.773**	**58.964**	**1,00**	**0,72**	**927.851.925**	**1.044.066.168**	**750.626.434**	**-116.214.243**	**-13%**	**177.225.490**	**19%**

Table 9 (cont.). Allowance Distribution Table

HOSPITALS ACCORDING TO ROLE GROUPS	NUMBER OF CASES	TOTAL RELATIVE VALUE	CMI	TRANSFORMED CMI	GLOBAL BUDGET ALLOWANCE SENT FOR INPATIENT	ALLOWANCE TO BE SENT ACCORDING TO DRG	ALLOWANCE TO BE SENT ACCORDING TO DRG/CMI	DIFFERENCE ACCORDING TO DRG (TRY)	DIFFERENCE ACCORDING TO DRG (%)	DIFFERENCE ACCORDING TO DRG/CMI (TRY)	DIFFERENCE ACCORDING TO DRG/CMI (%)
C-1	506	483	0,96	0,69	24.732.662	8.560.898	5.628.883	16.171.765	65%	19.103.779	77%
C-2	1.315	1.712	1,30	0,94	12.398.110	30.313.737	27.157.347	-17.915.627	-145%	-14.759.236	-119%
C-3	3.989	3.206	0,80	0,58	74.625.975	56.762.250	31.389.855	17.863.725	24%	43.236.121	58%
C-4	2.105	1.792	0,85	0,61	47.805.105	31.737.189	18.595.975	16.067.916	34%	29.209.130	61%
C-5	3.123	2.753	0,88	0,63	38.564.990	48.755.399	29.580.641	-10.190.409	-26%	8.984.349	23%
C-6	730	712	0,98	0,70	32.106.335	12.610.095	8.465.410	19.496.240	61%	23.640.925	74%
C-7	5.205	4.488	0,86	0,62	85.753.615	79.476.747	47.162.147	6.276.867	7%	38.591.468	45%
C-8	2.362	2.105	0,89	0,64	53.514.021	37.277.479	22.863.741	16.236.542	30%	30.650.280	57%
C-9	2.182	1.816	0,83	0,60	35.147.620	32.161.799	18.422.987	2.985.822	8%	16.724.633	48%
C-10	1.444	880	0,61	0,44	30.812.929	15.584.488	6.536.602	15.228.440	49%	24.276.327	79%
TOTAL	**22.961**	**19.949**	**0,87**	**0,62**	**435.461.363**	**353.240.081**	**215.803.587**	**82.221.281**	**19%**	**219.657.775**	**50%**
GRAND TOTAL	**477.009**	**664.018**	**1,39**	**1,00**	**11.757.652.410**	**11.757.652.410**	**11.757.652.410**	**-**	**0%**	**0**	**0%**

CMI 1,39 corresponding to total allowance of TRY 11,757,652,410 sent from the global budget, and the global budget allowance which the hospitals should receive according to the total relative values and CMIs were recalculated while calculating Table-7. The allowance to be received for the relative value was obtained by multiplying the unit base price obtained by dividing the total actual global budget allowance sent for the inpatient into the total relative value with the relative value of the hospital. The actual global budget allowance which the hospitals received was redistributed according to CMIs in the allowance distribution according to CMIs. Here, the CMIs were transformed and calculated according to the CMI average in the province. The calculations were transformed by dividing the CMI of the concerned hospital into provincial average CMI. The calculations were performed paying attention that the allowances to be distributed after CMIs and the total distributed global budget allowance are the same.

For example for A1 role group 1st hospital;

Base Price = (Global Budget Allowance) / (Total Relative Value) = (11.757.652.410) / 664.018

Allowance to be received according to the relative value=Base price*relative value of the hospital

In this context; it was calculated as follows:

Allowance to be received according to the relative value = 42782,91*17707 = TRY 757.549.668

The transformed CMI was found as follows: **CMI = 1,25 / 1,39 = 0,90** The transformed CMI was multiplied with the actual allowance and the allowance to be received according to DRG/CMI was calculated. However, the correction coefficient of 1,04393107278404 was applied for all hospitals with the assumption that the total distributed global budget is the same. In this manner the allowance to be received by all hospitals became the same as the total global budget allowance.

Allowance to be received according to DRG-CCI 919.976.459*0,90/1,043393107278404=650.260.778

was calculated as above.

The global budget allowance to be distributed was calculated for hospitals on the basis of the results both according to the relative value and according to the CMI.

Examining Table-9 27 hospitals included in the scope of the research were given allowance of TRY 11.757.652.410 from the global budget in 2023. The indebtedness condition of the hospitals is taken into consideration by the Ministry of Health while performing planning. Comparing the actually planned global allowance and the global allowances to be received according to the relative value according to the role groups; it is observed that AI and B role group hospitals were affected negatively at the amounts of TRY 412.177.357 and TRY 116.214.243 respectively. If planning were performed according to DRG; the A1 and B role group hospitals would have received higher global budget allowance. The hospitals in the other role groups were affected positively by failure to perform planning according to DRG. If CMIs were added to this distribution model as well, the A1 role group hospitals would be affected more positively and receive allowance of TRY 925.883.572 higher. On the other hand, the negative condition in the B role group would become positive and it would receive allowance of TRY 177.225.490 less.

It is observed that dealing with complex cases and naturally service provision costs in the services offered by the hospitals are disregarded while planning allowance from the global budget. Examining Table-9 carefully, it is observed that allowances of the A1 role group hospitals were shifted particularly to B and C role group hospitals. If a distribution were performed according to DRG-CMI the hospitals in the A role group would have received allowance higher approximately 11% of the total allowances. On the other hand, B and C role group hospitals received allowance approximately 19% and 50% higher respectively from the global budget allowances.

When assessment is performed specific to the role groups according to DRG-CMI;

It was calculated that the biggest difference in

the AI role group was in AI role group 3rd hospital with approximately TRY 1.732.066.368 million. There was a difference of approximately TRY 302.093.958 million in the AI role group 2nd hospital. The other hospitals were affected positively in allowance distribution according to global budget. It was observed that the number of cases of the hospitals affected positively were quite lower than that of the hospitals affected negatively. AI branch role group hospitals shall receive allowance higher approximately 11% in total when allowance planning is performed according to DRG-CMI.

It is observed that the AI branch role group hospitals were affected positively by performance of planning for AI branch role group hospitals according to global budget. If planning were performed according to DRG-CMI, AI branch role group hospitals would have received allowance lower approximately 22% in total.

On the other hand; AII, AII branch, B and C role group hospitals were affected positively by performance of planning according to DRG-CMI in the global budget allowance planning performed in 2023. Only the B role group 1st hospital experienced a negative effect of approximately 46%. Furthermore, particularly C role group 10th hospital gained 79% positive allowance benefit since planning was not performed according to DRG-CMI. If allowance planning were performed according to DRG-CMI for C role group hospitals an allowance loss of 50% would be in question.

In summary, A role group hospitals among the hospitals located in Ankara were affected negatively as role group by failure to perform planning according to DRG-CMI in 2023. C role group hospitals are the hospitals most positively affected. If planning were performed according to DRG-CMI the allowance distribution table of the hospitals would be formed very differently. Since the allowance planning performed according to DRG-CMI takes into consideration the complexity and costs of the cases, it shall lead to a more correct allowance distribution for A group hospitals compared to B and C group hospitals.

DISCUSSION

The role of financing is major for continuity of financial sustainability against the services which hospitals offered. The role of the reimbursing party is very significant in the process of transfer of financing sources to hospitals. Although various methods are used in the reimbursement systems, most reimbursement methods contain various advantages and disadvantages within themselves. DRG data and CMIs of 27 hospitals located in the province of Ankara were analyzed in our paper with respect to the method of DRG per case. There are not many researches in the Turkey with respect to this field when the literature is looked through. When the researches performed are examined;

Avcil et al. found the CMI average as 1,56 in their study conducted in using 2011 data in 3 education and research hospitals and 1 state hospital (16). The CMI of the hospital was found as 0,89 in the study performed by Özkan and Ağırbaş at a university hospital in Ankara using March 2015 data (25).

January data of Adana Numune Hospital and Ankara Education and Research Hospital were compared by Aydemir and Ağırbaş and CMIs of 1,34 and 1,22 were obtained respectively (3). In this study, 2023 average CMIs of the A role group hospitals of the province of Ankara are found 1,48.

CMI values of B group hospitals were found as 0,88 in the DRG group studies performed by Özkan and Ağırbaş all over Turkey (25). Sayar and Ağırbaş found the CMI value as 0,81 in 235 B group role affiliated with the Ministry of Health in the study they performed in (26). CMI was detected as 1,00 for B group hospitals in the province of Ankara in this study. It is observed in the studies performed as B role group that B group hospitals in the province of Ankara have high CMI, and this indicates that B group hospitals in the province of Ankara dealt with higher number of complex cases.

CMI of the hospitals in AII role group was detected as 1,01 in the study conducted by Işıkçelik, Öztürk and Ağırbaş (14). In this study, CMI value

of AII-1 hospital, AII hospital of the province of Ankara was found as 1,10. High CMI value of AII-1 hospital could be interpreted as the need of the concerned hospital for more source requirement.

As a consequence, the Ministry of Health hospitals in the province of Ankara were analyzed by being divided according to the DRG data and role groups and CMI calculations were performed in this study. As expected, CMI values of A role group hospitals were found higher compared to the B and C group hospitals. In the case that reimbursement is made according to DRG-CMI, it is observed that A role group hospitals dealt with more severe and complex cases compared to the other role groups and needed higher allowance in terms of source requirement.

It is considered that reimbursement according to DRG-CMI shall play significant role for measurement of costs and controlling the same. It is considered that payments to be made to the hospitals according to DRG-CMI shall be more realistic and fair. Furthermore, hospitals shall be ensured to assess clinical transactions and cost factors jointly thanks to DRG and take rational decisions in this manner. On the other hand, it is considered that cost analyses should regularly be performed at hospitals and relative values should be updated for the treatment transactions applied.

Global budget practice is currently available at Ministry of Health and university hospitals in Turkey and reimbursement is performed by SGK, the third party payer according to the lump sum protocol. Majority of the expenses of the hospitals of Ministry of Health is personnel and supplementary payment expenses. It is suggested paying personnel and supplementary payment expenses which are fixed expenses using again with the global budget; and paying the expenses of operation, medical material, medical drug and laboratory constituting the variable expenses according to DRG-CMI while making reimbursement to hospitals. It is considered that the reimbursement model to be applied in this manner shall provide cost control in clinic transactions and shall prevent unnecessary actions and transactions. Furthermore, it is considered thinking about the general economic conditions that the healthcare service financing shall be more efficient and it shall be an effective reimbursement model for saving.

It is suggested making reimbursement according to DRG-CMI in the Ministry of Health hospitals and University hospitals and private hospitals in Turkey. The burden of patients with high cost and complex cases all over the country shall be undertaken jointly by all health institutions in the system of reimbursement made in this manner.

ETHICS COMMITTEE APPROVAL

* The study was approved by the Ankara University, Presidency of Ethics Board (Date: 13.05.2024 and Number: 83).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

Does donating blood cause oxidative stress?

Kan bağışı oksidatif strese yol açar mı?

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ABSTRACT

Objective: Blood donation is a life-saving activity. However, it is indispensable to protect the health of blood donors during and after the donation process. This study aimed to evaluate investigate the effect of blood donation on thiol/disulfide homeostasis by the new automated colorimetric method that measures native thiol (NT), total thiol (TT), disulfide and ischemia-modified albumin levels.

Methods: A total of 30 voluntary blood donors who were eligible to donate blood were enrolled in this study. Peripheral venous blood samples were collected just before and after 30 minutes blood donation. Demographic data (age, sex, weight, height), and the number of blood donations within one year period of donors, were recorded. Individuals were classified into four age groups: Group 1, 18-30; Group 2, 31-40; Group 3, 40 and over. A repeat donor was defined as a donor who has donated blood at least once in the past. Body-Mass Index (BMI) measurement was calculated by use of the formula weight (kg)/height (m2). Donors were classified according

ÖZET

Amaç: Kan bağışı hayat kurtarıcı bir işlemdir. Bununla birlikte bağış süreci ve sonrasında kan bağışçılarının sağlığının korunması zorunludur. Bu çalışmada, bağış işleminin oksidatif strese neden olup olmadığının yeni bir otomatik yöntem kullanılarak native tiyol (NT), total tiyol (TT), disülfid ve iskemik-modifiye albümin seviyeleri ölçümü aracılığı ile değerlendirilmesi amaçlandı.

Yöntem: Bu çalışmaya kan bağışı yapmaya uygun 30 gönüllü kan bağışçısı dahil edildi. Kan bağışından hemen önce ve 30 dakika sonra periferik venöz kan örnekleri alındı. Bağışçıların demografik verileri (yaş, cinsiyet, kilo, boy) ve bir yıllık dönemde yapılan kan bağışı sayıları kayıt altına alındı. Katılımcılar Grup 1, 18-30; Grup 2, 31-40; Grup 3, 40 ve üzeri olacak şekilde yaş gruplarına ayrıldı. Mükerrer bağışçı, geçmişte en az bir kez kan bağışlayan bağışçı olarak tanımlandı. Vücut Kitle İndeksi (VKİ), kilogram cinsinden vücut ağırlığının, metre cinsinden boyun karesine bölünmesiyle hesaplandı. Bağışçılar VKİ değerlerine göre <25 ve ≥25 olarak

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to their BMI values as <25 and ≥25. Test parameters of the dynamic thiol/disulfide homeostasis (TDH) were measured by the automated spectrophotometric method.

Results: Nineteen of the individuals were first-time donor and the others were repeat donors. There were no significant differences in TDH parameters after blood donation. The IMA levels of repeat blood donors were statistically significantly higher after blood donation while other parameters of THD were not (p=0.04). Statistically significant decreases were found in the levels of native and total thiol in the 18-30 age group donors (p=0.03).

Conclusion: Test results of this study showed that blood donation does not cause oxidative stress. Further studies designed with the participation of more blood donors needed to be conducted to present more clearly the association between blood donation and oxidative stress. Considering that blood donation has no adverse effects on human health is considerably valuable in terms of encouraging people to donate blood.

Key words: Blood donors, blood donation, oxidative stress, body-mass index, thiol/disulfide homeostasis

sınıflandırıldı. Dinamik tiyol/disülfit homeostazının (TDH) test parametreleri otomatik spektrofotometrik yöntemle ölçüldü.

Bulgular: Katılımcılardan on dokuzu ilk defa kan bağışlayan, geri kalanları ise mükerrer bağışçıydı. Kan bağışından sonra TDH parametrelerinde anlamlı bir fark yoktu. Mükerrer kan bağışçılarının IMA seviyeleri, kan bağışından sonra istatistiksel olarak anlamlı derecede yüksek iken, THD'nin diğer parametreleri açısından anlamlı fark yoktu (p=0.04). 18-30 yaş grubu bağışçılarda nativ ve total tiyol düzeylerindeki azalma istatistiksel olarak anlamlıydı (p=0.03).

Sonuç: Bu çalışmanın test sonuçları kan bağışının oksidatif strese neden olmadığını göstermektedir. Kan bağışı ile oksidatif stres arasındaki ilişkiyi daha net ortaya koymak için daha fazla kan bağışçısının katılımını sağlayan ileri çalışmaların yapılması gerekmektedir. Kan bağışının insan sağlığına olumsuz etkisinin olmadığının ortaya konulması, insanları kan bağışına teşvik etmesi açısından oldukça değerlidir.

Anahtar Kelimeler: Kan bağışçıları, kan bağışı, oksidatif stres, vücut kitle indeksi, tiyol/disülfit homeostazı

INTRODUCTION

Blood is a life-saving biological vital fluid, the source of which is only human. Over 118 million blood donations are donated globally every year (1). It is essential to ensure the health and safety of donors during blood donation. Some criteria have been identified to avoid the harmful effects of blood donation on donors such as the minimum donor weight for whole blood donation should be at least 50 kg, and the volume of whole blood donated should not exceed 13% of the total blood volume (2).

Since it can only be supplied through the voluntary donors and has a limited shelf life, there may be a shortage of blood and blood components time to time. Therefore, it is crucial to increase the number of regular and volunteer non-remunerated blood donors to ensure a reliable supply of safe blood for patients whose lives depend on it. In order to encourage people to donate blood regularly, it is important to explain to them that blood donation does not harm their bodies as well as the benefits of blood donation to the health.

The balance between oxidant-antioxidant mechanisms is crucially important for maintaining a healthy life. Oxidative stress is defined as the deterioration of this balance as a result of an increase in production and accumulation of oxygen reactive species (ROS) such as hydroxyl radical, superoxide radical, and hydrogen peroxide formed during cellular metabolism and the inadequacy of the antioxidant system to detoxify these reactive products. Aerobic organisms have evolved integrated enzymatic and non-enzymatic antioxidant systems that are effective in preventing the harmful effects of these indigenously generated ROS. Primary antioxidants, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), are enzymatic antioxidants, which all contain the amino acid cysteine in their active centers (3,4). Thiols are one of the most important organic compounds of the non-enzymatic antioxidant system, which are also known as secondary antioxidants. Thiols are functional sulfur-containing compounds that react with reactive oxygen radicals and form disulfide bonds. These bonds can be reduced back to thiol groups to maintain dynamic thiol/disulfide homeostatic status. Dynamic thiol/disulfide homeostasis (TDH) plays a key role in several biochemical processes such as antioxidation, cellular signal transduction, apoptosis, and enzymatic regulation (5). As a novel marker of oxidative stress, it has been studied in several diseases, including diabetes mellitus, inflammatory bowel diseases, hypertension, Behçet disease, migraine, Familial Mediterranean Fever, basal cell carcinoma, etc.(6-12).

As authors, we wanted to search whether the donation process causes oxidative stress, even though the limited volume of blood that does not endanger donors' health is collected. In this study, we aimed to investigate the effect of blood donation on thiol/disulfide homeostasis by the new automated colorimetric method that measures native thiol (NT), total thiol (TT), disulfide, and ischemia-modified albumin levels.

MATERIAL and METHOD

Study design

According to statistical power analysis, it was determined that at least 30 participants were required for the study. A total of 30 voluntary blood donors who were eligible to donate blood in accordance with the National Guide to Preparation, Use, and Quality Assurance of Blood and Blood Components were included in this study. Blood donation and sampling processes have been performed at the Regional Blood Center of Gülhane Training and Research Hospital. Peripheral venous blood samples were collected to measure biochemical values and thiol-disulfide parameters just before blood donation and 30 minutes after blood donation, and placed into serum tubes (The BD Vacutainer® SST™ II Advance). Whole blood samples were centrifuged at 1600 x g for 10 minutes at room temperature. Sera were then stored at -80°C until required for analysis. Demographic data (age, sex, weight, height), and the number of blood donations within one year period of donors, were recorded. Individuals were classified into four age groups: Group 1, 18-30; Group 2, 31-40; Group 3, 40 and over. A repeat donor was defined as a donor who has donated blood at least once in the past. Body-Mass Index (BMI) measurement was calculated by use of the formula weight (kg)/height (m2). Donors were classified according to their BMI values as 25< and ≥25.

Blood sampling and measurement of study parameter

Measurement of TDH parameters: TDH tests were measured by the automated spectrophotometric method described by Erel & Neselioglu. Primarily, disulphide bonds were reduced to constitute free functional thiol groups with sodium borohydride. Unused reductant sodium borohydride was consumed and removed with formaldehyde to prevent reduction of DTNB (5,5'-dithiobis-(2-nitrobenzoic) acid), and all of the thiol groups including reduced

and native thiol groups were determined after the reaction with DTNB. The dynamic disulfide amount was calculated by subtracting the total thiols from the native thiols amount and dividing the results by two. After the determination of native and total thiols, disulfide amounts, disulfide/total thiol percent ratios (SS/SH+SS), disulfide/native thiol percent ratios (SS/SH), and native thiol/total thiol percent ratios (SH/SH+SS) were calculated (13). Venous blood samples accepted within 1 hour after collection were kept at room temperature for 30 minutes and then centrifuged at 3500 rpm for 5 minutes. Serum samples were transferred to Eppendorf tubes and stored at -80°C until tests were performed. Measurement of the ischemia-modified albumin (IMA): Venous blood samples accepted within 1 hour after collection were stored at room temperature for 30 minutes and then centrifuged at 3500 rpm for 5 minutes. The serum samples were transferred into Eppendorf tubes and stored at -80°C until tests were performed. The existence of IMA was detected using Albumin Cobalt Binding Test. This test was performed by adding 50 mL 0.1% cobalt (II) chloride ($CoCl_2$,$6H_2O$) (Sigma-Aldrich Chemie GmbH Riedstrasse 2, Steinheim, Germany) to the patient's serum. Following the mixing stage, a 10-minute incubation period was allowed for albumin cobalt binding. Subsequently, 50 mL of a solution containing 1.5 mg/mL dithiothreitol was added. Following a further mixing stage and a two-minute incubation period, 1.0 mL of a 0.9% sodium chloride solution was added in order to reduce the binding capacity. The blank preparation was conducted in a similar manner, with distilled water replacing the dithiothreitol. The absorbance of samples at 470 nm was detected with a spectrophotometer. The results of the analyses were shown as absorbance units (ABSU) (14).

Statistical analysis

All analyses were performed using SPSS version 18 for Windows (SPSS Inc, Chicago, IL, USA). Analysis of data was primarily descriptive for continuous variables using standard deviations, ranges, mean, and median values. Data were analyzed for normality of distribution using the Kolmogorov-Smirnov test. Differences in continuous variables were compared using a dependent samples t-test or a Wilcoxon rank-sum test based on the normality of distribution. For categorical variables, the chi-square test was used. P value ≤ of 0.05 was statistically significant.

The study was approved by the University of Health Sciences Ankara City Hospital Ethics Committee (Date: 02.03.2022 and Number: 2022-E2-22-1243). This study complies with the Declaration of Helsinki and informed consent was obtained from participants in the study.

RESULTS

A total of 30 volunteer blood donors, 29 male, and 1 female, were included in this study. The mean age of the blood donors was 35.2 ± 9.8 years. Nineteen of the individuals were first-time donors and the rest were repeat donors. There were no significant differences in TDH parameters after blood donation (Table 1). The IMA levels of repeat blood donors were statistically significantly higher after blood donation while other parameters of THD were not (p =0.043) (Table 2). There was no statistical difference between the groups with regard to BMI. A statistically significant decrease was detected in the levels of native and total thiol in the 18-30 age group donors (Table 3).

DISCUSSION

Blood donation is a crucial part of healthcare in worldwide for over a century. The need for blood and blood components is increasing in all around the world. It can be associated with the improvement in the health services provided to patients in both routine and emergency medical conditions.

Table 1. Thiol/disulfide homeostasis values of blood donors pre- and after donation.

	Pre-blood donation	After blood donation	p-value*
Native thiol (µmol/L)	495.4±70.2	476.9±71.7	0.231
Total thiol (µmol/L)	537±73.6	516.7±71	0.192
Disulphide (µmol/L)	20.7±6,2	19,8±7	0.550
Disulphide/total thiol percent ratio (SS/SH+SS)	4,2±1.3	4.3±1,7	0.921
Disulfide/native thiol percent ratio (SS/SH)	3.8±1.1	3.9±1.4	0.874
Native thiol/total thiol percent ratio (SH/SH+SS)	92.2±2.2	92.1±2.9	0.921
Ischemia-modified albumin	0.79±0.16	0.83±0.15	0.231

* dependent samples t-test

Table 2. Thiol/disulfide homeostasis values of blood donors according to the donation status.

Parameters	**Donation status**					
	First-time (n=19)			Regular (n=11)		
	Pre-blood donation	After-blood donation	p value*	Pre-blood donation	After-blood donation	p value*
Native thiol (µmol/L)	486.5±63.7	493.2±65.2	0.604	510.9±80.9	448.9±76.7	0.787
Total thiol (µmol/L)	527.4±61.7	532±62.9	0.723	553.6±91.6	490.3±79.3	0.755
Disulphide (µmol/L)	20.4±5	19.4±5.3	0.483	21.3±8.1	20.7±9.4	0.842
Disulphide/total thiol percent ratio (SS/SH+SS)	4.3±1.3	4±1.1	0.455	4.1±1.2	4.7±2.1	0.441
Disulfide/native thiol percent ratio (SS/SH)	3.9±1.2	3.7±1.2	0.435	3.8±1.1	4.2±1.7	0.484
Native thiol/total thiol percent ratio (SH/SH+SS)	92.1±2.3	92.5±2.4	0.438	92.4±2.2	91.4±3.5	0.484
Ischemia-modified albumin	083±0.14	0.80±±0.14	0.337	0.72±0.18	0.88±0.17	0.041

* dependent samples t-test

Table 3. Thiol/disulfide homeostasis values of blood donors according to the age groups

	18-30 years (n=10)			31-40 years (n=10)			≥41 years (n=10)		
	Pre-blood donation	After-blood donation	p value*	Pre-blood donation	After-blood donation	p value**	Pre-blood donation	After-blood donation	p value*
Native thiol (μmol/L)	534.6±64.3	493.3±31.8	0.031	482,5±74.5	456.1±93.8	0.551	463.4±48.7	492.2±63.7	0.171
Total thiol (μmol/L)	576.1±75	534.7±31.2	0.032	553.6±91.6	525.1±78.1	0.382	503.1±37.2	532.1±65.4	0.172
Disulphide (μmol/L)	20.7±5.8	20.7±8.5	0.872	21.2±6.6	19.2±5.7	0.345	19.8±6.9	19.9±7.7	0.956
Disulphide/total thiol percent ratio (SS/SH+SS)	3.8±0.6	4,2±1.8	0.447	4.4±1.3	4.4±1.8	0.706	4.4±1.9	4.1±1.6	0.612
Disulfide/native thiol percent ratio (SS/SH)	3.5±0.5	3.8±1.5	0.447	4±1.1	4±1.5	0.606	4±1.6	3.7±1.4	0.618
Native thiol/total thiol percent ratio (SH/SH+SS)	92.9±1.1	92.2±3	0.519	91.8±2.3	91.8±3	0.608	91.9±3.2	92.4±2.8	0.614
Ischemia-modified albumin	0.74±0.13	0.80±0.05	0.128	0.79±0.1	0.87±0.2	0.348	0.8±0.1	0.79±0.9	0.351

* dependent samples t-test
** wilcoxon rank-sum test

In 2017, the global blood need was over 304 million while the global blood supply was approximately 272 million, and of the 195 countries, 119 (61%) did not have sufficient blood supply to meet their need (15). Presenting the changes that occur in the body during the blood donation process can be a source of motivation for people to encourage them to donate blood. Our data showed that TDH as an indicator of oxidative stress was not affected by the blood donation process and there was also no significant difference among the individuals when they were grouped by BMI and age. However, IMA values were found significantly higher in repeat blood donors than in first-time donors.

Several methods and approaches have been used to measure oxidative stress in clinical samples: (i) direct measurement of ROS through the use of fluorogenic probes, (ii) indirect measurement of oxidative damage formed by ROS in the cell lipids, proteins, and nucleic acids, (iii) assessment of total antioxidant status (TAS) (16). A new spectrophotometric method developed by Erel and Neşelioğlu is used for assessing TAS (13). Recently, this method has been widely used to reveal the role of oxidative stress in several clinical conditions including inflammatory diseases, cancer, infectious disease, and rheumatologic disorders. In this study, using this new method, it was investigated whether 450 ± 10 ml of blood lost during blood donation causes oxidative stress.

The action of sulfur-containing compounds, which was called thiol groups, is crucial for the antioxidant system since they act as electron donors and extinguishing unstable free radicals. Disulphid compounds, which were the earliest indicator of oxidation created by ROS, are formed during this oxidation process. This transformation may also lead to a decrease in thiol levels. Thus, native thiol is considered as an antioxidant indicator while disulfide is considered as an oxidative stress indicator (17,18). In this study, thiol levels were found to be lower after blood donation than before donation. Although this decrease is not significant, it may be associated with oxidative stress generated through the blood donation process.

The relationship between blood donation and oxidative stress has been examined in a limited number of studies. Yunce et al. investigated the effect of blood donation on oxidative stress markers by measuring the activities of superoxide dismutase (SOD), myeloperoxidase (MPO), and the levels of malondialdehyde (MDA), nitric oxide (NOx). The results of this study showed that the level of oxidative markers (MPO and MDA) was reduced and the activity of the antioxidant enzyme SOD significantly increased 24 h. after blood donation (19). Mehrabani et al searched the association between blood donation frequency, antioxidant enzymes (glutathione peroxidase (GPX), SOD), and MDA level. They found a negative association between BMI, SOD activity, and the number of blood donations in a year (20). In our study, we found that THD parameters were not significantly altered after blood donation in blood donors. Our results show that blood donation does not cause oxidative stress or imbalance of thiol/disulfide homeostasis similar to previous studies.

Ischemia-modified albumin (IMA) is considered as a marker for elevated free radical-induced protein oxidative injury. This biomarker is associated with oxidative stress in various clinical conditions including ischaemic events such as stroke, acute mesenteric ischaemia, and some cancer types (21-23). In this study, IMA values were found to be significantly higher in repeat blood donors than in first-time blood donors. It is very well known that high donation frequency increases the risk of depletion of iron stores and iron-deficiency anemia. As a result of one donation, whole blood donors lose 8% (men) to 81% (menstruating women) of their total iron stores (24). Therefore various strategies have been developed to monitor the iron status of blood donors such as hemoglobin-guided donation intervals, ferritin-guided donation intervals, and iron supplementation (25). World Health Organization (WHO) also advises the monitoring of serum ferritin to detect iron deficiency (2). Although the serum ferritin levels in this study were not been investigated, the higher IMA level in repeat blood donors may be associated with the ischemia resulting from the depletion of iron stores.

Obesity may lead to high amounts of circulating glucose and lipids overflowing into adipose and non-adipose cells, resulting in adipocyte hypertrophy and adipose tissue hypoxia which might induce the production of ROS(26,27). Mehrabani et al. reported that as the BMI of the donors increased, the activity of SOD is decreased which is one of the antioxidant enzymes (20). In our study, although the decrease in thiol levels shows an inadequate antioxidant response, this difference was not statistically significant compared to pre-donation in obese donors. We classified the donors into three age groups. The native and total thiol levels as antioxidant indicators were found to be significantly lower after blood donation only in the 18-30 years old age group. To the best of our knowledge, this is the first study that investigates the oxidative stress of donors through the dynamic thiol/disulfide homeostasis by a new spectrophotometric method. The relationship between blood donation and oxidative stress will be better demonstrated by the studies which investigate other variables such as BMI and age in larger groups.

The blood donation process does not cause oxidative stress according to the results of our study.

However, regular blood donation may have a negative impact on THD. Further studies designed with the participation of high number of blood donors need to be conducted to prove an association between blood donation and oxidative stress. In conclusion, demonstrating that blood donation has no adverse effects on human health is extremely valuable in terms of encouraging people to donate blood.

ETHICS COMMITTEE APPROVAL

* The study was approved by the University of Health Sciences Ankara City Hospital Ethics Committee (Date: 02.03.2022 and Number: 2022-E2-22-1243).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

Healthcare-associated infections in a rehabilitation hospital in Türkiye: A six-year retrospective analysis of incidence, pathogens and risk factors

Türkiye'de bir rehabilitasyon hastanesinde sağlık hizmeti ilişkili enfeksiyonlar: İnsidans, patojenler ve risk faktörlerinin altı yıllık retrospektif analizi

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ABSTRACT

Objective: This study aimed to evaluate the incidence, risk factors, causative microorganisms, and antibiotic resistance patterns of healthcare-associated infections (HAIs) over a six-year period in a tertiary physical medicine and rehabilitation hospital.

