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On behalf of General Directorate of Public Health

Prof. Dr. Sedat KAYGUSUZ, 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.

The authors must fully obey the ethics of research and publication.

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IV) OPEN ACCESS POLICY

The Turkish Bulletin of Hygiene and Experimental Biology has adopted open access policy. Open Access Policy is based on rules of Budapest Open Access Initiative (BOAI). According to BOAI, Open Access states, "Scientific articles that have been evaluated by the referee, via the Internet; be freely accessible, readable, downloadable, copied, distributed, printed, scanned, linked to full texts, indexed, transmitted as data and used for any legal purpose, without financial, legal and technical barriers. https://www.budapestopenaccessinitiative.org/read

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The Turkish Bulletin of Hygiene and Experimental Biology instant open access to the articles is provided to make the articles published in journals freely available online all over the world. There is no need to subscribe to access articles. All articles in the system can be accessed and read without being a journal user. There is no fee for article submission, evaluation and publishing.

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

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 20 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".

Turkish Bulletin of Hygiene and Experimental Biology requires that each author, reviewer, and editor must disclose to the editor-in-chief any conflict of interest related to family, personal, financial, political or religious issues as well as any competing interest outlined above at the WAME's definition. Whether or not a conflict of interest and financial support exist, they must be declared at the end of the manuscripts.

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. 20 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.

Turkish Bulletin of Hygiene and Experimental Biology

General Directorate of Public Health

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TÜRK HİJYEN VE DENEYSEL BİYOLOJİ DERGİSİ

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- Bütün yazarlarca isim sırasına göre imzalanmış telif hakkı devir formu eksiksiz olarak doldurulduu.
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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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TURKISH BULLETIN OF HYGIENE AND EXPERIMENTAL BIOLOGY

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- Copyright transfer form is completed in full and signed by all authors according to the name order.
- Author names are written clearly.
- Affiliated institutions of the all authors are given on the title page by the number stated after the author's name.
- The name, address, phone-fax numbers and mail address of the author responsible for correspondence are given.
- Turkish, English titles and short title are written.
- The number of words in Turkish and English abstracts (between 300-500) is checked.
- Turkish and English keywords (according to MeSH) are given.
- All abbreviations are reviewed and non-standard abbreviations are corrected
- Original Latin names of microorganisms are written in italic.
- Symbols are mentioned according to the units in the Système International (SI).
- The article is written in passive mode and given one of the "past perfect, present perfect or past " tenses.
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İÇİNDEKİLER / CONTENTS

Araştırma Makalesi / Original Article

Derleme / Review

Araştırma Makalesi/Original Article
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Türk Hijyen ve Deneysel Biyoloji Dergisi

In vitro efficacy of *Hypericum perforatum* and *Urtica dioica* on *Leishmania tropica*

Hypericum perforatum ve *Urtica dioica*'nın *Leishmania tropica* üzerindeki in vitro etkinliği

Fatma İSLAMOĞLU[1] (ID), Mehmet Sami İSLAMOĞLU[2] (ID), Murat HÖKELEK[3] (ID)

ABSTRACT

Objective: *Leishmania* parasites cause a wide range of human diseases from localized self-healing cutaneous lesions to fatal visceral disease. Failure of the drugs used in the treatment of leishmaniasis, side effects, and drug resistance has increased the need for new drugs. As an alternative to treatment under these requirements, the use of herbal extract comes up. The aim of this study is to research the anti-leishmanial effect of herbs that *Urtica dioica* and *Hypericum perforatum*.

Methods: Our study was designed ın vitro efficacy of herbal products. In this study, the promastigotes these are growth and passaged RPMI-1640 medium with L-glutamine buffered and supplemented with 10% Fetal calf serum (FCS) are inoculated in mixed solutions prepared with different herbal drug extract concentrations and medium. Parasites were allowed to incubate at + 26 °C for 72 hours. End of time, the parasites were incubated in the plate are counted on the Thoma slide. The 50% inhibitory concentrations are calculated.

ÖZET

Amaç: *Leishmania* cinsi parazitler, kendiliğinden iyileşebilen lokalize cilt lezyonlarından, ölümcül visseral hastalıklara kadar bir grup insan hastalığına neden olabilir. Leishmaniasis tedavisinde kullanılan ilaçların yetersizliği, yan etkileri ve direnç sorunu yeni ilaçlara olan gereksinimi arttırmıştır. Bu gereksinimler doğrultusunda tedaviye alternatif olarak bitkisel ekstraktların kullanımı gündeme gelmektedir. Bu çalışmanın amacı *Urtica dioica* ve *Hypericum perforatum* bitkilerinin anti-leishmanial etkinliklerini araştırmaktır.

Yöntem: Yaptığımız çalışmada, %10 Fetal calf serum (FCS) eklenmiş L-glutaminli RPMI-1640 besiyerinde çoğaltılan ve pasajlanan promastigotlar, değişik konsantrasyonlarda hazırlanan bitkisel ilaç ekstraktı-besiyeri karışımına inoküle edildi. 72 saat boyunca +26°C'de inkübasyona bırakıldı. Bu süre sonunda plakta inkübe edilen parazitler thoma lamında sayıldı ve %50 inhibitör konsantrasyon değerleri (IC50) hesaplandı.

Bulgular: *U. dioica* kök tentürünün 0,07 mg/ml konsantrasyonunda promastigotların üremesini inhibe etmediği, 9,37 mg/ml konsantrasyonunda üremeyi

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Results: It was observed that concentration of *U. dioica* root tincture 0.07 mg/ml doesn't inhibit promastigote proliferation, in case 9.37 mg/ml inhibits growth completely, the concentration of *U. dioica* leaf fluid extract 250 mg/ml inhibitors growth completely The concentration of *H. perforatum* essential oil 0.02 mg/ml don't inhibit promastigote proliferation, in case 3.12 mg/ml inhibits growth completely. The 50% inhibitory concentration (IC50) values for root and leaves of *U. dioica* were calculated that respectively 579.93 µg/ml, 244.16 µg/ml, and *H. perforatum* essential oil was calculated that 189.88 µg/ml.

Conclusion: It was found that *U. dioica* and *H. perforatum* had inhibitory effects on *Leishmania tropica* promastigotes *in vitro*. *H. perforatum* essential oil has the best antileishmanial activity of the drugs used in this study. The high effect of the *H. perforatum* essential oil, *U. dioica* root, and leaf extracts, against *Leishmania* promastigotes has been reported with this study, so this issue will lead to other studies. It was thought that both of them can be used as an alternative treatment option in the future because they are less toxic than many drugs in routine.

Key Words: Leishmania, *Hypericum perforatum*, *Urtica dioica*, antileishmanial effect, herbal treatment

tamamen inhibe ettiği, *U. dioica* yaprak ekstraktının 250 mg/ml konsantrasyonda üremeyi tamamen inhibe ettiği görüldü. *H. perforatum* esansiyel yağının ise 0,02 mg/ml konsantrasyonda promastigotların üremesini inhibe etmezken, 3,12 mg/ml konsantrasyonda üremeyi tamamen inhibe ettiği görüldü. *U. dioica* kök ve yaprakları için IC50 değerleri sırasıyla 579,93 µg/ml, 244,16 µg/ml olarak hesaplanmıştır. *H. perforatum* esansiyel yağı için ise IC50 değeri 189,88 µg/ml olarak hesaplanmıştır.

Sonuç: *U. dioica* ve *H. perforatum*'un *Leishmania tropica* promastigotlarına karşı *in vitro* ortamda anti-leishmanial aktivitelerinin varlığı tespit edilmiştir. Çalışmamızda kullandığımız ilaçlar arasında *Hypericum perforatum* esansiyel yağı en etkili anti-leishmanial aktivitesi olan ilaçtır. *H. perforatum* esansiyel yağının, *U. dioica* kök ve yaprak ekstraktlarının, *Leishmania* promastigotlarına karşı yüksek düzeyde etki gösterdiğinin ilk defa bu çalışma ile rapor edilmiş olması, bu konuda yapılacak diğer çalışmalara öncülük edecektir. Bu ekstraktlarn rutin kullanımdaki birçok ilaçtan daha az toksik olmaları nedeniyle ileride alternatif tedavi seçeneği olabilecekleri düşünülmektedir.

Anahtar Kelimeler: Leishmania, *Hypericum perforatum*, *Urtica dioica*, antileishmanial etki, bitkisel tedavi

INTRODUCTION

Leishmaniasis is a vector-borne infectious disease caused by the *Leishmania* species that can lead to different clinical conditions (1). More than twenty-one species of *Leishmania* transmitted by the sand fly vector can cause leishmaniasis (2). Leishmaniosis, whose two main clinical forms are visceral leishmaniasis and cutaneous leishmaniasis, is seen in approximately 100 endemic countries, with 0.7-1 million new cases reported annually (3). In endemic regions where the poorest segments of the global population reside, the risk of infection increases due to poor housing conditions, insufficient environmental cleanliness, a lack of personal protective measures, work, and migration, depending on the economy (4). World Health Organization (WHO) defines leishmaniosis as a neglected tropical disease due

to the difficulty and cost of diagnosis, treatment, and follow-up, lack of control tools, insufficient investment in research, and the fact that the affected people live in rural areas where access to treatment is inadequate (5).

Pentavalent antimonials, amphotericin B, paromomycin, and miltefosine are used as first-line therapies in the treatment of leishmaniosis and these medicine may lead to adverse events such as renal failure, cardiotoxicity, pancreatitis, and impaired liver functions. However, increased parasite resistance against these chemotherapeutics can be observed (6). There is no proven vaccine against the disease in humans, and less costly immunotherapy approaches that have limited side effects and do not create parasite resistance are emphasised (1). Given the increased demand for herbal products, for which there is insufficient data on their efficacy and safety profile, studies have been conducted on their use in the treatment of leishmaniosis (7). Most studies have been performed using promastigote forms of *Leishmania* in vitro. Further studies need to be conducted in vivo and in host macrophages (8).

Among natural products, essential fatty acids showed significant activity against *Leishmania* promastigotes (9). With their hydrophobic structure, they can penetrate cells and show a broad spectrum of biological activity (10). The chemical composition and the pharmacologically antidepressant, antinociceptive, and inhibition of monoamine oxidase, antiviral, antibacterial, antifungal, and anti-proliferative activity of *Hypericum perforatum* (*H. perforatum*) , known as Saint John's Wart, is a genus of Hyperceae (11,12). *H. perforatum*, which grows in Europe, North America, North Africa, and East Asia, and has more than 450 species, is the most important of the *Hyperium* species due to its pharmacological activity (13) (Figure 1-2).

Figure 1. *Hypericum perforatum* flowers

Figure 2. *Hypericum perforatum* leaf

Stinging Nettle (*Urtica dioica* (*U. dioica*)), is known to be in the *Urticaceae* plant family, can be seen in different parts of the world, such as India, Iran, Malaysia, and the United States. It has long been known as a fibre and medicinal plant and has been used for the control of hypertension, the regulation of blood sugar, prostatic hyperplasia, and anti-inflammatory treatment (14,15) (Figure 3).

In our study, we aimed to show the in vitro anti-leishmanial effect of *H. perforatum* essential oil and anti-leishmanial effect of *U. dioica* root and leaf extract due to their inhibitory effects on *Leishmania* promastigotes.

Figure 3. *Urtica dioica*

MATERIAL and METHOD

Preparation of the Medium

Roswell Park Memorial Institute (RPMI) 1640 (Sigma Chemical Co, USA) broth containing L-glutamine and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was used as the medium for sensitivity and passages in the production of *Leishmania*. The PH value of the medium was adjusted to 7.3-7.4. To prevent contamination, 80 µg/ml of gentamicin was added to the medium to be used for passage. The medium mixture was sterilised by filtering it into sterile bottles with 0.45 µm filters and stored at +4°C until use. Before use, the medium was brought to room temperature, and 10% inactivated fetal calf serum (FCS) (Pan Biotech, Germany) was added.

Obtaining *Leishmania tropica* Promastigotes

The study was conducted at the İstanbul Aydın University Faculty of Medicine Medical Microbiology Research Laboratory. In the study, *L. tropica* promastigotes, which were isolated from a cutaneous leishmaniasis patient and produced by passage into RPMI 1640 medium with HEPES containing 10% inactivated FCS and gentamicin in a culture flask, were used.

The culture medium was checked with an inverted microscope to look for promastigotes.

Herbal Medicine Solutions

As an active ingredient, *H. perforatum* essential oil (Bristol Botanicals, UK), *U. dioica* leaf liquid extract (Bristol Botanicals, UK), and *Urtica dioica* root tincture (Bristol Botanicals, UK) were used. *H. perforatum* essential oil (1,000 mg/ml) was dissolved in 10% dimethylsulfoxide (DMSO, 10% solution in PBS) at a ratio of 1/10, and subsequent dilutions were made with RPMI medium at a ratio of 1:2 each time.

Urtica dioica leaf extract (1,000 mg/ml, 25% ethanol) and root tincture (300 mg/ml, 25% ethanol) were prepared in an RPMI medium in 1/2-1/4096 dilutions.

Inoculation of Parasites in Herbal Medicine Solutions

Parasite cultures in an RPMI 1640 medium and 2% formol-PBS solution were taken in equal amounts and mixed in a micro-test tube, and the promastigotes were immobilised. Parasites were counted on Thoma slides. Serial dilutions of herbal medicine extracts were prepared in

96-well plates. After the dilutions were prepared, promastigotes were inoculated into each well, the number of which was determined by counting on the Thoma slide.

A total of 200 µl of parasite-herbal medicine in dilutions of 500-0.244 mg/ml for the *U. dioica* leaf extract, 150-0.07 mg/ml for the *U. dioica* root tincture, and 50-0.02 mg/ml for *H. perforatum* essential oil extract mixtures were obtained.

Evaluation of Incubation and Growth

The inoculated plate was incubated at 26-26.5 ºC for 72 hours. At the end of the incubation, the contents of the wells in the plate were transferred to the Thoma slide, and a parasite count was performed. Tables were created by determining the inhibition and growth rates for each concentration, and herbal medicine extract-promastigote growth inhibition curves were drawn. Concentration values (IC50) inhibiting 50% of promastigotes were calculated online (16).

Experiments were run at least three times for each herbal medicine extract. The drug concentrations causing 50% inhibition (IC50) are shown in the graph drawn with the % inhibition values calculated against the herbal drug extract concentrations.

RESULTS

When the growth and inhibition rates were determined by counting the parasites on Thoma slides in the cultivations made on the media, after 72 hours of incubation at +26°C, the *U. dioica* root tincture did not inhibit the growth of promastigotes at concentrations of 0.07 mg/ml but it was observed that it inhibited reproduction by 100% at concentrations of 9.37 mg/ml (Table 1).

Table 1. Promastigote growth rates and inhibition rates for *Urtica dioica* root tincture

U. dioica root tincture Dilutions (mg/ml)	Growth rate (%)	İnhibition rate (%)
Control	100	0
0,07	100	0
0,14	77	23
0,29	61	39
0,58	42	58
1,17	23	77
2,34	13	87
4,68	3	97
9,37	0	100*
18,75	0	100
37,5	0	100
75	0	100
150	0	100

**U. dioica* root tincture inhibited reproduction of 100% at concentrations of 9.37 mg/ml

The IC50 value for the *U. dioica* root tincture was calculated as 579.93 µg/ml (Figure 4). Dilutions were prolonged because inhibition continued even in the last dilution with the *U. dioica* leaf extract. It was observed that the *U. dioica* leaf extract inhibited the growth of promastigotes by 100% at a concentration of 250 mg/ml (Table 2).