Methods: In this retrospective study, the medical records of 9,139 patients hospitalized between January 1, 2017, and December 31, 2022, in a 210-bed tertiary physical medicine and rehabilitation hospital were reviewed. HAIs were defined according to the National Healthcare-Associated Infections Surveillance Diagnostic Guideline of the Directorate General of Public Health, Ministry of Health of the Republic of Türkiye.

Results: During the study period, 179 patients developed HAIs. The incidence of HAIs was 1.95, and the incidence density was 0.56. The majority of patients were male (82.7%), with a median age of 40 years. The

ÖZET

Amaç: Bu çalışmada, üçüncü basamak bir fizik tedavi ve rehabilitasyon hastanesinde altı yıllık dönemde gelişen sağlık hizmeti ile ilişkili enfeksiyonların (SHİE) insidansı, risk faktörleri, etken mikroorganizmaları ve antibiyotik direnç paternlerinin değerlendirilmesi amaçlandı.

Yöntem: Bu retrospektif çalışmada, 1 Ocak 2017 - 31 Aralık 2022 tarihleri arasında, 210 yataklı üçüncü basamak bir fizik tedavi ve rehabilitasyon hastanesinde yatan 9.139 hastanın tıbbi kayıtları incelendi. SHİE'ler, Türkiye Cumhuriyeti Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü Ulusal Sağlık Hizmeti ile İlişkili Enfeksiyonlar Sürveyans Tanı Rehberine göre tanımlandı.

Bulgular: Çalışma döneminde 179 hastada SHİE gelişti. SHİE insidansı 1,95 ve insidans dansitesi 0,56 saptandı. Hastaların çoğunluğu erkekti (%82,7) ve medyan yaş 40 idi. Hastaların en sık tanıları spinal

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most common diagnoses were spinal cord injury (62%) and stroke/traumatic brain injury (15.2%). Of the HAIs, 92.2% were urinary tract infections (UTIs) and 7.8% were skin and soft tissue infections. The most frequently isolated microorganism was *Escherichia coli* (*E. coli*) (52.5%). Multidrug-resistant organisms accounted for 27.9% of isolates. Over the six-year period, a decreasing trend in HAI incidence density was observed; the incidence density of catheter-associated UTIs decreased by 67%, while the standardized infection ratio (SIR) declined from 1.6 in 2017 to 0.6 in 2022. This reduction was associated with infection control measures such as regular staff training, improved compliance with hand hygiene, implementation of national infection prevention bundles, antimicrobial stewardship, daily catheter assessments, and timely removal of unnecessary catheters.

Conclusion: HAIs are common in physical medicine and rehabilitation centers, and this is particularly pronounced among patients with neurological disorders. The most frequent type of infection is UTI, with urinary catheter use being the main modifiable risk factor. *E. coli* was the most frequently isolated pathogen, and multidrug resistance remains a major concern. The decline in infection rates underscores the importance of infection control measures that are implemented effectively and continuously in rehabilitation hospitals.

Key Words: Healthcare-associated infections, rehabilitation hospital, spinal cord injury, urinary tract infection, multidrug resistance, infection control

kord yaralanması (%62) ve inme/travmatik beyin hasarı (%15,2) idi. SHİE'lerin %92,2'si üriner sistem enfeksiyonu (ÜSE), %7,8'i ise deri ve yumuşak doku enfeksiyonlarıydı. En sık izole edilen mikroorganizma *Escherichia coli* (*E. coli*) (%52,5) idi. Çok ilaca dirençli organizmalar izolatların %27,9'unu oluşturdu. Altı yıllık dönemde SHİE insidans dansitesinde azalma eğilimi izlendi; kateter ilişkili-ÜSE insidans dansitesi %67 oranında düşerken, standardize enfeksiyon oranı (SIR) 2017'de 1,6'dan 2022'de 0,6'ya geriledi. Bu düşüş; düzenli personel eğitimi, el hijyenine uyumun artırılması, ulusal enfeksiyon önleme paketlerinin uygulanması, antimikrobiyal yönetim, kateterlerin günlük değerlendirilmesi ve gereksiz kateterlerin zamanında çıkarılması gibi enfeksiyon kontrol önlemleri ile ilişkiliydi.

Sonuç: Fizik tedavi ve rehabilitasyon merkezlerinde SHİE'ler yaygın olup, bu durum özellikle nörolojik hastalığı olan hastalarda daha belirgindir. En yaygın enfeksiyon tipi ÜSE olup, üriner kateter kullanımı başlıca değiştirilebilir risk faktörüdür. *E. coli* en sık izole edilen patojen olup çoklu ilaç direnci önemli bir sorun olmaya devam etmektedir. Enfeksiyon oranlarındaki azalma, fizik tedavi ve rehabilitasyon hastanelerinde etkin ve sürekli şekilde uygulanan enfeksiyon kontrol önlemlerinin önemini vurgulamaktadır.

Anahtar Kelimeler: Sağlık hizmeti ile ilişkili enfeksiyonlar, rehabilitasyon hastanesi, spinal kord yaralanması, üriner sistem enfeksiyonu, çoklu ilaç direnci, enfeksiyon kontrolü

INTRODUCTION

Healthcare-associated infections (HAIs), previously termed "nosocomial infections," have been defined under this name by the Centers for Disease Control and Prevention since 2008. The National Healthcare Safety Network describes HAI as a localized or systemic clinical condition caused by infectious agents or toxins that were neither present nor incubating at the time of hospital admission (1). In Türkiye, the Ministry of Health's National Healthcare-Associated Infections Surveillance Guideline also defines HAIs within this framework and provides standardized criteria for their monitoring and

reporting (2). Bacteria originating from the hospital environment and the human microbiota are the most common pathogens, although fungi and viruses may also be responsible. The most frequently encountered types of HAIs include bloodstream infections, urinary tract infections (UTI), pneumonia, and surgical site infections (3).

HAIs represent one of the most significant complications of modern medical care. Major risk factors include advanced age, comorbidities, increasing use of invasive devices, and inappropriate use of antibiotics. These infections increase morbidity and mortality in hospitalized patients while also prolonging the length of hospital stay and escalating healthcare costs (4). Studies from developed countries have reported that 5% to 15% of patients, particularly those in intensive care, transplantation, and surgical units, develop HAIs (5,6). However, HAIs are not limited to high-risk units and are also observed in wards where surgical procedures are not performed (7). The risk of developing HAIs increases with prolonged hospital stays, which are most commonly required in rehabilitation units. Rehabilitation services typically care for patients who have stabilized after acute medical processes such as intensive care treatment or surgical procedures, many of whom present with multiple comorbidities and require long-term follow-up (8). Surveillance data from Europe indicate that HAIs cause more deaths than any other infectious disease under monitoring and occur with similar frequency in long-term care facilities as in acute care hospitals (7). The incidence of HAIs in long-term care facilities, including rehabilitation hospitals, has been reported to range from 1.8 to 13.5 infections per 1,000 patient-days (9).

In the rehabilitation setting, HAIs further prolong hospitalization, delay recovery, worsen prognosis, increase disability, and deepen dependency in activities of daily living (10). Despite these critical outcomes, the number of studies focusing specifically on HAIs in rehabilitation hospitals remains limited. Existing research has largely concentrated on specific patient populations or has evaluated rehabilitation hospitals together with other long-term care institutions, such as nursing homes (7,11). Therefore, in line with both international definitions and the Ministry of Health's National Healthcare-Associated Infections Surveillance Guideline, we aimed to provide up-to-date and comprehensive data on HAIs among adult patients in a rehabilitation hospital.

MATERIAL and METHOD

This retrospective study evaluated the records of 9,139 patients hospitalized between January 1, 2017, and December 31, 2022, in a 210-bed tertiary rehabilitation hospital.

An HAI was defined as an infection occurring on or after the third day of hospital admission. The earliest date on which site-specific diagnostic criteria were fulfilled was considered the 'date of infection'. Diagnostic criteria were determined according to the National Healthcare-Associated Infections Surveillance Guideline of the General Directorate of Public Health, Ministry of Health of the Republic of Türkiye (2).

Our hospital operates a prospective, hospital-wide, laboratory-based surveillance system for the detection and monitoring of HAIs. All culture results obtained throughout the hospital were reviewed daily by the infection control team. Potential cases were systematically evaluated and discussed within the hospital infection control committee. Cases identified as HAIs were recorded and reported on a quarterly basis.

For each patient, the following variables were collected: age, sex, primary diagnosis, admitting department, risk factors, type of HAI, isolated microorganisms with resistance profiles, antibiotics administered, and length of hospital stay. Physical examination findings, laboratory results, and treatment protocols were obtained from hospital surveillance forms and the electronic medical records database.

The control group included 185 patients without HAIs, hospitalized in the same clinics during the study period, selected through simple random sampling. There was no significant difference between the study and control groups in terms of age and sex.

Microorganisms isolated from patients were identified by the hospital microbiology laboratory. Standard laboratory methods were used for quantitative urine and tissue cultures. Antimicrobial susceptibility testing was performed using the disk diffusion method on Mueller-Hinton agar in accordance with the guidelines and breakpoint values of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Plates were incubated at 36 °C for 16-20 hours; for tissue cultures, incubation was extended up to 48 hours when required. Inhibition zones were measured and classified as susceptible, intermediate, or resistant according to EUCAST criteria, and antimicrobial susceptibilities of bacteria were determined accordingly (12). MDR microorganisms were defined as non-susceptible to at least one agent in three or more antimicrobial categories (13).

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (IBM SPSS Inc., Chicago, IL). Normality of data distribution was assessed with the Kolmogorov-Smirnov test. Continuous variables were expressed as mean ± standard deviation, and categorical variables as frequencies and percentages.

Comparisons between two groups were performed using the Student's t-test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables. Comparisons among three or more groups were conducted using ANOVA for normally distributed variables and the Kruskal-Wallis test for non-normally distributed variables. Pairwise comparisons were adjusted using the Bonferroni correction.

Categorical variables were compared with the Chi-square test. Longitudinal changes were assessed using mixed-effects models. A two-sided p-value <0.05 was considered statistically significant.

The study was approved by the Ankara Bilkent City Hospital Clinic Researchers Ethics Committee (Date: 01.03.2023 and Number: E2-23-3470).

RESULTS

Between 2017 and 2022, a total of 9,139 patients were hospitalized in our rehabilitation hospital, corresponding to 316,685 patient-days. HAIs developed in 179 patients, yielding an overall incidence of 1.95 and an incidence density of 0.56. Among patients with HAI, 148 (82.7%) were male and 31 (17.3%) were female, with a median age of 40 years (range: 18-93).

Regarding primary diagnoses, most patients with HAIs had spinal cord injury (SCI, n=111, 62.0%), followed by stroke or traumatic brain injury (TBI, n=27, 15.2%). Other diagnoses included malignancy, fracture, or amputation (n=21, 11.7%), neurological disorders such as Guillain-Barré syndrome, transverse myelitis, multiple sclerosis, or encephalitis (n=11, 6.1%), and degenerative joint or intervertebral disc disease (n=9, 5.0%). HAIs were particularly common in the SCI rehabilitation department, where they accounted for 50.3% of all cases.

The majority of infections were UTIs (n=165, 92.2%), followed by skin and soft tissue infections (SSTIs) (n=14, 7.8%). The overall incidence of UTI was 1.8. Of these, 133 (74.3%) were non-catheter-associated urinary tract infections (non-CAUTI, symptomatic UTI), while 32 (17.9%) were catheter-associated urinary tract infections (CAUTI, symptomatic UTI). All SSTIs (n=14, 7.8%) were identified as infected pressure ulcers. No cases of asymptomatic bacteremic UTI or pneumonia were detected during the study period.

HAIs were evaluated according to the method of bladder drainage. Clean intermittent catheterization (CIC) was used in 92 patients (51.7%), indwelling catheterization (IC) in 31 patients (17.4%), while 56 patients (30.9%) had voluntary bladder control.

Escherichia coli (*E. coli*) was the most frequently isolated pathogen in healthcare-associated urinary tract infections (HA-UTIs) (n=94, 52.5%). Pathogens identified in SSTIs included *Klebsiella* spp. (n=5), *Acinetobacter* spp. (n=4), *Pseudomonas* spp. (n=3), methicillin-resistant *Staphylococcus aureus* (MRSA) (n=1), and *Enterobacter* spp. (n=1).

Among the 179 HAIs, 50 (27.9%) were caused by multidrug-resistant (MDR) microorganisms. Evaluation of resistance profiles revealed that 23.4% of isolates were extended-spectrum ß-lactamase-producing (ESBL+) and 4.5% demonstrated carbapenem resistance. MRSA was identified in one patient, whereas no vancomycin-resistant enterococcus was detected.

The most frequently used antibiotics were fluoroquinolones (29%), nitrofurantoin (20.1%), dual antibiotic therapy (16.8%), ß-lactam/ß-lactamase inhibitor combinations (10.6%), carbapenems (8.9%), cephalosporins (5.0%), and fosfomycin (4.0%). Other antibiotics, including tetracyclines, sulfonamides, and oxazolidinones, were prescribed in 5.6% of cases. The clinical and demographic characteristics of the patients are presented in Table 1.

Table 1. Demographic and clinical characteristics of the patients

Variable	n (%)
Age (median, range)	40 (18-93)
Gender	
Male	148 (82.7)
Female	31 (17.3)
Clinic/Ward	
SCI Rehabilitation	90 (50.2)
Acute Rehabilitation	48 (26.8)
Brain Injury Rehabilitation	17 (9.5)
Rheumatologic Rehabilitation	15 (8.4)
Orthopedic Rehabilitation	9 (5.1)
Type of HAI	
Non-CAUTI	133 (74.3)
CAUTI	32 (17.9)
SSTIs	14 (7.8)
Bladder drainage method	
CIC	92 (51.7)
IC	31 (17.4)
Voluntary bladder control	56 (30.9)

Table 1 (cont.). Demographic and clinical characteristics of the patients

Variable	n (%)
Isolated microorganisms	
E. coli	94 (52.5)
Klebsiella spp.	42 (23.5)
Pseudomonas spp.	13 (7.2)
Enterobacter spp.	10 (5.6)
Two types of microorganisms	7 (3.9)
Acinetobacter spp.	4 (2.2)
Proteus spp.	3 (1.7)
Enterococcus spp.	3 (1.7)
S. aureus / Coagulase-negative staphylococci	3 (1.7)
Antibiotic treatment	
Fluoroquinolones	52 (29.0)
Nitrofurantoin	35 (20.1)
Dual antibiotic therapy	30 (16.8)
ß-lactam/ß-lactamase inhibitor combinations	20 (10.6)
Carbapenems	16 (8.9)
Cephalosporins	9 (5.0)
Fosfomycin	7 (4.0)
Other (tetracycline, sulfonamide, oxazolidinone)	10 (5.6)

Note: n = number of patients, % = percentage. SCI: Spinal cord injury; HAI: Healthcare associated infections; non-CAUTI: non-catheter-associated urinary tract infections; CAUTI: catheter-associated urinary tract infections; SSTI: Skin and soft tissue infection; CIC: Clean intermittent catheterization; IC: Indwelling catheterization.

HAIs occurred significantly more often in patients with SCI and in those with stroke or TBI ($p<0.001$). The distribution of HAI types by primary diagnosis is presented in Table 2.

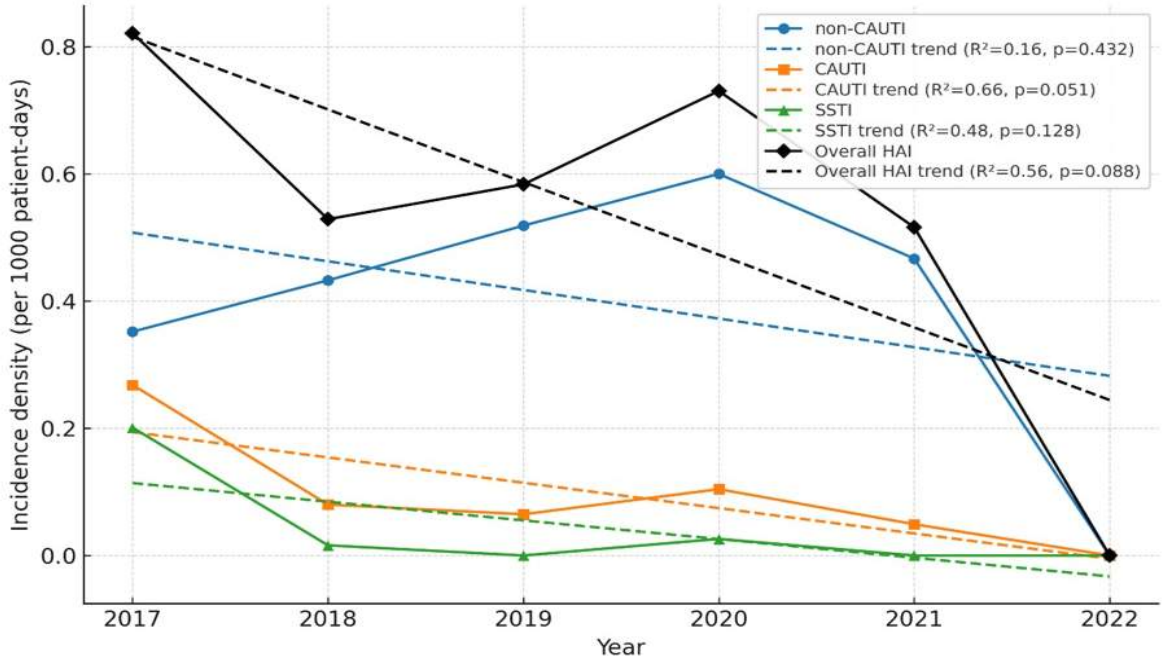
Over the six-year period, incidence density showed a declining trend across most infection types. A reduction was observed for CAUTI ($R^2=0.66$, $p=0.051$), SSTI ($R^2=0.48$, $p=0.128$), and overall HAI incidence density ($R^2=0.50$, $p=0.091$). In contrast, non-CAUTI showed a slight downward trend that did not reach statistical significance ($R^2=0.16$, $p=0.432$). Annual incidence densities and regression trend lines are displayed in Figure 1. Additionally, CAUTI rates and standardized infection ratios (SIRs) were analyzed separately due to their clinical importance as a quality indicator (Figure 2).

When the distribution of the most frequently isolated pathogens was compared by bladder drainage method, significant differences were observed among the groups (p<0.001). *E. coli* predominated in the CIC group (79.7%) and in patients with voluntary bladder control (60.5%), whereas the IC group showed a more heterogeneous profile with *Klebsiella* spp. (38.1%), *E. coli* (33.3%), and *Pseudomonas* spp. (28.6%) (Table 3). In patients who developed HAIs, both urinary catheter utilization rates and length of hospital stay were significantly higher compared with the control group without HAIs (p<0.001).

Table 2. Distribution of HAI types by clinical diagnosis

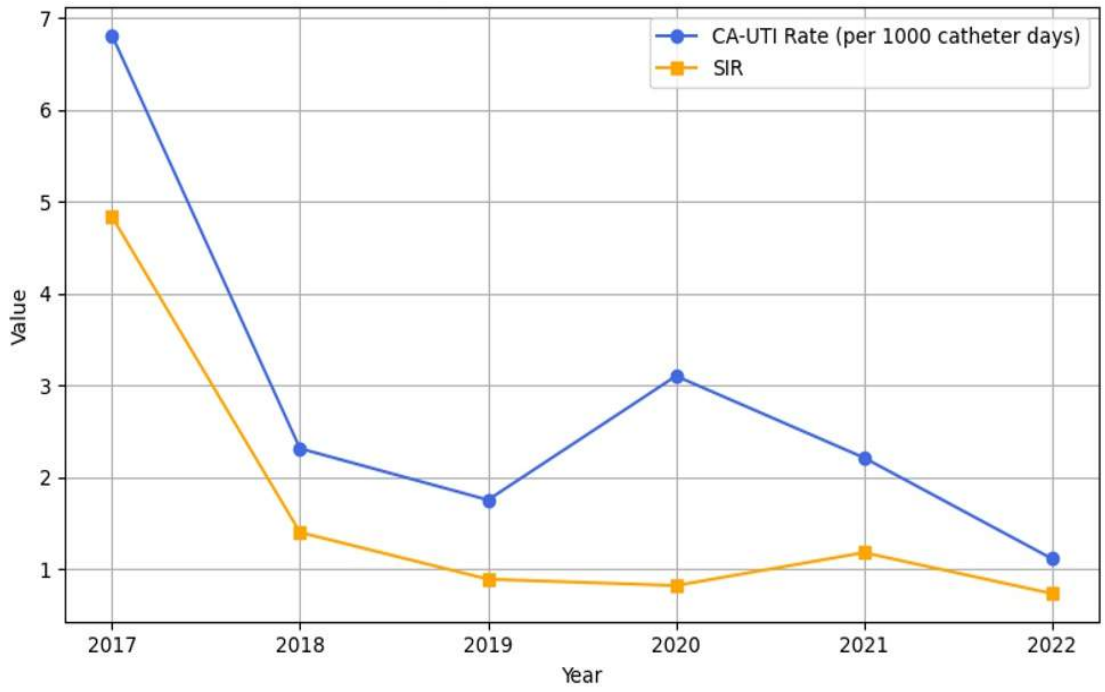
HAI type n(%)	SCI (n=111)	Stroke or traumatic brain injury (n=27)	Other neurological diseases* (n=11)	Degenerative joint and intervertebral disc anomalies (n=9)	Other diseases** (n=21)	p
Non-CAUTI	88 (79.3%)	22 (81.4%)	8 (72.7%)	8 (88.9%)	7 (33.3%)	<0.001 [a]
CAUTI	19 (17.1%)	4 (14.8%)	3 (27.3%)	1 (11.1%)	5 (23.8%)	
SSTIs	4 (3.6%)	1 (3.7%)	--	--	9 (42.9%)	

SCI: Spinal cord injury; non-CAUTI: non-catheter-associated urinary tract infection; CAUTI: catheter-associated urinary tract infection; SSTI: skin and soft tissue infection.
*Other neurological diseases: Guillain-Barré syndrome, transverse myelitis, multiple sclerosis, encephalitis.
**Other diseases: malignancy, fracture, amputation, etc.
a: Chi Square Test, (n / %)



HAI: healthcare-associated infection; CAUTI: catheter-associated urinary tract infection; non-CAUTI: non–catheter-associated urinary tract infection; SSTI: skin and soft tissue infection.

Figure 1. Annual incidence density trends of HAIs between 2017 and 2022



CAUTI: catheter-associated urinary tract infection; SIR: standardized infection ratio.

Figure 2. Annual CAUTI rates and SIR values, 2017-2022

DISCUSSION

Previous studies conducted in rehabilitation and long-term care settings have reported similar incidence rates of HAIs. For instance, a point prevalence study from Germany found an incidence of 1.7%, while an orthopedics and rehabilitation clinic reported 1.98% (14,15). Likewise, a multicenter study in Italy involving brain-injured patients reported an incidence of 1.93% (16). Similar to the literature, the incidence of HAI was found to be 1.95 in our study.

Over the six-year study period, a gradual decline was observed in the incidence density of HAIs, including CAUTIs, SSTIs, and overall infection rates. Specifically, CAUTI incidence density decreased from 0.27 in 2017 to 0.09 in 2022, corresponding to an approximate 67% reduction, while the SIR declined from 1.6 to 0.6, reflecting a 62% decrease. We believe that these improvements were closely linked to the strengthening of infection control practices following the appointment of a full-time infectious diseases specialist in 2018. During this period, regular staff training was intensified, national infection prevention bundles were implemented, antimicrobial stewardship with feedback mechanisms was reinforced, daily catheter indications were evaluated with prompt removal of unnecessary catheters, and hand hygiene practices were closely monitored. In a study conducted in India, a 60% reduction in CAUTI SIR was reported through similar infection control measures (17). Overall, our findings are consistent with international evidence and highlight the critical

role of comprehensive and sustained infection control strategies in reducing infection rates in rehabilitation hospitals.

The etiopathogenesis of infectious diseases in physical therapy and rehabilitation hospitals varies according to the primary diagnosis of patients. SCI predisposes individuals to multiple complications in organs below the neurological lesion level. Owing to respiratory dysfunction—such as atelectasis and impaired secretion clearance—the risk of pneumonia is increased in these patients (18). Neurogenic bladder dysfunction leads to elevated intravesical pressure and bladder overdistension, which can cause ischemia and mucosal damage, thereby predisposing to urinary tract infections (19). In immobilized patients, sustained pressure over bony prominences compromises local blood flow and facilitates the development of pressure ulcers, which in turn increase the risk of secondary SSTIs, often aggravated by urinary or fecal incontinence (20). Additionally, patients with neurological disorders may be at increased risk of developing HAIs because of altered mental status and a higher tendency for aspiration. Immune suppression and a greater likelihood of concomitant trauma also contribute to this increased vulnerability (21). Several studies have shown that post-stroke immunosuppression contributes significantly to the increased risk of infections in this patient population (22,23). In a study conducted in the physical therapy and rehabilitation service, Demirdal et al. reported that 58.3% of HAIs occurred in patients with SCI, whereas 33% were observed in patients with stroke (24). Consistent with these findings, our study also demonstrated that HAIs were predominantly observed in patients with SCI or other neurological disorders, while they were significantly less frequent among patients with non-neurological conditions such as degenerative joint disease and intervertebral disc anomalies ($p<0.05$).

In a study from Germany, UTIs were the most common HAIs, accounting for approximately 50% of cases, followed by respiratory, SSTIs (15). Similarly, a ten-year study from the Netherlands identified UTIs as the most frequent HAIs in long-term care centers, with a prevalence of 1.7% (95% CI: 1.6–1.8) (25). In contrast, a multinational prevalence study from Europe found that respiratory tract infections were the most common HAIs in long-term care centers (21.4% pneumonia and 4.3% other lower respiratory tract infections), followed by UTIs (18.9%) and surgical site infections (18.4%) (7). In our study, the most common HAIs were UTIs (92.2%), followed by SSTIs (7.8%). In the study where respiratory tract infections were the most common HAIs, the lower frequency of UTIs was attributed to the fact that most of the evaluated patients were over the age of eighty-five (7). In contrast, the absence of healthcare-associated respiratory tract infections in our study may be explained by the younger age of our patient population, the high prevalence of neurogenic bladder dysfunction, and the widespread use of CIC or IC.

In our study, urinary catheter use was statistically significantly higher in patients with HAI compared to those without HAI. This association has also been emphasized in previous research: a study from Italy reported that 59.5% of patients with HAI in post-acute clinics and all patients with HAI in long-term rehabilitation centers had a urinary catheter (16), while another Italian study showed that one-third of catheterized rehabilitation patients developed HAIs (25). Another study from the Netherlands reported that 20% of HA-UTIs were catheter-related (26). In our study, this rate was 17.9%. Collectively, both our findings and previous reports highlight urinary catheterization as a major, yet preventable, risk factor for HAIs in rehabilitation settings.

Patients who undergo CIC are typically those with SCI and neurogenic bladder dysfunction. Compared to IC or suprapubic catheterization, CIC has been reported to be safer and associated with lower rates of complications and UTIs (27,28). In our study, 18.2% of patients were managed with an IC, 54.5% with CIC, and 27% had voluntary bladder control.

The higher frequency of HA-UTIs among patients undergoing CIC may be related to infection control policies: CIC was preferred over IC when clinically appropriate, and IC users were transitioned to CIC whenever feasible. Furthermore, the Spinal Cord Medical Consortium guidelines state that evidence on infection risk is mixed and does not clearly favor one bladder management method over another. Similarly, a recent systematic review emphasized that the hypothesis suggesting ICs cause more UTIs than CICs is not supported by current scientific evidence (29,30).

In our study, *E. coli* was the most frequently isolated microorganism across all diagnostic groups. Consistently, studies in similar patient populations have also reported *E. coli* as the leading pathogen in urinary tract infections (7,11,26). Our analysis showed that patients managed with CIC or voluntary voiding predominantly harbored common pathogens, whereas IC users exhibited a higher proportion of more resistant organisms such as Klebsiella and Pseudomonas. The literature also suggests that long-term catheterization may provide a favorable environment for colonization by more heterogeneous and resistant microorganisms, and that polymicrobial infections can be more frequent in this patient group (31,32). These findings support the importance of considering bladder management methods when selecting empirical antibiotic therapy.

In a multicenter study of patients with severe acquired brain injury, the prevalence of MDR microorganisms was reported as 30.3% (16). Likewise, a European point-prevalence study reported MDR in 28% of isolates and carbapenem resistance in 4.2% of isolates from long-term care centers (7). In our study, MDR microorganisms accounted for 27.9% of isolates, and carbapenem resistance was 4.5%. MRSA prevalence is generally low in rehabilitation hospitals, and in our study only one case was detected. Although the overall prevalence of resistant pathogens was comparable to reports from European rehabilitation and long-term care facilities, even a single MRSA case remains clinically significant, as it may trigger outbreaks with serious consequences in vulnerable patients with prolonged hospital stays (33).

When the antibiotics used in patients who developed HAI were examined in our study, it was noteworthy that, fluoroquinolones were the most frequently prescribed, accounting for 29%. Similarly, in Germany, fluoroquinolones were reported as the most commonly used agents in 31.3% of patients with HA-UTI (14). In our cohort, 16.8% of patients required dual antibiotic therapy, particularly in cases of polymicrobial growth or suspected highly resistant organisms. Recent evidence also shows that for carbapenem-resistant Gram-negative infections, combination therapy can reduce mortality and improve outcomes compared with monotherapy (34). Nevertheless, such regimens should be carefully balanced with antimicrobial stewardship principles, favoring targeted therapy guided by susceptibility testing (35).

Although research on HAIs and MDR HAIs in rehabilitation and neurorehabilitation settings remains limited, recent studies have consistently shown that these infections negatively affect rehabilitation outcomes and significantly prolong hospital stay, particularly in patients with severe brain injury (36). For example, a multicenter study in Italian neurorehabilitation centers reported a mean length of hospital stay of 88.3 ± 55 days in patients with HAI, compared with 56 ± 50.7 days in those without infection ($p < 0.05$) (37). Similar findings have been reported in other studies (11,16,36). In our study, the mean length of hospital stay was significantly longer in patients with HAI (56.6 ± 31.9 days) compared to those without HAI (30.8 ± 18.6 days) ($p < 0.001$).

Pressure ulcers are also frequent complications in individuals with SCI, occurring from the acute hospitalization phase through to community reintegration. They are associated with poorer quality of life, longer hospital stays, and increased morbidity and mortality (38). Reported prevalence rates during hospitalization vary, with some studies citing values

between 12% and 16.1% (39,40). In our study, 7.8% of patients developed healthcare-associated SSTIs, all of which presented as infected pressure ulcers.

This study has several limitations. First, it was conducted retrospectively in a single tertiary rehabilitation hospital, which may restrict the generalizability of the findings. Second, due to the retrospective design, certain clinical details—such as symptom severity and functional outcomes—could not be systematically evaluated. Third, detailed molecular typing of resistant organisms was not performed; therefore, the epidemiological characterization of MDR pathogens remains incomplete.

In conclusion; in this six-year study, HAIs were shown to be a frequent complication in rehabilitation hospitals, particularly among patients with neurological disorders. HA-UTIs were the predominant infection type, with urinary catheterization identified as the main modifiable risk factor. Strengthened infection control practices were associated with a decline in overall HAI incidence and CAUTI SIR, emphasizing the need for comprehensive and sustained prevention strategies in rehabilitation settings.