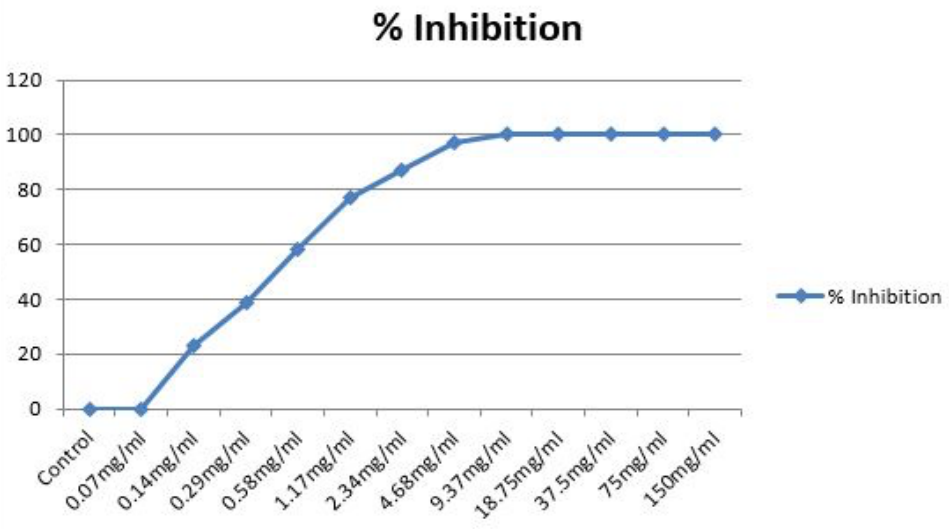

Figure 4. Effect of *U. dioica* root tincture on the growth of *Leishmania tropica* promastigotes

Table 2. Promastigote growth rates and inhibition rates for *Urtica dioica* leaf extract

U. dioica leaf extract dilution (mg/ml)	Growth rate (%)	Inhibition rate (%)
Control	100	0
0,003	92	8
0,007	72	28
0,015	67	33
0,030	77	23
0,061	62	38
0,122	43	57
0,244	48	52
0,488	29	71
0,976	25	75
1,953	24	76
3,906	24	76
7,812	24	76
15,625	18	82
31,25	18	82
62,5	8	92
125	3	97
250	0	100*
500	0	100

* *U. dioica* leaf extract inhibited the growth of promastigotes of 100% at a concentration of 250 mg/ml

The IC50 value for the leaf extract of the *U. dioica* was calculated as 244.16 µg/ml (Figure 5). It was observed that *H. perforatum* essential oil did not inhibit the growth of promastigotes at a concentration of 0.02 mg/mL and inhibited growth by 100% at a concentration of 3.12 mg/ml (Table 3). The IC50 value for *H. perforatum* essential oil was calculated as 189.88 µg/ml (Figure 6).

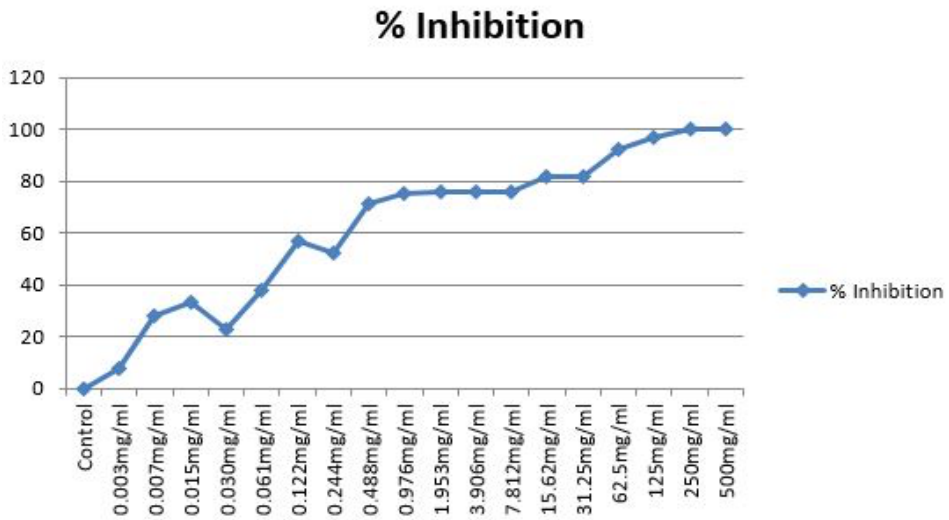

Figure 5. Effect of *U. dioica* leaf extract on the growth of *Leishmania tropica* promastigotes

Table 3. Promastigote growth rates and inhibition rates for *Hypericum perforatum* essential oil

H. perforatum essential oil dilution (mg/ml)	Growth rates (%)	Inhibiton rates (%)
CONTROL	100	0
0,02	100	0
0,04	84	16
0,09	82	18
0,19	47	53
0,39	37	63
0,78	18	82
1,56	5	95
3,12	0	100*
6,25	0	100
12,5	0	100
25	0	100
50	0	100

* *Hypericum perforatum* essential oil inhibited the growth of promastigotes at a concentration of 3.12 mg/ml

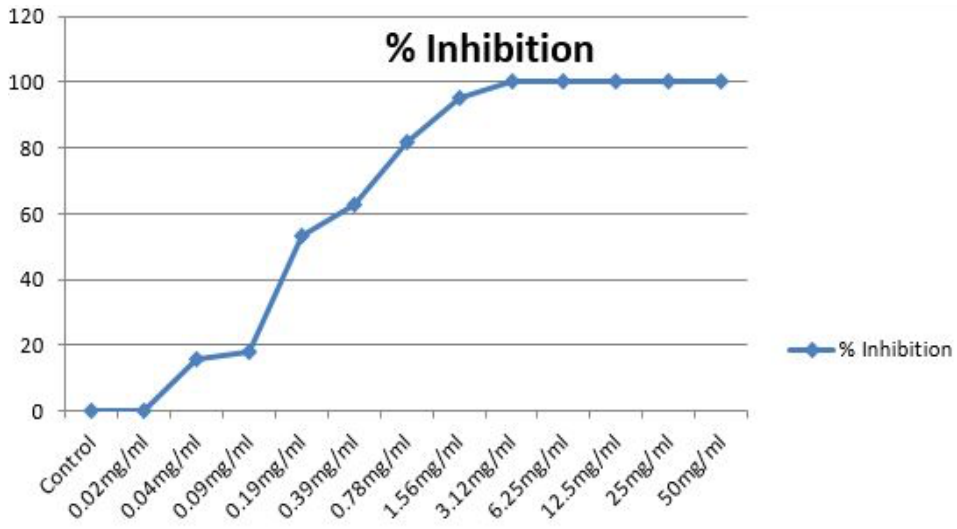

Figure 4. Effect of *H. perforatum* essential oil on the growth of *Leishmania tropica* promastigotes

Amphotericin B was used as a positive control. It inhibited growth at a concentration of 2µg/ml. The contribution of 25% ethanol in the content of the *U. dioica* plant solutions to the inhibitory effect on promastigotes was investigated. It was determined that the inhibitory effect detected in the dilutions made with *U. dioica* did not persist in the dilutions made with ethanol. The contribution of 10% DMSO, which we used to dissolve the essential oil, to the inhibitory effect on promastigotes was also investigated. No inhibitory effect of DMSO on promastigotes was detected.

DISCUSSION

In our study, the presence of the anti-leishmanial activities of *H. perforatum* and *U. dioica* against *L. tropica* promastigotes was determined. The IC50 value was the lowest for the *H. perforatum* essential oil, which was found to be more effective than the others.

Leishmaniasis is a disease caused by the obligate intracellular parasite *Leishmania* and *Leishmania* exists in two forms in its life cycle: as promastigote in a vector and amastigote in the mammalian host (17,18). Leishmaniasis can occur in various clinical forms, ranging from self-healing skin lesions to severe fatal diseases with organ involvement (19). Meglumine antimoniate and pentamidine, which are used in the treatment of leishmaniosis because of without effective vaccine, require long injections. New drugs are needed because of the toxic effects of amphotericin B and pentamidine (20). A total of 65% of the 15 antiparasitic drugs approved between 1981 and 2006 consisted of natural products or the derivatives of natural products (21). In the treatment of leishmaniosis, many plants with different medicinal effects collected from the tropical region are used (18). Chincinella-Carmona et al. investigated the anti-Leishmanial effect of 67 fresh or dried extracts of plants measuring % 50 inhibitory concentration (IC50) in their study and found that 16 plants were more effective than others (22). Montesino et al. investigated the in vitro activities of 58 herbal extracts against *Leishmania*, *Trypanosoma*, and *Plasmodium* and found that 16 extracts were effective (23).

Previous studies have noted the effectiveness of *U. dioica* root tincture in allergic rhinitis, arthritis, cardiovascular diseases, and prostate diseases. Badirzadeh et al. conducted the only study in the literature on anti-Leishmania activity (15). They investigated the in vivo mice infected with *Leishmania* major and in vitro activity of *U. dioica* aqueous extract against *Leishmania* and determined that *U. dioica* was effective against the *Leishmania* parasite.

In our study, besides determining the in vitro activity of the *U. dioica* root tincture against *Leishmania* promastigotes, we used the *U. dioica* leaf extract for *Leishmania* activity for the first time. When their efficacy was evaluated according to IC50 values, we found that the *U. dioica* leaf extract was more effective against *Leishmania* than the *U. dioica* root tincture.

Hypericum perforatum, which grows in many parts of the world and is accepted as a nutritional supplement, is an invasive species, especially in Asia and Europe. It has antidepressive, anxiolytic effects and is used in the treatment of depression, postmenopausal symptoms, obsessive-compulsive disorder, behavioural disorders, and psoriasis (24). Some bioactive molecule derived from Hypericum species is studied for leischmanicidal activity by measurement of IC50. For example a study by Dagnino et al. indicated that substances obtained from the *Hypericum* species may be high leischmanicidal activity against promastigot by inducing mitochondria and reactive oxygen compounds in *Leishmania* promastigotes (25). In their other study, Dagnino et al. found that the lipophilic extracts of four *Hypericum* species were effective against *L. amazonensis* (26). In this study most effective types are *H. carinatum, H. linoides* and *H. polyanthemum*. Studies have shown that caffeic acid, ferulic acid, syringic acid, 4-hydroxybenzoic acid, and chemicals found in herbal extracts exhibit parasiticidal effects against *L. tropica* (27). Studies have also shown that herbal extracts, such as *Zatara multiflora* oil, *Pistacia vera* essential oil, and *Myrrutus communis* oil, are effective against *L. tropica*, which is the causative agent of cutaneous *Leishmania* (28,30). *H. perforatum* has not previously been used in the treatment of *L. tropica*. Thus, our study was the first to establish that *H. perforatum* was effective against *L. tropica*.

In recent years, herbal products have been used to treat a wide range of infectious diseases as well as other diseases. We detected the presence of the antileishmanial activities of *H. perforatum* and *U. dioica*, which are less toxic than many drugs routinely used against *L. tropica* promastigotes. We think that examining the substance or substances responsible for the antiLeishmanial effect of the plant solutions we used in our study, conducting toxicity studies, and developing the results of these studies with in vivo models may be useful for future clinical studies.

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
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Türk Hijyen ve Deneysel Biyoloji Dergisi

Molecular detection of leptospirosis from genital system in mares

Kısrakların genital sisteminden leptospirosisin moleküler tespiti

Derya KARATAŞ YENİ[1] (ID), Aslı BALEVİ[2] (ID), Ayten GÖK[2] (ID)

ABSTRACT

Objective: Leptospirosis is a worldwide zoonotic disease and well recognized infectious disease of horses. Equine leptospirosis is to cause the birth of weak foals, neonatal deaths and abortion after pregnancy period. Studies on leptospirosis in horses are generally investigations of urine samples and serological studies. Although leptospirosis causes reproductive disorders, genital sample studies that may be a source of infection have been ignored. The aims of this study were to study the prevalance of *Leptospira* by PCR using vaginal swab from apparently healthy horses.

Methods: A total of 92 vaginal swab samples were collected and transferred to the bacterial diagnosis laboratory of Selcuk University Veterinary Faculty. The vulva and vagina were cleaned before swab samples were taken. All samples were stored in the refrigerator at -20°C and taken to the laboratory for processing. These samples were sent to the laboratory under cold chain conditions. DNA was extracted from suspicious samples and conventional PCR was used to detect Leptospira spp. Specific primers were selected and PCR was finalized for *Leptospira* spp.

ÖZET

Amaç: Leptospirosis, dünya çapında zoonotik olan ve atların iyi bilinen bir enfeksiyöz hastalığıdır. Atlarda leptospirosis, zayıf tayların doğumuna, yenidoğan ölümlerine ve gebelik sonrası abortlara sebep olmaktadır. Atlarda leptospiroz ile ilgili çalışmalar genellikle idrar örneklerinin araştırılması ve serolojik çalışmalardır. Leptospirosis, reprodüktif bozukluklara yol açmasına rağmen, enfeksiyon kaynağı olabilecek genital örnek çalışmaları göz ardı edilmiştir. Bu çalışmanın amacı, herhangibir klinik semptom göstermeyen kısraklardan alınan vajinal sürüntü örneklerinde PCR ile *Leptospira* prevalansını incelemekti.

Yöntem: Toplam 92 adet vajinal sürüntü örneği toplanarak Selçuk Üniversitesi Veteriner Fakültesi bakteriyel tanı laboratuvarına aktarılmıştır. Sürüntü örnekleri alınmadan önce vulva ve vajina temizlenmiştir. Tüm örnekler -20°C'de buzdolabında saklanmış ve işlenmek üzere laboratuvara götürülmüştür. Bu numuneler soğuk zincir koşullarında laboratuvara gönderilmiştir. Şüpheli örneklerden DNA ekstrakte edilmiş ve *Leptospira* spp.'yi saptamak için spesifik primerler seçilmiş ve konvansiyonel PCR kullanılmıştır.

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Results: As a result of the study, it was found that out of 92 mare's vaginal swab samples 7 (7.6%) were positive for *Leptospira* spp. This study is the first report from our country due to the detection of Leptospira spp DNA in asymptomatic mares.

Conclusion: It revealed that the Leptospira PCR positive mare were not showing any signs and symptoms. When the results and observations were evaluated, it was thought that Leptospira PCR positive mares could play a role as a carrier in the transmission of leptospirosis. Our study is one of the rare studies on mares carrying the possible causative agent. Detection of leptospirosis by PCR can be considered as a reliable method for early detection of *Leptospira* shedding in asymptomatic animals. In addition, molecular studies from vaginal swab samples were observed as a rapid and definitive diagnostic option, considering the difficulty of isolation of the *Leptospira* agent and possible contaminations. According to the results of our study, it is recommended to reevaluate the control measures against the disease and to carry out molecular characterization and vaccination studies in risky areas.

Key Words: Leptospirosis, mare, PCR

Bulgular: Çalışmanın sonucunda, 92 kısrak vajinal sürüntü örneğinden 7 (%7,6)'sinde *Leptospira* spp. pozitif olduğu saptanmıştır. Bu çalışma, asemptomatik kısraklarda Leptospira spp DNA'sının tespit edilmesi sebebiyle ülkemizden ilk rapordur.

Sonuç: Leptospira PCR testi pozitif bulunan kısrakların herhangi bir belirti ve semptom göstermediği belirlendi. Sonuçlar ve gözlemler değerlendirildiğinde, Leptospira PCR testi pozitif kısrakların leptospirosis bulaşmasında taşıyıcı olarak rol oynayabileceği düşündürdü. Çalışmamız, olası etkeni taşıyıcı kısraklar üzerine yapılan ender çalışmalardan biridir. Leptospirosisin PCR ile tespiti, asemptomatik hayvanlarda *Leptospira* saçılımının erken tespiti için güvenilir bir yöntem olarak kabul edilebilir. Ayrıca, *Leptospira* etkeninin izolasyon güçlüğü ve gelişebilecek kontaminasyonlar göz önüne alındığında, vajinal sürüntü örneklerinden moleküler çalışmalar, hızlı ve kesin bir tanı seçeneği olarak gözlemlendi. Çalışmamızın sonuçlarına göre, hastalığa karşı kontrol tedbirleri yeniden değerlendirilip riskli alanlarda moleküler karakterizasyon ve aşılama çalışmaları yapılması önerilmektedir.

Anahtar Kelimeler: Leptospirozis, kısrak, PCR

INTRODUCTION

Leptospirosis is a zoonotic infectious disease with multi-organ involvement caused by *Leptospira* spp., a gram-negative, non-sporeless, non-encapsulated and aerobic bacterium (1). Leptospiras are bacteria belonging to the Leptospiraceae family in the class Spirochaetales. Spirochetes are approximately 0.1 µm in diameter and 6-20 µm in length (1).

Leptospirosis is a zoonosis that has gained great importance in terms of public health. Transmission of the disease to humans occurs through animal reservoirs, contact with urine, and exposure to contaminated environments. In addition, floods that may occur as a result of natural disasters such as excessive precipitation and earthquakes contribute to the spread of the disease (2).

Diagnosis of leptospirosis is done by clinical signs, autopsy findings, microscopy, culture, animal experiments, serology and Polymerase Chain Reaction (PCR) (3). Microscopic Agglutination Test (MAT) is the most widely used serological test in reference laboratories of our country and known as the "gold standard" test (4). MAT has good specificity. However, the sensitivity of MAT in the early period is low (4). There is a high probability of false negative cases. Apart from these tests, complement fixation and immunofluorescence tests are also used in the

diagnosis of *Leptospira*. However, all these tests are not sufficient to identify subclinically infected animals (5). There is dire need for rapid diagnosis of the disease to detect carrier animals that do not show any symptoms at early stage of infection are possible with polymerase chain reaction (PCR). PCR is based on the molecular detection of amplified bacterial gene fragments found in pathogen. For the diagnosis of *Leptospira*, 16S rRNA target genes and real-time quantitative PCR has been used successfully (6).

This infection has been commonly observed in areas with tropical climates with a rainy season and high temperatures (7). Bacteria can survive in water or soil for days or months (8). The prevalence of infection is increasing due to flooding of many areas, poor hygiene and improper maintenance (8).

Leptospirosis can be controlled by adapting precautionary measures, e.g. stay away from animals and/ or areas contaminated with their urine. In addition, rodent control, treatment of carriers, occupational hygiene and routine vaccination of healthy animals are among the other recommended control methods (9). Doxycycline and penicillin options are the leading antibiotic treatment options and are considered to be more effective if the treatment is started within the first 3-4 days (9).

Leptospira spp. can causes reproductive disorders in animals. Especially, this disease symptoms have been observed in domestic animals such as cattle, sheep, pig and horse (10-12).

Molecular methods based on detection of DNA have higher sensitivity/ specificity rates compared to other diagnostic methods. Therefore, these methods are increasingly being used in the diagnosis of leptospirosis (13).

This study was designed to detect molecular prevalence of Leptospirosis in mares. Suspected samples were collected from mares and PCR was used to detect the DNA of *Leptospira*. PCR is considered to be rapid, more specific and sensitive method as compare to the microbial culture, isolation and serological studies.

MATERIAL and METHOD

Sampling and storage

A total of 92 vaginal swab samples were collected and transported to the Selçuk Üniversity Faculty of Veterinary Medicine, bacterial diagnosis laboratory.. For this study, samples were taken from mares aged 2-5 years in individual horse farms from rural settlements in Konya. The vulva and vagina were cleaned before swab samples were taken. All samples were kept at -20°C in a refrigerator and taken to the laboratory for processing. These samples were sent to the laboratory under cold-chain conditions.

Polymerase chain reaction

DNA was extracted from all swab samples using the Wizard® Genomic DNA Purification Kit (Promega, USA) following the manufacturer's instructions. The DNA samples were stored at −20°C until PCR analysis. A total of 92 DNA extractions and positive control were used in PCR. As positive control, *L. interrogans serovar pomona* (Leptovac 5®/Vetal, Turkey) were used in PCR.

PCRs were performed for *Leptospira* spp. using specific primers (14). The thermal conditions were as follow; 95°C 15 min, 40 (94°C 1 min, 50°C 1 min, 72°C 1 min) and a final extension at 72°C 10 min. The detection limit was 10 ng/µL of extracted DNA in all reactions. The PCR assays were carried out according to the conditions listed in Table 1.

All PCRs were performed using 5 µL 5× FIREPol®Master Mix (Solis Biodyne, Estonia), 20 pmol of each primer, and DNA template (50 ng/µL), and 1 µL water (negative control). Positive control DNA was used in each PCR series. PCR products were analyzed by electrophoresis on 1.5% agarose gels at 60 mA for 1 h, stained with ethidium bromide and visualized under UV illumination. A 100 bp DNA ladder (Thermo Scientific, SM0373) was used for comparison of DNA sizes.

The study was approved by the SÜVDAMEK Local Ethics Committee (Date: 16.02.2021 and Number: 2021/07).

Table 1. PCR primers, cycle conditions, and product sizes

Microorganism	Target gene	Primer sequences (5'--3')	Termal conditions	Product (bp)	References
16S rRNA	*Leptospira* spp.	GGCGGCGCGTCTTAAACATG TTCCCCCCATTGAAGCAAGATT	95°C 15 min, 40 (94°C 1 min, 50°C 1 min, 72°C 1 min) 72°C 10 min	331	Tramuta et al., 2011

RESULTS

The vaginal swab samples were collected from apparently healthy mares. The mares (92) were observed/ examined clinically and no signs or symptoms of the disease were observed. DNA was extracted from vaginal swabs and PCR was performed to detect the asymptomatic mares. The primers used were derived from 16S rRNA target gene (16S) of *Leptospira* spp. *Leptospira* spp. were detected in seven vaginal swab samples (Figure 1). Results revealed that 7.6% mares (7/92) were positive as carriers and shedding pathogen (*Leptospira* spp.).

DISCUSSION

Leptospirosis is responsible for several chronic infection in domestic and wild mammals in many countries of the World. Colonization of *Leptospira* has been widely reported in various animals via the renal route. Particularly, canine leptospirosis is characterized by an acute or chronic illness. Some dogs are known as asymptomatic carriers by urinary excretion (15). It has been shown that the bacterium can also be found in the female genital tract in ruminant animals and can be transmitted sexually between animals by these means (16). Abortion, stillbirth and neonatal death has been

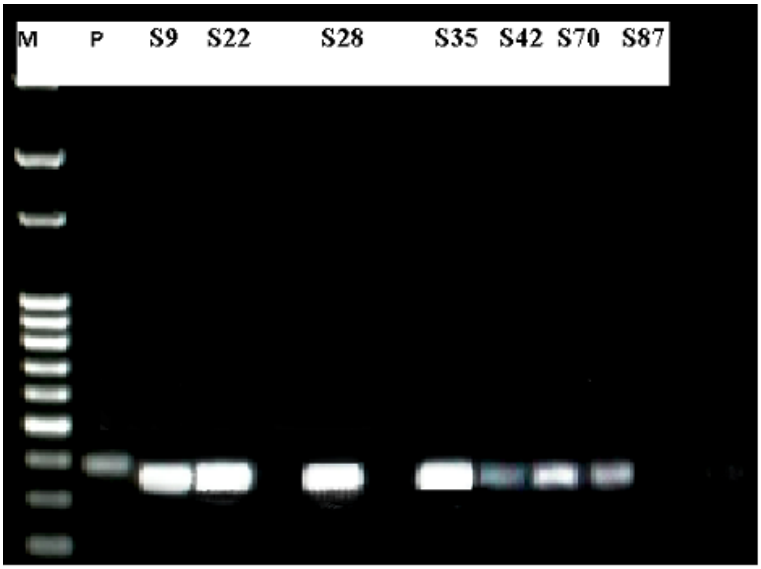

Figure 1. An ethidium bromide-stained agarose gel of PCR products that shows the sensitivity of the assay. M: DNA marker (100bp); P: positive control; S9-S22-S28-S35-S42-S70-S87:positive sample numbers)

noticed in mares with *Leptospira* infection in different studies (17-19). Till now, there have been no reports of evidence of *Leptospira* in the vaginal fluid of healthy mares (11). During this study DNA of leptospira spp was detected for the first time in vaginal fluid of apparently healthy mare.

"PCR is a very sensitive and specific test in the acute phase of leptospirosis, it detected 50% of cases that were negative with other serological tests. In the light of various studies, it has been shown that the PCR method has advantages over other serological tests in the early diagnosis of leptospirosis. Rapid diagnosis of Leptospirosis with a correctly applied molecular method is necessary for the efficient implementation of both animal and public health measures (10). Erol et al.compared the results of real time PCR, FAT and MAT in mare abortion cases and found that molecular methods are effective diagnostic method in the diagnosis of leptospiral abortion (20).

During this study we analyzed a total of 92 mare vaginal swab samples by conventional PCR assay using specific primers. Results revealed the presence of *Leptospira* especially in asymptomatic animals. During our study, none of the animals developed abortion while *Leptospira* spp. DNA was detected in seven samples.

The most important outcome of this study was evidence of leptospiral DNA in vaginal swab samples from mares with absolutely no clinical/ reproductive symptoms. Previously, It has been reported by researchers that leptospirosis can be transmitted between animals through direct or indirect contact with the urine of carriers. (16,21).

Therefore, detection of *Leptospira* spp. DNA in an apparently healthy mare's genital tract sample is a significant/ important finding. So, it is suggested that the carrier mares may be associated with spread of leptospiral infection through mucous membranes (11,22).

This study also revealed the possibility of sexual transmission of leptospirosis in horses (female to male). Early detection of asymptomatic carriers may play an important role in prevention and control of the disease. Di Azevedo et al. reported a systematic review of data on equine genital infection of leptospirosis (23). As a result of the study, it was reported that Leptospirosis can cause sexually transmitted "Equine genital leptospirosis" in horses. They emphasized the inadequacy of serological diagnosis for this disease and the contribution of molecular studies. This inference is consistent with our study. In order to adequately recognize this syndrome, clinical findings and molecular studies should be evaluated simultaneously, especially in mares.

Moreover, the results suggested that comprehensive further studies are required to investigate the disease in mares.

It was concluded that 7.6% apparently healthy mares were carriers of the leptospira, which is a great concern of animal and public health. Moreover, PCR was found to be a suitable method for early and rapid detection of leptospirosis in carrier animals. As a suggestion, genotypic characterization of *Leptospira* strains affecting mares by molecular methods should also be defined. At the same time, it will be evaluated in terms of public health and vaccinating horses in risky areas will make important contributions to protection and control measures.

ETHICS COMMITTEE APPROVAL

* The study was approved by the SÜVDAMEK Local Ethics Committee (Date: 16.02.2021 and Number: 2021/07).

CONFLICT OF INTEREST

The author declares no conflict of interest.

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Araştırma Makalesi/Original Article
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Türk Hijyen ve Deneysel Biyoloji Dergisi

Halk Sağlığı Laboratuvarlarının mikrobiyoloji alanındaki kalite gelişmelerinin dış kalite çalışmaları ve akreditasyon açısından değerlendirilmesi

Evaluation of quality improvements in the field of microbiology of Public Health Laboratories in terms of external quality studies and accreditation

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ÖZET

Amaç: Halk Sağlığı Laboratuvarı, toplum sağlığının korunması ve iyileştirilmesi kapsamında birey ve toplum sağlığını etkileyen ve/veya etkileyebilecek etmenleri inceleyen ve görev alanıyla ilgili klinik ve klinik dışı laboratuvar hizmetleri sunan laboratuvardır. TS EN ISO/IEC 17025 standardının gerekliliklerini sağlayarak akredite olmak isteyen laboratuvarlar için kalite kontrol çalışmaları son derece önemlidir. Böylelikle laboratuvarlar, kalite gerekliliklerini yerine getirerek doğru sonuç üretip kaliteli hizmet sunduklarını ispat etmiş olurlar. Bu çalışmanın amacı; 2012-2021 yılları arasında Halk Sağlığı Laboratuvarlarında yürütülen kalite ve akreditasyon süreçleri ile katılım sağlanan Dış Kalite Değerlendirme programlarının, mikrobiyolojik analiz alanındaki kalite üzerine yaptıkları etkilerini araştırmaktır.

Yöntem: Laboratuvarlarda yürütülen tüm kalite çalışmaları grafiksel ve ortalama değerler olarak irdelendi. Uygulanan Dış Kalite Değerlendirme çevrim

ABSTRACT

Objective: Public Health Laboratory is the laboratory that examines the factors that affect and/or may affect individual and public health within the scope of protection and improvement of public health, provides clinical and non-clinical laboratory services related to their field of duty. Quality control studies are extremely important for the laboratories wishing to be accredited by meeting the requirements of the TS EN ISO/IEC 17025 standard. In this way, laboratories prove that they produce correct results and provide quality service by fulfilling quality requirements. The aim of this study is to investigate the effects of the quality and accreditation processes carried out in Public Health Laboratories between 2012-2021 and External Quality Assessment programs attended, on quality in the field of microbiological analysis.

Methods: All quality studies carried out in laboratories were analyzed as graphical and average values. ISO 13528:2005 Robust Analysis Method was used

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sonuçlarının Z değeri hesaplanması işlemi için ISO 13528:2005 Robust Analiz Yöntemi kullanıldı. Her bir laboratuvarın performansı ISO 13528 standardı ve IUPAC Protokolü ile uyumlu olarak Z değeri cinsinden ifade edildi.

Bulgular: 2012-2021 yılları arasında kalite çalışmaları ile Dış Kalite Değerlendirme çevrimlerinden elde edilen veriler değerlendirilmiş, kalite ve akreditasyon üzerine pozitif etkileri belirlenmiştir. 2015-2018 yılları arasında kurumsal olarak uygulanan Dış Kalite Değerlendirme çevrimleri incelendiğinde, programlara katılan tüm laboratuvarların "Koliform bakteri", "*Escherichia coli*" ve "Intestinal enterokok" parametrelerinde %90-100 başarılı, "*Clostridium perfringens*" ve "*Pseudomonas aeruginosa*" parametrelerinde ise %80-90 oranında başarılı oldukları görülmüştür. Kalite çalışmaları sonrasında TS EN ISO/IEC 17025 standardına göre akredite olan Halk Sağlığı Laboratuvarları, Dış Kalite Değerlendirme programlarında da başarılı Z değerleri elde etmişlerdir. Bu çalışmalar sayesinde, laboratuvarlarda yıllar içinde akredite parametre sayısı ve çalışılan numune sayısında belirli düzeyde artış sağlandığı görülmüştür.

Sonuç: Halk Sağlığı Laboratuvarları, kalite altyapıları geliştirilip kalite güvence sistemleri kurularak iç ve dış denetim mekanizmaları ile doğru sonuç üreten laboratuvarlar haline gelmişlerdir. Sonuçta Dış Kalite Değerlendirme programlarından başarılı sonuçlar alan ve kendini ispatlayan 19 adet L1 hizmet tipi laboratuvarın ilgili standartlar doğrultusunda akredite olduğu görülmektedir.

Anahtar Kelimeler: Dış kalite değerlendirme, kalite gelişimi, mikrobiyoloji, su, halk sağlığı

for calculating the Z score of External Quality Assessment cycles results. The performance of each laboratory was expressed in terms of Z score in accordance with the ISO 13528 standard and IUPAC Protocol.

Results: The data collected from the Quality and External Quality Assessment were evaluated between 2012-2021, their positive effects on quality and accreditation were determined. When the External Quality Assessment cycles which were institutionally applied between 2015-2018 are examined, it is seen that all laboratories participating in the programs are 90-100% successful in "Coliform bacteria", "*Escherichia coli*" and "Intestinal enterococci" parameters, and 80-90% successful in "*Clostridium perfringens*" and "*Pseudomonas aeruginosa*" parameters. After the quality studies, Public Health Laboratories accredited according to the TS EN ISO/IEC 17025 standard have also obtained successful Z scores in the External Quality Assessment programs. Thanks to these studies, it has been observed that the number of accredited parameters and the number of samples studied have increased to certain level in laboratories over the years.

Conclusion: Public Health Laboratories have become laboratories that produce accurate results with internal and external control mechanisms by improving quality infrastructures and establishing quality assurance systems. As a result, it is seen that 19 L1 service type laboratory, which achieved successful results from the External Quality Assessment programs and proved themselves, are accredited in line with the relevant standards.

Key Words: External quality assessment, quality improvement, microbiology, water, public health

GİRİŞ

Halk Sağlığı Laboratuvarı (HSL), toplum sağlığının korunması ve iyileştirilmesi kapsamında birey ve toplum sağlığını etkileyen ve/veya etkileyebilecek etmenleri inceleyen ve görev alanıyla ilgili klinik ve klinik dışı laboratuvar hizmetleri sunan laboratuvardır. HSL, halkı sağlık tehlikelerinden korumada hayati

bir rol oynamaktadır. Bu laboratuvarlar ayrıca özel organizmalar için laboratuvar doğrulamasını sağlar ve halk sağlığının hastalık gözetim işletmesinin bir parçasıdır, salgınlar sırasında bulaşıcı organizmaların veya toksinlerin doğru, zamanında tespitini sağlar (1).

HSL'ler, Halk Sağlığı Genel Müdürlüğü (HSGM), Tüketici Güvenliği ve Halk Sağlığı Laboratuvarları Dairesi Başkanlığı (TGHSLDB) koordinasyonunda 81 ilde 19 adet L1 ve 65 adet L2 tipi olmak üzere 84 merkezde yapılanmış olup, hizmet tipine göre L1 ve L2 tipi olmak üzere ikiye ayrılmaktadır. Merkezde ise Ulusal Halk Sağlığı Referans Laboratuvarı ile hizmet verilmektedir.

Laboratuvarlarda kalite sistemlerinin kurulması ve kalite alt yapılarının güçlendirilmesi için gerekli desteği vermek, kalite sistemlerini ve akreditasyon süreçlerini izlemek, değerlendirmek ve bu konuda koordinasyonu sağlamak TGHSLDB'nin en önemli görevlerindendir (2). Referans ve L1 Hizmet Tipi HSL, hizmet kapsamındaki ilgili standartlara göre akredite olan ve DKD testleri konusunda test numunesi hazırlayabilen laboratuvarlar olup kapsamları çerçevesinde bu çalışmaları yürütmeleri beklenir (3).

Akreditasyon, yetkili bir kuruluşun, laboratuvarın inceleme yapmaya yetkin olduğunun resmi olarak tanınmasını sağlamak için laboratuvarların açık kalite yönetim kriterlerini karşılamasını sağladığı bir hakem değerlendirme sürecidir (4). EN ISO 17025'in uygulanması, günlük laboratuvar uygulamalarının sürekli iyileştirilmesi için bir sistem sağlar (5). Akreditasyon, ilgili standartlara göre doğru, güvenilir sonuçlar üretilmesini ve sonuçta uluslararası tanınırlığı sağlayan bir yaklaşımdır (6).