ETHICS COMMITTEE APPROVAL

* The study was approved by the Ankara Bilkent City Hospital Clinic Researchers Ethics Committee (Date: 01.03.2023 and Number: E2-23-3470).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

Apoptotic effect of doxorubicin delivering micelles on MDA-MB-231 triple negative breast cancer cells

Doksorubisin taşıyan misellerin MDA-MB-231 üçlü negatif meme kanseri hücreleri üzerindeki apoptotik etkisi

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ABSTRACT

Objective: Many anticancer agents exhibit limited therapeutic efficacy due to high systemic toxicity or rapid elimination by the reticuloendothelial system. Therefore, nanocarrier systems that enable drug targeting directly to tumor tissue have become a significant research area in recent years. The aim of this study was to prepare and characterize methoxy polyethylene glycol-block-polycaprolactone (mPEG-b-PCL)-based doxorubicin (DOX)-loaded micelles (DOX_{ld}-M) and micelles formed by covalent conjugation of DOX (DOX_{conj}-M), and to compare the biological effects of these systems on MDA-MB-231 breast cancer cells.

Methods: DOX was either loaded directly onto mPEG-b-PCL micelles (DOX_{ld}-M) or covalently conjugated to hydrazide-functionalized mPEG-b-PCL (DOX_{conj}-M), resulting in micelle formation. The physicochemical properties and drug release profiles of the micelles were determined. At the cellular level, their effects

ÖZET

Amaç: Birçok antikanser ajanı, yüksek sistemik toksisite veya retiküloendotelyal sistem tarafından hızlı eliminasyon nedeniyle terapötik etkinliğini sınırlı düzeyde gösterebilmektedir. Bu nedenle, ilacın doğrudan tümör dokusuna hedeflenmesini sağlayan nano-taşıyıcı sistemler son yıllarda dikkat çekici bir araştırma alanı haline gelmiştir. Bu çalışmanın amacı, metoksi polietilen glikol-blok-polikaprolakton (mPEG-b-PCL) esaslı doksorubisin (DOX) yüklü miseller (DOX_{ld}-M) ile DOX kovalent konjugasyonu ile oluşturulan misellerin (DOX_{conj}-M) hazırlanması, karakterizasyonu ve bu sistemlerin MDA-MB-231 meme kanseri hücreleri üzerindeki biyolojik etkilerinin karşılaştırılmasıdır.

Yöntem: BDOX, ya doğrudan mPEG-b-PCL misellerine yüklenmiş (DOX_{ld}-M) ya da hidrazid fonksiyonlu mPEG-b-PCL'ye kovalent olarak bağlanarak konjuge edilmiş (DOX_{conj}-M) ve sonrasında misel yapısı oluşturulmuştur. Misellerin fizikokimyasal özellikleri ve ilaç salım profilleri belirlenmiştir. Hücresel düzeyde

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on cytotoxicity, cell internalization, cell migration, and colony formation were evaluated in MDA-MB-231 cells. Apoptotic responses were investigated using flow cytometry, qRT-PCR, mitochondrial transmembrane potential measurements, and reactive oxygen species (ROS) assays. Furthermore, the anti-angiogenic effects of both micelle formulations were investigated in a co-culture system with HUVEC cells.

Results: Both micelle types exhibited faster drug release under acidic conditions compared to neutral conditions, offering a therapeutic advantage given the acidic nature of the tumor microenvironment. DOX conjugation provided more controlled and slower drug release compared to loading. Drug-free micelles did not exhibit any cytotoxicity. The natural fluorescence of DOX confirmed that the micelles were effectively internalized by the cells. All DOX-containing micelles significantly suppressed proliferation and migration in MDA-MB-231 cells. In particular, DOX_{ld}-M was characterized by an increase in the proportion of apoptotic cells, a significant increase in Bax and p53 gene expression, a significant decrease in Bcl-2 and Bcl-xL expression, and an increase in ROS production. Both micelle formulations decreased mitochondrial membrane potential and triggered apoptosis induction.

Conclusion: The results indicate that DOX_{ld}-M was more effective than DOX_{conj}-M in inducing apoptosis and inhibiting proliferation of MDA-MB-231 cells. These findings suggest that mPEG-b-PCL-based DOX-loaded micelles may be promising nanocarrier systems for cancer therapy.

Key Words: Doxorubicin, hydrazone, drug loading, breast cancer, apoptosis

ise, MDA-MB-231 hücrelerinde sitotoksisite, hücre içine alımı, hücre göçü ve koloni oluşumu üzerine etkileri değerlendirilmiştir. Apoptotik yanıtlar; akış sitometrisi, qRT-PCR, mitokondriyal transmembran potansiyel ölçümleri ve reaktif oksijen türleri (ROS) tayini ile incelenmiştir. Ayrıca, HUVEC hücreleriyle yapılan ko-kültür sisteminde her iki misel formülasyonunun anti-anjiyogenik etkileri araştırılmıştır.

Bulgular: Her iki misel türü de asidik koşullarda nötr ortama kıyasla daha hızlı ilaç salımı göstermiş olup bu durum, tümör mikroçevresinin asidik karakteri açısından terapötik avantaj sunmaktadır. DOX konjugasyonu, yüklemeye kıyasla daha kontrollü ve yavaş ilaç salımı sağlamıştır. İlaç içermeyen miseller herhangi bir sitotoksisite göstermemiştir. DOX'un doğal floresansı, misellerin hücreler tarafından etkin biçimde internalize edildiğini doğrulamıştır. Tüm DOX içeren miseller MDA-MB-231 hücrelerinde proliferasyon ve göçü belirgin şekilde baskılamıştır. Özellikle DOX_{ld}-M, apoptotik hücre oranında artış, Bax ve p53 gen ifadesinde belirgin yükseliş, Bcl-2 ve Bcl-xL ifadesinde anlamlı azalma ve ROS üretiminde artış ile karakterize edilmiştir. Her iki misel formülasyonu da mitokondriyal membran potansiyelinde azalmaya yol açmış ve apoptoz indüksiyonunu tetiklemiştir.

Sonuç: Sonuçlar, DOX_{ld}-M'nin apoptoz indüklemede ve MDA-MB-231 hücrelerinin proliferasyonunu engellemede DOX_{conj}-M'ye kıyasla daha etkili olduğunu göstermektedir. Bu bulgular, mPEG-b-PCL tabanlı DOX yüklü misellerin kanser tedavisinde umut vadeden nano-taşıyıcı sistemler olabileceğini ortaya koymaktadır.

Anahtar Kelimeler: Doksorubisin, hidrazon, ilaç yükleme, meme kanseri, apoptoz

INTRODUCTION

Breast cancer is the most common cancer type in women, and approximately 20% of these patients present with metastasis. It is the second leading cause of cancer-related death after lung cancer (1). Breast cancer is classified into several subtypes, including estrogen receptor-positive breast cancer (ERBC), progesterone receptor-positive breast cancer (PRBC), human epidermal growth factor receptor 2-positive

breast cancer (HER2-BC) and triple negative breast cancer (TNBC). TNBC accounts for about 20% of all breast cancers, which lacks a cell surface receptor and is highly aggressive. Due to the absence of targetable receptors, cancer-targeted drug delivery systems are generally ineffective against this subtype (2). Moreover, TNBC exhibits resistant to radiotherapy, making its treatment particularly challenging (3).

Anthracycline antibiotics are commonly used in cancer treatment. These agents act as alkylating agents, intercalate into DNA, inhibit topoisomerase activity, and generate free radicals, thereby causing oxidative damage to cellular proteins. Doxorubicin (DOX), a widely used anthracycline antibiotic, binds directly to DNA, induces single- and double-strand breaks, and can crosslinks DNA. DNA. However, its clinical use is limited by dose-dependent cardiotoxicity (4).

Cancer cells preferentially utilize glycolysis over oxidative phosphorylation to meet the high energy demand required for rapid proliferation. This metabolic shift leads to hypoxia within the tumor microenvironment, causing pH values to decrease to 6.5-7.2 in the extracellular space, 5.0-6.5 in endosomes, and 4.5-5.0 in lysosomes (5). pH sensitive drug delivery systems, typically exploit this feature by linking drugs to polymers through pH-responsive linkers such as hydrazone, disulfide, azo, acetal, ortho ester, vinyl ether, amine, or imine bonds (6). Hydrazone linkages are particularly promising, as they remain stable under physiological conditions (pH 7.4) but are cleaved in acidic environments (7). For instance, Etrych et al. (2014) conjugated DOX to N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers via hydrazone bonds and reported faster drug release at pH 5.0 compared to pH 7.4 (8). Similarly, Lale et al. (2015) demonstrated that DOX conjugated to a pentablock copolymer via hydrazone bonds released 89% of the drug at pH 5.0, whereas only 29% was released at pH 7.4 (9). More recently, Zhang et al. (2022) conjugated DOX to a supramolecular organic framework via hydrazone bonds, reporting DOX release levels of 80%, 45%, and 15% at pH 4.5, 5.6, and 7.4, respectively, after 72 hours. The amounts of the released DOX were 80%, 45% and 15% at pH 4.5, 5.6 and 7.4, respectively after 72 hours (10). Collectively, these findings confirm that hydrazone linkages are pH-responsive, enabling accelerated drug release under acidic conditions compared to physiological environments.

Polymeric micelles are nano-sized particles composed of a hydrophilic shell and a hydrophobic core in aqueous media. Hydrophobic anticancer drugs can be encapsulated within the hydrophobic core, thereby improving drug bioavailability, reducing systemic side effects, and offering advantages in both in vitro and in vivo studies (11–14). These systems are relatively easy to prepare, can be synthesized with small and uniform particle sizes, and provide high drug-loading capacity as well as controlled drug release. Methoxy poly(ethylene glycol)-block-polycaprolactone (mPEG-b-PCL) is a synthetic, amphiphilic, biocompatible and biodegradable polymer approved by FDA (15). Poly(ethylene glycol) (PEG) and polycaprolactone (PCL) are biocompatible polymers which are widely used in drug delivery systems and tissue engineering (16). However, PCL's highly hydrophobic nature, slow degradation rate, and immunogenicity limit its application (17). On the other hand, PEG is hydrophilic and helps nanoparticles evade immune recognition. Therefore, di-block or tri-block copolymers of PEG and PCL have been developed to produce nanoparticles or micelles, which have shown promising in vitro and in vivo results as drug delivery systems (18–21).

The aim of the present study was to prepare DOX-loaded and DOX-conjugated mPEG-b-PCL micelles and compare their anticancer effects on MDA-MB-231 breast cancer cells. To develop pH responsive controlled delivery systems, DOX-conjugated micelles (DOX_{conj}-M) were synthesized using hydrazide-functionalized mPEG-b-PCL, which was covalently linked to DOX via pH-sensitive hydrazone bonds. In parallel, DOX-loaded micelles (DOX_{ld}-M) were prepared using unmodified PEG-b-PCL copolymer. The

physicochemical properties of the micelles, as well as their cytotoxic and apoptotic effects on MDA-MB-231 cells, were systematically evaluated and compared.

MATERIAL and METHOD

Materials

Adriamycin (trade name of DOX) was purchased from Deva Holding (Turkey) as lyophilized powder of 10 mg of Doxorubicin hydrochloride in injection vials. ε-caprolactone (ε-CL) was purchased from Acros Organics, USA. Methoxy poly(ethylene glycol) (mPEG) (Mn=5000 Da), tin(II) 2-ethylhexanoate, succinic anhydride, dimethylaminopyridine, dicyclohexylcarbodiimide, N-hydroxysuccinimide, hydrazine hydrate, glycine, sodium bicarbonate, trinitrobenzene sulfonic acid (TNBSA), polyvinyl alcohol, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethylsulfoxide (DMSO), paraformaldehyde, crystal violet, and oil red O were the products of Sigma-Aldrich, Germany. Tetrahydrofuran, diethyl ether, methanol, trifluoroacetic acid, concentrated sulfuric acid, acetone, and ethanol were purchased from Merck, Germany. Leibovitz's L-15 medium, fetal bovine serum (FBS), gentamicin, and penicillin-streptomycin were the products of Biological Industries, Israel.

Methods

Synthesis and Characterization of Hydrazide Functionalized mPEG-b-PCL Copolymer (mPEG-b-PCL-CO-NH-NH_2)

mPEG-b-PCL copolymer was synthesized by ring-opening polymerization of ε-caprolactone (CL) according to the method previously reported by our group (22). In our previous study, the hydrazide functionalized copolymer was synthesized in two steps (23):

1. Introduction of a carboxylic acid group at the PCL end of the copolymer using succinic anhydride in the presence of dimethylaminopyridine (DMAP) and tetrahydrofuran (THF).

2. Subsequent incorporation of the hydrazide group at the terminal carboxylic acid moiety using dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), and THF.

The synthesized copolymer was characterized with ^{1}H NMR, and FT-IR. The primary amine amount was calculated with trinitrobenzene sulfonic acid assay as previously described (23).

Conjugation of DOX to mPEG-b-PCL-CO-NH-NH_2

DOX was conjugated to mPEG-b-PCL-CO-NH-NH_2 following the method reported by our group (23). Briefly, both mPEG-b-PCL-CO-NH-NH_2 copolymer and DOX were dissolved in methanol, a drop of TFA was added, and the solution was incubated overnight at 60°C. After methanol removal under vacuum, the residue was suspended in water and dialyzed against deionized water at RT. The DOX-conjugated copolymer (mPEG-b-PLC-CO-NH-NH_2-DOX) was obtained after lyophilization. Conjugation efficiency was calculated using a calibration curve constructed for DOX, following its dissolution in concentrated sulfuric acid at RT for 5 min (23).

Critical Micelle Concentration (CMC) of DOX Conjugated mPEG-b-PCL-CO-NH-NH_2

The CMC of DOX-conjugated copolymers was determined using pyrene as a fluorescent probe. For this purpose, 20 mg of polymer was dissolved in 5 mL of THF, followed by the addition of 40 mL of deionized water. The solution was left in a fume hood for THF evaporation. Pyrene solutions of varying concentrations ($0.5x10^{-3}$ - 0.5 g/L) in 0.6 mM acetone were prepared. Subsequently, 10 µL of pyrene solutions were added to 1 mL of polymer solutions and the mixtures were incubated at 37°C overnight. Excitation spectra were recorded between 250-360 nm at a fixed emission wavelength of 390 nm with a 1 nm bandwidth using a microplate reader. The intensity ratio at 338 and 333 nm (I_{338}/I_{333}) was plotted

against the logarithm of polymer concentrations. CMC was determined from the intersection of the two tangent plots (24).

Preparation of DOX Conjugated (DOX_{conj}-M) and DOX Loaded (DOX_{ld}-M) Micelles

Micelles were prepared using co-solvent evaporation method (25). For DOX_{conj}-M, 30 mg of mPEG-b-PCL-CO-NH-NH_2-DOX copolymer was dissolved in 0.5 mL of acetone. For DOX_{ld}-M, 30 mg of mPEG-b-PCL-CO-NH-NH_2 copolymer and 3 mg of DOX were dissolved in 0.5 mL of acetone containing 5 µL of triethylamine. To each solution, 3 mL of 1% PVA was added dropwise under vigorous agitation. The resulting micelle suspensions were mixed at 1100 rpm for 5 h to allow solvent evaporation and micelle formation. Micelles were collected by centrifugation at 14,000 rpm for 20 min at 4°C. For purification, micelles were washed twice by resuspension in distilled water and centrifugation. The final micelles were suspended in 5 mL of distilled water, passed through a 0.22 µm syringe filter, and freeze-dried (FreeZone 6 Plus, Labconco Corp., USA) for 24 h before storage in dry form. Before freeze-drying, the hydrodynamic diameter and zeta potential of these micelles (M) (having no drugs) were determined using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., MA). All measurements were performed in triplicates (n=3) to ensure reproducibility.

Encapsulation efficiency and drug loading capacity of DOX_{conj}-M and DOX_{ld}-M were calculated using the equations given below. Briefly, weighed amounts of freeze-dried micelles were dissolved in concentrated sulfuric acid, and DOX absorbance was measured at 543 nm using a UV-visible spectrophotometer (Hitachi U-2800A, Hitachi Ltd., Japan). DOX content was quantified using the previously established calibration curve (23). Blank micelles (without DOX) dissolved in concentrated sulfuric acid were used as background controls. All measurements were performed in triplicates (n=3) to ensure reproducibility.

$$Encapsulation\ efficiency\ (\%) = \frac{(The\ amount\ of\ drug\ inside\ the\ micelles)}{(The\ initial\ amount\ of\ drug\ added)} \times 100$$

$$Drug\ loading\ capacity\ (\%) = \frac{(The\ amount\ of\ drug\ inside\ the\ micelles)}{(The\ amount\ of\ micelles)} \times 100$$

Release of DOX from DOX_{conj}-M or DOX_{ld}-M

The release of DOX from DOX_{conj}-M or DOX_{ld}-M micelles was evaluated in three different media with pH values of 7.4, 6.8 and 5.0. For this study, 10 mg of DOX_{conj}-M or DOX_{ld}-M was suspended in 1 mL of phosphate-buffered saline (PBS, 0.1 M and pH 7.4) or 1 mL of citrate-phosphate buffer (CPB, 0.15 M; pH 5.0 or pH 6.8) (n = 6). Free DOX was dispersed in PBS-7.4 or CPB-5.0 or CPB-6.8 (1 mg DOX/ mL) and used as control groups. Micelle solutions and free DOX solutions were transferred into dialysis bags (MWCO is 3500 Da; Spectrum Laboratories, USA), placed in 4 mL of the corresponding release medium, and incubated in a thermostatic shaker (37°C, 80 rpm) (Nüve ST-30, Türkiye). At predetermined intervals, 2 mL of the release media was withdrawn and replaced with fresh buffer. Collected samples were freeze-dried, dissolved in ethanol, and analyzed using a microplate reader (SpectraMax iD3, Molecular Devices, USA) at an excitation wavelength of 480 nm and an emission wavelength of 590 nm. The amount of DOX in the release medium was quantified using a calibration curve constructed from DOX solutions in ethanol. To investigate the release mechanism, the data were fitted to zero order (cumulative percent release of DOX versus time plot), first order (log cumulative percent of DOX remaining versus time plot), Higuchi model (cumulative percent of DOX release versus square root of time plot), and Korsmeyer-Peppas (log cumulative percent release of DOX versus log time plot) kinetic models.

In Vitro Cell Culture Experiments

Determination of IC_{50} of DOX on MDA-MB-231 Cells

The cytotoxic effect of different concentrations of DOX on MDA-MB-231 triple negative breast cancer cells

was evaluated to determine the IC_{50} value. Cells were cultured in Leibovitz's L-15 medium supplemented with 15% FBS, 0.1% gentamicin and 0.1% penicillin-streptomycin at 37°C in a 5% CO_2 atmosphere. Then, cells were seeded in 96-well plates at a density of 2500 cells/well in 100 µL medium and allowed to adhere overnight. The following day, the medium was replaced with fresh medium containing DOX at concentrations ranging from 0 nM to 90 nM (prepared from 400 µM DOX stock solution) in 0.4% water, and cells were incubated for 48 hours. Subsequently, 10 µL of MTT solution (5 mg/mL) was added to each well., cells treated with 0.4% water-containing medium were used as the negative control. After 4 h of incubation, the solutions were discarded, and the formazan crystals were solubilized in 50 µL DMSO for 20 min on an orbital shaker. The solutions were then transferred to a new 96-well plate, and absorbance was measured at 570 nm (SpectraMax iD3, Molecular Devices, USA). Cell viability was calculated as the percentage of the negative control, and the IC50 value was calculated using GraphPad Prism 8 (GraphPad Software, USA) (n=4).

Cytotoxicity of DOX_{conj}-M and DOX_{ld}-M on MDA-MB-231 Cells

MDA-MB-231 triple negative breast cancer cells treated with DOX_{conj}-M, or DOX_{ld}-M were incubated at 37°C in 5% CO2 atmosphere to compare their cytotoxic effects. Empty micelles without any drug (M) were also used to show cytocompatibility. Cells were seeded in 96-well plates at a density of 2500 cells/well in 100 µL medium and allowed to adhere overnight. The next day, the medium was replaced with fresh medium containing micelles (1.5 mg/mL) dispersed in complete medium. Free DOX containing medium was used to check the effectiveness of micelles compared to free drug. Cytotoxicity was evaluated by MTT assay, as described previously. Cells incubated in pure medium served as positive controls and were considered 100% viable (n=4).

Determination of Cell Internalization of Micelles

The intrinsic fluorescence of DOX was used to assess the internalization of free DOX, DOX_{conj}-M, and DOX_{ld}-M in MDA-MB-231 cells. Cells were seeded on coverslips at a density of 1x105 cells/well in 1 mL medium and allowed to adhere overnight. The next day, the medium was replaced with fresh medium containing free DOX (80 nM), DOX_{conj}-M (1.5 mg/mL), or DOX_{ld}-M (1.5 mg/mL) in complete medium. Samples were incubated for 1, 2, 4 and 6 h at 37ºC. At each time point, cells were washed with cold PBS, fixed with 4% paraformaldehyde (w/v) at RT, and stained with DAPI. Internalization was observed by confocal microscopy (Zeiss Cell Observer SD, Germany).

Cell Migration Assay

Cell migration was assessed using transwell chambers for 24-well plates with 8.0 µm pores (Greiner, Austria) (26). MDA-MB-231 cells were seeded in the inserts at a density of $6x10^4$ cells/insert in 200 µL of serum-free medium containing free DOX (80 nM), M (1.5 mg/mL), DOX_{conj}-M (1.5 mg/mL), or DOX_{ld}-M (1.5 mg/mL). Untreated cells were used as the control group. The lower chambers were filled with 500 µL serum containing medium. After 48 h, non-migrated cells were removed with a cotton swab, while migrated cells were fixed with 4% paraformaldehyde (w/v) and stained with 0.2% crystal violet (w/v). Chambers were washed with water and left to dry. Images were acquired using an inverted phase-contrast microscope (Nikon Eclipse TS100, Nikon Corp, USA), and migrated cells were quantified in three random fields using ImageJ software. The percentage of migrated cells was normalized with respect to the control (n=3).

Colony Formation Assay

Colony formation was evaluated after treatment with M, DOX_{conj}-M, or DOX_{ld}-M. MDA-MB-231 cells were seeded in 6-well plates at a density of 2000 cells/well and cultured for 24 h. Then, cells were treated with free DOX (80 nM), M (1.5 mg/mL), DOX_{conj}-M (1.5 mg/

mL), or DOX_{ld}-M (1.5 mg/mL) for one week. Thereafter, colonies were fixed with 4% paraformaldehyde (w/v) for 20 min, stained with 0.2% crystal violet (w/v), and counted manually. The experiment was performed in triplicates (n=3) (26). Untreated cells were used as controls.

Determination of Lipid Droplets in MDA-MB-231 Cells

To assess apoptosis, lipid droplet formation was evaluated in cells treated with free DOX, M, DOX_{conj}-M, or DOX_{ld}-M. Cells were seeded in 24-well plates at a density of $1x10^5$ cells/well in 1 mL medium and allowed to adhere overnight. The next day, cells were treated with free DOX (80 nM), M (1.5 mg/mL), DOX_{conj}-M (1.5 mg/mL), or DOX_{ld}-M (1.5 mg/mL) in complete medium and incubated for 48 h. Cells were then washed with PBS, and fixed with 4% paraformaldehyde (w/v) for 20 min. After removing the paraformaldehyde, fixed cells were treated with 60% isopropanol for 5 min. Lipid droplets were stained with Oil Red O for 30 min, followed by counterstaining with Weigert's hematoxylin for 10 min. Finally, cells were washed three times with water, and lipid droplets were visualized and photographed using an inverted phase-contrast microscope (Nikon Eclipse TS100, Nikon Corp, USA) (27).

Detection of Apoptosis in Cancer Cells with qRT-PCR

Apoptotic gene expression was evaluated in MDA-MB-231 cells treated with M, DOX_{conj}-M, or DOX_{ld}-M. Cells were seeded in 6-well plates at a density of $1x10^6$ cells/well in 3 mL medium and allowed to adhere overnight. The following day, cells were treated with free DOX (80 nM), M (1.5 mg/mL), DOX_{conj}-M (1.5 mg/mL), or DOX_{ld}-M (1.5 mg/mL) and incubated for 24 h. Untreated cells were used as controls. Total RNA was extracted using the Roche High Pure RNA Isolation Kit (Switzerland), and cDNA was synthesized with the Roche Transcriptor High Fidelity cDNA Synthesis Kit (Switzerland), following the manufacturer's instructions. qRT-PCR was performed using LightCycler® FastStart DNA Master SYBR Green I (Switzerland). The relative expression of pro-apoptotic (Bax, and p53) and anti-apoptotic (Bcl-2, and Bcl-xL) genes was quantified relative to the reference ß-actin gene using ΔΔCT method. All measurements were performed in triplicates (n = 3) to ensure reproducibility. Primer sequences are listed in Table 1.

Table 1. Primers used in qRT-PCR studies

Primer	Sequence
Bax F	CCCGAGAGGTCTTTTTCCGAG
Bax R	CCAGCCCATGATGGTTCTGAT
p53 F	CCTCAGCATCTTATCCGAGTGG
p53 R	TGGATGGTGGTACAGTCAGAGC
Bcl-2 F	GGTGGGGTCATGTGTGTGG
Bcl-2 R	CGGTTCAGGTACTCAGTCATCC
Bcl-xL F	GAGCTGGTGGTTGACTTTCTC
Bcl-xL R	TCCATCTCCGATTCAGTCCCT
β-*actin* F	ATGTGGCCGGAGGACTTGATT
β-*actin* R	AGTGGGGTGGCTTTTAGGATG

F: Forward primer sequence, R: Reverse primer sequence.

Determination of Cell Apoptosis with Annexin V-FITC Assay

The apoptotic effects of M, DOX_{conj}-M, and DOX_{ld}-M on MDA-MB-231 cells were evaluated using an FITC Annexin V-PI kit. Briefly, cells were seeded in 6-well plates at a density of $1x10^6$ cells/well in 3 mL of complete medium and allowed to adhere overnight. The next day, the medium was replaced with fresh medium containing free DOX (80 nM), M (1.5 mg/mL), DOX_{conj}-M (1.5 mg/mL), or DOX_{ld}-M (1.5 mg/mL) and cells were incubated for 24 h. Cells treated with DOX at its IC_{50} value (80 nM) were used as the negative control. After incubation, the medium was discarded, and cells were washed with sterile PBS (0.1 M, pH 7.4), detached using a cell scraper, and collected by centrifugation at 2000 rpm for 5 min. Cells were then stained with FITC Annexin V and propidium iodide according to FITC Annexin V-PI kit protocol (BioLegend, USA). Apoptosis analysis was performed using flow cytometry (BD Accuri C6, USA). First, cells from each group were counted and dilutions were made to obtain $1x10^6$ cells/mL for each group. Data were gated to exclude debris using unstained cell suspensions. Cells stained with Annexin V-FITC and propidium iodide were analyzed, and the proportions of live and apoptotic cells were calculated relative to the untreated control group (n=3).

Mitochondrial Transmembrane Potential Detection

Changes in mitochondrial transmembrane potential ($\Delta\Psi m$) are an early hallmark of apoptosis. To assess this, 3,3'-Dihexyloxacarbocyanine iodide (DiOC6), a membrane permeable fluorescent probe that dyes mitochondria, was used. DiOC6 selectively stains mitochondria in healthy cells; however, in apoptotic cells with disrupted mitochondrial membrane integrity, DiOC6 uptake is impaired. MDA-MB-231 cells were seeded in 6-well plates at a density of $1x10^6$ cells/well in 3 mL of medium and allowed to adhere overnight. The next day, the medium was replaced with fresh medium containing free DOX (80 nM), M (1.5 mg/mL), DOX_{conj}-M (1.5 mg/mL), or DOX_{ld}-M (1.5 mg/mL) in complete medium, and cells were incubated for 24 h. Cells treated with DOX at its IC_{50} value (80 nM) were used as the negative control. After incubation, the medium was discarded, cells were washed with sterile PBS (0.1 M, pH 7.4), detached with a cell scraper, and collected by centrifugation. Cells were then incubated with 40 nM DiOC6 for 30 min, and analyzed by flow cytometry (BD Accuri C6, USA). First, cells from each group were counted and dilutions were made to obtain $1x10^6$ cells/mL for each group. Data were gated to exclude debris using unstained cell suspensions. Loss of mitochondrial transmembrane potential was determined by a reduction in the percentage of cells in the V1-R region compared with the control group (n=3) (28).

Reactive Oxygen Species (ROS) Determination

Intracellular ROS levels, which increase during apoptosis, were determined using the Cellular ROS Assay Kit (Abcam, UK). The fluorescent probe of 2',7'-Dichlorofluorescin diacetate (DCFDA) was employed to detect ROS in cells. MDA-MB-231 cells were seeded either on coverslips in 24-well plates at a density of $1x10^5$ cells/well in 1 mL medium for confocal microscopy imaging, or in 96-well plates at a density of 2500 cells/well in 100 µL medium for ROS quantification. After overnight adhesion, cells were treated with medium containing free DOX (80 nM), M (1.5 mg/mL), DOX_{conj}-M (1.5 mg/mL), or DOX_{ld}-M (1.5 mg/mL) in complete medium for 24 h. After that, the medium was discarded, and cells were incubated with 20 µM DCFDA solution for 30 min, followed by washing with PBS. Cells on coverslips were examined with confocal microscopy (Zeiss Cell Observer SD, Germany), while fluorescence intensity in 96-well plates was measured using a microplate reader (SpectraMax iD3, Molecular Devices, USA) (n=4). Medium containing 0.4% ethanol (v/v) was used as the negative control, while tert-butyl hydroperoxide (TBHP, 50 µM for 4 h) was used as the positive control prior to DCFDA staining.

Endothelial Cell Tube Formation Assay

The anti-angiogenic effects of the micelles were investigated using an endothelial cell tube formation assay (29). Human umbilical vein endothelial cells (HUVECs) were cultured in endothelial cell (EC) medium supplemented with 10% FBS. First, 50 µL of Matrigel solution (Corning, USA) was added to 96-well plates and incubated for 30-60 min at 37°C to allow polymerization. Then, $4x10^4$ HUVECs/well and/or $4x10^4$ MDA-MB-231 cells/well were seeded onto the Matrigel. Next, 150 µL of medium containing free DOX, DOX_{conj}-M, or DOX_{ld}-M was added, and cells were incubated for 2, 4, 8, and 24 h. Images were captured at each time point using an inverted microscope (Nikon Eclipse TS100, Nikon Corp, USA), and were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The total endothelial tube lengths and number of nodes were quantified using the Angiogenesis Analyzer plugin in ImageJ software. Results were averaged from three independent experiments (n=3). The experimental groups and media compositions are summarized in Table 2.

Statistical Analysis

All data are presented as mean ± standard deviation. Results were analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test.