Çalışmamızın amacı, HSL'lerde mikrobiyoloji laboratuvarlarının yıllar içerisinde kalite alanında sağladığı gelişmeleri ve izlediği yol haritasında ne oranda başarılı olduğunu incelemektir.

GEREÇ ve YÖNTEM

Bu çalışmada 2012-2021 yılları arasında HSL'lerde yürütülen, Dış Kalite Değerlendirme ve Yeterlilik Testi (DKD) çalışmalarının, mikrobiyolojik kalite, standardizasyon ve akreditasyon faaliyetlerinin gelişimi üzerine etkileri ele alınarak grafiksel ve ortalama değerler olarak incelendi.

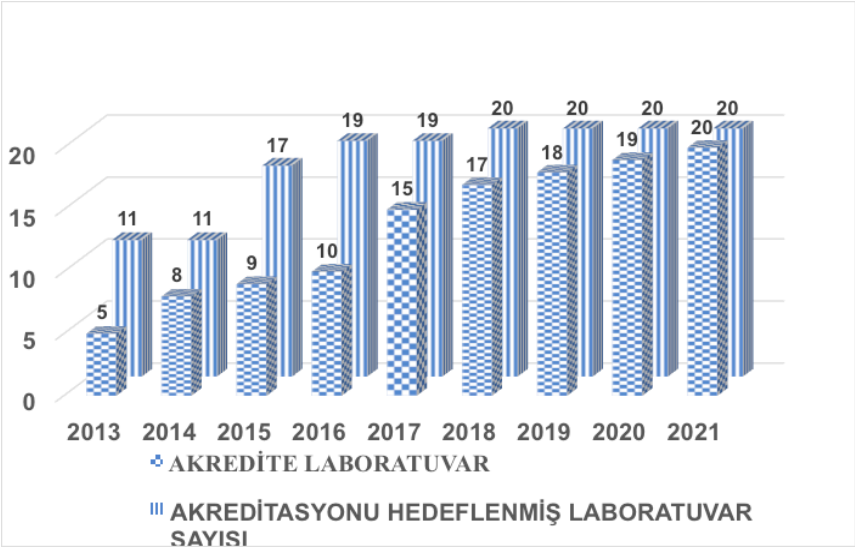
DKD çevrim sonuçlarının Z değeri hesaplanması işlemi için, ISO 13528:2005 Robust Analiz Yöntemi esas alınarak, hesaplanan ortalama Robust ortalama (atanmış değer) olup, hesaplanan standart sapma Robust standart sapmadır. Daha sonra atanmış değerin ölçüm belirsizliği ve Z değeri hesaplanır (7). Her bir laboratuvarın performansı ISO 13528 standardı (8) ve IUPAC Protokolü ile uyumlu olarak Z değeri cinsinden ifade edilir (9).

Kalite sistem çalışmalarının her aşamada elde edilen sayısal verileri derlendi, toplu grafikleri oluşturuldu ve bunun kalite gelişimi üzerindeki etkileri değerlendirildi.

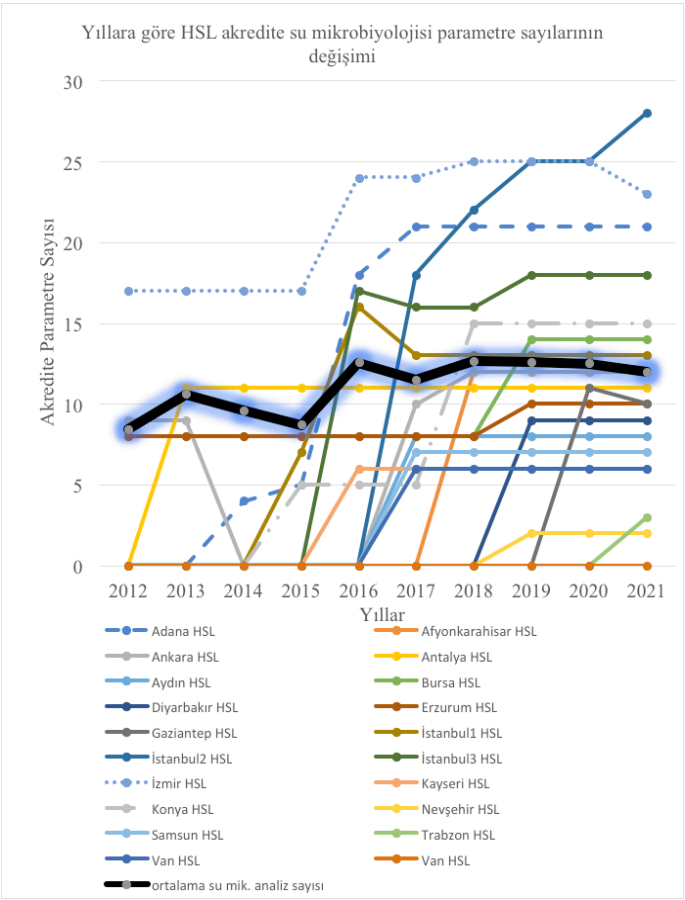
BULGULAR

Standardizasyon ve akreditasyon çalışmalarına, 5 akredite HSL ile başlanmış 2021 yılı sonunda Şekil 1'de de görüldüğü gibi tüm L1 hizmet tipi HSL'lerin TS EN ISO/IEC 17025 standardı akreditasyonu hedefine ulaşılmıştır (10). 2016 yılına kadar laboratuvar bilgi düzeyi ile tecrübesinin artmasından sonra akreditasyon süreci başarı ile tamamlanmıştır. Tablo I' de ilgili standartlara göre akredite olan HSL'ler gösterilmektedir (11).

2016 yılından sonra HSL'lerde çalışılan mikrobiyolojik su akredite parametre sayısında ortalama olarak %44-46 oranındaki artış Şekil 2'de görülmektedir. Yine Şekil 3'de 2016 yılı sonrasında toplam akredite parametre sayılarında ortalama %30 kadar bir artış görülmektedir. Daha sonra bu artış oranı ortalama %40 düzeyinde dengelenmiştir. Şekil 4'de ortalama numune sayıları incelendiğinde 2017 ve 2018 yılında numune sayılarında %11-13 artış belirlenirken 2020 yılına kadar sabit kalan ortalama numune sayılarının 2020-2021 yıllarında 2016 yılına göre %54 oranında arttığı saptanmıştır.



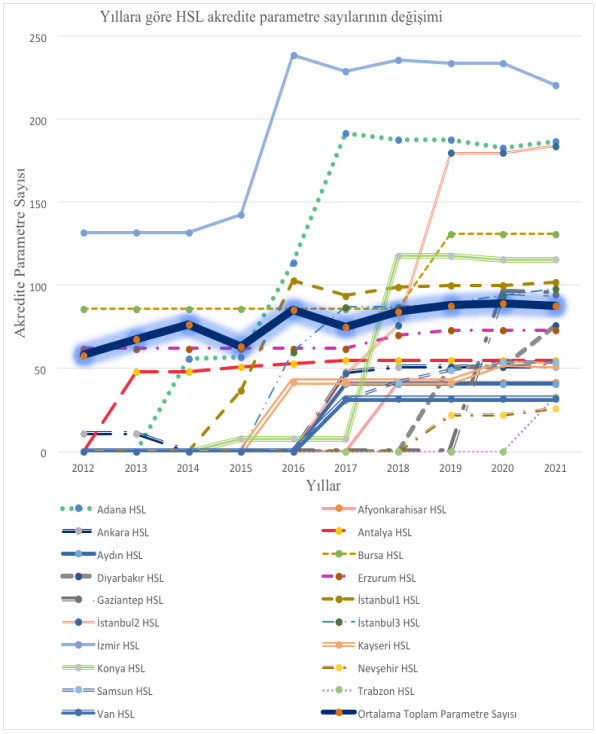
Şekil 1. L1 HSL'lerin TS EN ISO/IEC 17025 kapsamında hedeflenmiş ve tamamlanmış akreditasyon durumu



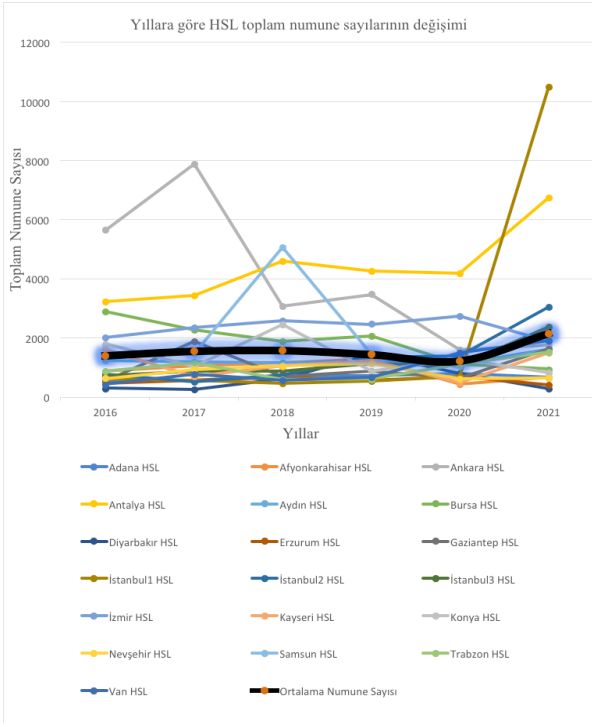
Şekil 2. Yıllara göre HSL akredite su mikrobiyolojisi parametre sayılarının değişimi

Tablo 1. TS EN ISO/IEC 17025 kapsamında akredite olan HSL'ler, parametre sayıları ve geçerlilik süreleri (Temmuz 2023)

HSL	Akredite Parametre Sayısı	Akreditasyon Tarihi	Akreditasyon No	Geçerlilik Süresi
		TS EN ISO/IEC 17025:2017		
ADANA	185	2.01.2014	AB-653-T	10.05.2026
AFYONKARAHİSAR	42	18.01.2018	AB-1276-T	17.01.2026
ANKARA	52	30.01.2017	AB-1075-T	29.01.2025
ANTALYA	55	14.11.2014	AB-0791-T	3.03.2027
AYDIN	41	2.10.2017	AB-1225-T	1.10.2025
BURSA	180	22.04.2011	AB-445-T	13.11.2023
DİYARBAKIR	51	25.04.2019	AB-1401-T	23.04.2027
ERZURUM	138	5.03.2008	AB-205-T	29.09.2024
GAZİANTEP	95	20.03.2020	AB-1502-T	19.03.2024
İSTANBUL 1 NOLU	96	10.02.2015	AB-828-T	28.09.2023
İSTANBUL 2 NOLU	215	21.03.2017	AB-1167-T	19.03.2025
İSTANBUL 3 NOLU	89	26.04.2016	AB-1005-T	25.04.2024
KAYSERİ	52	18.11.2016	AB-1073-T	17.11.2024
KONYA	119	4.08.2015	AB-860-T	04.11.2023
NEVŞEHİR	22	25.06.2019	AB-1462-T	23.10.2023
SAMSUN	30	11.05.2015	AB-834-T	05.09.2023
TRABZON	33	22.04.2022	AB-1706-T	22.04.2026
VAN	30	27.03.2018	AB-1244-T	26.03.2026
ULUSAL HALK SAĞLIĞI REFERANS LAB. (Mikrobiyolojik ve Kimyasal Analiz Lab.)	84 (sabit kapsam)	30.12.2015	AB-949-T	29.12.2023
ULUSAL HALK SAĞLIĞI REFERANS LAB. (Kimyasal Savaş Ajanları Tanı ve Doğrulama Lab.)	30 (esnek kapsam)	26.12.2019	AB-949-T	25.12.2023
		TS EN ISO/IEC 15189:2014		
ADANA	3	12.10.2017	AB-0041-TL	11.10.2025
İSTANBUL 3 NOLU	11	25.06.2020	AB-0058-TL	24.06.2024
		TS EN ISO/IEC 17043:2013		
İSTANBUL 3 NOLU	79	01.12.2022	AB-0026-YT	30.11.2026
		OECD İyi Laboratuvar Uygulamaları		
ULUSAL HALK SAĞLIĞI REFERANS LAB. (Biyosidal Ürün Lab.)	İLU uygunluk beyanı	04.07.2019	AB-0001-IL	03.07.2027
		TS EN ISO/IEC 17020:2012 | C tipi Muayene Kuruluşu		
ULUSAL HALK SAĞLIĞI REFERANS LAB. (Sterilite Kontrol L)	14	15.12.2017	AB-0368-M	14.12.2025



Şekil 3. Yıllara göre HSL akredite parametre sayılarının değişimi



Şekil 4. Yıllara göre HSL toplam numune sayılarının değişimi

2015 yılında DKD çevrim programına katılım sağlayan 31 adet laboratuvardan; "Koliform bakteri" ile "Intestinal enterokok" parametrelerinde tüm laboratuvarların %100 başarılı olduğu, "*Escherichia coli*" parametresinde başarı oranının %90,3 olarak gerçekleştiği belirlenmiştir. Sadece üç adet laboratuvarın Z değerinin > ± 2 olduğu görülmektedir (12).

2016 yılında DKD çevrimine katılan 82 adet laboratuvardan; "Koliform bakteri", "*E. coli*" ve "Intestinal enterokok" parametrelerinde tüm laboratuvarların %100 başarılı; "22°C'de Toplam Koloni Sayımı" ve "36°C'de Toplam Koloni Sayımı" parametrelerinde başarı oranının %98,73 olarak gerçekleştiği, sadece bir laboratuvarın Z değerinin > ± 3 olduğu görülmektedir (13).

2018 yılında DKD çevrimine katılan 121 adet laboratuvardan; "Toplam Koliform bakteri", "*E. coli*" ve "36°C'de Toplam Koloni Sayımı" parametrelerinde tüm laboratuvarların %100 başarılı; "İntestinal enterokok" parametresinde %97,09 ve "22°C'de Toplam Koloni Sayımı" parametresinde ise %96,33 başarı elde ettikleri, sadece üç adet laboratuvarın Z değerinin > ± 3 olduğu görülmektedir. *Clostridium perfringens* parametresi için başarı oranı %83,75 ve *Pseudomonas aeruginosa* parametresi için başarı oranı %80,67'dir (14).

TARTIŞMA

2012 yılından sonra HSL'lerde yeniden yapılanma ile gelişen kaliteli hizmet sunumu, mikrobiyoloji ve tüm akredite parametrelerde artış ile kendini göstermektedir. Şekil 2 ve Şekil 3'de görüldüğü gibi 2016 yılından sonra mikrobiyoloji ve tüm akredite parametrelerde bir artış olmuştur. Bu artış ve süreklilik, kalite yapısının akreditasyon geleneğinin yerleştiğini kanıtlamıştır. Özellikle numune sayıları Şekil 4'de görüldüğü gibi akreditasyon süreçleri sonrasında artmış ve ortalama numune sayıları incelendiğinde belli bir dengeye ulaştığı görülmüştür. 2018–2020 yılları arasında ortalama numune sayılarında görülen sabitlik, akreditasyon şartlarının ilk yıllarda laboratuvarlarda yerleşmesi ve kazanılan ilk yıl deneyimlerinden kaynaklanmaktadır. Nitekim bu sayı, 2020 yılından sonra artarak belli bir dengeye ulaşmıştır. Bu, HSL'lerin hem kamu hem de özel sektör tarafından tercih edilir olduklarını göstermektedir.

Laboratuvarların akreditasyon çalışmalarında, DKD çevrim programlarına katılmak, uygun sonuçlar almak ve bunun devamlılığını sağlayabilmek büyük önem taşımaktadır. Mikrobiyoloji laboratuvarları, akredite olduğu parametrelerde ilgili standardın gereklerine ve Türk Akreditasyon Kurumu'nun (TÜRKAK) ilgili dokümanına göre bir dış kalite kontrol programına katılım sağlar (10). İlgili yönetmeliğe göre, Dış kalite kontrol değerlendirmelerinde kantitatif analizlerde, test sonucunun $|Z| \leq 2$ olması başarılı sayılır. (3) Yine TÜRKAK'ın ilgili dokümanına göre test sonuçları değerlendirildiğinde, $Z \leq \pm 2$ Başarılı performans, $\pm 2 < Z \leq \pm 3$ Sorgulanabilir performans, $Z > \pm 3$ Başarısız performans olarak kabul edilir (15).

Kurumsal olarak 2015 yılında 31 adet HSL ile düzenlenen, sularda mikrobiyolojik analizlere yönelik DKD çevrimi (12), 2016 yılında tüm HSL'ler için organize edilmiş ve başarı ile gerçekleştirilmiştir (13). 2018 yılında DKD çevrimleri, HSL'lerin tümü ile Yüzme Suyu Analizi Konusunda Yetkilendirilmiş Özel Laboratuvarlar olmak üzere toplam 121 adet laboratuvarı kapsayacak şekilde Türkiye çapında düzenlenmiştir (14). Bu çevrimlerde laboratuvarların çoğu başarılı Z değerleri elde etmişlerdir. Bütün bu DKD çalışmaları yıllara göre incelendiğinde; hem başarı oranları yükselmiş hem de katılımcı laboratuvarların sayısı ve katılım sağlanan parametre sayısı artmıştır.

Uras, Türkiye'de tıbbi laboratuvarlarda standardizasyon, akreditasyon ve kalite bilincinin artmaya devam ettiği, ISO 15189 standardının düzenlemelere dahil edilmesinin yararlı olacağını vurgulamıştır (16). Gaunt ve ark., laboratuvar performansının zaman içinde değiştiği ve DKD çevrimlerine düzenli katılımın uzun vadeli iyileştirmeler sağladığını belirtmiştir (17).

Berwouts ve ark.'na göre, Dış Kalite Kontrol (DKK) verileri, Avrupa'da kalite güvencesinin önemi konusunda farkındalığın arttığını göstermektedir. DKD planının çıktıları, çalışmalarda laboratuvar raporlarının kalite güvencesinin yıllar içinde geliştiğini göstermektedir. Ayrıca, DKK'ya daha sık katılımın, sonuçların yorumlanmasında faydalı olduğu görülmektedir. Hem akreditasyon hem DKK çalışmalarında alınan sonuçların, test hizmetlerinin kalitesinin artırılmasında iyi araçlar olduğunu belirtmektedirler (18).

Rautemaa-Richardson ve ark., Kalite güvence sistemi olarak ele alındığında, standart yöntemlerle çalışmanın, laboratuvarda kullanılan proseslerin, malzeme ile yöntemlerin etkinliğini ve güvenliğini değerlendiren DKK'nın önemini vurgulamıştır (19). Buchta ve ark. çalışmalarında, DKD verileri ve DKD hizmeti sunanlardan elde edilecek bilgilerin, laboratuvarlara ve sağlık yetkililerine faydalı olacağını belirtmiştir (20).

Kakc ve ark., DKD programlarının klinik mikrobiyoloji alanında düzenlenmesi, Sri Lanka'daki mikrobiyoloji hizmetleri üzerinde önemli bir olumlu etki yaptığını belirtmiştir. Bu çalışmaya göre, DKD uygulamalarının geliştirilmesinin Sri Lanka Ulusal DKD programına faydalı olacağı düşünülmektedir (21). Przybył-Hac ve Ciechowicz, 2021 yılında uygulanan DKD programlarında, doğru sonuç yüzdesinin bazı kriterlere göre 2020 yılına göre biraz daha yüksek olduğunu belirlemişlerdir. Ayrıca 2021'de uygulanan programlardaki yıllık kalite verilerinin de 2020'de değerlendirilen sonuçlarla karşılaştırılabilir düzeyde olduğu görülmüştür (22). Başka bir çalışmada, DKK programlarına katılan laboratuvarların, mikrobiyolojik çalışmalarda başarılı olduğu gösterilmiştir. Elde edilen sonuçların, mikrobiyolojik iç kalite kontrol çalışmalarında da, yol gösterici olduğu vurgulanmıştır (23).

DKD çalışmaları ile katılımcı laboratuvarlar tecrübelerini arttırarak kendilerini geliştirmiş, test güvenirliklerini ispat ederek doğru sonuç ürettiklerini ispatlamışlardır. Akredite HSL'lerin artması, akreditasyon bilincinin oluşması ve bunun devamlılığı, yapılan tüm çalışmaların bir sonucudur.

DKD çalışmaları, standardizasyon ve kalite sistemlerine çok büyük katkı sağlamıştır. Laboratuvarlarca üretilen hizmetlerin doğruluğu ve kalitesi sağlanmıştır. Aynı zamanda bu çevrimler, sistem içi denetim mekanizmalarına katkıda bulunmuştur. Başarılı Z değerleri, laboratuvarların analiz yetkinliğine sahip olduğunu vurgulamaktadır.

Mursaloğlu Kaynar incelemesinde laboratuvar akreditasyonunun önemini açıklamış, TS EN ISO/IEC 17025 standardı revizyonundan sonra vurgulanan risk temelli düşünce, karar kuralı, ölçüm belirsizliği, meteorolojik izlenebilirlik gibi terimlere açıklık getirmiştir (24).

Akreditasyon bir yetkinlik göstergesidir. Yine Şekil 2, Şekil 3 ve Şekil 4'de akredite parametre ile numune sayılarının artışı olarak görülen yukarı yönlü ivme bize kalite anlayışında devamlılık ve sürdürülebilirlik anlamında da değerli bilgiler vermektedir. HSL'ler, DKD programları ve kalite güvence sistemleri kurularak doğru laboratuvar sonucu ürettiklerini kanıtlamışlardır. Tüm çalışmalardan sonra ilgili standartlar doğrultusunda akredite olarak uluslararası normlarda hizmet veren laboratuvarlar haline gelmişlerdir.

Sonuç olarak, tüm akredite HSL'ler ve L2 hizmet tipi HSL'lerin çoğunluğunun analiz yetkinliğine sahip olduğu görülmektedir. Ancak sonuçlarında sapma görülen laboratuvarlarda, neden-sonuç analizi ile sorunların kaynağını belirlenecek ve uygulanacak düzeltici faaliyetler sayesinde sonuç kalitesinin iyileştirilmesi sağlanacaktır. Akredite olunan parametrelerin ihtiyaçlar doğrultusunda arttırılması, dış kalite kontrol çalışmalarının aktif olarak sürdürülmesinin HSL'lerin sundukları hizmet kalitesine katkı sağlayacağı kanaatine varılmıştır.

TEŞEKKÜR

* Bu çalışmada emeği geçen HSGM, Tüketici Güvenliği ve Halk Sağlığı Laboratuvarları Dairesi Başkanlığı, Mikrobiyoloji Referans Laboratuvarları ve Biyolojik Ürünler Dairesi Başkanlığı ile HSL personeline teşekkür ederiz.

ETİK KURUL ONAYI

* Bu çalışma, Etik Kurulu onayı gerektirmemektedir.

ÇIKAR ÇATIŞMASI

Yazarlar bu makale ile ilgili herhangi bir çıkar çatışması bildirmemişlerdir.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

Kırklareli İli içme-kullanma sularının organoleptik özelliklerinin koliform kirliliğiyle ilişkisinin değerlendirilmesi

Evaluation of the relationship of organoleptic properties of drinking waters of Kırklareli Province with coliform pollution

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ÖZET

Amaç: Bu çalışma ile Kırklareli ilinde koagülant ajan ile arıtım olmaksızın insani tüketime sunulan içme-kullanma sularının organoleptik özelliklerinden olan renk, koku ve bulanıklık parametrelerinin değişiminde koliform bakterilerin etkisinin ölçülmesi ve bu parametrelerdeki değişimlerin birbirleriyle ilişkilerinin istatistiki olarak belirlenmesi amaçlanmıştır.

Yöntem: Araştırma kapsamında Kırklareli ili genelinde Kasım 2019 - Kasım 2021 tarihleri arasında 218 noktadan toplam 1022 tane içme-kullanma suyu numunesi alınmıştır. Numunelerin *Escherichia coli* ve diğer koliform bakteri analizi numunelerin membran filtrasyon işleminden sonra geleneksel kültür metoduyla 100 ml'de kantitatif olarak; renk, koku ve bulanıklık parametreleri ise 98/83/EC normu doğrultusunda tüketicilerce kabul edilebilir durumda olduğunun uygunluğuna göre kalitatif olarak değerlendirilmiştir.

Bulgular: 1022 numune içerisinde *E. coli* ve diğer koliform bakteri üremesi görülen numune sayısı sırasıyla 229 ve 244 olup (toplam koliform üreyen numune 352)

ABSTRACT

Objective: In this study, it was aimed to measure the effect of coliform bacteria on the change of organoleptic characteristics of the color, odor and turbidity parameters of drinking water offered for human consumption without treatment with coagulant agents in Kırklareli province and to determine the statistical relationship between the changes in these parameters.

Methods: Within the scope of the research, a total of 1022 drinking water samples were taken from 218 points throughout the province of Kırklareli between November 2019-2021. *Escherichia coli* and other coliform bacteria (OCB) analysis of samples were quantitatively in 100 ml by traditional culture method after membrane filtration process of samples; color, odor and turbidity parameters were evaluated qualitatively according to the conformity of being acceptable to consumers in line with the 98/83/EC norm.

Results: The number of samples with *E. coli* and OCB growth in 1022 samples is 229 and 244, respectively (total coliform growing sample is 352) and the number

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rengi, kokusu ve bulanıklığı tüketicilerce kullanıma uygun olmayan numune sayısı sırasıyla 79, 51 ve 145'tir. *E. coli* ve diğer koliform bakteri üremelerinin suyun rengi, kokusu ve bulanıklığı üzerindeki etkisi %95 güven aralığında ikili (binomial) lojistik regresyon analizi ile araştırılmıştır. Buna göre ortaya çıkan regresyon modeli "Koku" parametresi için anlamlı bulunmuş olup (Hosmer&Lemeshow p>0,05), renk ve bulanıklık parametreleri için anlamlı değildir. Araştırmanın bağımsız değişkenleri suyun kokusu üzerindeki değişimlerin yüzde 8,9'unu açıklamaktadır (Nagelkerke R2 =0,089; Omnibus Ki Kare = 30,265; Sd =2; p<0,001). Ayrıca analiz sonucu ikili kategorik olarak değerlendirilen renk, koku ve bulanıklık parametrelerinin birbirleriyle ilişkisini belirlemeye yönelik gerçekleştirilen Ki Kare (X^2) analizine göre; bulanıklık-renk, bulanıklık-koku ile renk-koku parametreleri arasında %95 güven aralığında anlamlı bir ilişki bulunmaktadır (Sırasıyla X^2 = 260,690; 53,492; 94,352. Hepsi p<0,001). Suların bulanıklığı ile rengi arasındaki ilişki güçlü, bulanıklığı ile kokusu ve rengi ile kokusu arasındaki ilişki ise zayıftır (Sırasıyla Cremers V = 0,533; 0,229; 0,034. Hepsi p<0,001).

Sonuç: Çalışmamızda incelenen su örneklerinin organoleptik özellikleriyle mikrobiyal kirliliği arasında ilişki olabileceğinin tespit edilmiş olması nedeniyle, içme-kullanma sularında organoleptik yöntemlerle uygunsuzluk saptandığında, bakteriyolojik kirlilik de olabileceğinin daima göz önüne alınması ve aksi ispatlanıncaya kadar bakteriyolojik kirlilik açısından dikkatli olunması gerektiği söylenebilir.

Anahtar Kelimeler: İçme suyu, organoleptik, koliform basil, *Escherichia coli*, su mikrobiyolojisi

of samples whose color, odor and turbidity are not suitable for use by consumers are 79, 51 and 145, respectively. The effect of *E. coli* and OCB growth on the color, odor and turbidity of water was investigated by binary logistic regression analysis at 95% confidence interval. Accordingly, the resulting regression model was found to be significant for the "Odor" parameter (Hosmer&Lemeshow p>0.05), but not for the color and turbidity parameters. It explains 8.9% of the changes in the odor of water in the independent variables of the research (Nagelkerke R2 =0.089; Omnibus Chi-Square =30.265; Sd =2; p<0.001). In addition, according to the Chi-Square (X^2) analysis carried out to determine the relationship between color, odor and turbidity parameters, which are evaluated as binary categorical analysis; There is a significant relationship between turbidity-color, turbidity-odor and color-odor parameters at the 95% confidence interval (X^2=260,690; 53.492; 94.352, all p<0.001). The relationship between turbidity and color of water is strong, while the relationship between turbidity and odor and color and odor is weak (Cremers V=0.533; 0.229; 0.034, all p<0.001).

Conclusion: Since it has been determined in our study that there may be a relationship between the organoleptic properties of the water samples and their microbial pollution, it can be said that when non-suitable condition is detected with organoleptic methods in drinking water, the possibility of bacteriological pollution should always be taken into account and should be careful in terms of bacteriological pollution until proven otherwise.

Key Words: Drinking water, organoleptic, coliform bacilli, *Escherichia coli*, water microbiology

GİRİŞ

Su; tüm organik dokularda bulunan tatsız, kokusuz ve mükemmel derecede çözücü bir sıvıdır. Su insan vücudu hacminin yaklaşık %75'ini oluşturur ve bilindiği üzere yaşam susuz düşünülemez. Bu nedenle, sürekli bir içme suyu kaynağına ihtiyacımız bulunmakta ve bu içme sularının insan sağlığını bozmamasını sağlamak amacıyla sürekli olarak kontrollerinin yapılması gerekmektedir (1). Diğer yiyecek ve içecekler gibi, tüketiciler de musluk suyunu öncelikle duyusal nitelikleriyle değerlendirir.

İçme suyunun içerik olarak güvenli olmasının yanında aynı zamanda fiziksel özellikleri bakımından da kabul edilebilir olması tüketicilerce yüksek öncelik oluşturmaktadır. Tüketiciler genellikle kendi içme sularının güvenliğini kendi kendilerine yargılamak için fiziksel olarak değerlendirmek haricinde başka bir araca sahip değildir. Tüketicilerin bulanık, rengi farklı görünen ya da hoş olmayan bir tada veya kokuya sahip suya şüpheyle bakmaları doğaldır, ancak bu özellikler kendi başlarına suyun sağlığa uygun olup olmadığını belirlemek için doğrudan bir gösterge olmayabilir. İçme suyunda insan sağlığında olumsuz herhangi bir etki oluşturmayacak düzeyde bulunan bir etkenin, suyun tüketicilerce kullanılmasını etkileyecek kadar fiziksel özelliklerini değiştirebileceği gibi, insan sağlığında olumsuz etki yaratabilecek bir etkenin ise suyun fiziksel özelliklerini değiştirmeden içeriğinde bulunabilmesi mümkündür. Bileşenlerin tüketiciler için sakıncalı olduğu konsantrasyon değişkendir. Ayrıca topluluğun alıştığı suyun kalitesi çeşitli sosyal, çevresel ve kültürel hususlar dahil olmak üzere bireysel ve yerel faktörlere de bağlıdır. Olumsuz sağlık etkileriyle doğrudan bağlantısı olmayan ancak su kalitesini etkileyen bileşenler için referans değerler oluşturulmamıştır. Ancak içme sularında fiziksel etkiye neden olabilecek bazı maddeler için tüketicilerin bunları duyuları ile algılama yetenekleri çok geniş bir aralıkta olduğundan dolayı düşük konsantrasyonlarda referans değerler oluşturulmuştur. Bu referans değerler uluslararası standartlar ve yerel dinamikler doğrultusunda şekillendirilmektedir. Su arıtma ve dağıtım uygulamalarının içme suyunun tüketicilerce kabul edilebilirliğini ve sağlık açısından sorun riskini en aza indirgemek için yönetimini sağlamak önemlidir. Örneğin, uygun şekilde yönetilmeyen kloraminasyon işlemi kabul edilemez tat veya kokuya neden olabilen trikloraminlerin oluşumuna yol açabilir. Ayrıca dağıtım sistemlerinde akış bozulduğunda veya değiştiğinde tüketicilere ulaştırılan suyun görüntüsünde bozulmalar meydana gelmiş olabilir. Koku, renk, bulanıklık gibi suyun fiziksel parametreleri doğal organik ve inorganik maddeler, sentetik kimyasallar, korozyon ve kloraminasyon gibi dağıtım ve arıtım işlemlerinden kaynaklı problemler, doğal biyolojik kaynak (akuatik mikroorganizmalar) ya da prosesler veya fekal mikrobiyal kontaminasyon gibi birçok etkenden ötürü çeşitlilik gösterebilir (2).