Table 2. Groups for endothelial cell tube formation assay

Group name	Cells	Medium and treatment
Control-1	HUVEC	EC medium
Control-2	HUVEC	EC:L15 medium (1:1)
Free DOX-1	HUVEC	EC medium, free DOX
Free DOX-2	HUVEC	EC:L15 medium (1:1), free DOX
Co-culture control	HUVEC:MDA-MB-231	EC:L15 medium (1:1)
Co-culture free DOX	HUVEC:MDA-MB-231	EC:L15 medium (1:1), free DOX
HUVEC1-DM1	HUVEC	EC medium, DOX_{conj}-M
HUVEC1-DM2	HUVEC	EC medium, DOX_{ld}-M
HUVEC2-DM1	HUVEC	EC:L15 medium (1:1), DOX_{conj}-M
HUVEC2-DM2	HUVEC	EC:L15 medium (1:1), DOX_{ld}-M
HUVEC1-EM	HUVEC	EC medium, M
HUVEC2-EM	HUVEC	EC:L15 medium (1:1), M
Co-culture DM1	HUVEC:MDA-MB-231	EC:L15 medium (1:1), DOX_{conj}-M
Co-culture DM2	HUVEC:MDA-MB-231	EC:L15 medium (1:1), DOX_{ld}-M
Co-culture EM	HUVEC:MDA-MB-231	EC:L15 medium (1:1), M

RESULTS

Synthesis and Characterization of Hydrazide Functionalized mPEG-b-PCL Copolymer (mPEG-b-PCL-CO-NH-NH_2)

The synthesized copolymer was characterized with ^{1}H NMR, and FT-IR spectroscopy. The presence of primary amine groups, confirming the hydrazide functionalization of the copolymer, was detected as described previously (23). The hydrazide content of mPEG-b-PCL-CO-NH-NH_2 polymer was determined to be 45.6 mole% (100 moles of polymer contains 45.6 moles of -NH_2 as hydrazide group.) (23).

Conjugation of DOX to mPEG-b-PCL-CO-NH-NH_2

DOX conjugation was verified using ^{1}H NMR spectroscopy (23). The yield of DOX-conjugated polymer synthesis was 78.9%, with a conjugation efficiency of 36.04%, as reported in our previous study (23).

Critical Micelle Concentration (CMC) of DOX Conjugated mPEG-b-PCL-CO-NH-NH_2

Critical micelle concentration (CMC) is a key parameter for assessing the stability of micellar formulations. Pyrene was used as a fluorescent probe to determine the CMC. Upon micelle formation, the excitation wavelength of pyrene shifts from 333 nm to 338 nm and the fluorescence intensity ratio at 338 nm and 333 nm (I_{338}/I_{333}) was plotted against the logarithm of polymer concentration (24). In our previous study, the CMC values of mPEG-b-PCL, and mPEG-b-PCL-CO-NH-NH_2 copolymers were found as 14.1×10^{-3} mg/mL, and 9.8×10^{-3} mg/mL, respectively (30). The CMC of DOX-conjugated mPEG-b-PCL-CO-NH-NH_2 copolymers was found as 7.1×10^{-3} mg/mL (Figure 1A). These results indicate that increased hydrophobicity in the copolymer decreases the CMC value, thereby enhancing the stability of micellar formulations.

Characterization of DOX Conjugated Micelles (DOX_{conj}-M) and DOX Loaded Micelles (DOX_{ld}-M)

DOX_{conj}-M were obtained from covalently DOX-conjugated copolymers, while DOX_{ld}-M were prepared from copolymers using the co-solvent evaporation method without sonication. All micelles were characterized by hydrodynamic diameter and zeta potential measurements. Figure 1B shows the size distribution of micelles. The hydrodynamic mean diameter of DOX_{conj}-M (Figure 1B) was 121.6±16.3 nm (n=3) with a polydispersity index (PDI) of 0.206, and the zeta potential was -6.61±1.49 (n=3). In contrast, DOX_{ld}-M had a hydrodynamic diameter of 265.3±25.6 nm (n=3) (Figure 1C), a PDI of 0.353, and a zeta potential of -10.75±0.78 (n=3). TEM micrographs (Figure 1D and 1E) confirmed that the micelles were uniform and spherical in shape. Compared to DOXconj-M, DOXld-M exhibited a larger hydrodynamic diameter, higher PDI, and more negative zeta potential.

The drug loading capacities of the micelles were found as 13.54% (±1.13) for DOX_{conj}-M (n=3), and 8.62% (±0.47) DOX_{ld}-M (n=3). Meanwhile, the encapsulation efficiency (EE) of DOX in DOX_{ld}-M was calculated as 83.32% (±4.54). The lower drug loading capacity of DOX_{ld}-M may be attributed to the micelle preparation technique, which affects stability and consequently the drug loading capacity of the micelles (31). For example, the use of water or polyvinyl alcohol (PVA) in the aqueous phase during preparation influences micelle stability. In most cases, the addition of PVA, which acts as a surfactant and reduces surface tension, enables the formation of more stable micelles compared to using pure water (31).

Moulahoum et al. (2022) reported DOX loading into micelles prepared from carboxylic acid-terminated mPEG-b-PCL copolymers. They achieved 38% EE with micelles having a hydrodynamic diameter of 145.6 nm (PDI = 0.386) and a zeta potential of -37.2 mV (32). In comparison, the micelle sizes obtained in our study differed: DOX_{conj}-M were smaller while DOX_{ld}-M were

larger than those reported. Furthermore, the zeta potentials of both micelles (DOX_{conj}-M, and DOX_{ld}-M) in our study were lower than the literature values, likely due to differences in the copolymers used. Specifically, Moulahoum et al. (2022) synthesized a copolymer with a molecular weight of 2510 Da (32), whereas the copolymer in our study had a molecular weight of 16 kDa. On the other hand, the EE of DOX_{ld}-M in our study was higher than previously reported.

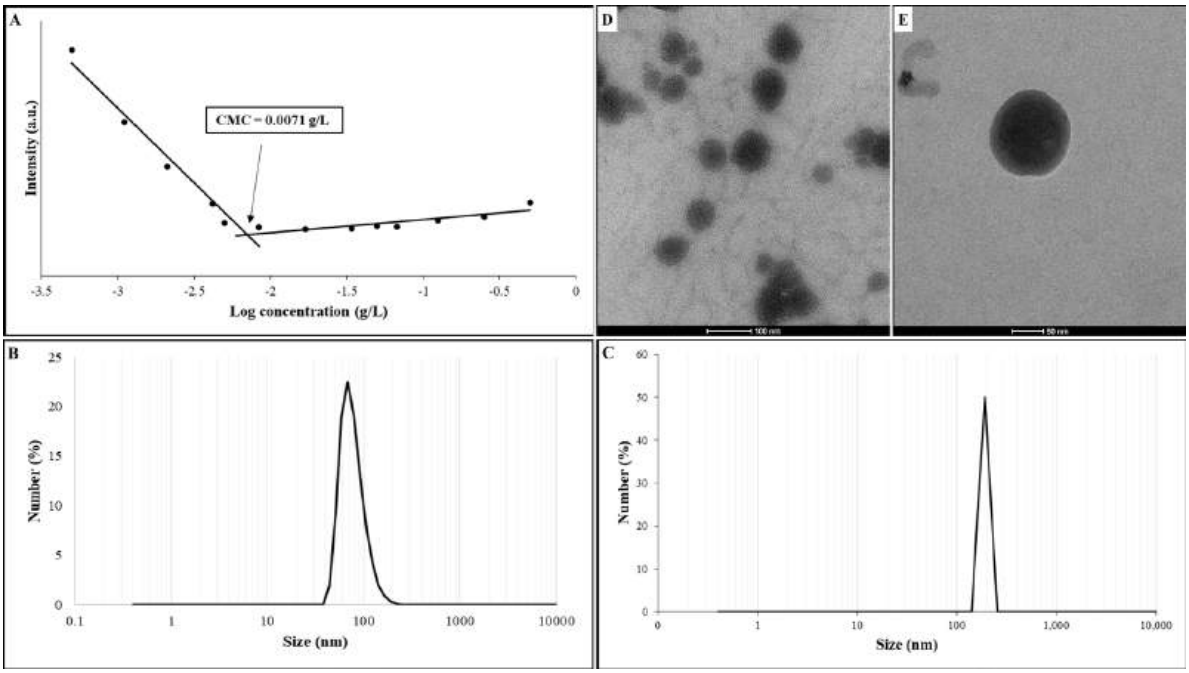

Figure 1. A) Plot of intensity ratio (I_{338}/I_{333}) of the excitation spectra of pyrene against log concentration of DOX conjugated copolymers (DOX_{conj}-M). The CMC of DOX-conjugated mPEG-b-PCL-CO-NH-NH_2 copolymers was found as 7.1×10^{-3} mg/mL. Size distribution charts of B) DOX_{conj}-M (121.6±16.3 nm, n=3) and C) DOX_{ld}-M (265.3±25.6 nm, n=3) prepared with 1% PVA – acetone. TEM images of D) DOX_{conj}-M, and E) DOX_{ld}-M prepared by co-solvent evaporation method without sonication using 1% PVA as water phase.

Release of DOX from DOX_{conj}-M or DOX_{ld}-M

In acidic media, the DOX release rate was higher for both DOX_{conj}-M and DOX_{ld}-M compared to neutral media (Figure 2).

For DOX_{conj}-M, no significant difference was observed between the release profiles at pH 7.4 and pH 6.8 in the first 2 days (Figure 2A). However, after this period, a significant increase in the release rate was observed at pH 6.8 ($p<0.05$). Moreover, DOX release was significantly faster at pH 5.0 than at the other tested conditions after 2 h ($p<0.05$). This shows that pH sensitive hydrazone bonds break in acidic environments, thereby increasing the amount of DOX released.

For DOX_{ld}-M, no significant difference was observed between the release profiles up to 2 h. Afterward, the DOX release rate significantly increased at pH 5.0 and pH 6.8 compared to physiological pH ($p<0.05$) (Figure 2B). Moreover, after 5 h, the release was significantly higher at pH 5.0 compared to pH 6.8 ($p<0.05$). This may be attributed to the increased solubility of DOX in acidic media.

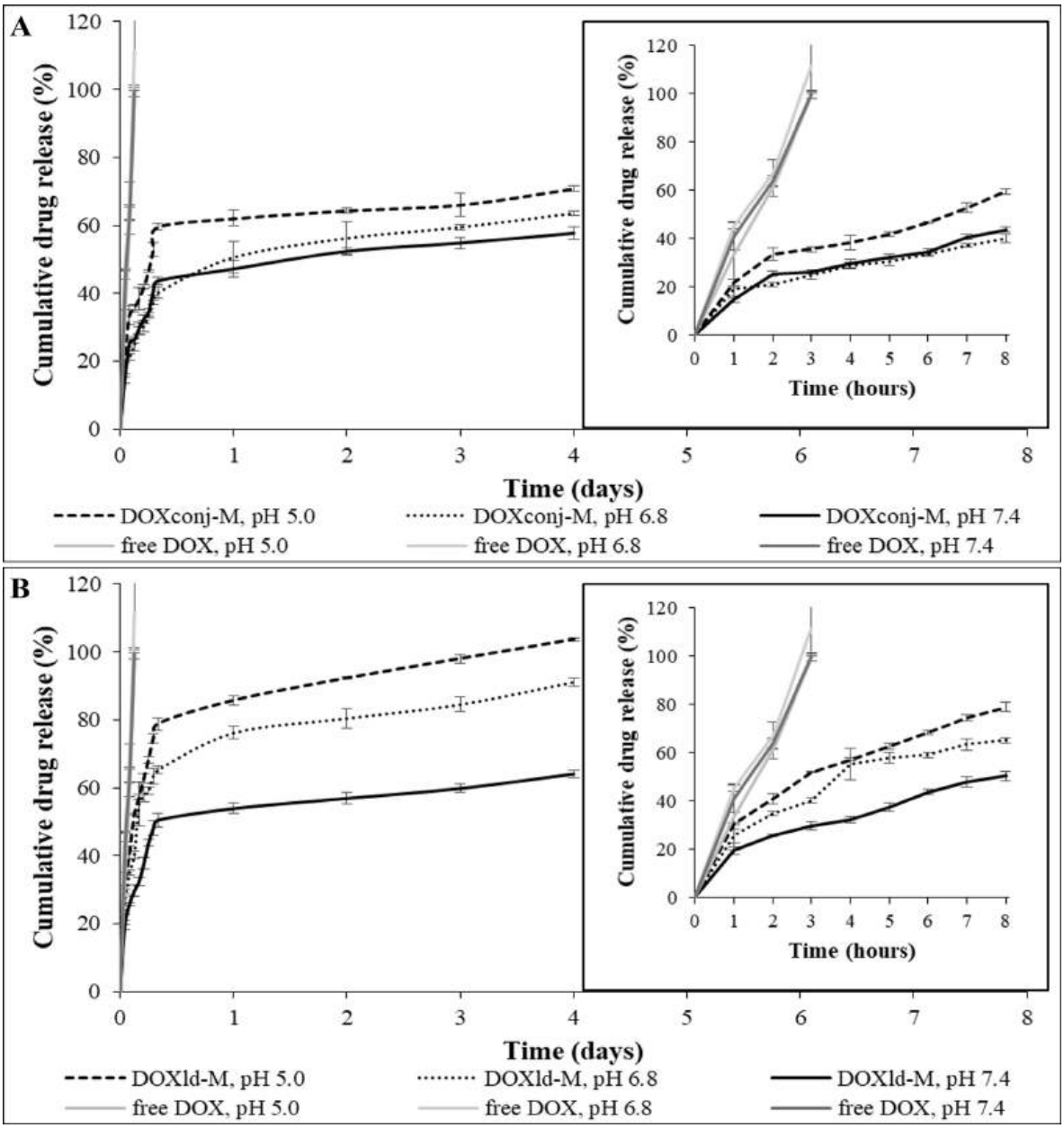

Figure 2. Cumulative drug release profiles from dialysis membrane of A) free DOX and DOX_{conj}-M; B) free DOX and DOX_{ld}-M under physiological conditions (PBS, 0.1 M and pH 7.4) and acidic conditions (CPB-pH 5.0 and CPB-pH 6.8; 0.15 M) at 37°C (n=6).

The release of free DOX through a dialysis membrane was also examined, showing much faster release compared to micelle formulations. Similar results showing fast release in acidic conditions were reported in the literature. For example, Liao et al. (2021) conjugated DOX to poly(2-(diethylamino) ethyl methacrylate)-polypropargyl methacrylate via hydrazone bonds and prepared micelles from the conjugated polymer. In acidic environments, the diethylamino groups of poly(2-(diethylamino) ethyl

methacrylate) become protonated, facilitating faster DOX release, while hydrazone bonds are also cleaved under acidic conditions. Thus, they achieved a dual pH responsive system combining hydrazone-bonded DOX and charge-reversible polymers. They reported DOX release rates of 63.43% at pH 5.0, 28.48% at pH 6.8, and 13.18% at pH 7.4 after 48 h (33).

In our study, higher burst release was observed in DOX_{ld}-M compared to DOX_{conj}-M. Within the first 8 h at pH 5.0, DOX release values were 60% for DOX_{conj}-M and 79% for DOX_{ld}-M. At pH 6.8, the release was 40% for DOX_{conj}-M and 61% for DOX_{ld}-M, while at pH 7.4, it was 41%, and 51%, respectively. The slower release of DOX_{conj}-M is likely due to the covalent attachment of DOX via hydrazone bonds, which reduces the release rate. After 48 h, 52% and 64% of DOX were released from DOX_{conj}-M at physiological pH and pH 5.0, respectively, compared to 57% and 92% from DOX_{ld}-M. At pH 5.0, complete DOX release (100%) occurred from DOX_{ld}-M within 4 days, whereas only ~70% release was achieved from DOX_{conj}-M (Figure 2).

Qi et al. (2018) prepared DOX-loaded pH-sensitive micelles with PEG-dihydrazone-PLA. They also prepared DOX-loaded PLA-PEG-PLA micelles to compare the effectiveness of dihydrazone bonds under acidic conditions. Their results showed that at pH 7.4, DOX release was 40% from PLA-PEG-PLA micelles and 38% from PEG-dihydrazone-PLA micelles, while at pH 5.0 the values were 40% and 75%, respectively. The reported results showed a pH sensitive drug delivery system with dihydrazone bonds (34).

Table 3. *In vitro* release kinetic parameters of DOX_{conj}-M and DOX_{ld}-M.

Micelles	Release Kinetic Models		pH 7.4	pH 6.8	pH 5.0
DOX_{conj}-M	Zero order	K_0	0.617	0.683	0.557
		R^2	-1.387	-1.741	-3.210
	First order	K_1	-0.009	-0.008	-0.006
		R^2	0.616	0.679	0.933
	Higuchi model	K_H	7.8552	0.111	9.885
		R^2	0.240	0.778	-0.028
	Korsmeyer-Peppas model	K_P	0.163	0.198	0.199
		n (exponent of release)	0.232	0.203	0.130
		R^2	0.926	0.951	0.478
DOX_{ld}-M	Zero order	K_0	0.739	1.012	1.140
		R^2	-1.669	-1.577	-0.794
	First order	K_1	-0.008	-0.008	-0.10
		R^2	0.612	0.598	0.919
	Higuchi model	K_H	8.791	0.069	14.225
		R^2	0.097	0.647	0.095
	Korsmeyer-Peppas model	K_P	0.208	0.278	0.254
		n (exponent of release)	0.215	0.222	0.238
		R^2	0.920	0.913	0.518

Our findings similarly showed that DOX release was faster under acidic conditions, and DOX conjugation (DOX_{conj}-M) resulted in slower drug release compared to DOX loading (DOX_{ld}-M). These results confirm the pH-responsive nature of DOXconj-M due to hydrazone bonds, which cleave in acidic environments, as expected. DOXld-M also showed pH-sensitive behavior, likely due to the higher solubility of DOX under acidic conditions compared to physiological conditions.

The release data were fitted to various kinetic models to determine the most suitable model for describing the release profile of DOX from the micelles. The rate constants (K0, K1, KH, and KP for zero-order, first-order, Higuchi, and Korsmeyer-Peppas models, respectively), the release exponent (n) values, and coefficient of determination (R2) were presented in Table 3. The Korsmeyer-Peppas model, which shows that drug release occurs from a polymeric system, provided the best fit for the release profiles of both micelles at pH 7.4 (PBS, 0.1 M and pH 7.4) and pH 6.8 (CPB, 0.15 M and pH 6.8). In contrast, the first-order model, which reflects a faster release behavior, was the best fit for the release of DOX from both micelles under acidic conditions (CPB, 0.15 M and pH 5.0).

In Vitro Cell Culture Experiments

Determination of IC_{50} of DOX on MDA-MB-231 Cells

Figure 3A presents the cell viability results of MDA-MB-231 cells. The IC50 value of DOX was calculated using GraphPad Prism 8 software and was determined to be 79.98 ± 4.37 nM. In the literature, IC50 value

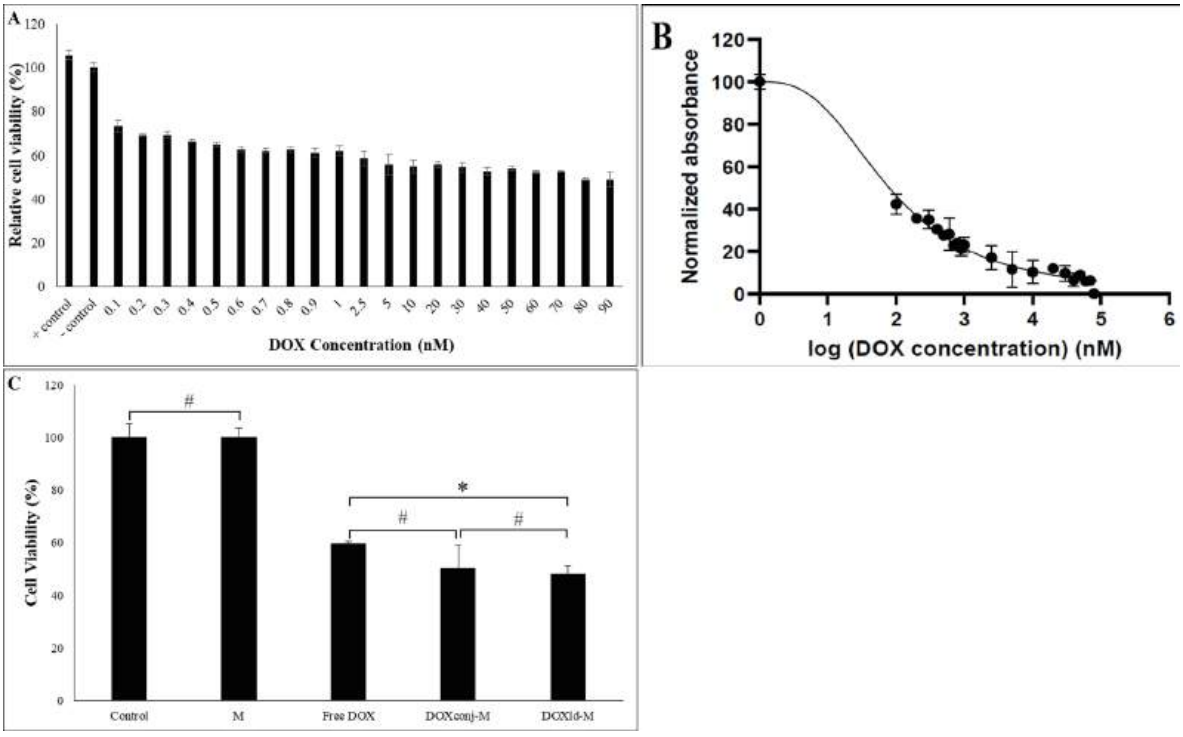

Figure 3. A) Cell viability results of MDA-MB-231 cells after treatment with different concentrations of DOX (n=4). B) Normalized absorbance vs. DOX concentration charts of MDA-MB-231 cells after data fitting using GraphPad Prism 8 software. C) Cytotoxic effect of DOX_{conj}-M and DOX_{ld}-M compared to free DOX (80 nM) on MDA-MB-231 cells (n=4). * shows the significant difference between the groups and # shows the non-significant difference between the groups ($p<0.05$; n=4)

of DOX on MDA-MD-231 cells has been reported as 1262 nM (35). The lower IC50 value observed in our study may be attributed to differences cell passage numbers or variations in cell line characteristics during culture. Figure 3B shows the normalized absorbance versus DOX concentration curves for MDA-MB-231 cells. The coefficient of determination (R^2) was 0.9575, indicating that the data closely fit the regression line (Figure 3B).

Cytotoxicity of DOX_{conj}-M and DOX_{ld}-M on MDA-MB-231 Cells

The viability of MDA-MB-231 cells was assessed after treatment with DOX_{conj}-M and DOX_{ld}-M to examine the effects of different drug loading mechanisms in the micelles (Figure 3C). The results showed that both DOX_{conj}-M and DOX_{ld}-M reduced cell viability, with no significant difference between the two groups. Although 1.5 mg/mL of DOX_{conj}-M and DOX_{ld}-M contained 374 µM and 238 µM of DOX, respectively, this did not result in a difference in cell viability. This outcome is consistent with the IC_{50} study of free DOX, where increasing the DOX concentration above the IC_{50} (80 nM) did not further reduce cell viability.

Cancer cells possess several mechanisms of drug resistance, including drug efflux. Due to the high mutation rate in cancer cells, multidrug resistance transporter proteins may actively expel drugs from the cell to prevent cytotoxicity (36,37). When DOX enters the cell via endocytosis, it can be recognized and removed by efflux mechanisms. Although the MDA-MB-231 cells used in this study are not classified as DOX resistant, prolonged passage numbers such as 25–30 may have contributed to acquired resistance. Ciocan-Cartita et al. (2020) studied the effect of passage number on mutagenicity of MDA-MB-231 cells and showed that mutations increased in passages 12 and 24 compared to passage 0. They also showed that increasing number of passages increased expression of drug resistance genes (38).

In our study, DOX_{ld}-M caused a significant decrease in cell viability compared to free DOX. However, DOX conjugation or loading had similar effects on MDA-MB-231 cells. DOX_{conj}-M had a higher drug loading capacity but a slower release rate than DOX_{ld}-M, which may explain the comparable cell viability observed for these groups.

Determination of Cell Internalization of Micelles

Cell internalization of free DOX began after 1 h and reached maximum levels at 4 h (Figure 4A). Similarly, DOX_{conj}-M was internalized after 1 h and reached peak internalization at 4 h (Figure 4B). Figure 4C shows the internalization of DOX_{ld}-M, which localized in the cell nuclei within 1 h. Overlap of DOX fluorescence with DAPI (dye for cell nuclei) staining in the images resulted in a purple coloration of the nuclei.

DOX_{ld}-M showed a different internalization pattern compared to DOX_{conj}-M and free DOX. As shown in the release studies, DOX release from DOX_{ld}-M was faster than from DOX_{conj}-M suggesting that after internalization, DOX_{ld}-M can deliver more DOX into the nuclei. Similar observations were reported by Cai et al. (2014) using DOX-conjugated hyaluronan nanoparticles (39) and Okur et al. (2016) with DOX-loaded PEG-dextran nanoparticles (40). These studies both reported cell nucle localization of DOX loaded nanoparticles. It is also known that DOX cause DNA damage in cells (41,42), so cell nuclei internalization of DOX_{ld}-M shows that these micelles may have high therapeutic potential in breast cancer treatment.

Cell Migration Assay

To simulate cancer cell migration in vitro, a transwell migration assay was carried out (Figure 4D and 4E). The results showed that the cell number at the bottom of the transwell decreased after treatment with free DOX, DOX_{conj}-M, and DOX_{ld}-M. While the cell numbers were similar for DOX_{conj}-M and DOX_{ld}-M treatments, both groups exhibited a significantly greater inhibition of migration compared to free DOX.

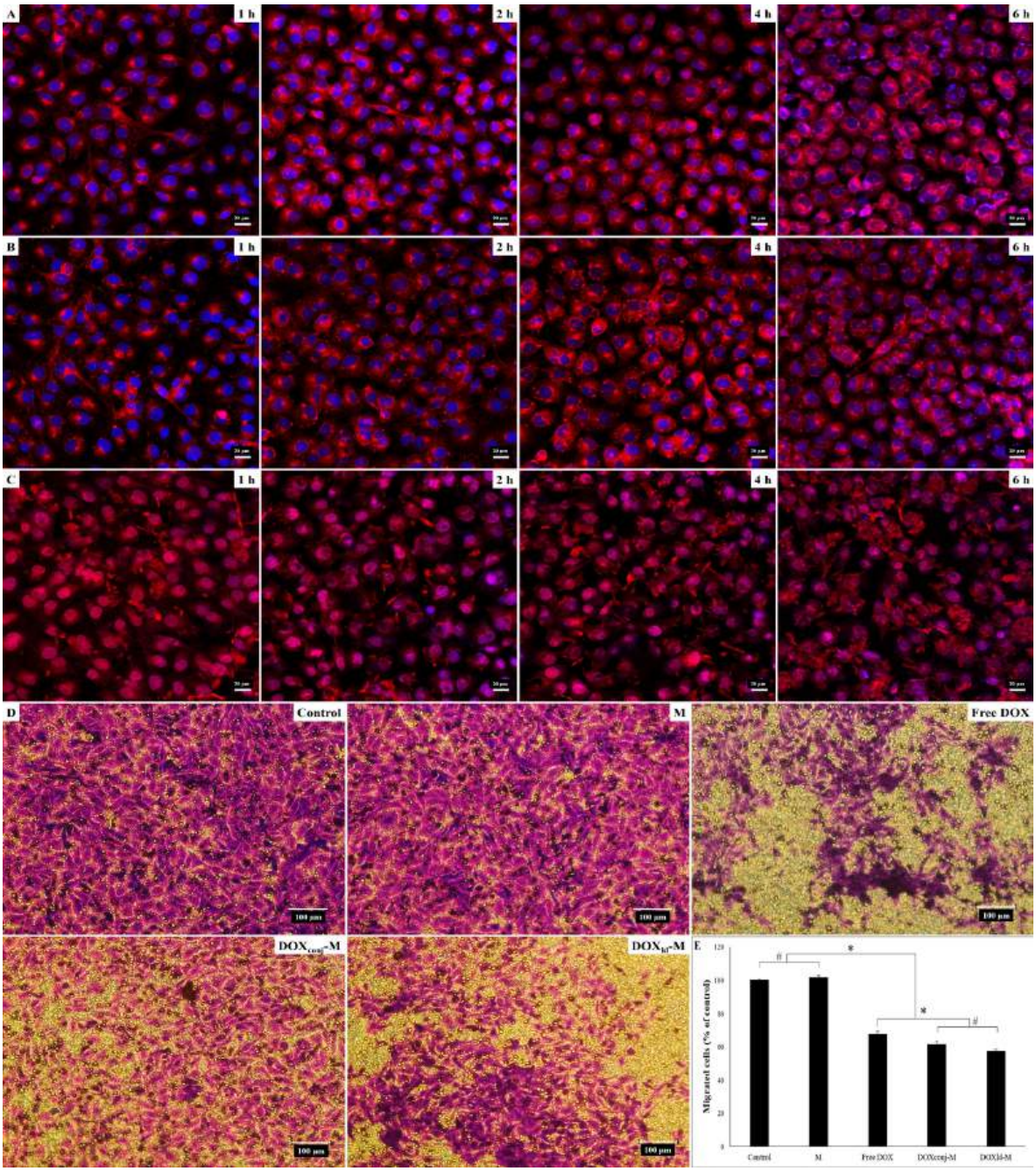

Figure 4. Cell internalization after 1h, 2h, 4h and 6h of A) free DOX, B) DOX_{conj}-M, and C) DOX_{td}-M. Cell nuclei was stained with DAPI. Blue is DAPI, and red is DOX in images. D) Images of MDA-MB-231 cells migrated from the top of the transwell to the bottom of the transwell after treatment. E) Normalized plot of migrated cells after transwell migration assay. IC_{50} concentration of DOX (80 nM) was used in free DOX group. Transwell migration assay was carried out for 48 hours and then, cells were fixed with 4% paraformaldehyde and stained with 0.2% crystal violet. After that, cells were counted from three independent experiments. * shows the significant difference between the groups and # shows the non-significant difference between the groups ($p<0.05$; n=3).

Colony Formation Assay

To evaluate the anti-proliferative effects of micelles (DOX_{conj}-M, and DOX_{ld}-M), a colony formation assay was carried out (Figure 5A). The results showed that both micelle formulations showed similar anti-proliferative effects to free DOX, while the control (untreated) and M (empty micelles) groups displayed the highest proliferation. Xie et al. (2019) co-loaded DOX and miR-34a into micelles prepared from polyethylenimine-polycaprolactone and gadolinium(III)-conjugated polyethyleneglycol-polycaprolactone. In their study, colony formation assays on MDA-MB-231 cells showed that micelles containing 15 μM DOX reduced colony numbers by approximately 30%, whereas micelles containing both 15 μM DOX and miR-34a reduced colony numbers by approximately 55% (43).

In our study, DOX_{conj}-M and DOX_{ld}-M contained 374 μM and 238 μM DOX, respectively, which are considerably higher amounts of DOX compared to the micelles prepared by Xie et al. (2019). Consistently, both DOX_{conj}-M and DOX_{ld}-M decreased the colony-forming ability of MDA-MB-231 cells by 50%. This enhanced inhibition may be attributed to the higher DOX content of our micelles, despite the absence of miR-34a co-loading.