Halk ve çevre sağlığının korunması, güvenli içme suyu sağlanmasıyla mümkündür. Suyun "güvenli" olarak kabul edilmesinin en önemli şartlarından biri de patojenik bakterileri içermemesidir. Su kaynaklarında yayılan patojenler arasında en sık rastlananları enterik patojenlerdir. Bu nedenle insan kullanımına ayrılmış sulardaki fekal kirlilik kaynakları sıkı bir şekilde kontrol edilmelidir (3). İçme sularında bulunan koliformlar, tüm dünyada kirlilik göstergesi olarak kabul görmektedir. Toplam koliform terimi, 35–37 °C'de 24 saatte üreyebilen gram negatif, geniş aralıklı aerob ya da fakültatif anaeorab, spor oluşturmayan, laktozu fermente edip asit veya aldehit oluşturabilen ve yüksek konsantrasyonlardaki safra tuzunda üreyebilen bakteri grubunun tanımlanması için kullanılmaktadır. *Escherichia coli* ve termotolerant koliformlar, yüksek sıcaklıklarda laktoz fermente edebilen toplam koliform grubunun bir alt kümesidir. Laktoz fermantasyonunun bir parçası olarak, toplam koliformlar β-galaktosidaz enzimini üretir (2). Patojenik bakterilerden daha yüksek konsantrasyonlarda tespit edilen koliformlar, su ortamlarında entero-patojenlerin potansiyel varlığının bir indeksi olarak kullanılır. Koliform grubunun ve daha spesifik olarak *Escherichia coli*'nin mikrobiyolojik su kalitesinin bir göstergesi olarak kullanımı, 19. yüzyılın sonunda dışkıdan ilk izolasyonlarından kaynaklanmaktadır. Koliformların bazıları çevresel kökenli olduklarından, çeşitlendirilmiş doğal ortamlarda da rutin olarak bulunur, ancak içme suyu onlar için doğal bir ortam olarak değerlendirilmemelidir. İçme suyundaki mevcudiyetleri sağlığı tehdidi olarak, mikrobiyolojik su kalitesi bozulmasının göstergesi olarak düşünülmelidir. Genellikle koliform içermeyen bir içme suyunda, arıtma ve dezenfeksiyon kaynaklı problemler, kontamine su bulaşı ya da dağıtım

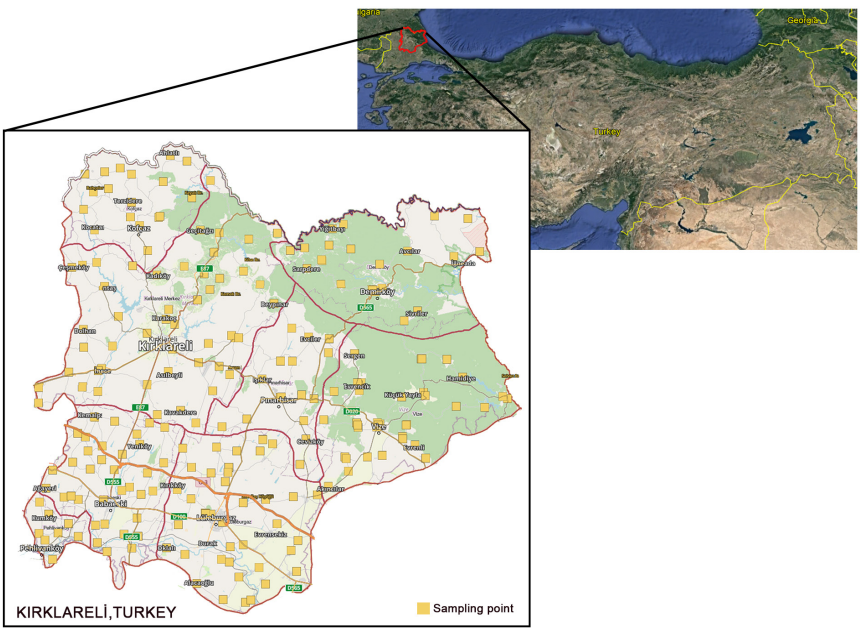
sistemlerindeki pasif koliformun aktif hale gelmesi gibi nedenlerden ötürü tekrar koliform varlığı görülebilir. Bu nedenle hanelere dağıtılan içme suları sürekli kontrol altında tutulmalıdır (4-6).

İnsani tüketime sunulan sular gerek ulusal gerekse uluslararası düzeyde regülasyonlara tabii tutulmaktadır. Bu mevzuatta da belirtildiği üzere suların mikrobiyolojik, kimyasal, radyoaktif ve fiziksel kontrolleri düzenli olarak yapılmalıdır. Bu parametreler arasından koliform bakterilerin tespiti ve suların organoleptik özelliklerinin kontrolleri sık sık gerçekleştirilmektedir. Buna göre ülkemizde yürürlükte olan ve 98/83/EC Avrupa Birliği direktifine (güncel olarak 2020/2184 direktifi) paralel olarak hazırlanan İnsani Tüketim Amaçlı Sular Hakkında Yönetmelik kapsamında suda koliform bakterilerin varlığı istenmezken, suyun organoleptik özelliklerinde ise (tat, koku, bulanıklık, renk) suyun kalitesine uygun olarak tüketicilerce kabul edilebilir olması ve anormal değişiklik olmaması istenir. Bu çalışma kapsamında Kırklareli ilinde koagülant ajan ile arıtım olmaksızın insani tüketime sunulan suların organoleptik özelliklerinden olan renk, koku ve bulanıklık parametrelerinin değişiminde koliform bakterilerin etkisinin istatistiki olarak değerlendirilmesi amaçlanmaktadır.

GEREÇ ve YÖNTEM

Su Örneklerinin Toplanması

Analiz edilecek su örnekleri Kasım 2019- Kasım 2021 tarihleri arasında Kırklareli ilinin sekiz ilçesinde yaklaşık olarak eşit sayıda numune sayısına sahip toplam 218 numune alma noktasından insani olarak tüketime sunulan musluk suları arasından elde edilmiş olup toplam 1022 adettir (Şekil 1). Numuneler mikrobiyolojik analiz için 1000 ml'lik sodyum tüyosülfatlı steril şişelerde, organoleptik analiz için ise 1500 ml'lik steril saydam plastik şişelerde aseptik şartlara uygun olarak alınmış olup soğuk zincirde en geç 8 saat içerisinde laboratuvara ulaştırılmış ve analize başlanmıştır. Araştırmanın konusu ve materyalleri Etik Kurul Onayı kapsamında değildir.



Şekil 1. Kırklareli ilinin Türkiye'deki konumunu ve il içerisindeki numune alım noktalarını gösteren harita

Koliform Bakterilerin Analizi

Numunelerdeki Koliform bakterilerin varlığı TS EN ISO 9308-1: 2014 standardına uygun olarak tespit edilmiştir. Analiz için membran filtrasyon yöntemi (0,45 µm por çaplı Sartorius™ marka selüloz nitrat filtre kağıdı) kullanılmıştır. 100 ml numuneler, membran filtrasyon işleminden sonra filtreler kromojenik koliform besiyerine (Sartorius™) yerleştirilmiş ardından 36±2 °C' de 18-24 saat inkübasyona bırakılmıştır. İnkübasyon sonrasında üreme görülen koyu lacivert (ß-D-galaktosidaz ve ß-D-glukuronidaz reakiyonu pozitif) koloniler *Escherichia coli* olarak tespit edilmiş, pembe-leylak rengi (sadece ß-D-galaktosidaz reaksiyonu pozitif) koloniler ise şüpheli koliform olarak belirlenerek oksidaz testi ile doğrulamalarının yapılabilmesi için pasajlanmış, bu maksatla Triptik Soy Agar (TSA) (Merck™) besiyerine inoküle edilerek 37°C'de 24 saat inkübasyona bırakılmıştır. İnkübasyon sonrasında TSA'da gelişen şüpheli koliform bakteri kolonileri oksidaz testine alınmış, oksidaz negatif olan koloniler koliform olarak değerlendirildikten sonra *E. coli* sayısı da eklenerek toplam koliform sayısı elde edilmiştir.

Organoleptik Parametrelerin Analizi

1500 ml'lik saydam steril şişelerde alınan numuneler tamamen duyusal olarak kokusuna, rengine ve bulanıklığına bakılarak en az iki analist ile çift kör yöntemiyle analiz edilmiş olup, estetik özelliklerinin İnsani Tüketim Amaçlı Sular Hakkında Yönetmelik doğrultusunda tüketicilerce kabul edilebilir durumda olduğunun uygunluğuna göre "Uygun" ya da "Uygun Değil" olarak sonuçlandırılmıştır. Analistler arası uyumsuzluklarda üçüncü bir analist devreye alınarak karar verilmiştir.

İstatiksel Analiz

Kantitatif olarak sonuçlandırılan *E. coli* ve diğer koliform bakteri (*E. coli* olmayan, sadace sadece ß-D-galaktosidaz reaksiyonu pozitif olan koliform bakteriler) verileri ile aynı numuneye ait ikili kategorik veri olarak sonuçlandırılan renk, koku, bulanıklık parametreleri istatistiksel analize alınmıştır. İçme sularında indikatör olarak tespiti yapılan toplam koliform parametrelerinin (*E. coli* ve diğer koliform bakteri) %95 güven aralığında suyun rengi, kokusu ve bulanıklığındaki değişimine etkisi ayrı ayrı olarak ikili lojistik regresyon analizi ile araştırılmıştır. Ayrıca suların renk, koku ve bulanıklık parametrelerinin birbirleriyle bağımsız ilişkisi Ki Kare (x^2) testi ile analiz edilmiştir. Bulunan sonuçların istatiksel açıdan değerlendirilmesinde, *p* değeri 0,05'in altında olan bulgular anlamlı kabul edilmiştir. İstatiksel analizler için IBM SPSS 22 programı ile Python yazılımı üzerinde çalışan Pandas ve Seaborn kütüphanelerinden yararlanılmıştır.

BULGULAR

Kırklareli ili geneli 218 noktadan alınan toplam 1022 adet arıtımsız su numunesinin gerçekleştirilen mikrobiyolojik analizlere göre elde edilen verilerin özeti Tablo 1'de gösterilmiştir.

Tablo 1. Numunelere ait mikrobiyolojik analiz verileri (N=1022)

Parametre	Ortalama kob*	Standart Sapma
Toplam Koliform Bakteri	27,68	129,407**
Esherichia coli	16,60	110,502**
Diğer Koliform Bakteri	11,08	65,134**

*kob = koloni oluşturan bakteri (100 ml numune için)
**mod=0, medyan=<0,001

1022 numunenin 352 tanesinde toplam koliform bakteri üremesi, 229'inde ise *E. coli* üremesi görülmüştür. *E. coli* ile birlikte diğer koliform bakteri üremesi görülen numune sayısı 121'dir. Numunelerde görülen minimum ve maksimum üreme sayıları 100 ml için sırasıyla 1 ila 1000 kob olmak üzere değerlendirmeye alınmıştır. Numunelerde tespit edilen mikrobiyolojik koloni üreme sayılarına göre numune sayılarının sınıflandırması Tablo 2'de gösterilmiştir.

Analiz edilen toplam 1022 numunenin renk, koku ve bulanıklık parametrelerinin değerlendirmesinde "Uygun" ya da "Uygun Değil" olarak sonuçlandırılan numune sayısının çapraz olarak sınıflandırılması Tablo 3'te gösterilmiştir.

Tablo 2. Numunelerin görülen mikrobiyolojik üreme sayı aralığına göre sınıflandırılması (N=1022)

Parametre/ kob*	0	1-10	11-50	50-100	101-500	>500
Toplam Koliform Bakteri	670	137	126	49	23	17
Escherichia coli	793	134	54	22	6	13
Diğer Koliform Bakteri	778	101	101	26	12	4

*100 ml'de görülen koloni üreme sayısı

Tablo 3. Numunelere ait organoleptik analiz verilerinin çapraz tablo olarak gösterimi (N=1022)

Parametre	Değerlendirme	Renk		Koku		Toplam
		Uygun	Uygun Değil	Uygun	Uygun Değil	
Bulanıklık	Uygun	860	17	851	26	877
	Uygun Değil	83	62	120	25	145
Koku	Uygun	914	57	-	-	971
	Uygun Değil	29	22	-	-	51
Toplam		943	79	971	51	1022

Analiz sonucu ikili kategorik olarak değerlendirilen renk, koku ve bulanıklık parametrelerinin birbirleriyle ilişkisini belirlemeye yönelik gerçekleştirilen analize göre; bulanıklık-renk, bulanıklık-koku ile renk-koku parametreleri arasında %95 güven aralığında anlamlı bir ilişki bulunmaktadır (Sırasıyla x^2 = 260,690; 53,492; 94,352. Hepsi p<0,001). Suların bulanıklığı ile rengi arasındaki ilişki güçlü, bulanıklığı ile kokusu ve rengi ile kokusu arasındaki ilişki ise zayıftır (Sırasıyla Cremers V = 0,533; 0,229; 0,034. Hepsi p<0,001). Ayrıca tüm organoleptik parametreler açısından tüketicilerce kullanıma uygun olarak belirlenmiş 838 numune içerisinde 100 ml'de toplam koliform üremesi görülen numune sayısı 281 olmakla birlikte bu numuneler arasından 171'inde *E. coli* üremesi, 207'sinde ise *E. coli* harici diğer koliform üremesi gözlenmiştir.

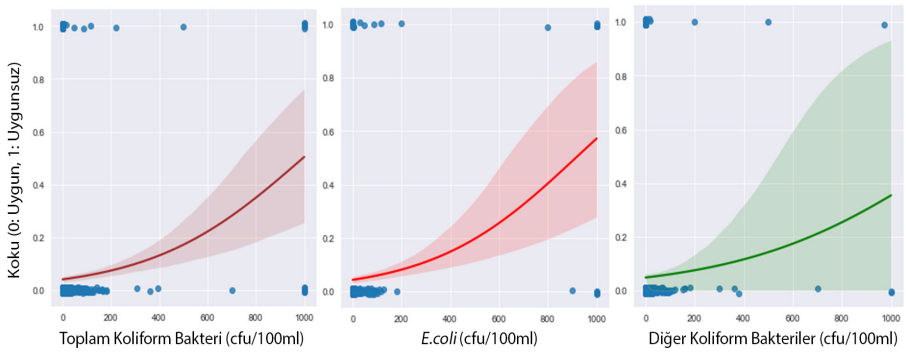
Kırklareli ili genelinde halkın kullanımına sunulan içme-kullanma sularında görülen *E. coli* ve diğer koliform bakteri varlığının, suyun organoleptik parametrelerine yönelik etkisinin hesaplanması amacıyla %95 güven aralığında ikili(binomial) lojistik regresyon analizi test edilmiştir (Tablo 4).

Gerçekleştirilen binomial (ikili) lojistik regresyon analizi testi sonucunda ortaya çıkan regresyon modeli koku parametresi için anlamlı bulunmuş olup, renk ve bulanıklık parametreleri için anlamlı değildir. Araştırmanın bağımsız değişkenleri olan

E. coli ve diğer koliform bakteriler suyun kokusu üzerindeki değişimlerin %8,9'unu açıklamaktadır. Modelin içme-kullanma sularında koku parametresini gözlenen verilere göre "Uygun" olarak tahmin etme yüzdesi %99,4 iken "Uygun Değil" olarak tahmin etme yüzdesi ise %13,7; tahmin doğruluğu genel yüzdesi ise %95,1'dir. Bulanıklık ve Renk parametrelerinin verileri test varsayımları sağlanamadığından ötürü değerlendirmeye alınmamıştır. Ayrıca elde edilen analiz verilerinin doğrulamasının yapılması maksadıyla toplam koliform parametresinin tekil olarak koku, renk ve bulanıklık paramatreleri üzerindeki etkisi %95 güven aralığında binomial (ikili) lojistik regresyon analizi gerçekleştirilmiş, *E. coli* ve diğer koliform bakterilerinn etkisinin ölçüldüğü analiz sonuçlarına benzer şekilde koku üzerine etkisi anlamlı bulunmuş (Nagelkerke R2 =0,087; Omnibus Ki Kare = 29,656; Sd =1; p<0,001; Hosmer&Lemeshow = p>0,05), diğer parametreler üzerindeki etkisi ise anlamlı bulunmamıştır (Hosmer&Lemeshow = p<0,05) (Şekil 2).

Tablo 4. *E.coli ve* diğer koliform bakteri parametrelerinin organoleptik parametreler üzerindeki etkisi (N=1022)

Parametre		B	S.E.	Wald	Sd	*p*	Exp(B)	Exp (B) %95 Güven Aralığı	
								Düşük	Yüksek
Koku	D.Koliform	0,002	0,001	4,587	1	0,032	1,002	1,000	1,005
	E.coli	0,003	0,001	32,680	1	<0,001	1,003	1,002	1,005
	Sabit	-3,154	0,159	392,633	1	<0,001	0,043	-	-
	Nagelkerke R2 =0,089; Omnibus Ki Kare = 30,265; Sd =2; p<0,001; **Hosmer&Lemeshow = p>0,05**								
Renk	D.Koliform	-0,002	0,003	0,314	1	0,575	0,998	0,992	1,005
	E.coli	0,003	0,001	28,098	1	<0,001	1,003	1,002	1,004
	Sabit	-2,583	0,126	420,504	1	<0,001	0,076	-	-
	Nagelkerke R2 =0,062; Omnibus Ki Kare = 27,034; Sd =2; p<0,001; **Hosmer&Lemeshow = p<0,05**								
Bulanıklık	D.Koliform	-0,002	0,002	0,638	1	0,425	0,998	0,993	1,003
	E.coli	0,003	0,001	17,610	1	<0,001	1,003	1,001	1,004
	Sabit	-1,848	0,094	385,468	1	<0,001	0,158	-	-
	Nagelkerke R2 =0,032; Omnibus Ki Kare = 18,642; Sd =2; p<0,001; **Hosmer&Lemeshow = p<0,05**								



Şekil 5. Kırklareli ili İçme-kullanma sularındaki koliform bakteri varlığının suyun organoleptik parametrelerinden olan koku parametresi üzerindeki %95 güven aralığında anlamlı etkisini gösterir lojistik regresyon grafiği

TARTIŞMA

WHO'nun da rehberinde bahsettiği üzere tüketicilerce kullanıma sunulan suların duyular aracılığıyla algılanan renk, bulanıklık ve koku gibi organoleptik özellikleri su kaynağının coğrafik özellikleri, suların kimyasal ya da mikrobiyal kontaminasyonu ya da kloraminasyon gibi arıtım ve dağıtım aşaması sürecindeki işlemler de dahil olmak üzere çeşitli faktörlerden etkilenmektedir. Literatür detaylı olarak incelendiğinde ise bu konuda gerçekleştirilen çalışmalar içme-kullanma sularında görülen fekal mikrobiyal kontaminasyonun suyun organoleptik özellikleri üzerindeki etkisinin önemini vurgulama konusunda yetersiz kaldığı düşünülmektedir.

Koçak ve Güner tarafından 2009 yılında Erzurum'da yapılan bir çalışmada, toplamda 70 numunenin incelendiği ve bunlardan yalnızca bir tanesinde bulanıklık değerinin izin verilen maksimum değerden düşük olduğu, diğer numunelerin hepsinin (%98,5) izin verilen değerin üzerinde olduğu bildirilmiştir. Buna karşın numunelerin %48,5'inde (n=34), çalışmanın yapıldığı tarihlerde yürürlükte olan mevzuata göre bakteriyel uygunsuzluk olduğu belirtilmektedir (7). Bu bulgular suların bulanıklığı ile bakteriyel kirliliği arasında birebir olmasa dahi bir ilişki olabileceğini düşündürmektedir. Buna mukabil yine Erzurum'da 2019 yılında Gökçen ve Atasever tarafından yapılan bir çalışmada, il merkezi ve ilçe merkezlerinden alınan numunelerin %8,6'sında bakteriyel uygunsuzluk olduğu bildirilmiştir. Bu çalışmada ise alınan numunelerin tamamının gerek bulanıklık ve renk değerleri, gerekse koku ve tat değerleri açısından yürürlükteki mevzuata uygun olduğu belirtilmektedir (8). Brooks ve arkadaşları tarafından, Batı Kenya'da hanelere dağıtılan depolanmış içme-kullanma sularının kullanıcılarla algılanan parametreleri(organoleptik) ile genel kalitelerinin *E. coli* üremeleri arasında anlamlı bir ilişki olup olmadığını tespit etmeye yönelik gerçekleştirdikleri çalışma neticesinde Colilert aracılığıyla tespit edilen *E. coli* kontaminasyonu (>1 MPN/100 ml) varlığı ile suların tat veya kokusu açısından mükemmelden daha az olarak değerlendirenler arasında istatistiksel olarak anlamlı bir ilişki olduğu belirtilmiştir. Ayrıca CBT (Compartment Bag Test) ile gerçekleştirdikleri *E. coli* analizleriyle ise tat ve koku algıları için benzer sonuçlar bulmuş, fakat bu ilişkinin istatistiksel olarak anlamlı olmadığını ifade etmişlerdir (9). Kamboçya'da ise yapılan bir çalışma kapsamında Orgill ve arkadaşları *E. coli* ile organoleptik parametreler arasında istatistiki olarak bir ilişki saptayamamıştır (10). ABD'nin Alabama eyaletinde gerçekleştirilen bir çalışmada ise Wedgworth ve arkadaşları hanelere sunulan suların estetik parametreleri ile toplam koliform verileri arasında anlamlı bir ilişki gözlenmediğini ifade etmiştir (11). Dianty ve arkadaşları ise içme sularındaki toplam koliform ve *E. coli* mevcudiyetinin insanlarca algılanan parametreler üzerindeki etkisini anlamlı bulmamışken tüketicilerin bireysel tercihlerine göre sonucun farklılaştığını belirtmişlerdir (12). Son yıllarda ise Hu ve arkadaşlarının gerçekleştirdiği bir çalışma sonucunda, özellikle koliform üremeleri nispeten yüksek olan (ortalama 8 kob/100 mL) içme sularının kokusunun tüketicilerce uygun bulunmadığı anket ve istatistik çalışmaları sonucu açıkça ifade edilmiştir (13).

Literatür kapsamlı olarak incelendiğinde içme-kullanma sularının gerek estetik, gerekse organoleptik olarak değerlendirilen bulanıklık, renk ve koku parametrelerinin değişiminin her zaman tek faktörle açıklanamayacağı, bazen de çeşitli maddelerin sinerjik etkisiyle ya da coğrafi farklılıklara göre değişim göstereceğini ortaya koymaktadır (14). Stauber tarafından kaleme alınan bir editöryel makalede ise içme suyu kalitesinin organoleptik algısı üzerine yapılan araştırmaların çoğunlukla gelişmiş ülkelerde tüketici tercihini anlamaya yönelik karmaşık yaklaşımlardan oluşmakta olduğunu, suyun tüketicilerce algılanan özellikleri üzerine metodoloji geliştirilmesinin ihtiyacını ve su kalitesinin değerlendirilmesinde organoleptik

parametrelerin birincil olarak değerlendirilmesi gerektiği ifade edilmiştir (15).

Ülkemizde değişik bölgelerde yapılan pek çok çalışma sonuçları birlikte değerlendirildiğinde, verdiğimiz Erzurum'da yapılan iki farklı çalışma örneğinde olduğu gibi bazı çalışma sonuçları içme-kullanma amacıyla kullanılan suların bulanıklık, tat, koku gibi özellikleriyle, bakteriyel kirliliği arasında ilişki olabileceğini düşündürürken, bazı çalışmalar da tersine bulgular içermektedir (16-18). Ancak yapılan literatür incelemesinde, ülkemizde yapılmış içme-kullanma sularının organoleptik özellikleriyle mikrobiyal kirliliği arasında ilişki olup olmadığını değerlendiren bir çalışmaya rastlanmamıştır.

Çalışmamızda, Kırklareli ili genelinde halkın kullanımına sunulan içme-kullanma sularının organoleptik özelliklerinden koku parametresinin değişiminde, yapılan lojistik regresyon analizine göre FIO parametrelerinden *E. coli* ve diğer koliform bakterilerin istatistiki olarak %95 güven aralığında %8,9 oranında yordayıcı olduğu belirlenmiştir. Ayrıca suyun organoleptik parametrelerinin değişimlerinin birbirleriyle olan ilişkisi analiz edildiğinde suyun bulanıklığı ile renginin değişiminin güçlü şekilde ilişkili olduğu gözlenmiştir. Ancak çalışmamızın en önemli kısıtlılığı belli bir bölge ve zaman dilimini kapsayan bir kesitsel çalışma olmasıdır. Ayrıca yine çok önemli kısıtlılık da, çalışmamızda organoleptik parametrelerle yalnızca koliform bakteri varlığının etkisinin karşılaştırılmış olmasıdır. Numunelerde koliform dışında bulunabilecek ve organoleptik özellikleri değiştirebilecek bir flora olup olmadığı, yani karıştırıcı faktörler bu çalışma kapsamında değerlendirilmemiştir. Bu itibarla benzer çalışmaların ülke genelinde yapılması konu hakkında daha kapsamlı bilgi birikiminin oluşması açısından gereklidir.

Çalışmamızın en önemli sonucu, incelenen su örneklerinin organoleptik özellikleriyle mikrobiyal kirliliği arasında ilişki olabileceğinin tespit edilmiş olmasıdır. Bu nedenle, içme-kullanma sularında organoleptik yöntemlerle uygunsuzluk saptandığında, bakteriyolojik kirlilik de olabileceğinin daima göz önüne alınması ve aksi ispatlanıncaya kadar bakteriyolojik kirlilik açısından dikkatli olunması gerektiği söylenebilir.

ETİK KURUL ONAYI

* Bu çalışma, Etik Kurulu onayı gerektirmemektedir.

ÇIKAR ÇATIŞMASI

Yazarlar bu makale ile ilgili herhangi bir çıkar çatışması bildirmemişlerdir.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

Günübirlik tedavi ünitesinde ayaktan parenteral antibiyotik tedavisi alan hastaların değerlendirilmesi

Evaluation of patients receiving outpatient parenteral antibiotic therapy in the outpatient unit

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ÖZET

Amaç: Artan antimikrobiyal direnç nedeniyle geniş spektrumlu parenteral antibiyotik kullanımı giderek artmaktadır. Bu yüzden ayaktan parenteral antibiyotik uygulamalarının yapılacağı günübirlik tedavi ünitelerinin (GTÜ) önemi yükselmektedir. Bu çalışma ile hastanemiz bünyesinde hizmet veren GTÜ'nün çalışmaları değerlendirilerek, enfeksiyon hastalıkları tedavisindeki yerinin belirlenmesi ve öneminin vurgulanması amaçlandı.

Yöntem: Çalışmamız retrospektif kesitsel çalışma olarak planlandı. Aralık 2021-Şubat 2022 tarihleri arasında ünitemizde parenteral antibiyotik alan 18 yaş üstü hastalar çalışmamıza dahil edildi. Hasta bilgileri GTÜ defter kayıtlarından ve hastane bilgi işlem sisteminden tarandı. Hastaların demografik özellikleri, eşlik eden hastalıkları, hastaların üniteye yönlendirildiği birim, enfeksiyon odağı, etken mikroorganizma, antimikrobiyal direnç özellikleri, kullanılan antibiyotik, tedavi süresi ve yanıtı incelendi.

Bulgular: Çalışmaya, belirlenen kriterleri taşıyan toplam 101 hasta dahil edildi. Çalışmamıza dahil edilen

ABSTRACT

Objective: Due to increasing antimicrobial resistance, the use of broad-spectrum parenteral antibiotics is increasing. For this reason, the importance of outpatient parenteral antibiotic treatment (OPAT) is increasing. With this study, it was aimed to evaluate the OPAT unit of our hospital, to determine its use in the treatment of infectious diseases and to emphasize its importance.

Methods: Our study is a retrospective cross-sectional study. Patients over the age of 18 who received parenteral antibiotics in our OPAT unit between December 2021 and February 2022 were included in our study. Patients data were obtained from medical records of hospital. The demographic characteristics of the patients, their comorbidities, the unit they were referred to, the site of infection, the causative microorganism, antimicrobial resistance characteristics, antibiotics used, duration of treatment and response were examined.

Results: One hundred and one patients were included in the study. The mean age of the patients included in our study was 58.4±15.51 years, and 54.5% (n=55) of the

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hastaların ortalama yaşı 58,4±15,51 olup, hastaların %54,5 (n=55)'i erkekti. Hastaların %62,4 (n=63)'ünde ek hastalık mevcuttu. En sık eşlik eden hastalık Diyabetes mellitus (%18,8, n= 19) idi. Hastalarda en sık enfeksiyon odağı üriner sistem enfeksiyonu (n=53, %52,3), en sık kullanılan antibiyotikler ise sırası ile ertapenem (n=57,%56,4) ve teikoplanin (n=37, %36,6) idi. *Escherichia coli* en sık görülen Gram negatif etken iken, *Staphylococcus aureus* en sık görülen Gram pozitif etken idi (Tablo 4). *Enterobacteriaceae* ailesinde genişletilmiş spektrumlu beta laktamaz (GSBL) direnç oranı %96,36, *Staphylococcus* spp.'lerde metisilin direnç oranı %100 idi. Antibiyotik başlanan hastaların tamamında herhangi bir yan etki oluşmadan tedavileri tamamlandı. Bir hasta dışında tedavi alan tüm hastalar şifaya kavuştu.

Sonuç: Hastanemizde GTÜ hem poliklinik hastalarının tedavilerinde hem de yatan hastaların ardışık tedavilerinde önemli bir yer tutmaktadır. Dirençli mikroorganizmaların artışı nedeniyle oral antibiyotik seçeneklerinin kısıtlanması parenteral antibiyotik tedavisinin artışına neden olmuştur. Bu çalışma ile üç aylık kısa bir süre zarfında bile GTÜ sayesinde 101 hastanın hastane yatışı olmadan tedavisinin tamamlandığı görülmüştür. Bu nedenle ülkemizde bu tür ünitelerin yaygınlaşması önemlidir.

Anahtar Kelimeler: Antibiyotik, direnç, GSBL, parenteral, üriner

patients were male. Comorbidity was present in 62.4% (n=63) of the patients. The most common comorbid disease was Diabetes mellitus (18.8%, n= 19). The most common focus of infection was urinary tract infection (n=53, 52.3%). The most commonly used antibiotics were ertapenem (n=57, 56.4%) and teicoplanin (n=37, 36.6%), respectively. *Escherichia coli* was the most common Gram-negative agent, while *Staphylococcus aureus* was the most common Gram-positive agent (Table 4). Extended-spectrum beta-lactamase (ESBL) resistance rate in *Enterobacteriaceae* family was 96.36%, and methicillin resistance rate in *Staphylococcus* spp. was 100%. All of the patients who were started on antibiotics were treated without any side effects. Except for one patient, all patients who received treatment recovered.

Conclusion: In our hospital, OPAT has an important place both in the treatment of outpatients and in the consecutive treatments of inpatients. The limitation of oral antibiotic options due to the increase in resistant microorganisms has led to the increase in parenteral antibiotic therapy. With this study, it was observed that even in a short period of three months, 101 patients completed their treatment without hospitalization through OPAT units. For this reason, it is important that these units become widespread in our country.

Key Words: Antibiotics, ESBL, parenteral, resistance, urinary

GİRİŞ

Ayaktan parenteral antibiyotik tedavisi (APAT), antibiyotik tedavisi dışında herhangi bir klinik desteğe ihtiyaç duymayan hastalara uygulanan bir tedavi rejimidir. Dirençli mikroorganizmaların artışı nedeniyle gerek hastane kaynaklı enfeksiyonlarda, gerekse toplum kökenli enfeksiyonlarda geniş spektrumlu parenteral tedavi seçeneklerine ihtiyaç duyulmaktadır (1). Bu durum hastalara gereksiz yatış endikasyonu doğurmakta ve sosyoekonomik problemleri beraberinde getirmektedir. Bu nedenle APAT uygulamaları iyi bir seçenek olarak karşımıza çıkmaktadır. APAT ilk defa 1974 yılında Amerika Birleşik Devletleri'nde uygulanmıştır (2). Yaklaşık 50 yıldır da birçok farklı ülkede APAT uygulamaları devam etmektedir. Günübirlik tedavi üniteleri (GTÜ) ülkemizde çok yaygın değildir. Ancak artan antimikrobiyal direnç oranları ve ekonomik maliyetler nedeniyle, APAT uygulamalarının yapılabilmesi için GTÜ'ye giderek daha fazla ihtiyaç duyulmaktadır. Çalışmamızda hastanemiz bünyesinde hizmet veren

GTÜ'nün verileri değerlendirilerek, enfeksiyon hastalıkları tedavisinde yerini belirlemek ve önemini vurgulamak amaçlandı.

GEREÇ ve YÖNTEM

Çalışmamız retrospektif kesitsel bir çalışma olarak planlandı. Hastanemizde GTÜ Mart 2019 tarihinden beri hizmet vermektedir. Hastanemiz 3. basamak bir hastane olarak hizmet vermektedir. GTÜ bünyesinde dermatoloji, nöroloji ve enfeksiyon hastalıkları uzmanlarının gözetiminde ilgili kliniklere ait parenteral tedaviler bu birim için görevlendirilen hemşireler tarafından uygulanmaktadır.

Hastanemiz GTÜ biriminde Aralık 2021-Şubat 2022 tarihleri arasında on sekiz yaş üzeri, parenteral antibiyotik tedavisi alan hastalar çalışmaya dahil edildi. Bu hastalar hastane yatış endikasyonu bulunmayan, polikliniklerden birime yönlendirilen veya hastane yatış sonrası genel durumu düzelip yatış endikasyonu kalmayan, ardışık tedavi için yönlendirilen hastalardı. Hastalara ait ilk başvuru anındaki veriler değerlendirmeye alındı.