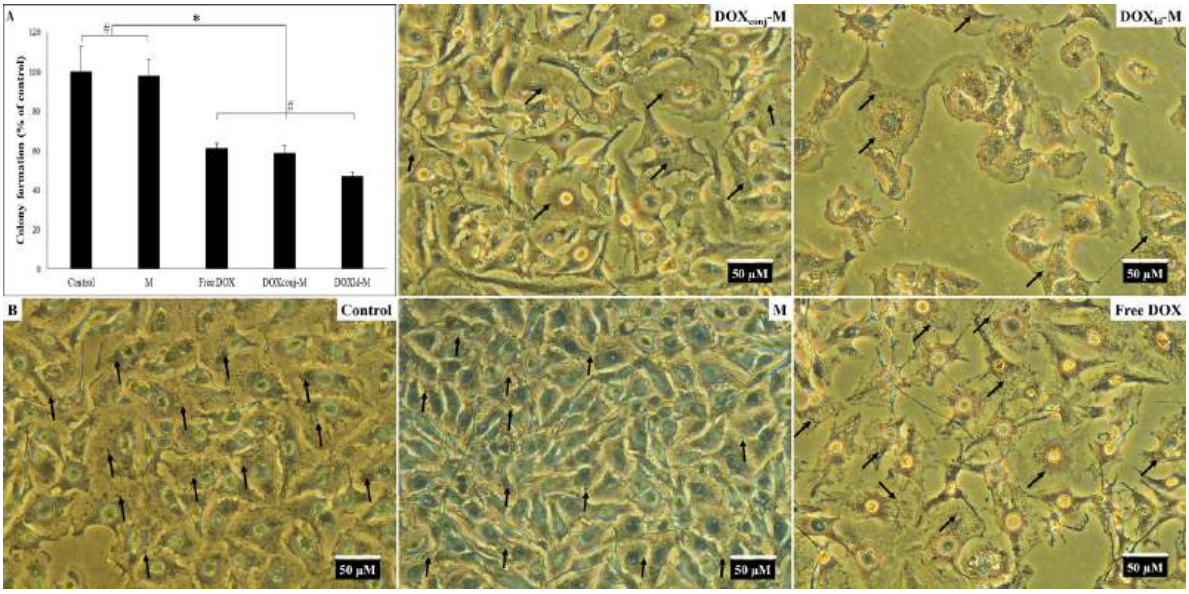

Figure 5. A) Normalized plot of colonies after colony formation assay. IC_{50} concentration of DOX (80 nM) was used in free DOX group. Colony formation assay was carried out for a week and then, colonies were counted manually from three independent experiments. * shows the significant difference between the groups and # shows the non-significant difference between the groups ($p<0.05$; n=3). B) Images of MDA-MB-231 cells stained for lipid droplets with oil red after treatment. Hematoxylin was used to counterstain the cells. IC_{50} concentration DOX (80 nM) was used in free DOX group. Black arrows show the lipid droplets. They are seen as small gray dots inside the cells (in the cytoplasm).

Determination of Lipid Droplets in MDA-MB-231 Cells

Lipid droplet formation shows the proliferation ability of breast cancer cells. To assess the lipid droplet formation in MDA-MB-231 cells, the cells were stained with oil red O and hematoxylin after incubation with free DOX, M, DOX_{conj}-M, and DOX_{ld}-M for 48 h (Figure 5B). The results showed that none of the treatments altered lipid droplet formation, although changes in cell morphology were observed.

Cells in the DOX-treated groups appeared enlarged compared to the control and M groups. Similar findings have been reported in the literature: Mehdizadeh et al. (2017) treated hepatocellular carcinoma and colorectal cancer cells with DOX and after oil red O staining, observed that treatment with DOX did not decrease lipid droplet formation, but, on the contrary, increased it (44).

Detection of Apoptosis in Cancer Cells with qRT-PCR

During apoptosis, the expression of pro-apoptotic genes such as Bax and p53 increases, while the expression of anti-apoptotic genes Bcl-2 and Bcl-xL decreases. Therefore, we investigated the apoptotic effect of free DOX, DOX_{conj}-M, and DOX_{ld}-M on MDA-MB-231 cells (Figure 6A, 6B, 6C, and 6D). Free DOX demonstrated higher Bax gene expression compared to all other groups (Figure 6A). However, there was no significant difference in Bax expression between DOX_{conj}-M and DOX_{ld}-M (Figure 6A). In all groups, Bax expression was higher compared to the control (untreated cells) and M groups (Figure 6A). Free DOX also yielded the highest p53 expression (Figure 6B). Both DOX_{conj}-M and DOX_{ld}-M increased p53 expression compared to the control and M groups, with DOX_{conj}-M inducing greater expression than DOX_{ld}-M (Figure 6B). Free DOX and DOX_{conj}-M both elevated Bcl-2 (Figure 6C) and Bcl-xL (Figure D) expressions, whereas DOXld-M significantly reduced their expression. Abdel-Hakeem et al. (2020) similarly reported that DOX-loaded chitosan-protamine nanoparticles reduced Bcl-2 expression in MDA-MB-231 cells more effectively than free DOX (45). In our study, cell internalization results showed that DOXld-M localized in the cell nuclei, potentially causing greater DNA damage due to DOX release within the nucleus compared to DOX_{conj}-M. Lower Bcl-2 and Bcl-xL expressions observed with DOX_{ld}-M may therefore result from this higher DNA damage. Although both micelle types promoted apoptosis, DOXld-M was found to be more effective than DOX_{conj}- M.

Determination of Cell Apoptosis with Annexin V-FITC Assay

Apoptosis of MDA-MB-231 cells after treatment with free DOX, DOX_{conj}-M, and DOX_{ld}-M was evaluated using the Annexin V-FITC assay (Figure 6E, and 6F). All three treatments significantly increased apoptosis compared to the control and M groups. The highest number of apoptotic cells was observed in the DOX_{ld}-M group, while no significant difference was detected between free DOX and DOX_{conj}-M. In line with our findings, Gao et al. (2017) prepared DOX- and honokiol-containing mPEG-b-PCL micelles and evaluated their effects on apoptosis. Honokiol, known for its antioxidative, anti-inflammatory, and potential anticancer properties, did not induce apoptosis in C6 glioma cells when delivered in micelles. In contrast, DOX-loaded and DOX-honokiol co-loaded micelles significantly increased apoptosis (46). In our study, all DOX-containing formulations induced apoptosis, which is consistent with our qRT-PCR and ROS generation results.

3.6.9. Mitochondrial Transmembrane Potential Detection

DiOC6 was used as the fluorescent probe which binds to intact mitochondria, where a decrease in fluorescence intensity indicates loss of mitochondrial transmembrane potential, ultimately leading to apoptosis. In Figure 6G, mitochondrial transmembrane potential analysis results are given. Treatment with free DOX, DOXconj-M, and DOXld-M decreased mitochondrial transmembrane potential by 44.4 ± 0.7%, 41.8 ± 2.8%, and 43.0 ± 1.7%, respectively. These findings indicated that all DOX-containing micelles caused mitochondrial dysfunction, which can trigger apoptosis through the release of apoptotic proteins. Similarly, Zeng et al. (2014) demonstrated that DOX-loaded nanoparticles prepared from Boltorn® H30-based hyperbranched dendritic-linear copolymers reduced mitochondrial transmembrane potential in MCF-7 cells (47).

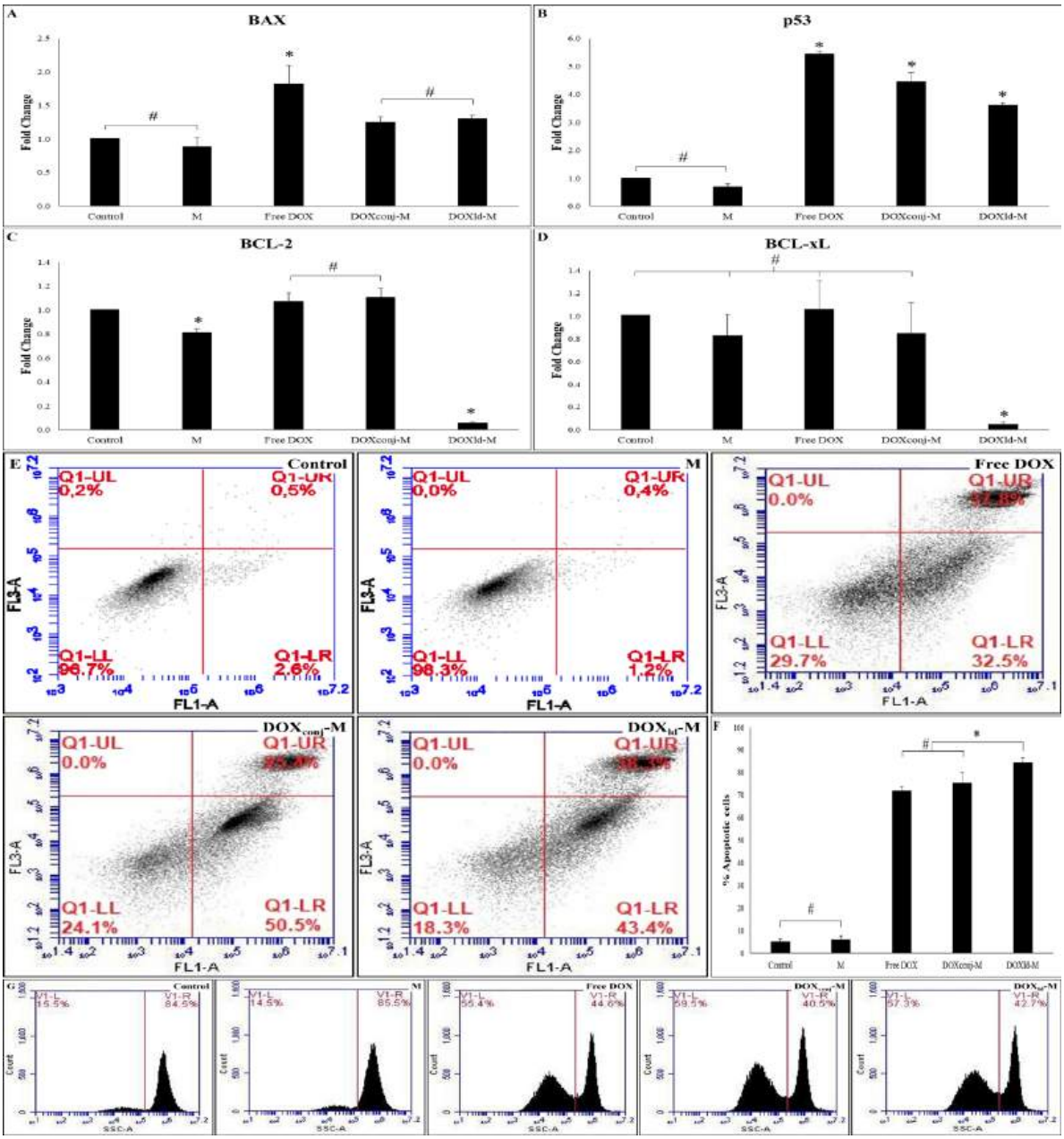

Figure 6. qRT-PCR results of A) Bax, B) p53, C) Bcl-2, and D) Bcl-xL genes of MDA-MB-231 cells after free DOX, DOX_{conj}-M, DOX_{ld}-M, and M treatment for 24 hours (n=3). IC_{50} concentration of DOX (80 nM) was used in free DOX group. * shows the significant differences between the groups ($p<0.05$) and # shows non-significant differences ($p>0.05$). E) Annexin-V/FITC apoptosis assay results of MDA-MB 231 cells without treatment (control group: untreated cells), after treatment with M, free DOX, DOXconj-M, and DOXld-M for 24 h. Q1-LL: viable cells. Q1-UL: dead cells. Q1-UR: late apoptotic cells. Q1-LR: early apoptotic cells. F) Percentage of apoptotic cells after 24 h incubation (n=3). IC_{50} concentration of DOX (80 nM) was used in free DOX group. * shows the significant differences ($p<0.05$) between the groups and # shows the nonsignificant differences ($p>0.05$). G) Mitochondrial transmembrane potential measurements of MDA-MB-231 cells without treatment (control group: untreated cells) using DiOC6, after treatment with M, free DOX, DOX_{conj}-M, and DOX_{ld}-M for 24 h using DiOC6.

Reactive Oxygen Species (ROS) Determination

DCFDA, a fluorescent probe, was used to detect intracellular ROS. In this study, ROS fluorescence was determined with confocal microscopy, and the results showed that free DOX, DOX_{conj}-M, DOX_{ld}-M, and TBHP all increased ROS levels compared to the control and M groups (Figure 7A). TBHP served as a positive control since it generates radical species that elevate ROS levels in cells. ROS levels were further quantified by measuring fluorescence intensity (Figure 7B). Among the groups, DOX_{conj}-M induced the highest ROS generation. DOX_{ld}-M significantly increased ROS generation compared to free DOX group. These results showed that DOX-conjugated and DOX-loaded micelles enhanced ROS generation more effectively than free DOX, potentially contributing to stronger apoptotic activity, as ROS generation is an early hallmark of apoptosis. Cheng et al. (2020) prepared DOX-loaded mixed micelles from Pluronic F127 and phenylboronic ester-grafted Pluronic P123.

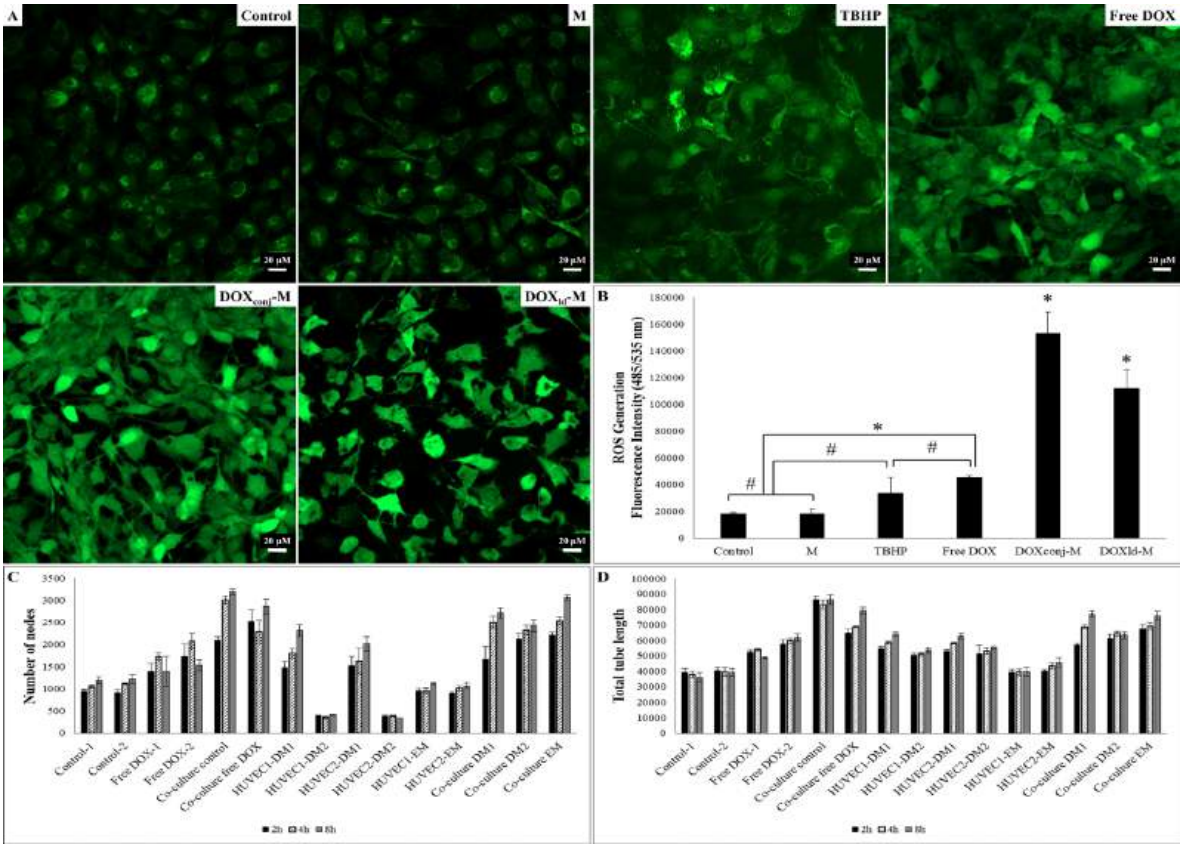

Figure 7. CA) ROS determination with confocal microscopy after treatment with TBHP, M, free DOX, DOX_{conj}-M, and DOXld-M for 24 h (TBHP: Tert-butyl hydroperoxide). B) ROS determination by measuring fluorescence intensity using microplate reader after treatment with TBHP, M, free DOX, DOX_{conj}-M, and DOX_{ld}-M for 24 h (n=4). * shows the significant differences between the groups ($p<0.05$) and # shows non-significant differences ($p>0.05$). DCFDA is a green fluorescent dye that stains ROS in cells. C) Quantification of tube formation in terms of number of nodes. D) Quantification of tube formation in terms of total tube lengths. IC_{50} concentration (80 nM) was used in free DOX group. Images of HUVECs were taken after 2, 4, and 8 hours of incubation at 37°C from three independent experiments and number of nodes and total tube length were measured using ImageJ software (n=3). 1: EC medium, 2: EC:L15 medium (1:1), EM: mPEG-b-PCL micelles (M), DM1: DOX_{conj}-M, DM2: DOX_{ld}-M

Phenylboronic ester was used to give ROS sensitivity to micelles. They reported that ROS-insensitive and ROS-sensitive DOX-loaded mixed micelles increased ROS generation in DOX-resistant MCF7 cells by 5.49- and 8.96-fold, respectively, compared to free DOX (48). In our study, DOX_{conj}-M and DOX_{ld}-M increased ROS generation compared to free DOX by 3.37-fold and 2.47-fold, respectively. These results showed that DOX-containing micelles can increase the effectiveness of DOX in resistant cells. Furthermore, as shown in Figure 7A, DOX_{ld}-M decreased cell numbers more than DOX_{conj}-M, and morphological changes appeared more pronounced, suggesting that DOX_{ld}-M induced stronger ROS generation than DOX_{conj}-M.

Endothelial Cell Tube Formation Assay

Cancer cells overexpress vascular endothelial growth factors (VEGFs) to stimulate angiogenesis, thereby improving access to nutrients. Tube formation assay was carried out to determine the effects of DOX- containing micelles on angiogenesis. The results showed that co-culture of MDA-MB-231 cells with HUVECs significantly stimulated tube formation (Figure S1, Figure S2, Figure S3 and Figure S4). Free DOX further increased tube formation compared to control groups, whereas both DOXconj-M, and DOXld-M reduced tube formation relative to free DOX.

There were more meshes in co-cultures of MDA-MB-231 cells with HUVECs compared to the control groups (untreated cells) which means that cancer cells promoted the vessel formation of endothelial cells. Co-culture of endothelial cells with cancer cells increases angiogenesis of endothelial cells, because cancer cells overexpress some growth factors like VEGF. Breast cancer cells also overexpress VEGF, which results in new blood vessel formation and increased permeability of vessels, which causes metastasis. In brain, the vessels present in 'blood-brain barrier' decrease permeability. This prevents brain from components as well as bioactive agents and drugs in the circulating blood. Metastatic breast cancer cells increase the permeability of these vessels in the blood-brain barrier, thereby promoting brain metastasis.

Treatment with empty micelles (M) resulted in similar mesh formation as the control groups (untreated cells). Free DOX significantly increased the number of nodes and total tube length compared to both the control and co-culture control groups (Figure 7C, and 7D). Among the DOX-containing micelles, DOXconj-M caused in the highest number of nodes, whereas DOXld-M produced the lowest. Importantly, micelles containing DOX did not differ significantly from the control in terms of number of nodes and total tube length. In agreement with our findings, Shi et al. (2021) also showed that 500 nM of free DOX increased number of nodes compared to control cells (49).

No significant differences were observed in number of nodes between the co-culture control and co-cultures treated with empty micelles (M) after 2h and 8h of incubations. However, after 4h, co-culture control group had a significantly higher number of nodes than the co-culture group treated with empty micelles (M). Moreover, the total tube length of the co-culture control group was significantly higher than that of the groups treated with M at 2 h, 4 h, and 8 h.

DISCUSSION

In this study, a hydrazide-functionalized mPEG-b-PCL copolymer was synthesized, and micelles (M) were prepared using the solvent evaporation method without sonication. DOX was either physically loaded into these micelles or first covalently conjugated to the copolymer via pH-responsive hydrazone bonds before micelle formation, and then micelles were prepared. Characterization studies showed that all micelles exhibited a uniform shape with nanosized structures of approximately 100-200 nm, making them suitable for intravenous administration. Micelles were highly stable, as confirmed by zeta potential and polydispersity index analyses.

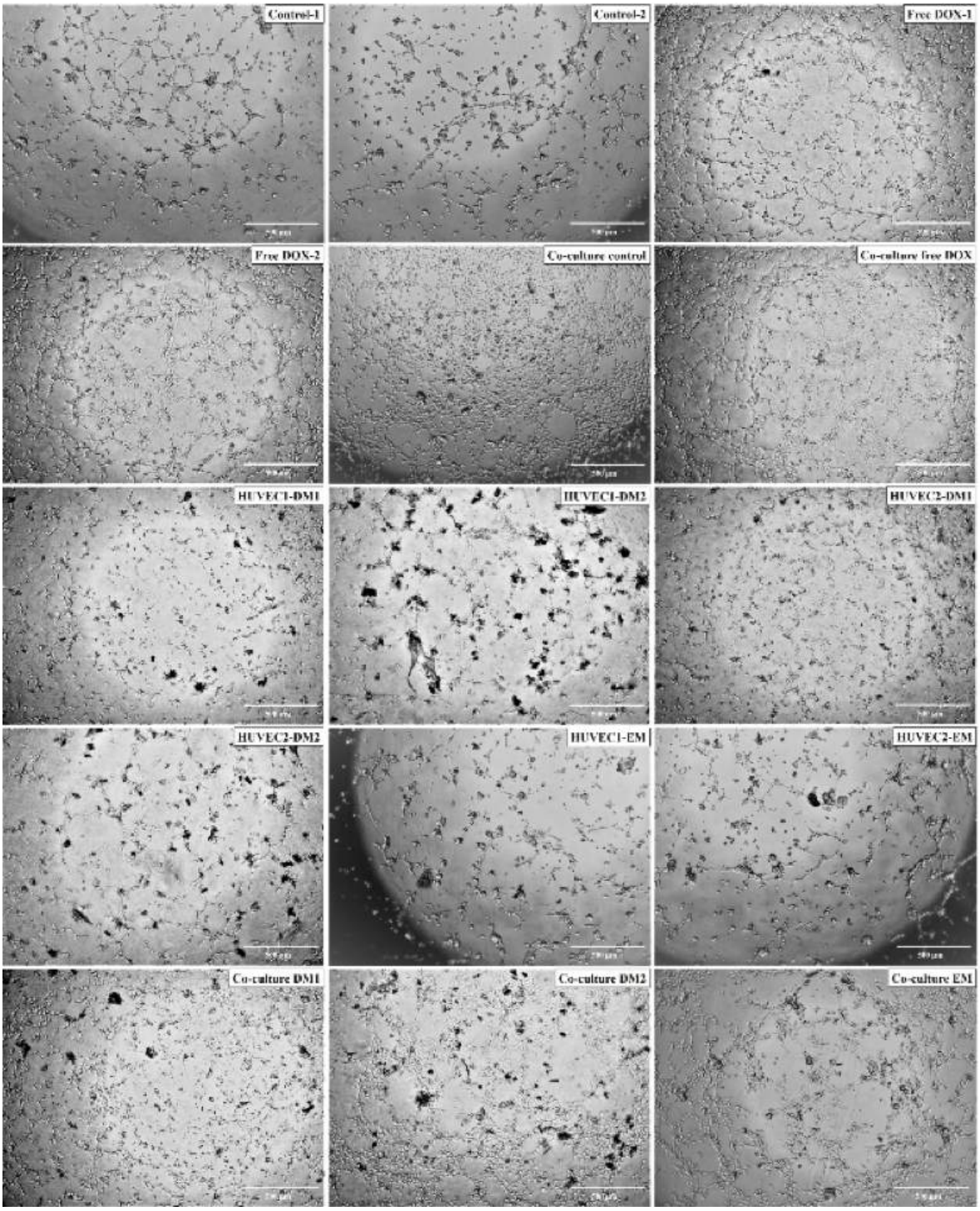

Figure S1. Images of tube formation assay after 2 hours of incubation. Scale bar is 500 µm. 40×10^3 HUVECs and 40×10^3 MDA-MB-231 cells were used in the experiments. 1: EC medium, 2: EC:L15 medium (1:1), EM: mPEG-b-PCL micelles (M), DM1: DOX_{conj}-M, DM2: DOX_{ld}-M

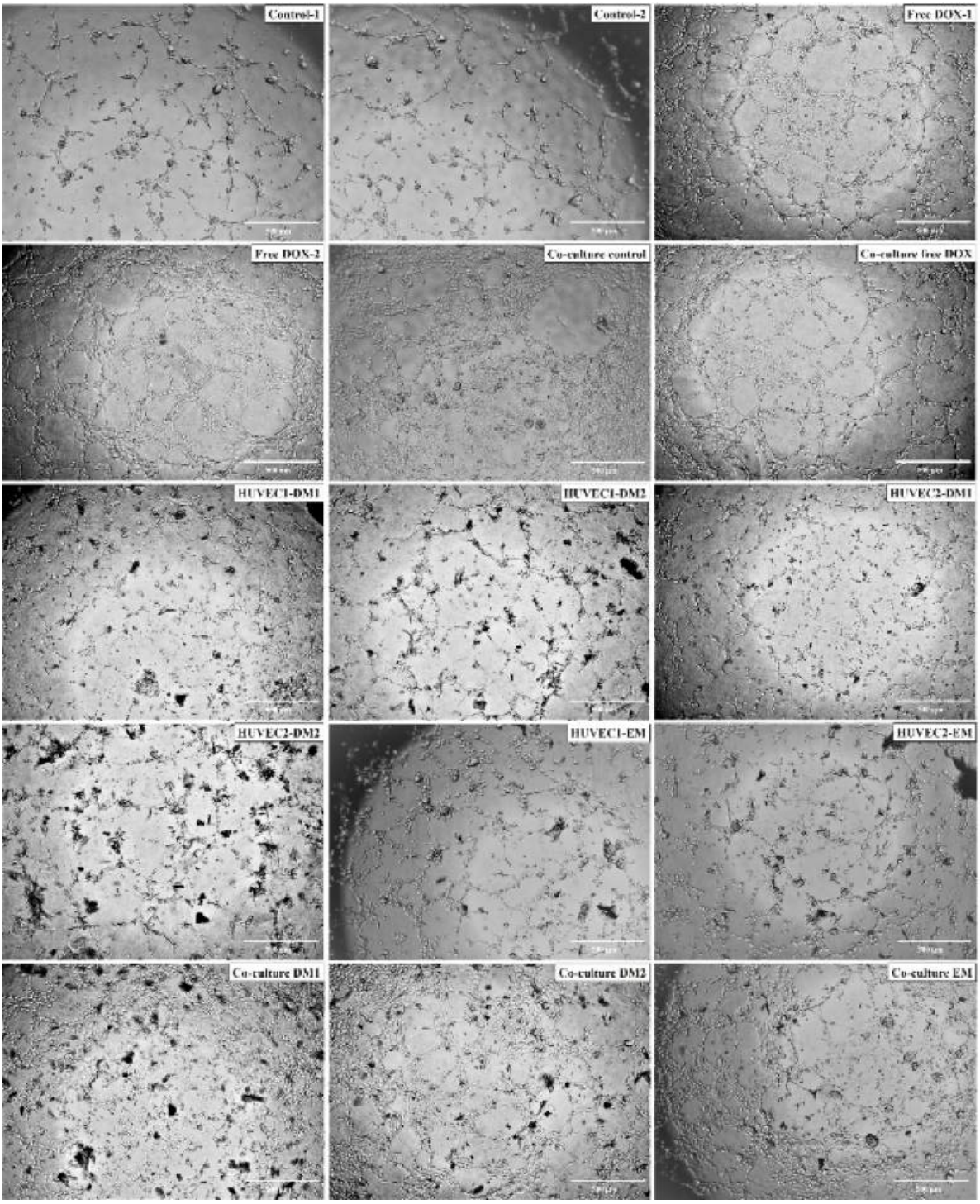

Figure S2. Images of tube formation assay after 4 hours of incubation. Scale bar is 500 µm. 40×10^3 HUVECs and 40×10^3 MDA-MB-231 cells were used in the experiments. 1: EC medium, 2: EC:L15 medium (1:1), EM: mPEG-b-PCL micelles (M), DM1: DOX_{conj}-M, DM2: DOX_{td}-M.

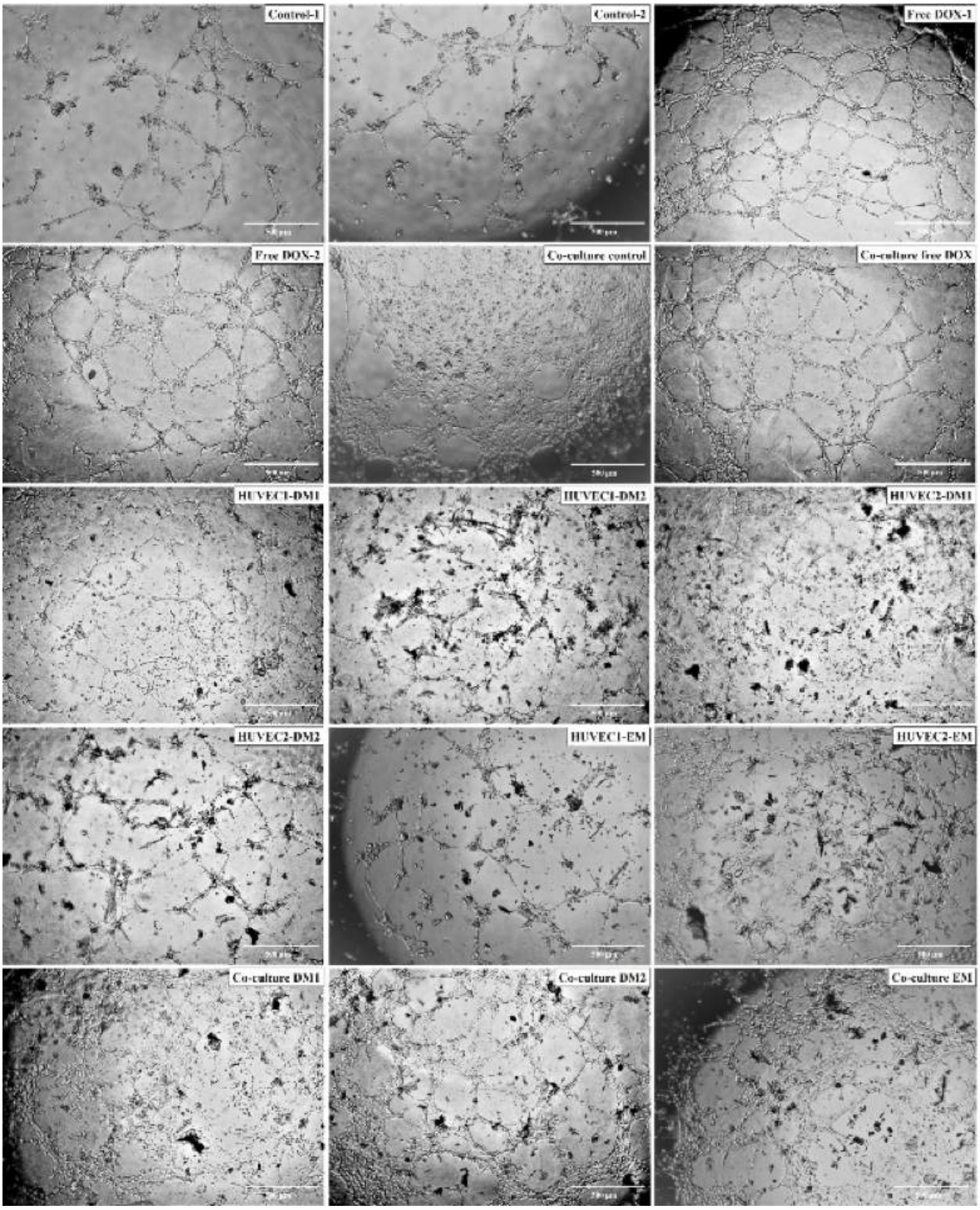

Figure S3. Images of tube formation assay after 8 hours of incubation. Scale bar is 500 µm. 40×10^3 HUVECs and 40×10^3 MDA-MB-231 cells were used in the experiments. 1: EC medium, 2: EC:L15 medium (1:1), EM: mPEG-b-PCL micelles (M), DM1: DOX_{conj}-M, DM2: DOX_{ld}-M.

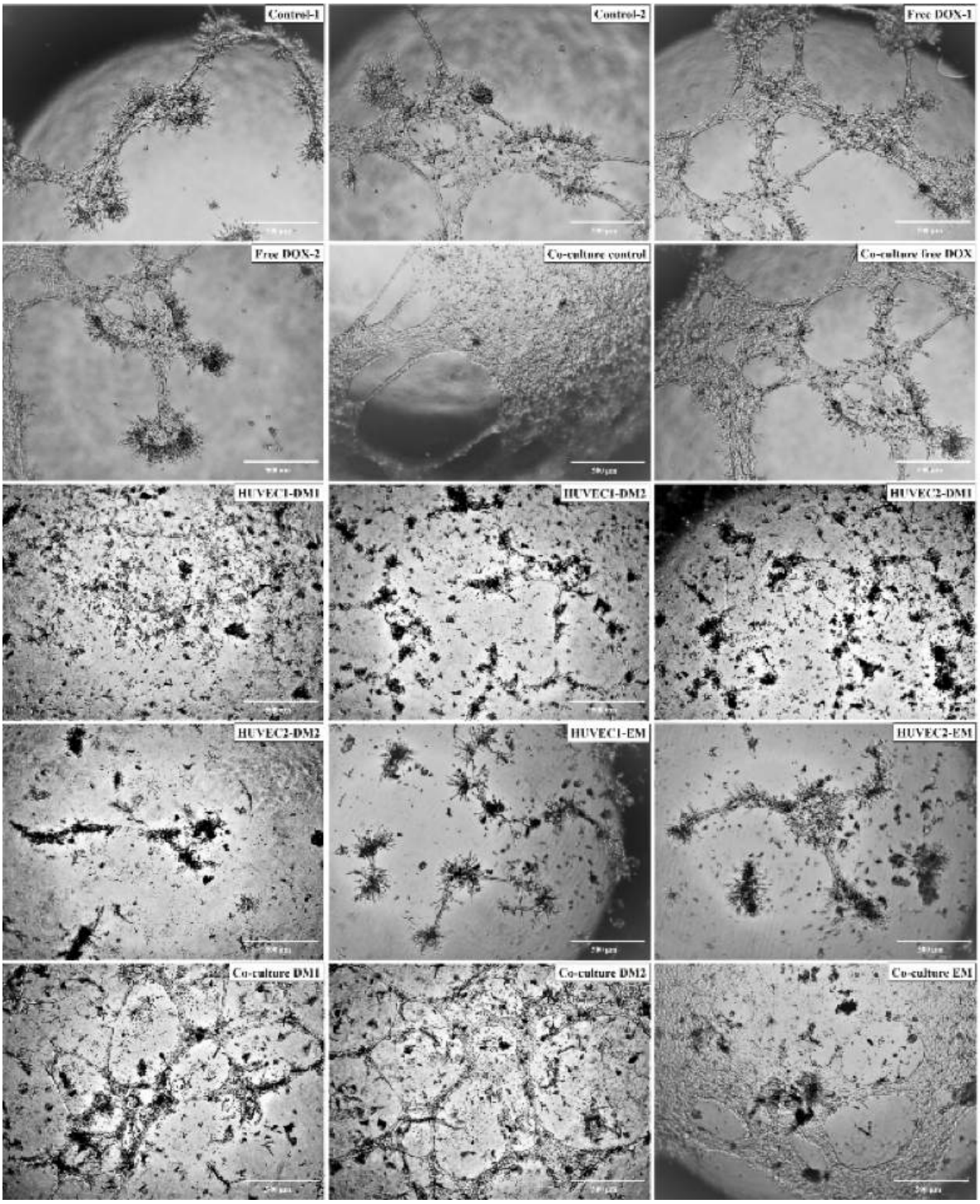

Figure S1. Images of tube formation assay after 24 hours of incubation. Scale bar is 500 µm. 40×10^3 HUVECs and 40×10^3 MDA-MB-231 cells were used in the experiments. 1: EC medium, 2: EC:L15 medium (1:1), EM: mPEG-b-PCL micelles (M), DM1: DOX_{conj}-M, DM2: DOX_{ld}-M.

Drug release studies showed pH-sensitive behavior, with DOX-conjugated micelles showing slower drug release compared to DOX-loaded micelles. All DOX-containing micelles demonstrated greater cytotoxic effects on MDA-MB-231 cells compared to the drug-free micelle control group. Furthermore, cell migration was reduced in all treatment groups. DOX_{ld}-M induced higher levels of apoptosis and reactive oxygen species (ROS) generation than DOX_{conj}-M. In addition, DOX-containing micelles caused a decrease in mitochondrial transmembrane potential, indicating apoptotic progression. Tube formation assays revealed that DOX_{ld}-M more effectively inhibited endothelial cell tube formation compared to DOX_{conj}-M.

In conclusion, pH-sensitive micelles were developed through both DOX conjugation via hydrazone linkage and DOX loading. Among these systems, DOX loading was found to be more effective against cancer cells than DOX conjugation. To show the effectiveness of these systems on cancer treatment, in vivo studies should be carried out.

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ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Araştırma Makalesi/Original Article
Makale Dili "İngilizce"/Article Language "English"

Türk Hijyen ve Deneysel Biyoloji Dergisi

In vitro evaluation of the cytotoxic effects (IC50) of climbazole-alcohol compound on various human cancer cell lines

Climbazole-alkol bileşiğinin farklı insan kanser hücre hatları üzerindeki sitotoksik etkisinin (IC50) *in vitro* değerlendirilmesi

Numan TAŞPINAR[1] (ID), Metin ÇALIŞKAN[2] (ID), Melek GÖKMEN KARAKAYA[3] (ID)

ABSTRACT

Objective: Cancer is one of the most significant health problems in both developed and developing countries today. Most of the currently available anticancer drugs can cause severe side effects and toxicities, which negatively affect patients' quality of life. Therefore, the development of safer and more selective anticancer agents remains one of the primary goals of contemporary oncology research. In recent years, the cytotoxic and antiproliferative effects of various pharmacological agents on different cancer cell lines have been extensively studied. This study aimed to investigate the potential anticancer effects of the Climbazole (CBZ) derivative, Climbazole-alcohol, on different human-derived cancer cell lines (HT-29, MKN28, A549, MDA-MB-231) and a normal control cell line (HEK293) under in vitro conditions.

Methods: CBZ was reduced to Climbazole-alcohol according to previously reported procedures, and the structure was confirmed by ^{1}H-NMR spectroscopy. Human cancer cell lines (HT-29, MKN28, A549, MDA-

ÖZET

Amaç: Kanser, günümüzde hem gelişmiş hem de gelişmekte olan ülkelerde en önemli sağlık sorunlarından biridir. Mevcut kanser ilaçlarının çoğu, hastaların yaşam kalitesini olumsuz etkileyen ciddi yan etkilere ve toksisitelere neden olabilir. Bu nedenle, daha güvenli ve daha seçici kanser önleyici ajanların geliştirilmesi, çağdaş onkoloji araştırmalarının temel hedeflerinden biri olmaya devam etmektedir. Son yıllarda, çeşitli farmakolojik ajanların farklı kanser hücre hatları üzerindeki sitotoksik ve antiproliferatif etkileri kapsamlı bir şekilde incelenmiştir. Bu çalışma, Climbazole (CBZ) türevi olan Climbazole-alkolün, farklı insan kaynaklı kanser hücre hatları (HT-29, MKN28, A549, MDA-MB-231) ve normal kontrol hücre hattı (HEK293) üzerindeki potansiyel kanser önleyici etkilerini in vitro koşullarda araştırmayı amaçlamaktadır.

Yöntem: CBZ, literatürde bildirilen yöntemlere göre indirgeme reaksiyonu ile Climbazole-alkol formuna dönüştürülmüş, bileşiğin yapısı ^{1}H-NMR analiziyle doğrulanmıştır. Hücre kültürü çalışmaları, ATCC kaynaklı

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Taşpınar N, Çalışkan M, Gökmen Karakaya M. *In vitro* evaluation of the cytotoxic effects (IC50) of climbazole-alcohol compound on various human cancer cell lines. Turk Hij Den Biyol Derg, 2025; 82(4): 651 - 658

MB-231) and the normal HEK293 cell line were cultured under standard conditions at 37°C in a humidified atmosphere containing 5% CO_2. Cells were treated with various concentrations of Climbazole-alcohol for 72 hours. Cytotoxicity was assessed using the MTT assay, and IC_{50} values were determined using GraphPad Prism software.

Results: Climbazole-alcohol induced a mild decrease in cell viability in all tested cell lines but did not exhibit a significant cytotoxic effect. The calculated IC_{50} values were 179.2 µM for HT-29, 168.1 µM for MKN28, 164.1 µM for A549, 158.6 µM for MDA-MB-231, and 169.8 µM for HEK293. No statistically significant difference was observed between cancer and normal cell lines ($p>0.05$).

Conclusion: Climbazole-alcohol did not display selective anticancer activity within the tested concentration range. The findings indicate that this compound lacks cancer-specific cytotoxicity, suggesting limited potential as an anticancer agent. Future studies should focus on structural modifications or combination therapies to enhance its anticancer properties.

Key Words: Cancer cell lines, Climbazole-alcohol, Cytotoxicity, HEK293, MTT assay

HT-29, MKN28, A549, MDA-MB-231 ve HEK293 hücre hatları üzerinde yürütülmüştür. Hücreler 37°C'de, %5 CO_2 atmosferinde uygun kültür ortamlarında çoğaltılmış ve 72 saat boyunca farklı Climbazole-alkol konsantrasyonlarına maruz bırakılmıştır. Sitotoksisite, MTT hücre canlılık testi ile değerlendirilmiş ve IC_{50} değerleri GraphPad Prism yazılımı kullanılarak hesaplanmıştır.

Bulgular: Climbazole-alkol, test edilen tüm hücre hatlarında düşük düzeyde hücre canlılığı azalmasına neden olmuş, ancak belirgin bir sitotoksik etki göstermemiştir. IC_{50} değerleri sırasıyla HT-29 için 179,2 µM, MKN28 için 168,1 µM, A549 için 164,1 µM, MDA-MB-231 için 158,6 µM ve HEK293 için 169,8 µM olarak hesaplanmıştır. Kanser hücre hatları ile kontrol hattı arasında anlamlı bir fark gözlenmemiştir ($p>0.05$).

Sonuç: Climbazole-alkol bileşiği, incelenen konsantrasyon aralıklarında seçici antikanser aktivite göstermemiştir. Bulgular, bu bileşiğin kanser hücrelerine özgü toksisite oluşturmadığını ve dolayısıyla antikanser ajan potansiyelinin sınırlı olduğunu ortaya koymaktadır. Gelecekte yapılacak çalışmalar, bileşiğin farklı türevlerinin veya kombinasyonlarının antikanser etkinlik açısından değerlendirilmesi yönünde olmalıdır.

Anahtar Kelimeler: Kanser hücre hatları, Klimbazol-alkol, Sitotoksisite, HEK293, MTT

INTRODUCTION

Cancer is one of the most significant health problems in both developed and developing countries today. The increasing global incidence and mortality rates of cancer have made it essential to develop new therapeutic approaches and chemotherapeutic agents. The fundamental characteristics of cancer include uncontrolled cell proliferation, evasion of apoptosis, the ability to metastasize, and the promotion of angiogenesis (1). These processes contribute to the emergence of various types of cancer in different organs and tissues.

Cell lines such as lung cancer (A549), breast cancer (MDA-MB-231), gastric cancer (MKN28), and colorectal cancer (HT-29) are widely used *in vitro* models for understanding cancer biology and developing new therapeutic strategies(1-3). Most of the currently available anticancer drugs can cause severe side effects and toxicities, which negatively affect patients' quality of life. Therefore, the development of safer and more selective anticancer agents remains one of the primary goals of contemporary oncology research (4, 5).

In recent years, the cytotoxic and antiproliferative effects of various pharmacological agents on different cancer cell lines have been extensively studied (6). Cell lines used in cancer research are of great importance for elucidating the molecular mechanisms of the disease, evaluating the cytotoxic and antiproliferative effects of drugs, and investigating resistance mechanisms in cancer cells. Particularly, the A549 lung cancer cell line, MDA-MB-231 triple-negative breast cancer cell line, MKN28 gastric cancer cell line, and HT-29 colorectal cancer cell line are among the most commonly studied human cancer cell lines in the literature (7-9).

In addition to these, normal human embryonic kidney cell line (HEK293) is often used as a control to assess the selectivity and specificity of toxic effects toward cancer cells. The use of cancer cell lines plays a crucial role in developing new anticancer agents and improving the efficacy of current treatment strategies (10).

Lung cancer is one of the leading causes of cancer-related deaths worldwide. The A549 cell line, derived from human lung adenocarcinoma, is one of the *in vitro* models frequently used to study the anticancer effects of cytotoxic agents and natural compounds (11, 12). Breast cancer is the most common cancer among women, and the MDA-MB-231 cell line is widely used as a model for triple-negative breast cancer (9, 13, 14).

Gastric cancer is one of the most common and fatal tumors of the gastrointestinal system. The MKN28, NCI-N87, and AGS cell lines are frequently used in gastric cancer research. Colorectal cancer is among the leading causes of cancer-related deaths worldwide, and the HT-29 cell line is widely used as an *in vitro* model for this cancer type (15-17).

Normal cell lines also play a critical role in toxicity evaluations alongside cancer cell lines. The HEK293 cell line, derived from human embryonic kidney cells, is widely used to represent normal cell physiology. The absence of toxicity in normal cell lines such as HEK293 when exposed to test compounds indicates selective anticancer potential (18).

In recent years, the development of targeted and selective anticancer agents has gained great importance. Compounds that exhibit high toxicity toward cancer cell lines but show no toxicity toward normal cells are considered potential anticancer agents (19). Developing such agents would enable the preservation of healthy tissues and minimize side effects during cancer treatment. Numerous studies have demonstrated that various natural and synthetic compounds exert significant cytotoxic and antiproliferative effects on cancer cells while remaining non-toxic to normal cells (15, 18).

In this study, the anticancer potential of Climbazole (CBZ) was investigated. Climbazole is an imidazole-based antifungal compound commonly used as an anti-dandruff and antimicrobial preservative in personal care products such as shower gels, toothpaste, hair conditioners, hair tonics, and shampoos (20). The toxicity of CBZ toward various organisms, including algae, crustaceans, fish, and plants, has been investigated (21); however, its potential cytotoxic effects on cancer cells have not been thoroughly studied. There is no study showing the anticancer effects of climbazole in the studies conducted so far.

In summary, the A549 lung cancer, MDA-MB-231 breast cancer, MKN28 gastric cancer, and HT-29 colorectal cancer cell lines are indispensable *in vitro* models for understanding cancer biology and developing novel anticancer agents. The use of normal control cell lines such as HEK293 in cytotoxicity assessments plays a critical role in determining the selective anticancer potential of tested compounds. Previous studies have shown that many natural and synthetic agents exhibit strong cytotoxicity in cancer cells but not in normal cells, suggesting their potential as anticancer agents (15, 18). These findings further emphasize the importance of selective toxicity in cancer treatment and the crucial role of cell culture models in developing new therapeutic agents.

MATERIAL and METHOD

Chemicals and Instruments

Analytical-grade chemicals from Sigma-Aldrich, Merck, and TCI were used as received. Reaction monitoring was performed via TLC under UV light (CAMAG). 1H NMR spectrum was recorded at 100 MHz in CDCl3 using a Varian spectrometer (Palo Alto, USA).

Synthesis of 1-(4-chlorophenoxy)-1-(1H-imidazol-1-yl)-3,3-dimethylbutan-2-ol

The compound was synthesized according to the previously reported procedure (20-22). Briefly, CBZ (1.00 g, 3.416 mmol) and sodium borohydride ($NaBH_4$) (0.388 g, 10.247 mmol) were dissolved in a mixture of tetrahydrofuran (20 mL) and water (20 mL) in a 100 mL round-bottom flask. The reaction mixture was stirred magnetically at room temperature for 24 h, and the progress of the reaction was monitored by thin-layer chromatography (TLC). After completion, the reaction mixture was extracted with ethyl acetate (2 × 30 mL), and the combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by recrystallization from ethyl acetate/hexane to afford 1-(4-chlorophenoxy)-1-(1H-imidazol-1-yl)-3,3-dimethylbutan-2-ol (CBZ-alcohol).

The reduction reaction is illustrated in Figure 1, consistent with the previously described transformation of CBZ to CBZ-alcohol (22).

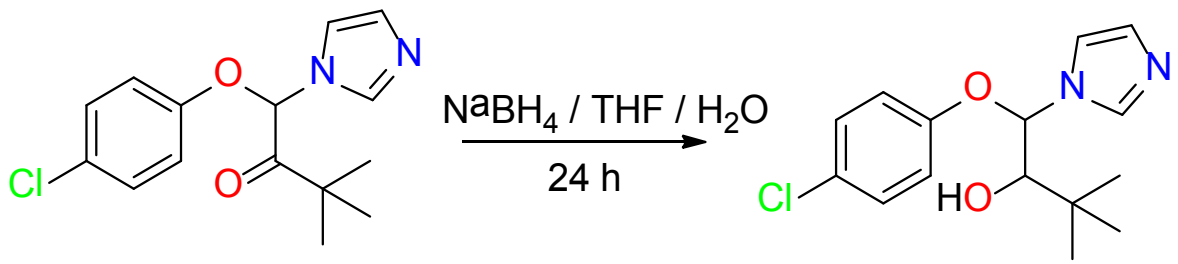

Figure 1. The schematic representation of the reduction of climbazole into climbazole-alcohol using sodium borohydride as the reducing agent (22)

Preparation of Climbazole-Alcohol Dilutions

From the compound (molecular weight 294.78 g/mol), 603.7 mg was weighed and dissolved in 10 mL of DMSO to prepare a 204.8 mM stock solution. This stock was diluted 1/100 with culture medium to obtain an intermediate concentration of 2048 µM. Serial dilutions with culture medium were then performed to prepare final concentrations of 1024, 512, 256, 128, 64, and 32 µM. These dilutions were used for cell treatment applications.

Cell Culture and Anticancer Activity Assay

Five different human cell lines were used in this study: human colorectal adenocarcinoma (HT-29, ATTC code; HTB-38), human gastric cancer (MKN28), human lung adenocarcinoma (A549/ CCL-185), human breast cancer (MDA-MB-231, ATTC code; HTB-26), and normal human embryonic kidney (HEK293, ATTC code; CRL-1573) as the control. All cell lines were obtained from the American Type Culture Collection (ATCC).

HEK293 and MDA-MB-231 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, high glucose, Gibco, USA), while A549, HT-29, and MKN28 cells were cultured in RPMI-1640 (Gibco, USA). All media were supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA). Cells were maintained in a humidified incubator at 37°C with 5% CO_2.

When cell confluence reached approximately

85-90%, the cells were detached using trypsin-EDTA (Sigma-Aldrich, USA), washed twice with PBS, and seeded into 96-well plates at a density of 1×10^4 cells per well. After 24 hours of incubation, the culture medium was replaced with 200 µL of different concentrations of Climbazole-alcohol solution (in triplicates), while control wells received only culture medium. Plates were then incubated for 72 hours.

MTT Cell Viability Assay

After incubation, the media were removed, and each well received 5 µL of 5 mg/mL MTT solution and 45 µL of culture medium (final volume 50 µL). Plates were incubated for 4 hours at 37°C. After incubation, 100 µL of 99.9% DMSO was added to dissolve the formazan crystals, and the plates were further incubated for 1 hour. Absorbance was measured at 570 nm using a Multiskan Sky Microplate Spectrophotometer (Thermo Fisher Scientific).

Cell viability (%) was calculated according to the following formula:

"Cell viability (%)"="Sample OD" /"Control OD" ×100

Determination of IC_{50} Values and Statistical Analysis

The IC_{50} value, representing the concentration required to inhibit 50% of cell viability, was determined using GraphPad Prism software. Statistical significance among viability percentages at the same concentrations was evaluated using one-way ANOVA followed by Tukey's post hoc test. A p-value of <0.05 was considered statistically significant.

RESULTS

Chemical Results

The compound 1-(4-chlorophenoxy)-1-(1H-imidazol-1-yl)-3,3-dimethylbutan-2-ol (CBZ-alcohol) was obtained by reduction of CBZ. The progress of the reaction was monitored by TLC, and the resulting product was purified by column chromatography. The physical and spectral data of the synthesized compound were found to be in good agreement with those previously reported in the literature (20-22).

Cell Viabilitiy Results

The anticancer activity of Climbazole-alcohol was evaluated against human colorectal (HT-29), gastric (MKN28), lung (A549), and breast (MDA-MB-231) cancer cell lines, along with the normal human embryonic kidney cell line (HEK293). According to MTT assay results, the compound caused a mild reduction in cell viability in a concentration-dependent manner but did not exhibit a marked cytotoxic effect overall.

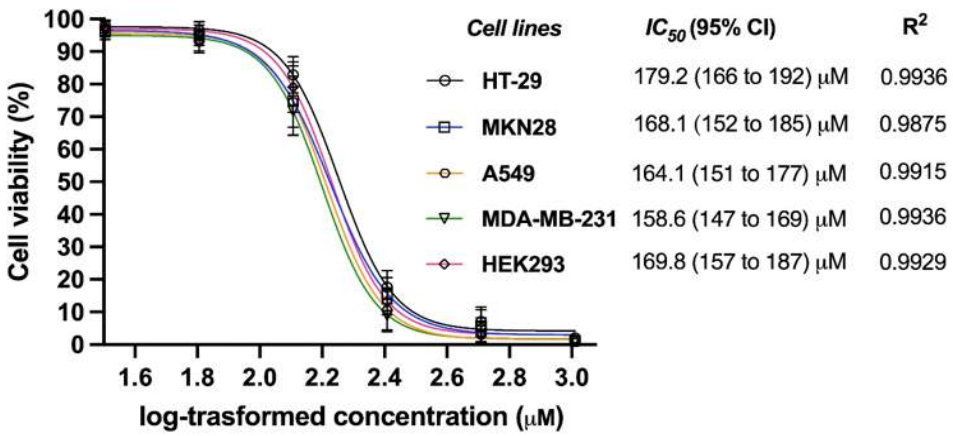

Figure 2. Dose-response curves of Climbazole-alcohol on HT-29, MKN28, A549, MDA-MB-231, and HEK293 cell lines. Cell viability (%) is plotted against logarithmically transformed extract concentrations (µg/mL). IC_{50} values, corresponding 95% confidence intervals (CI), and R^2 coefficients are indicated for each cell line

The high curve-fitting coefficients ($R^2 \geq 0.987$) confirmed the experimental reliability of the dose-response curves. The calculated IC_{50} values were 179.2 µM for HT-29, 168.1 µM for MKN28, 164.1 µM for A549, 158.6 µM for MDA-MB-231, and 169.8 µM for HEK293. These values were similar across all tested cell lines, with no statistically significant difference between the control (HEK293) and cancer cell lines ($p > 0.05$). This finding indicates that Climbazole-alcohol did not exhibit selective anticancer activity under the conditions of this study and produced comparable effects in all tested cell lines.

DISCUSSION

In this study, the in vitro cytotoxicity of Climbazole-alcohol compound was evaluated on four different human cancer cell lines (HT-29, MKN28, A549, MDA-MB-231) and the normal control cell line HEK293. The IC_{50} values obtained (HT-29: 179.2 µM; MKN28: 168.1 µM; A549: 164.1 µM; MDA-MB-231: 158.6 µM; HEK293: 169.8 µM) were within very similar ranges regardless of cell type and did not show any significant difference between cancer cell lines and normal cell lines ($p > 0.05$). This finding suggests that the compound does not exhibit selective anticancer activity, but on the contrary, has similar levels of activity in both cancer and normal cells.

The imidazole ring forms the structural basis of many biologically active molecules and has been described as a frequently used scaffold in the development of anticancer agents (23). In one study, average IC_{50} values of compounds containing an imidazole nucleus were obtained at low micromolar levels (e.g., 2.4 µM) in the NCI60 cell line panel (23). Such data suggests that imidazole-containing compounds can achieve high efficacy.

In contrast, the Climbazole-alcohol derivative used in our study exhibited relatively low cytotoxicity, with IC_{50} values in the range of 150-180 µM. This suggests that the compound requires structural modification or does not possess optimal intrinsic activity in target cells Another study reported that imidazole-chalcone derivatives had IC_{50} values in the range of 7-63 µM in the A549 cell line (24). This comparison shows that the activity of our compound is weak compared to imidazole derivatives that have shown activity at low micromolar levels in the literature.

The anticancer activities of imidazole derivatives have generally been shown to occur through apoptosis induction, cell cycle arrest (especially in the G2/M phase), and inhibition of signaling pathways (25). In this context, it may be thought that the Climbazole-alcohol structure cannot reach the relevant targets or its intracellular concentration is not sufficient. Furthermore, pharmacokinetic parameters such as lipophilicity/diffusion properties of the compound, permeation through the cell membrane, and metabolic stability may also contribute to the low efficacy.

There are several possible reasons for the low cytotoxicity of Climbazole-alcohol. Firstly, although the high lipophilicity of the compound allows it to easily cross the cell membrane, its low water solubility may have limited its effective distribution in the cell culture medium and thus its ability to reach intracellular targets. Second, the hydroxyl group in the compound's chemical structure may have weakened its pharmacophoric activity compared to the original Climbazole structure(26). Furthermore, although Climbazole-alcohol has been described as a potent antifungal agent, it appears unlikely to affect signaling pathways such as STAT3, BCL-2, or p53, key regulators of cancer cell proliferation. Some imidazole derivatives have previously been reported to induce apoptosis by inhibiting these signaling pathways (27, 28). However, MTT results indicate that Climbazole-alcohol does not exhibit such a mechanism of action.

The findings of the study demonstrate that Climbazole-alcohol does not induce selective toxicity and exhibits similar efficacy across all cell types. This suggests that the compound has a low anticancer potential. However, the imidazole ring in the Climbazole skeleton suggests that more potent anticancer derivatives could be obtained

through future structural modifications. It is anticipated that Climbazole analogs, particularly those containing halogenated or aromatic rings, may increase lipophilicity, facilitate permeation through cell membranes, and enhance interactions with target proteins (29). Furthermore, the use of Climbazole-alcohol in different combinations, such as with DNA synthesis inhibitors or oxidative stress inducers, may produce a synergistic effect (30). These approaches could be evaluated to enhance the weak anticancer activity of the compound alone.

In conclusion, this study demonstrates that the Climbazole-alcohol compound does not produce significant cytotoxic effects on various human cancer cell lines. IC_{50} values, which are close to each other and range from 158 to 179 µM, indicate that the compound does not exhibit selective activity against cancer cells, thus limiting its potential as a single anticancer agent. However, these results do not imply that Climbazole derivatives are completely ineffective; on the contrary, they suggest that pharmacological efficacy can be enhanced through structural optimization and combination strategies. Given that the imidazole backbone has been shown to have high potential in the literature (e.g., derivatives exhibiting nanomolar IC_{50}) (27), the Climbazole structure can be considered a lead compound and more effective derivatives can be developed through optimization studies.Future studies should aim to investigate in detail the effects of different chemical modifications of Climbazole derivatives on cell cycle regulatory proteins, oxidative stress responses, and apoptosis markers (e.g., BAX, BCL-2, CASPASE-3).

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Sağlıklı genç erişkinde *Streptococcus constellatus*'un neden olduğu nadir ampiyem vakası: Bir olgu sunumu

A rare case of empyema caused by *Streptococcus constellatus* in a healthy young adult: A case report

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ÖZET

Streptococcus constellatus (*S. constellatus*), Gram-pozitif, spor oluşturmayan, hareketsiz ve katalaz-negatif koklardır. CO_2 varlığında büyümeleri artarken, aerobik koşullarda büyüme azalır ve bazı suşlar optimal büyüme için anaerobik koşullara ihtiyaç duyar. *Streptococcus anginosus* grubunun (SAG) bir üyesi olan *S. constellatus*, doğal olarak oral kavite, gastrointestinal sistem ve ürogenital sistem florasında bulunur. Genellikle kommensal bir organizma olarak kabul edilmesine rağmen, bağışıklık sistemi zayıf bireylerde fırsatçı bir patojen haline gelebilir ve ciddi invaziv enfeksiyonlara neden olabilir. Bu enfeksiyonlar arasında ampiyem oldukça nadir görülmekte olup, literatürde sınırlı sayıda vaka rapor edilmiştir. Bu çalışma, Nahçivan bölgesinden bildirilen ilk olgu olma özelliği taşımaktadır.Bu çalışmada, sağlıklı bir bireyde *S. constellatus*'un neden olduğu nadir bir ampiyem vakası sunulmaktadır. Hasta, ateş, göğüs ağrısı ve

ABSTRACT

Streptococcus constellatus (*S. constellatus*) is a Gram-positive, non-spore-forming, immobile, and catalase-negative coccus. Its growth is enhanced in the presence of CO_2, while growth is reduced under aerobic conditions, with some strains requiring anaerobic conditions for optimal growth. A member of the *Streptococcus anginosus* group (SAG), *S. constellatus* is naturally found in the flora of the oral cavity, gastrointestinal system, and urogenital system. Although typically considered a commensal organism, it can become an opportunistic pathogen in immunocompromised individuals, leading to severe invasive infections. Among these infections, empyema is rare, with only a limited number of case reports in the literature. This study presents the first reported case from the Nakhchivan region. This study presents a rare case of empyema caused by *S. constellatus* in a healthy individual. The patient presented with

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nefes darlığı gibi klasik bakteriyel ampiyem belirtileri ile başvurmuştur. Detaylı tanı sürecinde mikrobiyolojik incelemeler yapılmış ve enfeksiyonun sebebinin *S. constellatus* olduğu doğrulanmıştır. "Mikrobiyolojik kültür analizlerinde izole edilen bakterinin beta-hemolitik özellik gösterdiği saptanmış; gerçekleştirilen antibiyotik duyarlılık testleri sonucunda ise hedefe yönelik ve etkili bir tedavi protokolü oluşturulmuştur. Uygun antibiyotik seçimi sayesinde enfeksiyon kısa sürede kontrol altına alınmış ve hastada klinik iyileşme hızla sağlanmıştır." Bu vaka, *S. constellatus*'un nadir bir ampiyem etkeni olarak değerlendirilmesi gerektiğini vurgulamakta ve bu tür durumlarda erken tanının önemine dikkat çekmektedir. Erken tanı, yalnızca enfeksiyonun kontrol altına alınmasında değil, aynı zamanda tedaviye yanıtın optimize edilmesinde de kritik bir rol oynamaktadır. Sonuç olarak, bu vaka raporu, literatüre nadir bir enfeksiyon sunarak *S. constellatus*'un ampiyem etkeni olarak tanınmasının önemini vurgulamaktadır. Ayrıca, bu enfeksiyonların tanı ve tedavisinde karşılaşılan zorlukları ele almakta ve klinik pratiğe önemli katkılar sağlamaktadır. Bu vaka, ampiyem gibi ciddi enfeksiyonların yönetiminde atipik patojenlerin dikkate alınması gerektiğini bir kez daha vurgulamaktadır.

Anahtar Kelimeler: *Streptococcus constellatus*, ampiyem, tanı, plevral efüzyon, antibiyotik seçimi

classical symptoms of bacterial empyema, including fever, chest pain, and shortness of breath. During the diagnostic process, microbiological investigations confirmed that the cause of the infection was *S. constellatus*. "Microbiological culture analysis revealed that the isolated bacterium exhibited beta-hemolytic properties; following antibiotic susceptibility testing, an effective targeted treatment protocol was established. With appropriate antibiotic selection, the infection was quickly controlled, and the patient experienced rapid clinical improvement." This case emphasizes the need to consider *S. constellatus* as a rare cause of empyema and highlights the importance of early diagnosis in such cases. Early diagnosis plays a critical role not only in controlling the infection but also in optimizing the response to treatment. In conclusion, this case report underscores the importance of recognizing *S. constellatus* as an agent of empyema, contributing a rare infection to the literature. Furthermore, it addresses the challenges encountered in the diagnosis and treatment of such infections and provides significant contributions to clinical practice. This case reiterates the necessity of considering atypical pathogens in the management of serious infections like empyema.

Key Words: *Streptococcus constellatus*, empyema, diagnosis, pleural effusion, antibiotic selection

GİRİŞ

S. constellatus, Gram-pozitif, spor oluşturmayan, hareketsiz ve katalaz-negatif koklardır. Bu bakteri, *S. anginosus* ve *S. intermedius* ile birlikte *S. anginosus* grubunu (SAG) oluşturur. Daha önce *S. milleri* grubu olarak adlandırılan SAG, orofarinks, üst solunum yolu, gastrointestinal sistem ve ürogenital sistem mukozasında yerleşik olarak bulunan kommensal bakterilerden oluşmaktadır (1,2). SAG bakterilerinin enfeksiyon etkeni olarak değerlendirilmesi çoğu zaman güçtür. Bununla birlikte, immünsüprese bireylerde veya invaziv işlemler geçiren hastalarda ciddi enfeksiyonlara neden olabilirler.

Ampiyem, plevral boşlukta gelişen bakteriyel enfeksiyon sonucu apse oluşumu ile karakterize ciddi bir klinik tablodur. Yüksek morbidite ve mortalite oranlarına sahip olan bu durumun, özellikle COVID-19 pandemi sürecinde olmak üzere, dünya genelinde insidansında artış gözlenmiştir (2,3) Ampiyem tedavisi sıklıkla torakotomi ile plevral dekortikasyon, göğüs tüpü ile drenaj ve uygun antibiyotik tedavisi gibi invaziv

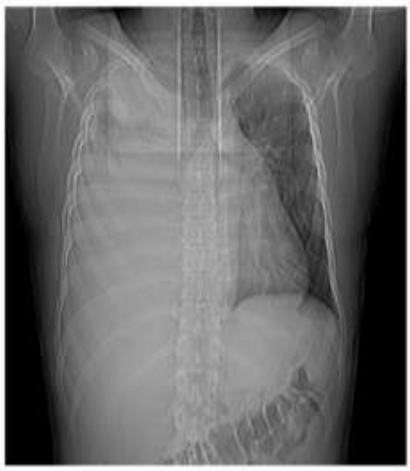
yaklaşımlar gerektirir. Erken tanı, başarılı tedavi süreci açısından kritik öneme sahiptir. SAG kaynaklı ampiyem vakaları, genellikle alkol bağımlılığı öyküsü bulunan, bağışıklık sistemi baskılanmış veya ciddi komorbiditeleri olan hastalarda ortaya çıkmaktadır. SAG üyeleri arasında *S. anginosus* en sık izole edilen tür olup, *S. intermedius* ise *S. constellatus*'a kıyasla daha invaziv bir klinik seyir göstermektedir (1-4). Bu çalışma, sağlıklı bir bireyde *S. constellatus* kaynaklı ampiyem gelişimini ele almakta ve tanı sürecindeki zorluklar ile enfeksiyonun klinik seyrini detaylı bir şekilde tartışmaktadır.

OLGU SUNUMU

On sekiz yaşında, erkek hasta, üç gündür süregelen ateş, nefes darlığı, kuru öksürük ve plevritik göğüs ağrısı şikâyetleri ile acil servise başvurmuştur. Hastanın özgeçmişinde sağlıklı bir çocukluk dönemi geçirdiği, üflemeli bir müzik aleti çaldığı ve son bir aydır ek iş olarak soğuk bir depoda çalıştığı bilgisi alınmıştır. Hastanın kronik hastalığı veya önceki hastaneye yatış öyküsü bulunmamaktadır. Son bir aydır kuru öksürük ve gece terlemeleri yaşadığı bildirilmiştir. Bu bulgular, sistemik bir inflamatuvar yanıta işaret etmektedir.

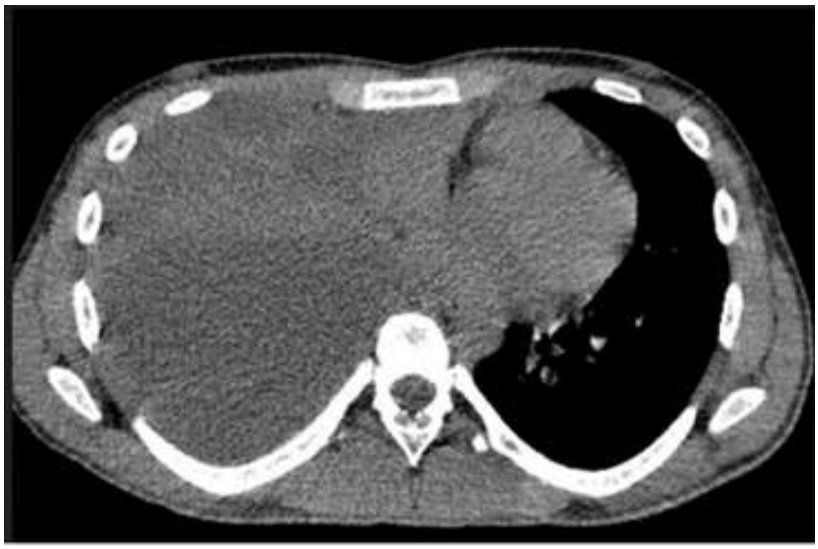
Fizik Muayene Bulguları: Hastanın vital bulguları değerlendirildiğinde, bilincinin açık olduğu, vücut sıcaklığının 38,5°C, kalp atım hızının 117/dakika, solunum sayısının 30/dakika ve periferik oksijen satürasyonunun %80 olduğu tespit edilmiştir. Fizik muayenede, genel durumunun orta derecede olduğu ve fiziki gelişiminin yaşına uygun olduğu gözlemlenmiştir. Cilt ve skleraların soluk olduğu dikkat çekmiştir. Akciğer oskültasyonunda sağ akciğerin tamamında solunum sesleri alınamamış, bu durum plevral bir etiyolojiyi düşündürmüştür. Kardiyak oskültasyonda normal kalp sesleri duyulmuş, herhangi bir üfürüm veya ek ses izlenmemiştir. Abdominal muayenede hassasiyet saptanmazken, kostovertebral açı hassasiyeti ve pretibial ödem gözlemlenmiştir. Hastanın ateşi, taşikardisi, takipnesi ve hipoksemisi sistemik bir inflamatuvar yanıta işaret etmektedir.

Radiyoloji Bulguları: Hastanın başvuru anında çekilen posteroanterior akciğer grafisinde, sağ hemitoraksta diffüz opasite artışı izlenmektedir. Bu opasite, sağ hemitoraksı neredeyse tamamen dolduran yaygın plevral effüzyona işaret etmektedir. Effüzyona bağlı olarak mediastinal yapılarda, özellikle trakeada sola doğru belirgin bir şift görülmektedir. Bulgular, parankimal volüm kaybından ziyade, plevral boşluktaki yüksek hacimli sıvının baskılayıcı etkisini düşündürmektedir (Şekil 1).



Şekil 1. Başvuru anında çekilen akciğer grafisinde, sağ hemitoraksı neredeyse tamamen dolduran yaygın plevral effüzyon izlenmiş; buna bağlı olarak trakeada ve mediastinal yapılarda sola belirgin şift gözlenmiştir

Toraks bilgisayarlı tomografisinde, sağ hemitoraksta 16 cm kalınlıkta ölçülen lokalize (loküle) plevral effüzyon izlenmektedir. Effüzyona komşu akciğer segmentlerinde kompresif atelektazi alanları mevcuttur. Ayrıca sağ kostal plevrada yaygın kalınlık artışı gözlenmiş olup, bu kalınlık en fazla 13 mm olarak ölçülmüştür. Mediastinal yapılarda sola doğru şift izlenmiş ve sağ akciğer hacminde belirgin azalma dikkat çekmiştir. Bu radyolojik bulgular enfeksiyöz/inflamatuvar süreci desteklemektedir (Şekil 2).



Şekil 2. Toraks BT'de sağ hemitoraksta 16 cm'lik loküle plevral effüzyon, kompresif atelektazi, 13 mm'ye ulaşan plevral kalınlık ve mediastinal yapılarda sola şift izlenmiştir.

Laboratuvar Bulguları: Lökosit sayısı 22.300/mm^3, nötrofil oranı %89, hemoglobin düzeyi 12,4 g/dL, hematokrit değeri %39,8 olarak ölçülmüştür. C-reaktif protein (CRP) seviyesi 309 mg/L, kreatinin düzeyi 0,8 mg/dL ve prokalsitonin düzeyi 0,8 ng/mL olarak belirlenmiştir. Anti-HIV, Anti-HCV ve HBsAg testleri negatif sonuçlanmıştır. Bu parametreler, ciddi bakteriyel enfeksiyon lehine yorumlanmıştır.

Torasentez Bulguları: Torasentez ile elde edilen plevral sıvı; LDH: 4824 IU/L, glukoz: 0,4 mg/dL, albümin: 3,18 g/dL değerleriyle eksüda özelliği taşıyordu. Glukozun ileri derecede düşük olması ve yüksek LDH düzeyi, komplike parapnömonik efüzyon veya ampiyemi düşündürmekteydi. Tanısal inceleme amacıyla örnekler mikrobiyoloji laboratuvarına gönderildi.

Tanısal amaçla plevral sıvıdan *Mycobacterium tuberculosis* kültürü, tüberküloz PCR testi, aerobik ve anaerobik kültür incelemesi için örnekler alınarak Mikrobiyoloji Laboratuvarı'na gönderilmiştir.

Mikrobiyolojik Bulgular: Hastadan alınan plevral sıvının 5'er mL'si aerobik ve anaerobik kan kültür şişelerine inoküle edilmiştir. Ayrıca periferik venöz kandan alınan birer adet aerobik ve anaerobik kültür şişesi ile birlikte tüm örnekler Biomerieux Bact Alert 3D cihazında inkübasyona alınmıştır.

Plevral sıvı ayrıca %5 koyun kanlı agar ve EMB agara ekilmiş; %10 CO_2 içeren inkübatörde, 37°C'de inkübe edilmiştir. Gram boyamada plevral sıvıda Gram-pozitif koklar gözlenmiştir. İnkübasyonun 48. saatinde tüm kültür şişelerinde pozitif sinyal saptanmış; mikroskobik incelemelerde Gram-pozitif kok morfolojisi doğrulanmıştır.

Kanlı agarda beta-hemolitik zonlar ve bu alanlarda koloni gelişimi izlenmiştir. Hemokültür pasajlarında kontaminasyon saptanmamıştır.

Saf koloniler, VITEK 2 Compact sisteminde tanımlanmış; VITEK® 2 GP (REF 21342) ve VITEK® 2 AST-ST03 (REF 421040) kartları kullanılmıştır. Sistem, izolatı %50 olasılıkla *S. constellatus*, %50 olasılıkla *S. intermedius* olarak tanımlamıştır (Şekil 3). Literatürde, belirgin beta-hemolizin *S. constellatus* için ayırt edici olduğu belirtilmektedir (7).

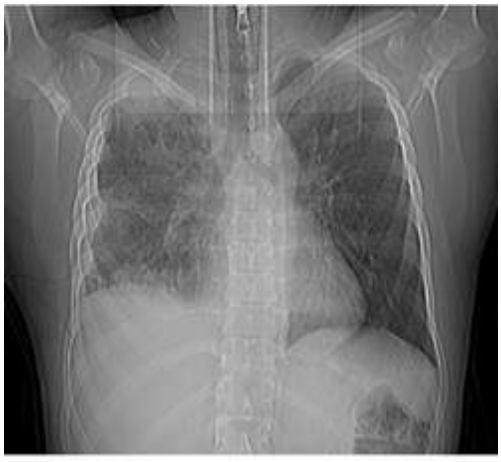
Antibiyotik duyarlılık testinde, makrolidlere, glikopeptitlere, penisilinlere ve diğer ß-laktam grubu antibiyotiklere karşı duyarlı olduğu saptanmıştır.

Aside dirençli boyama ve tüberküloz PCR testi negatif sonuç vermiştir.

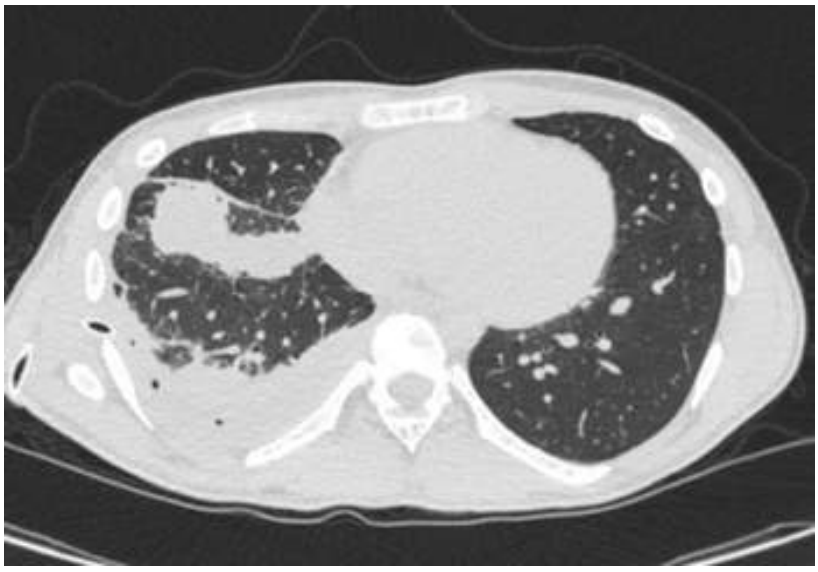
Şekil 3. Kanlı agarda *S. constellatus* kolonileri

Tedavi Süreci

Ampirik olarak başlanan ampisilin-sulbaktam tedavisine, mikrobiyolojik kültür sonuçlarının ardından da devam edilmiştir. Hastada tedavinin ilk 72 saati içerisinde ateşin düşmesi ve solunum semptomlarında gerileme ile belirgin klinik düzelme gözlenmiştir. On günlük intravenöz tedavi sürecinin ardından yapılan kontrol toraks bilgisayarlı tomografisinde (BT) akciğer parankiminin açık olduğu, ancak sağ alt lobda parsiyel atelektazi izlendiği raporlanmıştır (Şekil 4, 5).



Şekil 4. Hasta kabulünden 10 gün sonra yapılan X-Ray karşılaştırma da sağ tarafta plevral efüzyonda belirgin azalma izlenmekle beraber kardiyofrenik ve kostofrenik sinüsler hala kapalı olarak izlenmektedir



Şekil 5. Onuncu gün toraks BT karşılaştırmasında, sağ hemitoraksta en kalın yerinde 4 cm ölçülen rezidüel plevral effüzyon izlenmiş; önceki BT bulgularına göre effüzyon miktarında belirgin azalma kaydedilmiştir.

İzlemde oral antibiyotik tedavisine geçilerek amoksisilin-klavulanik asit başlanmış, toraks kateteri göğüs cerrahisi birimi kontrolünde çıkarılmıştır. Oda havasında rahat solunum yapan, klinik ve laboratuvar parametrelerinde belirgin iyileşme izlenen hasta, solunum egzersizlerinin ve antibiyotik tedavisinin devamı önerilerek, 10 gün sonra poliklinik kontrolü planlanacak şekilde taburcu edilmiştir.

TARTIŞMA

SAG (*Streptococcus anginosus* grubu), ilk kez 1956 yılında Guthof tarafından dental apselerden ve diğer oral inflamatuar lezyonlardan izole edilerek tanımlanmıştır (5). *S. constellatus*, SAG içerisinde yer alan, Gram-pozitif, fakültatif anaerob ve katalaz negatif bir kok bakteridir. Pek çok vücut sistemine ait doku ve organlarda kommensal olarak bulunan bu bakteri, genellikle patojen kabul edilmez ve özellikle altta yatan hastalığı bulunmayan bireylerde göz ardı edilebilir. Ancak, *S. constellatus* pyojenik enfeksiyonlara yol açma eğilimindedir ve farklı anatomik bölgelerde değişken klinik tablolarla kendini gösterebilir (6). Bu tür enfeksiyonların tedavisinde genellikle uzun süreli antibiyotik tedavisi ve/veya cerrahi müdahaleler gerekebilir. Ampiyem, plevral boşlukta irin birikimiyle karakterize edilen bir klinik tablodur. En sık görülen semptomlar arasında dispne, ateş, göğüs ağrısı ve öksürük yer almakta olup, hemoptizi nadiren eşlik eder. Laboratuvar bulguları genellikle nötrofil sayısında artış ve eksüda formunda plevral efüzyon varlığını göstermektedir. *S. anginosus* grubu'nun (SAG) virülansı, kapsüler yapılarından kaynaklanmakta olup, bu yapılar empiyemli hastalarda patojeniteye katkıda bulunmaktadır (7).

SAG kaynaklı ampiyem, oral sekresyonların aspirasyonu, travma veya cerrahi yoluyla doğrudan bulaş, komşu dokulardan yayılım ya da vücudun diğer bölgelerinden hematojen yol ile taşınma yoluyla akciğerlere ulaşabilmektedir (8). Pnömoni, torasik cerrahi, malignite, diyabet, nörolojik hastalıklar, alkol kullanımı ve mukozal hasar (sinüzit ve periodontal hastalıklar) ampiyem gelişimi için bilinen risk faktörleri arasındadır (9,10). Geleneksel olarak en baskın nedenin bakteriyel pnömoni olduğu düşünülmekle birlikte, ampiyemin bakteriyel

etiyolojisi pnömoni ile her zaman örtüşmemektedir. Ampiyeme en sık neden olan etkenler arasında S.pneumoniae, S. pyogenes ve Staphylococcus aureus yer almakla birlikte, son yıllarda SAG üyelerinin etken organizmalar olarak görülme sıklığında artış gözlemlenmektedir (11).

S. *anginosus* grubu (SAG) türleri arasında, enfeksiyonların büyük çoğunluğunda etken patojen S. anginosus olmakla birlikte, göğüs enfeksiyonlarında en sık izole edilen tür S. *constellatus*'tur. S. *intermedius* genellikle piyojenik enfeksiyonlarla ilişkilendirilirken, S. anginosus ve S. *constellatus* türleri ise daha çok bakteriyemi ile ilişkilidir (1,4). S. *constellatus* kaynaklı enfeksiyonların klinik seyri genellikle daha hafif olup, plevral drenaj ve antibiyotik tedavisi ile etkin bir şekilde kontrol altına alınabilmektedir. Ancak, immünkompromize bireylerde, S. *constellatus* enfeksiyonları dekortikasyon amacıyla cerrahi müdahale gerektirebilir (12). Özellikle altta yatan hastalığı bulunan bireylerde, S. *constellatus* enfeksiyonları hafife alınmamalıdır. S. *anginosus* grubu (SAG), sıklıkla diğer anaerob mikroorganizmalarla birlikte ko-enfeksiyon oluşturma eğilimi gösterir. Özellikle odontojenik enfeksiyonlu hastalarda, SAG ile zorunlu anaerob bakterilerin birlikte enfeksiyon oluşturduğu sıkça bildirilmektedir (13). Bu eş zamanlı enfeksiyon durumu, mikroorganizmaların virülansında artışa yol açmaktadır. Bu nedenle, SAG kaynaklı ampiyem olgularında diğer anaerob mikroorganizmaların varlığının saptanması gereklidir. Ampiyemli hastalarda, genellikle plevral dekortikasyon, drenaj ve antibiyotik tedavisini içeren invaziv girişimlere ihtiyaç duyulmaktadır.

Son dönemde yapılan çalışmalarda, supragingival dental plağın apselere yol açan enfeksiyöz patojenlerin kaynağı olabileceği belirtilmiştir. Bu çalışmalarda, dental plak örneklerinden izole edilen mikroorganizmaların büyük çoğunluğunu S. *anginosus* grubu (SAG), özellikle de S. *constellatus* türü olduğu gösterilmiştir (14).

Bu çalışmada, bağışıklık sistemi sağlam bir genç hastada gelişen ve S. *constellatus* kaynaklı ampiyem olgusu sunulmuştur; hastanın torasentez ve antibiyotik tedavisiyle başarılı bir şekilde iyileşme sağlanmıştır. Bu olgumuzda enfeksiyona neden olan S. *constellatus*'un kaynağı kesin olarak belirlenememiştir. İzole edilen bu suşun penisilin G başta olmak üzere, ß-laktam grubu antibiyotiklere, makrolidlere ve glikopeptitlere karşı tamamen duyarlı olduğu saptanmıştır.

S. *constellatus* kaynaklı ampiyem, altta yatan hastalığı olmayan hastalarda nadir görülse de, toplum kökenli ampiyemlerde dikkate alınmalıdır. Etkili tedavi için, hekimlerin erken plevral drenaj ve uygun antibiyotik tedavisini göz önünde bulundurması önemlidir. Bu yaklaşım, cerrahi dekortikasyon gerekliliğini en aza indirerek, uzun süreli hastanede yatışa bağlı komplikasyonları önleyebilir ve dolayısıyla hastanın prognozunu iyileştirebilir.

BİLGİLENDİRİLMİŞ ONAM FORMU

* Hastanın kendi imzaladığı Bilgilendirilmiş Onam Formu alınmıştır. (Tarih: 27.12.2024)

ÇIKAR ÇATIŞMASI

Yazarlar bu makale ile ilgili herhangi bir çıkar çatışması bildirmemişlerdir.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

Tek sağlık yaklaşımı ve halk sağlığında iyi uygulamalar

The one health approach and good practices in public health

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ÖZET

Tek Sağlık; insanların, hayvanların ve ekosistemlerin sağlığını sürdürülebilir bir uyum içinde en iyi hale getirmeyi amaçlayan bütüncül birleştirici bir yaklaşımdır. Bu yaklaşım; insan, hayvan ve çevre sağlığının birbirleriyle yakından ilişkili olduğunu kabul etmektedir. Bu yazı kapsamında Tek Sağlık yaklaşımının halk sağlığı için önemini bilerek konu ile ilgili Dünya'da iyi uygulama örneklerinin açıklanması amaçlanmıştır. Çalışmanın yapılabilmesi için PubMed ve Google Akademik veri tabanları taranmıştır. Konu ile ilgili WHO, CDC, FAO, UNEP, WOAH, PAHO, European Commission, FDA Türk Tabipleri Birliği, Veteriner Hekimler Derneği, Halk Sağlığı Uzmanları Derneği, Sağlık Bakanlığı ve Tarım Orman Bakanlığına ait kurumsal web siteleri incelenmiştir. Konu ile ilgili eylem planları ve öne çıkan küresel metinler incelenmiştir. Yeni ortaya çıkan bulaşıcı hastalıklar, yeniden önem kazanan bulaşıcı hastalıklar, vektör kaynaklı hastalıklar, antimikrobiyal ilaç direnci, gıda güvenliği ve güvencesi, çevre

ABSTRACT

One Health is a holistic and integrative approach aimed at optimizing the health of humans, animals, and ecosystems in a sustainable harmony. This approach recognizes the close interconnections between human, animal, plant, and environmental health. This paper seeks to highlight the significance of the One Health approach for public health and to discuss successful case studies worldwide. To conduct this study, a thorough review of literature and resources on PubMed and Google Scholar databases was undertaken. Institutional websites of major health and environmental organizations such as WHO, CDC, FAO, UNEP, WOAH, PAHO, the European Commission, FDA, the Turkish Medical Association, the Association of Veterinary Physicians, the Association of Public Health Specialists, the Ministry of Health, and the Ministry of Agriculture and Forestry were examined. Relevant action plans and prominent global texts on the subject were reviewed. In critical areas impacting public health—such as emerging infectious diseases, re-emerging infections, vector-borne diseases,

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sağlığı ve iklim değişikliği gibi halk sağlığını yakından ilgilendiren konularda Tek Sağlık yaklaşımı daha fazla bilginin bir arada değerlendirilmesini sağlayarak, riskleri önceden belirleyip önlem alarak, kontrol altında tutarak; konuyu daha geniş bir bakış açısıyla değerlendirip daha etkili çözümler üretilebilmektedir. İnsan, hayvan ve çevre sağlığının yakın ilişkisi ve sağlık sorunlarının karmaşık yapısı nedeniyle ortak sağlık sorunları ile ilgili kalıcı çözümler elde edebilmek için disiplinlerin konu ile ilgili ayrı ayrı çözümlerinden ziyade disiplinler üstü tümelci bir yaklaşım oluşturulmalıdır. Bu tümelci yaklaşımın gerçekleştirilebilmesi için kamu otoritesi önderliğinde kurulacak yerel ve ulusal yapıların sistemlerini entegre edecek ve toplum katılımını da sağlayacak yapıların oluşturulması gerekmektedir. Bu entegre yapıların ülkelerin Tek Sağlık kapsamında çözüm üretilebilecek öncelikli sorunlarını belirlemesi gerekmektedir. Tek Sağlık kapsamında belirlenen öncelikli sorunlarla ile ilgili olarak ilişkili disiplinlerden akademisyenlere de danışarak bir müdahale planı geliştirilmeli ve bu müdahale planları pilot bölgelerde gerçekleştirildikten sonra mevcut eksiklikler saptanıp ulusal düzeyde uygulanmalıdır. Bu yazıda Tek Sağlık kavramının gelişimine, halk sağlığı ile benzeşen ve tamamlayıcı yönlerine, iyi uygulama örneklerine yer verilmiştir.

Anahtar Kelimeler: Tek sağlık, halk sağlığı, hayvan sağlığı, zoonoz, çevre sağlığı

antimicrobial resistance, food safety and security, environmental health, and climate change—the One Health approach facilitates a comprehensive assessment of information, allowing for early risk identification, preventive measures, and enhanced control strategies. Due to the close interrelationship between human, animal, and environmental health, as well as the complex nature of health issues, achieving lasting solutions to common health problems requires a transdisciplinary, holistic approach rather than isolated solutions from individual disciplines. Sustainable solutions for shared health problems require moving beyond isolated, discipline-specific solutions to establish an integrative approach that involves multiple disciplines. For the successful implementation of this approach, it is essential to establish local and national frameworks, led by public authorities, to integrate systems and ensure community engagement. These integrated structures should prioritize the main issues that can be addressed under the One Health framework. An intervention plan, developed with insights from academics across relevant disciplines, should address these priority issues. Following implementation in pilot regions, gaps should be identified and adjustments made to expand the plan nationally. This paper explores the development of the One Health concept, its parallels and complementary aspects with public health, and highlights exemplary practices worldwide.

Key Words: One health, public health, animal welfare, zoonoses, environmental health

GİRİŞ

İnsan, hayvan, bitki ve çevre sağlığı yakın bir ilişki içindedir ve birbirlerinden etkilenmektedir (1). Bulaşıcı hastalıkların %60'ından fazlasının zoonotik kaynaklı olduğu, her yıl 2,5 milyar hastalığın ve 2,7 milyon ölümün zoonotik hastalıklar nedeniyle gerçekleştiği tahmin edilmektedir (2, 3). Diğer yandan doğal kaynakların yenilenme hızından daha hızlı tüketilmesi, tarımın sürdürülebilir yöntemlerle yapılmaması sağlık risklerini arttırmaktadır (4). Günümüzde, nüfusun artması ile daha geniş bir

coğrafyada yaşamın sürdürülmesi, hayvansal ürünlere daha fazla ihtiyaç duyulması, iklim değişikliği, kentleşme, ormanlık alanların azalması, artan tarımsal faaliyetler, artan madencilik, uluslararası ticaretin ve seyahatin artması nedeniyle insan, hayvan ve çevre etkileşimi artmıştır. İnsan, hayvan ve çevre etkileşiminin artması yeni salgınların ortaya çıkmasını kolaylaştırmaktadır (1, 5). Birden fazla sektörün bir araya gelerek ortak çalışmasını gerektiren bu alanlarda hiçbir sektör veya kuruluş tek başına hareket ederek bu sorunlara karşı etkili bir çözüm üretemez (6). Sağlık risklerine karşı önlem alabilmek, etkili çözümler üretebilmek için konu ile ilgili sektörlerin arasında iyi bir iletişim ve iş birliği gerekmektedir. Bu konuda bilimin farklı alanları eşgüdüm içinde hareket etmektedir ve geniş perspektifler sunmaktadır. Küresel sağlık, halk sağlığı, Tek Sağlık bu kapsamda öne çıkan kavramlardır. Tek Sağlık, dinamik ve güncel bir yaklaşım olup insanların, hayvanların ve çevre sağlığının uyumunu gözeterek en iyi hale getirmeyi amaçlayan, iş birliği gerektiren tümelci, birleştirici bir perspektif sunmaktadır (1, 5, 7).

Bu yazı kapsamında Tek Sağlık yaklaşımının halk sağlığı için önemini bilerek konu ile ilgili Dünya'da iyi uygulama örneklerinin açıklanması amaçlanmıştır. Bu amaca ulaşabilmek için çalışmada yaklaşımın tanımlanması, tarihsel gelişiminin açıklanması, Tek Sağlık ve halk sağlığı kavramlarının kesişimselliklerinin tanımlanması, Tek Sağlık ile ilgili halk sağlığı alanında Dünya'da iyi uygulama örneklerinin açıklanması, konu ile ilgili araştırma ve diğer gereksinimlerin tanımlanması ve geleceğe dair önerilerin açıklanması hedefler arasında yer almıştır. Çalışmanın yapılabilmesi için PubMed ve Google Akademik veri tabanları taranmıştır. Konu ile ilgili WHO, CDC, FAO, UNEP, WOAH, PAHO, European Commission, FDA Türk Tabipleri Birliği, Veteriner Hekimler Derneği, Halk Sağlığı Uzmanları Derneği, Sağlık Bakanlığı ve Tarım Orman Bakanlığına ait kurumsal web siteleri incelenmiştir. Konu ile ilgili eylem planları ve öne çıkan küresel metinler incelenmiştir.

Kavramlar ve İlkeler

Tek Dünya Tek Sağlık, Tek Tıp ve Tek Sağlık kavramları insan, hayvan ve çevre sağlığı ile ilgili çalışmalara yön veren yaklaşımlardır (8). Tek Tıp kavramı, insan ve hayvan sağlığının pek çok ortak nokta içermesi nedeniyle iki farklı disiplinin entegre edilmesinin insan ve hayvan sağlığına katkı sunacağı düşüncesiyle 1984 yılında ortaya çıkmıştır. Çevre sağlığının insan ve hayvan sağlığı ile yakın ilişkisinin zamanla daha iyi anlaşılması üzerine Tek Tıp kavramının daha geniş bir çerçevede ele alınmaya başlanmıştır (9, 10).

Tek Sağlık; insanların, hayvanların, bitkilerin ve ekosistemlerin sağlığını sürüdürülebilir bir uyum içinde en iyi hale getirmeyi amaçlayan tümelci ve birleştirici bir yaklaşımdır. İnsan, hayvan, bitki ve çevre sağlığının birbirleriyle yakından bağlantılı ve birbirine bağlı olduğunu kabul etmektedir. Farklı disiplinlerin, sektörlerin bir arada çalışmasını gerektirir; sağlıklı gıda, su, enerji ve hava gereksinimini vurgular. Yaklaşım beş temel ilkeye öncelik verir (Şekil 1) (10).

Tek Sağlık Kavramının Gelişimi

Tek Sağlık kavramının kökenine dair ilk örnekler milattan önce 300-400 yıllarına dayanmaktadır. Aristoteles'in çalışmalarında hayvan hastalıklarından, Hipokrat'ın temiz bir çevre ile halk sağlığının birbirlerini etkilemesinden bahsetmesi Tek Sağlık kavramının kökenine dair ilk örnekler olarak gösterilmektedir (11). Rudolf Virchow 19. yüzyılda yaptığı araştırmalar sırasında domuz paraziti Trichinella spiralis'in insanlarda da hastalık yaptığını keşfetmesi üzerine ilk kez zoonoz kavramını kullanmıştır ve insan sağlığı ve hayvan sağlığı kavramlarının ayrı ayrı değerlendirilmemeleri gerektiğini tıp ve veteriner hekimlikte gerçekleşecek ilerlemenin birbirlerine fayda sağlayacağını belirtmiştir (12, 13). 2004 yılında DSÖ, BM Gıda ve Tarım Örgütü (UN Food and Agriculture Organization - FAO), Amerika Birleşik Devletleri Hastalıkları Kontrol ve Korunma Merkezi (USA Centers for Disease Control - CDC) gibi pek çok kuruluşun katılımıyla gerçekleştirilen "Küreselleşen Dünyada

Şekil 1. Tek Sağlık yaklaşımının öncelik verdiği ilkeler (10)

Sağlıkta İnterdisipliner Köprüler Kurmak" başlıklı sempozyumda Manhattan İlkeleri yayınlanmıştır. Tek Dünya, Tek Sağlık sloganı ile yayınlanan Manhattan İlkeleri 12 maddeden oluşmaktadır. Manhattan İlkelerinde;

- İnsan ve hayvan sağlığının birbirini etkilediği ve Tek Sağlık yaklaşımıyla insan ve hayvan sağlığının bir arada değerlendirilebileceği bunu sağlayabilmek için de ortak bütüncül yaklaşımlar geliştirmek gerektiği
- Yeniden ve yeni ortaya çıkan bulaşıcı hastalıkların insanlarla beraber bütün canlılar için bir tehdit oluşturduğu
- İnsan ve hayvan sağlığı için biyolojik çeşitliliğin önemi
- Ekosistemlerin sürdürebilirliğinin sağlanması açısından toprağı işleme ve suyu kullanma ile ilgili kararların önemi
- Yabani yaşamın hastalıkları önlemede temel bir bileşen olduğu
- İnsan sağlığını korumaya yönelik programların önemi
- Türler arası bulaşıcı hastalıkları engelleyebilmek için yabani hayvan et ticaretine yönelik düzenlemeler yapmanın ve talebi azaltmanın önemi
- Küresel olarak insan ve hayvan sağlığına yönelik yatırımların arttırılması gerektiği
- Hükümetler, halk ve kâr amacı gütmeyen kuruluşlar arasında iş birliğinin sağlanmasının önemi
- Küresel erken uyarı sistemlerinin kurulabilmesi için gerekli desteğin sağlanmasının önemi
- Küresel anlamda farkındalığın arttırılması ve eğitimin desteklenmesi için yatırımların yapılmasının önemi vurgulanmıştır (14, 15).

Tarihçede yer alan önemli hatırlatmalar Tablo 1'de sunulmuştur.

Tablo 1. Tek Sağlık yaklaşımının tarihçesine ilişkin bazı önemli hatırlatmalar (14, 16)

Dönem/yüzyıl	Öne çıkan olay
19. yüzyıl	Virchow zoonoz kavramını ilk kez kullanmıştır.
1947	CDC bünyesinde Veteriner Halk Sağlığı bölümü kurulmuştur.
2004	Manhattan ilkeleri yayınlanmıştır.
2007	111 ülkenin katılımı ile Yeni Delhi'de gerçekleştirilen uluslararası bir konferansta *Tek Sağlık* yaklaşımının geliştirilmesi teşvik edilmiştir.
2008	FAO, WOAH, DSÖ iş birliğiyle *Tek Sağlık* uygulamalarına yönelik stratejik bir çerçeve oluşturulmuştur.
2009	*Tek Dünya, Tek Sağlık* başlığı altında temel öneriler geliştirilmiştir.
2011	1. Uluslararası *Tek Sağlık* kongresi düzenlenmiştir.
2024	8. Dünya *Tek Sağlık* kongresi düzenlemiştir.

Türkiye'de Refik Saydam Hıfzısıhha Enstitüsünün 1928 yılında kurulması, zoonotik hastalıklarla mücadele kapsamında farklı disiplinlerin bir arada çalışmasına olanak sağlamıştır. Tek Sağlık kavramının uluslararası alanda daha fazla yer bulmaya başlamasıyla beraber, Türkiye'de de 2008 yılında Halk Sağlığı Uzmanları Derneğinin ve yine aynı yıl Veteriner Hekimleri Mikrobiyoloji Derneğinin düzenlediği kongrelerde Tek Sağlık kavramından bahsedilmeye başlanmıştır. 2009 yılında ise Türk Tabipleri Birliği ve Türk Veteriner Hekimleri Birliği arasında "Tek Dünya, Tek Sağlık" sloganıyla ortak bir deklarasyon yayınlanmıştır (17). Ortak yayınlanan deklarasyon metninde ilgili kurumlarla ilgili iş birliğinin geliştirilmesinin, farklı kurumlardan elde edilecek verilerin birlikte değerlendirilebileceği bir yapı kurulmasının ve Veteriner Halk Sağlığı biriminin oluşturulmasının önemi vurgulanmıştır (18). Dünya Sağlık Örgütü'nün, Dünya Hayvan Sağlığı Örgütü'nün (WOAH), Birleşmiş Milletler Gıda ve Tarım Örgütünün (FAO) ve Çevre Programı'nın (UNEP) ortak çalışmasıyla tüm dünyada Tek Sağlık uygulamalarını yaygınlaştırabilmek için ülkelerde kurulmasını planladıkları koordinasyon yapısını Türkiye'de de kurulmasını sağlamak amacıyla 2023 yılında bu dörtlü yapının önderliğinde Türkiye'de Ulusal Tek Sağlık Koordinasyon Mekanizması Oluşturulmasına Yönelik Çalıştay yapılmıştır. Çalıştaya Sağlık Bakanlığı, Tarım Orman Bakanlığı, Sivil Toplum Kuruluşları ve konu ile ilgili akademisyenler ve pek çok meslek çalışanı katılım sağlamıştır (19).

Yaklaşımı Nerelerde Kullanalım?

Yeni salgınları önleyebilmek, kontrol altında tutabilmek, etkin bir şekilde tedavi edebilmek için her geçen gün önemi artan Tek Sağlık yaklaşımına daha fazla ihtiyaç duyulmaktadır. Bulaşıcı hastalıklar, antimikrobiyal direnç, vektörel hastalıklar, gıda güvenliği ve güvencesi, kronik hastalıklar, ruh sağlığı ve hastalıkları, biyolojik çeşitliliğin azalması, hava ve su kirliliği, iklim değişikliği gibi birçok halk sağlığı sorununa Tek Sağlık yaklaşımı ile çözümler üretilebilir birincil, ikincil ve üçüncül koruma etkili bir şekilde gerçekleştirilebilir (1, 5, 6, 20). Tek Sağlık yaklaşımı doktorlar, veteriner hekimler, halk sağlığı uzmanları, yaban hayatı uzmanları, çevre sağlığı uzmanları başta olmak üzere birçok farklı meslek grubunun bir arada çalışmasını gerektirir (1, 21). İnsan, hayvan ve

çevre sağlığının birbirleriyle olan karmaşık ilişkisi nedeniyle farklı disiplinlerden profesyonellerin bir arada uyum içinde çalışmasının ve elde edilen verilerin diğer disiplinler tarafından etkili bir şekilde kullanılmasının, verilerin bütüncül bir yaklaşımla değerlendirilebilmeleri için bir araya getirilmesinin gerekmesi kapsayıcı çözümleri ve sistemleri zorunlu hale getirmektedir (4). Farklı disiplinlerin uyum içinde çalışmasını sağlamak için yapılması gereken sağlık harcamaları ile ilgili olarak bütüncül bir yaklaşım sergilemeden sağlık sorunlarını ayrı ayrı çözmeye çalışmak, COVID-19 sürecinde de görüldüğü üzere daha fazla maliyete neden olmaktadır. Tek Sağlık kapsamında yapılacak sağlık harcamalarının, Tek Sağlık yaklaşımı sayesinde elde edilecek faydanın %10'undan daha az maliyetli olacağı öngörülmektedir (5).

Tek Sağlık yaklaşımı; insan, hayvan ve çevre sağlığını ayrı ayrı değerlendirmek yerine tümelci bir yaklaşımla Tek Sağlık başlığı altında hepsini bir arada değerlendirerek en iyi sağlık sonuçlarını elde etmeyi hedefler (5). Bu tümelci yaklaşımı sağlayabilmek için konu ile ilgili farklı disiplinlerden uzmanların bir arada çalışması, ayrıca farklı kurum ve kuruluşların kaynak ve bilgi paylaşımı ile birbirlerini desteklemesi gerekmektedir (22). İnsan, hayvan, bitki ve ekosistem bütünlüğünün birbirine bağlı olan yapısı ve sorunlarının karmaşıklığı geniş bir çerçevede planlanmış çözümleri gerekli kılar. Geniş bir çerçevede planlanmış çözümler ancak her disiplinin diğer disiplinlerden bağımsız süregelmiş çözümlerinin ötesinde tümelci bir yaklaşımla elde edilebilir. Bu da ancak Tek Sağlık yaklaşımının ilgili bütün sektörler tarafından benimsenmesiyle mümkündür. Tek Sağlık yaklaşımı ile, ilgili disiplinlerin bilgi toplaması ve toplanan bilgilerin paylaşılması, planlanan faaliyetlerin gerçekleştirilmesi ve kurumsal bir kimlikle sürdürülmesi, sektörler arasında sağlıkla ile ilgili uyarı ve tehditlere yönelik sürecin başarı ile yürütülmesini sağlayacak protokoller ve bütüncül çözümlerin elde edilebileceği kurumsal karar süreçlerinin sağlanması gerekmektedir (4). Bu yüzden Tek Sağlık yaklaşımı için farklı disiplinlerin bir arada tümelci yaklaşımlarını sergileyebilecekleri; iletişimi, eş güdümü ve kapasiteyi arttırabilecekleri yapısal değişiklikler gereklidir. Yapısal değişikliklerle; entegre bir sürveyans sistemi, erken uyarı sistemleri, rutin izlemlerin yapılabileceği ve acil durumlarda kullanılabilecek eş güdümü destekleyecek platformlar sağlanmalıdır (1, 5).

Gıda ve Tarım Örgütü, Birleşmiş Milletler Çevre Programı, Dünya Sağlık Örgütü, Dünya Hayvan Sağlığı Örgütü katılımıyla 2021 yılında Tek Sağlık Üst Düzey Uzman Paneli (One Health High-Level Expert Panel) gerçekleştirilmiş ve ardından Tek Sağlık Ortak Eylem Planı (One Health Joint Plan Of Action) yayınlanmıştır (23). Bu eylem planı, Tek Sağlık uygulamaları için bir çerçeve hazırlamak, Tek Sağlık faaliyetlerinin kalıcı bir büyümeyle geliştirebilmek için çeşitli faaliyetler önermektedir. Tek Sağlık Ortak Eylem Planı altı ana tema üzerine oluşturulmuştur.

- Tek Sağlık kapasitelerini geliştirerek, sağlık sistemlerini güçlendirmek
- Yeni ortaya çıkan ve yeniden önem kazanan zoonotik salgınlar ve pandemilerin neden olduğu riskleri azaltmak
- Endemik, tropikal ve vektör kaynaklı zoonotik hastalıkları kontrol altına almak ve ortadan kaldırmak
- Gıda güvenliği ile ilgili riskleri değerlendirmek, yönetimini güçlendirmek ve iletişimi desteklemek
- Antimikrobiyal direnç salgınını kontrol altına almak
- Çevreyi Tek Sağlık yaklaşımı ile uyumlu bir şekilde bütünleştirmek (4).

Halk sağlığı çalışmalarında da farklı disiplinlerin iş birliği içinde çalışması gerekir. Tek Sağlık yaklaşımı halk sağlığı sorunlarının çözümünde etkili bir şekilde kullanılabilir. İnsan, hayvan ve çevre sağlığını birlikte tehdit eden sağlık sorunlarına yönelik hiçbir disiplin tek başına çözüm üretemez. Bu sorunların çözümü için iyi kurgulanmış yapısal değişikliklerle desteklenmiş bütüncül yaklaşımlar sergileyen ortak

yaklaşımlara ihtiyaç vardır (1, 4, 5, 24).

Yeni ortaya çıkan ve yeniden önem kazanan zoonotik hastalıklar, endemik, tropikal ve vektör kaynaklı zoonotik hastalıklar, antimikrobiyal direnç, gıda güvenliği ve gıda güvencesi, çevre kirliliği, iklim değişikliği ve insan, hayvan, çevre sağlığını tehdit eden diğer hastalıklar ve sağlık sorunları Tek Sağlık yaklaşımı ile kontrol altına alınabilir (1, 25). Tek Sağlık perspektifinin kullanıldığı alanlara Şekil 2'de yer verilmiştir.

Şekil 2. Tek Sağlık perspektifinin kullanıldığı alanlar (1)

Son yüzyılda toplum sağlığını tehdit eden, sosyal ve ekonomik problemlere neden olan çok sayıda yeni ortaya çıkan veya yeniden önem kazanan zoonotik hastalık görülmektedir (26). Nüfusun hızla artması ve daha geniş coğrafyalara yayılması, artan nüfusla beraber hayvansal ürünlere olan ihtiyacın artmasıyla beraber artan insan-hayvan ilişkisi, iklim değişikliği, küresel hareketliliğin artması gibi pek çok sebep yeni ortaya çıkan ve yeniden önem kazanan hastalıkların daha fazla görülmesine ve daha hızlı yayılmasına neden olmaktadır (1, 5). Yeni ortaya çıkan hastalıklar, pandemilere neden olarak ciddi ekonomik kayıplara da neden olabilmektedir. SARS salgının yaklaşık 40 milyar dolar, kuş gribinin yaklaşık 30 milyar dolar domuz gribinin ise yaklaşık 50 milyar dolar ekonomik kayba neden olduğu tahmin edilmektedir (27). Yeni ortaya çıkan ve yeniden önem kazanan bulaşıcı hastalıkların birçoğu hayvanlara yakın temas yolu ile insanlara bulaşmaktadır. İnsan ve hayvan sağlığının yakın ilişkisi nedeniyle bu konuya Tek Sağlık yaklaşımı ile yaklaşmak, Tek Sağlık yaklaşımı ile hazırlanmış ulusal bir stratejiden yoksun ülkeler ulusal bir Tek Sağlık planı oluşturmaları, hali hazırda bu yaklaşıma sahip ülkelerde ise Tek Sağlık yaklaşımı ile müdahale kapasitelerini arttırmaları için desteklenmelidir(28).

Bulaşıcı hastalıkların %17'sini vektör kaynaklı hastalıklardan oluşurken vektör kaynaklı hastalıklar yılda 700.000'den fazla ölümden sorumlu tutulmaktadır. Sıtma her yıl 249 milyon kişiye bulaşıp 600.000'den fazla ölüme neden olurken dang ateşi geniş bir coğrafi alanda 132'den fazla ülkede etkili olmaktadır. Vektör kaynaklı hastalıkların, hastalığın bulaşma şekline göre insan, hayvan, çevre ve vektör olmak üzere pek çok bileşeni vardır (29, 30). Amerika'da Arizona eyaletinde 2018 yılında yerel kabilelerde Rocky dağları benekli ateşini kontrol altına almak için bir Tek Sağlık uygulaması gerçekleştirilmiştir. Uygulama kapsamında yerel örgütlerde çalışmaya dahil edilerek köpeklere kene tasmaları takılmış, evler ilaçlanmış, eğitim materyalleri hazırlanıp dağıtılmış, sağlık uzmanları eğitilmiş, çöpler toplanmış, hayvanlara yönelik kontrol çalışmaları yapılmıştır. Bu yolla bölgede vaka ve ölüm sayısı üçte ikiden fazla azaltılmıştır (31).

Mikroskobik canlılar, doğal süreçlerinin bir parçası olarak zamanla antimikrobiyal ilaçlara karşı direnç geliştirmektedir. Antimikrobiyal ilaçların hem insanlarda hem de hayvanlarda gereksiz veya fazla kullanılması mikroskobik canlıların antimikrobiyal ilaçlara karşı direnç geliştirmesini hızlandırmakta ve aynı zamanda yaygınlaştırmaktadır (32). Hayvanlarda antimikrobiyal ilaçların tedavi dozlarının altında ve uzun süre kullanılması mikroskobik canlıların antimikrobiyal ilaçlara karşı direnç geliştirmesini kolaylaştırmaktadır. Hayvanlarda direnç kazanan mikroskobik canlılar kontamine gıdalar veya çevre yolu ile insanlara bulaşabilmektedir. Ayrıca hayvanlarda kullanılan antimikrobiyal ilaçların insanlarda kullanılanlarla büyük benzerlikler göstermektedir. Antimikrobiyal ilaçlara karşı dirence karşı 68. Dünya Sağlık Asamblesinde beş ana hedeften oluşan bir eylem planı hazırlanmıştır. Bu beş ana hedef konu ile ilgili farkındalık oluşturmayı, daha fazla araştırmayı destekleyerek daha fazla kanıta ulaşmayı, bulaşıcı hastalıkları sanitasyon ve hijyen tedbirleriyle azaltmayı, antimikrobiyal ilaçların direnç gelişimini göz önünde bulundurarak en uygun şekilde kullanılmasını, yeni ilaç, aşı ve tanı araçlarına yönelik yatırımların arttırılmasını şeklindedir (33). Sorunun kaynağı ve çözümüne yönelik hazırlanan ana hedefler incelendiğinde bu konuyu Tek Sağlık yaklaşımı ile ele almanın önemi ortaya çıkmaktadır (29, 30).

Yukarıda bahsedilen yeni ortaya çıkan bulaşıcı hastalıklar, yeniden önem kazanan bulaşıcı hastalıklar, vektör kaynaklı hastalıklar, antimikrobiyal ilaç direnci gibi konuların yanı sıra gıda güvenliği ve güvencesi, çevre sağlığı ve iklim değişikliği gibi konular Tek Sağlık perspektifiyle değerlendirilmesinin yanı sıra aynı zamanda halk sağlığı ile de yakından ilişkilidir(29). Tek Sağlık kavramının önemli halk sağlığı konularına tümelci bir yaklaşım sağlaması, Tek Sağlık kavramının halk sağlığı için önemli bir araç olduğu şeklinde yorumlanabilir.

İyi Uygulama Örnekleri

Amerika Birleşik Devletleri'nde, Oregon'da 2020 yılında insanlarda hastalığa neden olan bir Salmonella türü yabani bir ispinozdan izole edilmiştir. Yine 2020 yılında Kaliforniya'da yabani kuşlarda Salmonella enfeksiyonlarının arttığına dair veriler paylaşılınca iki olay arasında bağlantı olabileceği düşünülmüş. Kışın göç eden kuşlarda Salmonella enfeksiyonu ve enfeksiyona bağlı ölümler olağan olmasına karşın bildirilen vaka sayısında 5 yıl önce gerçekleşen salgına göre üç katlık bir artış gözlenmiştir. Bu yabani kuşlarla veya yabani kuşların yaşam alanları ve kuş bakıcıları ile temasta bulunan hayvanlara ve el hijyenine dikkat etmeyen insanlara Salmonella bulaşabilir ve hastalık meydana gelebilmektedir. Bu gelişmeler üzerine CDC Halk sağlığı uzmanları aracılığıyla Salmonella typhimurium enfeksiyonu olan kişilerde yabani kuşlarla temas öyküsünü sorgulatmıştır. CDC farklı eyaletlerden kuruluşların bir araya gelerek ortak çalışmasını sağlamış diğer yandan kuş besleyicilerin eğitimi için literatür taraması gerçekleştirip ilgili uzmanlık alanlarına danışarak bölgesel kuruluşlar aracılığıyla kuş bakımı, ölü kuşlara nasıl yaklaşacağını içeren tavsiyelerde bulunmuştur. İlk kez bu yaban

kuşları ile insanlarda görülen Salmonella enfeksiyonu ilişkilendirilerek enfeksiyonun daha iyi anlaşılması sağlanmıştır. Yaban hayatı çalışanları kuş ölümlerinin insan sağlığıyla ilgili olabileceğini öğrenmiştir. CDC'nin olaya dahil olmasıyla farklı eyaletlerle iş birliği sağlanmış ve uygulamanın ulusal düzeyde yapılması planlanmıştır (34).

Dünya Sağlık Örgütü'nün 2030 yılında kuduz hastalığından sıfır ölüm taahhüdünde bulunan Çin kuduz ölüm hızında tüm dünyada ikinci sırada yer almaktaydı. Kuduza bağlı ölümlerin birçoğu köpek ısırığı sonrasında gelişmektedir. Kuduz riskli temastan sonra profilaksiye ulaşmanın kolaylaştırılması ölüm sayılarında ciddi bir düşüşe yol açsa bile köpek popülasyonunu kontrol altına almak, büyük ölçekli aşılama kampanyaları ve kuduz ortaya çıkmadan tanı koyabilmek gerekmektedir.

Çin'de kuduz problemine çözüm bulmak için CDC ve Çin ofisi 2019 yılında Kuduz Eliminasyonuna Adım Adım Yaklaşım Çalıştayını (Stepwise Approach to Rabies Elimination) gerçekleştirdi. Bu çalıştay ile kuduzun önlenmesine yardımcı olacak uygulamaları belirlemek için insan ve hayvan sağlığı disiplinlerinden ulusal ve yerel yetkililer ve uluslararası kuruluşların temsilcileri (Dünya Sağlık Örgütü, Birleşmiş Milletler Gıda ve Tarım örgütü ve Dünya Hayvan Sağlığı Örgütü) katılmıştır. Çalıştayda kuduzu ortadan kaldırmak için yedi temel alandan 115 sorudan oluşan bir araç kullanılmıştır. 0 ila 5 puan arasında puanlandırılan bu araçtan sıfır puan almak henüz kuduz çalışmalarına başlamamış, 5 puan alan ülkelerse köpeklerden bulaşan kuduzun ortadan kaldırıldığı ve bakım aşamasına geçildiği şeklinde yorumlanmaktadır. Bakım aşaması insanın kuduza maruz kalması ve hayvan kuduz vakalarının rutin olarak izlendiği aşamadır. Çalıştay sırasında katılımcıların aldığı ortalama puan 1,5 olarak belirlenmiştir. Bu puan Çin'de kuduz ölümlerinin araştırıldığı ve incelendiği mevcut sistemi, proflaksi uygulamalarının varlığı, kırsal kesimde köpek aşılamalarının kapsamı ve köpek popülasyonu yönetim stratejilerini yansıtmaktadır. Ayrıca Çin'de hükümet tarafından koordine edilen kuduzla ilgili çalışma yapan biri birimin olmadığı belirlenmiştir. Çalıştay sonucunda yedi değerlendirme alanı (verilerin toplanması ve analizi, kuduzunun önlenmesi ve kontrolü, köpek popülasyonunun belirlenmesi ve kontrolü, laboratuvar, sağlık eğitimi, iş birliği ve yasal düzenlemeler) ile ilgili öncelikli faaliyetler belirlenmiştir.

- Kuduz kaynaklı insan ve hayvan ölümlerine ilişkin verilerin entegrasyonunun iyileştirilmesi
- İnsanların kuduz riskli teması ve profilaksi ihtiyacının belirlenmesi için yeni yaklaşımların uygulanması
- Laboratuvar tanı kapasitelerinin arttırılması
- Merkezi hükümet önderliğinde sektörler arasındaki iş birliğini geliştirmek için bir çalışma grubu oluşturulması ve bu oluşturulan grubun köpek popülasyonunu tahmin etmek ve aşı kampanyalarını desteklemek için yapılacak pilot projelerin koordine edilmesine yardımcı olması (35)

CDC Kazakistan'ın Jambıl eyaletinde Jambıl halk sağlığı ve veterinerlik çalışanlarıyla birlikte Jambıl eyaletinde 2000 – 2013 yılları arasında artan Kırım-Kongo Kanamalı Ateşi (KKKA) vakaları hakkında bilgi edinmek ve bir müdahale tasarlamak için bir çalışma yürütmüştür.

KKKA'da salgın başlangıç noktası inek, keçi, koyun gibi çiftlik hayvanlarıdır. Hastalık insanlara enfekte hayvanların kanına temasla, keneler aracılığıyla veya hasta bireylerin vücut sıvılarına temasla bulaşmaktadır. Hastalık ölümcüldür ve herhangi bir aşısı veya tedavisi bulunmamaktadır. Enfekte kişilerin beşte dördünde herhangi bir semptom görülmemektedir ve bu durum hastalığın yayılmasını kolaylaştırmaktadır. Bölgede öncelikle insanlarda ve hayvanlarda hastalığın yaygınlığını ölçmek için bir tanı testi uygulanmış, toplumda özellikle hastalık açısından riskli bireylere de bilgi, tutum ve uygulama bileşenleri olan bir anket yapılmıştır. CDC bir çalışma protokolü oluşturmuş, veri toplama öncesi veri toplama ile ilgili bir eğitim düzenlemiş ve veri toplama aşamasını yerinde izlemiştir. Ekipler anket ve tanı testi için 30 köy ziyaretinde bulunmuş ayrıca test

için kene de toplamışlar. Tanı testi yapılan bin kişiden beşinin son dört ay içinde on dördünün ise dört aydan daha önce enfekte olduğu tespit edilmiştir. Son dört aydan daha önce enfekte olan on dört kişinin tamamının hayvanlarla direkt temas gerektiren işlerde çalışmaktaymış. Tespit edilen kişilerin birçoğunun hastalık bildiriminin yapılmadığı fark edilmiştir. Ankete katılan her beş kişiden yalnızca birinin riskli müdahaleler yapacağı zaman kişisel koruyucu ekipman kullandığı bulunmuştur. Hayvanlardan toplanan kenelerin %97'den fazlasının hastalıkta vektör olarak rolü bulunan Ixodidae ailesinden olduğu, hayvanların %1,2'sinde hastalık taşıyan bir kene varlığı ve kenelerde sadece Ivermectin'in etkili olduğu tespit edilmiştir. Bu çalışma ile bölge KKKA ile ilgili birçok bilgi toplanmıştır. Bölgede toplumun KKKA ile ilgili bilgi ve becerisini artırmak için çeşitli kampanyalar, insanlara ve hayvanlara yönelik tarama sayılarının arttırılması ve ilaca direnç ile ilgili daha fazla çalışma yapılması planlanmıştır (36).

SONUÇ

İnsan, hayvan ve çevre sağlığının yakın ilişkisi ve sağlık sorunlarının karmaşık yapısı nedeniyle ortak sağlık sorunları ile ilgili kalıcı çözümler elde edebilmek için disiplinlerin konu ile ilgili ayrı ayrı çözümlerinden ziyade disiplinler üstü tümelci bir yaklaşım oluşturulmalıdır (5, 37). Bu bütüncül yaklaşımın gerçekleştirilebilmesi için kamu otoritesi önderliğinde kurulacak yerel ve ulusal yapıların sistemlerini entegre edecek ve toplum katılımını da sağlayacak yapıların oluşturulması gerekmektedir(38). Bu entegre yapıların ülkenin Tek Sağlık kapsamında çözüm üretilebilecek öncelikli sorunlarını belirlemesi gerekmektedir. Tek Sağlık kapsamında belirlenen öncelikli sorunlarla ilgili olarak ilgili disiplinlerden akademisyenlere de danışarak bir müdahale planı geliştirilmeli ve bu müdahale planları pilot bölgelerde gerçekleştirildikten sonra mevcut eksiklikler saptanıp ulusal düzeyde uygulanmalıdır. Tek Sağlık yaklaşımı birçok farklı disiplin tarafından kabul görmüş ve çeşitli sağlık sorunlarında uygulanmış olsa bile (22) Tek Sağlık ile ilgili iyi kurgulanmış uygulamalardan elde edilecek sonuçlara yeni uygulamaların kurgulanması için ihtiyaç vardır. Bu yüzden Tek Sağlık ile ilgili uygulamalar yerel, ulusal ve uluslararası kaynaklarla desteklenmelidir.

Kısa dönemde kamu otoritesi farklı disiplinlerin katılımıyla gerçekleştirilecek bir çalışma ile ulusal düzeyde Tek Sağlık ile ilgili bilgi alışverişini kolaylaştıracak disiplinler üstü bir yapı planlanmalı, orta dönemde bu yapının ilk örneklerinin pilot çalışmalarla bölgesel öncelikleri belirlemesi sağlanmalı ve bu pilot yapı önceliklere yönelik uygulamaları planlayıp uygulamalıdır. Uzun dönemde ise bölgesel düzeyde yapılan uygulamalar değerlendirilip tüm bölgeleri kapsayacak şekilde uygulama genişletilmelidir. Konu ile ilgili olarak Tek Sağlık yaklaşımının halk sağlığı sorunları ile ilgili sağlayabileceği risklerden erken haberdar olmak, gerekli önlemleri daha erken ve daha geniş bir yelpazede alabilmek, kalıcı çözümler için farklı disiplinlerin eşgüdüm içinde çalışmasını sağlayabilmek gibi faydaları göz önünde bulundurulduğunda halk sağlığı uygulamaları için elverişli bir araç olarak kullanılması yararlı olacaktır. Her aşamada uluslararası vizyon ve uygulamaların izlenmesi, iş birlikleri öncelenmeli ve ulusal çalışmalara yansıtılmalıdır.

ÇIKAR ÇATIŞMASI

Yazarlar bu makale ile ilgili herhangi bir çıkar çatışması bildirmemişlerdir.

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