Hasta bilgileri GTÜ defter kayıtlarından ve hastane bilgi işlem sisteminden tarandı. Hastaların demografik özellikleri, eşlik eden hastalıkları, hastaların üniteye yönlendirilme durumu, enfeksiyon odağı, etken mikroorganizma, antimikrobiyal direnç özellikleri, kullanılan antibiyotik, tedavi süresi ve yanıtı incelendi. Hastalar GTÜ'de günlük olarak etkinlik ve yan etki gelişimi açısından enfeksiyon hastalıkları uzmanı ve ünite hemşiresi tarafından değerlendirildi. Her hasta uzman doktorun önerdiği aralıklarla tam kan, biyokimya, enfeksiyon odaklarından kültür ve radyolojik görüntüleme ile kontrol edildi. Tedavi bitiminde klinik semptomları gerileyen ve enfeksiyon hastalıkları uzmanı tarafından değerlendirilerek şifa düşünülüp tedavisi kesilen hastalarda APAT başarılı olarak belirlendi. APAT sürecinde yatış endikasyonu doğan, yan etki veya klinik/ laboratuvar yanıtı alınamayıp tedavisi kesilen hastalarda APAT tedavisi başarısız olarak tanımlandı. Klinik yanıt semptomların gerilemesi, laboratuvar yanıt ise lökositoz, C-reaktif protein (CRP), eritrosit sedimantasyon hızında (ESR) gerileme, kontrol kültürlerde üreme olmaması ve radyolojik görüntülemelerde düzelme olarak değerlendirildi.

Çalışma için istatistiksel analiz SPSS versiyon 26.0.0 paket programı kullanılarak yapıldı. Devamlı değişkenler ortalama ± standart sapma ve ortanca (minimum- maksimum) şeklinde bildirildi. Kategorik değişkenler olan hastalara ait cinsiyet, ek hastalıklar, enfeksiyon odağı, verilen antibiyotik, GTÜ'ne yönlendirilme şekli sayı ve yüzde olarak tanımlandı.

Bu çalışma, Ankara Şehir Hastanesi Klinik Araştırmalar Etik Kurul Başkanlığı onayı ile gerçekleştirilmiştir (Tarih: 11.01.2023, Karar no: E.Kurul-E1-23-3180).

BULGULAR

GTÜ'de çalışmamızın yapıldığı üç aylık süre içerisinde 354 hastaya parenteral tedavi uygulandı. İki yüz elli üç hasta antibiyotik dışı tedavi alması nedeniyle çalışma dışı bırakıldı ve toplam 101 hasta çalışmaya dahil edildi. Çalışmamıza dahil edilen hastaların ortalama yaşı 58,4±15,51 olup, hastaların %54,5 (n=55)'i erkekti. Hastaların %62,4 (n=63)'ünde ek hastalık mevcuttu. Diyabetes mellitus hastaların %18,8 (n= 19)'inde olup, en sık eşlik eden hastalıktı. Diyabetes mellitusu sırası ile kronik böbrek hastalığı (%11,8, n=12), böbrek taşı (%10,9, n=11) ve benign prostat hiperplazisi (%7,9 n=8) takip ediyordu (Tablo 1).

APAT'a yönlendirilen hastalarda en sık enfeksiyon odağı üriner sistem enfeksiyonuydu (n=53, %52,3). Deri yumuşak doku enfeksiyonu hastaların %17,8 (n=18)'inde görülürken, kemik eklem enfeksiyonları %21,7 (n=22)'sinde, kan dolaşımı enfeksiyonları %5,9 (n=6)'unda, intraabdominal enfeksiyonlar ise %1,9 (n=2)'unda görüldü. Bu endikasyonlarda antibiyotiklerin APAT ile verilme ortanca süresi 10 (3-30) gündü (Tablo 1).

Tablo 1. Günübirlik tedavi ünitesinde tedavi alan hastaların özellikleri (n=101)

Yaş, ortalama±SS*	58,4±15,508
Cinsiyet, n (%)	
Erkek	55 (54,5)
Kadın	46 (45,5)
Ek hastalık, n (%)	63 (62,4)
Diyabetes mellitus	19 (18,8)
Kronik böbrek hastalığı	12 (11,8)
Böbrek taşı	11 (10,9)
Benign prostat hiperplazisi	8 (7,9)
Malignite	5 (4,6)
Hipertansiyon	3 (2,9)
Böbrek transplantasyonu	3 (2,9)
Gebelik	2 (1,9)
Enfeksiyon odağı, n (%)	
Üriner sistem enfeksiyonu	53 (52,5)
Deri-yumuşak doku enfeksiyonu	18 (17,8)
Kemik- eklem enfeksiyonu *Osteomyelit* *Protez eklem enfeksiyonu*	22 (21,7) *17 (16,8)* *5 (4,9)*
Kan dolaşımı enfeksiyonu	6 (5,9)
İntraabdominal enfeksiyon	2 (1,9)
Tedavi süresi, ortanca (min-maks)	10 (3-30)

Hastaların %47,5 (n=48)'i enfeksiyon hastalıkları polikliniğine ayaktan başvuran hastalar iken, %43,6 (n=44)'sı yatarken antibiyotik başlanıp sonrasında ardışık parenteral antibiyotik tedavisi için yönlendirilen hastalardı. Acil servisten tarafımızca APAT için yönlendirilen hastalar ise hastaların %8,9 (n=9)'unu oluşturmaktaydı (Tablo 2).

GTÜ'de en sık kullanılan antibiyotikler sırası ile ertapenem (n=57,%56,4), teikoplanin (n=37, %36,6), amikasin (n=5, %4,9), daptomisin (n=3, %2,9), seftriakson (n=2, %1,9) ve vankomisin (n=2, %1,9) idi (Tablo 3).

Tablo 2. Hastaların günübirlik tedavi ünitesine yönlendirilme şekli, n (%)

Poliklinikten yönlendirme	48 (47,5)
Yatışı sonrası ardışık antibiyotik tedavisi amacıyla yönlendirilme	44 (43,6)
Acil servisten yönlendirilme	9 (8,9)

Tablo 3. Günübirlik tedavi ünitesinde kullanılan antibiyotikler (n=106)

Ertapenem	57 (56,4)
Teikoplanin	37 (36,6)
Amikasin	5 (4,9)
Daptomisin	3 (2,9)
Seftriakson	2 (1,9)
Vankomisin	2 (1,9)

Verilen antibiyotik tedavilerinin %80,2 (n=81)'si etkene yönelik iken, %19,8 (n=20)'i ampirik olarak başlanmıştı. *Escherichia coli* en sık görülen Gram negatif etkenken, *Staphylococcus aureus* en sık görülen Gram pozitif etkendi (Tablo 4). *Enterobacteriaceae* ailesinde genişletilmiş spektrumlu beta laktamaz (GSBL) üretimi oranı %96,36, *Staphylococcus* spp.'lerde metisilin direnci oranı %100 idi. Aynı şekilde *Enterococcus* spp.'lerde de ampisilin direncinin %100 olduğu görüldü.

GTÜ'de antibiyotik başlanan hastaların tamamı antibiyotik tedavisini tamamladı. Herhangi bir yan etki gelişmeyen hastalarda tedavi değişikliği yapılmadı. Protez enfeksiyonu nedeniyle planlanan süre boyunca antibiyotik tedavisi alan bir hastada tedavi bitiminde yanıt alınamadığına karar verildi ve tekrar opere olmak için ortopedi kliniğine yönlendirildi. Diğer 100 hastada şifa izlendi.

Tablo 4. Hastalarda kültür sonuçlarının değerlendirilmesi

Kültür	**n (%)**
Yok	20 (19,8)
Var	81 (80,2)
Gram-negatif etkenler	**n (%)**
Escherichia coli	51 (50,5)
Klebsiella pneumoniae	3 (2,9)
Pseudomonas aeruginosa	2 (1,9)
Morganella morgagnii	1 (0,9)
Gram pozitif etkenler	**n (%)**
Staphylococcus aureus	10 (9,9)
Koagülaz negatif stafilokok	6 (5,9)
Enterococcus spp.	6 (5,9)

TARTIŞMA

Antibiyotik direncinin giderek artması ile oral tedavi seçenekleri kısıtlanmış ve parenteral tedavi seçeneklerine daha fazla ihtiyaç duyulmuştur. Elli yılı aşkın süredir dünyada kullanımda olan APAT, ülkemizde 2010 yılından beri çeşitli merkezlerde uygulanmaktadır. Hastanemiz 2019 yılında açılmış olup, o günden itibaren GTÜ aktif olarak hizmet vermektedir. Bu çalışmada özellikle pandemi döneminde yatak sayılarının kısıtlanması ile işlev ve önemini bir kez daha hissettiğimiz GTÜ'nün retrospektif kesitsel bir değerlendirmesi yapılmıştır

Hastanemiz 3. basamak referans bir hastanedir. Polikliniğimize tedavi deneyimli, tedaviye yanıt alamamış hasta başvuruları sıklıkla olmaktadır. Bu nedenle poliklinik hastalarında da dirençli mikroorganizmalar sıklıkla izlenmektedir. Hastanemizde daha önce yapılan bir çalışmada polikliniğimize başvuran toplum kökenli üriner sistem enfeksiyonlarında GSBL tipi direnç *E. coli* için %48,3, *K. pneumoniae* için %60 oranında izlenmiştir (1). Yine enfeksiyon servisinde 65 yaş üstü üriner sistem enfeksiyonu ile yatış yapılan hastalarda GSBL tipi direnç *E. coli* için %56, *Klebsiella* spp. için %40 olarak bildirilmiştir (3). Bu durum üriner sistem enfeksiyonlarında oral tedavi seçeneklerimizin ne kadar kısıtlandığını gözler önüne sermektedir. Dünyada da ülkemizde olduğu gibi üriner sistem enfeksiyonlarında yıllar içinde giderek artan dirençli mikroorganizmalar nedeniyle GTÜ'de günde tek doz uygulama avantajı olan ertapenemin kullanımı artmaktadır (4,5). Bizim çalışmamızda da APAT tedavisine yönlendirilen hastaların %52,3'ünde enfeksiyon odağı üriner sistem, en sık kullanılan antibiyotik ise ertapenem olarak izlenmiştir. Ertapenem, APAT ile hem ayaktan izlenen hastalarda, hem de yatarken başlanan tedavinin ayaktan parenteral devamında sıklıkla tercih ettiğimiz bir antibiyotiktir. Ülkemizde 2020 yılında Baştuğ ve arkadaşlarının yapmış olduğu APAT'ın değerlendirildiği bir çalışmada da çalışmamızla benzer şekilde en sık enfeksiyon odağı üriner sistem olarak bildirilmiştir (6). APAT, hem dirençli alt üriner sistem enfeksiyonlarının tedavisinde, hem de yatarak izlediğimiz üst üriner sistem enfeksiyonlarının tedavisinde yatış endikasyonunun bitmesiyle birlikte parenteral tedavinin tamamlanmasına olanak sağlar. Özellikle geriyatrik yaş grubunda uzun hastane yatışı, hastalarda deliryum, sekonder enfeksiyonlar için risk oluşturmaktadır. Geriyatrik hasta grubunda da diğer popülasyonlarda olduğu gibi üriner sistem enfeksiyonlarında GSBL tipi direncin arttığı bildirilmiştir (3). Bu nedenle özellikle bu hasta grubunda klinik destek ihtiyacının bitmesi ile birlikte APAT'a geçilmesi büyük önem taşımaktadır.

Kemik eklem enfeksiyonları ile izlenen hastalar, bu enfeksiyonların tedavisi için uzun süreli parenteral antibiyotik gerekmesi nedeniyle sosyoekonomik açıdan problemler yaşayabilmektedir. Uygun hastalarda APAT seçeneği, hastalara rutin yaşamlarını devam ettirebilme şansı sunmaktadır. Bizim çalışmamızda da üriner sistem enfeksiyonlarından sonra APAT'ın en sık kullanıldığı hasta grubu kemik eklem enfeksiyonuyla izlenen hastalardı. Bu hasta grubunda Gram pozitif etkenlerin sık karşımıza çıkması nedeniyle gerek etkene yönelik tedavide gerekse ampirik tedavide teikoplanini sıklıkla tercih edilmektedir. GTÜ'de de teikoplaninin %36,6 oranında kullanıldığı görülmüştür.

Çalışmamızda hastaların tamamı enfeksiyon odağına yönelik öngörülen sürede APAT almıştır. Çalışmamız süresince APAT alan hastalarda herhangi bir yan etki izlenmemiştir. Hastalarda tedavi başarısı %99 olarak izlenmiştir. Daha önce yapılmış benzer çalışmalarda tedavi başarı oranları %91-92,4-98,5 olarak bildirilmişti (5-7). APAT'ta yüksek başarı için enfeksiyon hastalıkları uzmanı tarafından yakın izlem ve deneyimli bir hemşire tarafından uygulanan tedavi önerilmektedir (8). Hastanemizde de APAT uygulamaları enfeksiyon hastalıkları uzmanı kontrolünde ve bu konuda deneyimli hemşireler tarafından uygulanmaktadır. Çalışmalarda hastaların

laboratuvar değerleri etkinlik ve yan etki takibi için haftalık olarak takip edilmesi önerilmiştir (9). Bizim çalışmamız retrospektif olması nedeniyle hastaların %80'ninde takiplerin haftalık olarak yapıldığı ancak %20 hastada bu takiplerin aksadığı ve takip aralarının 10-15 güne kadar uzadığı görülmüştür. Hastaların hiçbirinde yan etki ile karşılaşılmamıştır. Protez enfeksiyonu ile takipli bir hastamızda öngörülen sürede antibiyotik tedavisini aldıktan sonra alınan laboratuvar testlerinde CRP değerinde artış izlenmesi nedeniyle antibiyotik tedavisi kesilerek enfeksiyon kaynak kontrolü için ortopediye yönlendirilmiştir. APAT ile yanıt alamadığımız hastada başlangıç tedavisinin etkene yönelik başlanmış olması ve yatarak alacağı antibiyotik tedavisinin de APAT ile aynı antibiyotik olacağı düşünüldüğünde tedavi başarısızlığı APAT ile ilişkilendirilememiştir.

APAT yüksek başarı oranları nedeniyle antibiyotik tedavisinde önemli bir alternatif olarak yerini almıştır. Hastane yatışının olmaması nedeniyle hastaların hastane kaynaklı ikincil enfeksiyonlara maruz kalmaması önemli bir avantaj sağlamaktadır. Bu çalışmada maliyet etkinlik hesaplaması yapılamamıştır. Bu durum çalışmanın bir kısıtlılığıdır. Ancak daha önce yapılmış çalışmalarda APAT 'ın, yatarak tedaviye kıyasla çok daha maliyet etkin olduğunu bildirilmiştir (6,10). Bu nedenlerle uygun hastalarda APAT tercih edilmesi avantaj sağlamaktadır. Ülkemizde hastanelerde GTÜ'ler son yıllarda giderek artmaktadır. Ancak GTÜ'lerin ülkeye ve hastaya kattığı faydalar göz önüne alınarak, bu ünitelerin sayılarının arttırılması ve etkin şekilde kullanılması gerektiği düşünülmüştür.

ETİK KURUL ONAYI

* Bu çalışma, Ankara Şehir Hastanesi Klinik Araştırmalar Etik Kurul Başkanlığı onayı ile gerçekleştirilmiştir (Tarih: 11.01.2023, Karar no: E.Kurul-E1-23-3180).

ÇIKAR ÇATIŞMASI

Yazarlar bu makale ile ilgili herhangi bir çıkar çatışması bildirmemişlerdir.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

Larvicidal and ovicidal effects of selected chemicals found in plant-derived essential oils for mosquito control

Sivrisineklerle mücadelede bitkisel kökenli esansiyel yağlarda bulunan çeşitli kimyasalların larvisidal ve ovisidal etkileri

Fatma BURSALI[1] (ID)

ABSTRACT

Objective: *Aedes aegypti* (L., 1762) (Diptera: Culicidae) is a vector for numerous viral diseases including dengue fever, Chikungunya, Zika fever, and yellow fever in densely populated urban areas. Mosquito control programs rely heavily on applying larvicides in breeding areas and applying insecticides targeting adult mosquitoes in the *Ae. aegypti* populations. Essential oils are primarily composed of terpenoids and phenylpropanoids and have a range of beneficial properties, including insecticidal activities.

Methods: This study primarily investigated the ovicidal and larvicidal activity of selected chemicals (verbenone, propionic acid, lactic acid, 1-butanol, 2-butanol and citronellol) identified in essential oils of plants against *Ae. aegypti* in wells of 24-well plates.

Results: Verbenone, propionic acid and lactic acid were observed to present a dose dependent effect on mosquito larvae. Verbenone emerged as the most effective against larvae with an LC_{50} value

ÖZET

Amaç: *Aedes aegypti* (L., 1762) (Diptera: Culicidae), dünya nüfusunun büyük bir kısmını etkileyen dang humması, Chikungunya, Zika humması ve sarıhumma gibi çok sayıda viral hastalığın vektörüdür. Sivrisinek kontrol programları büyük ölçüde üreme alanlarında larvasitlerin uygulanmasına ve *Ae. aegypti* ergin popülasyonlarını hedef alan böcek ilaçlarının uygulanmasına dayanmaktadır. Esansiyel yağlar temel olarak terpenoidler ve fenilpropanoidlerden oluşmaktadır ve böcek öldürücü aktiviteleri de dahil olmak üzere çeşitli faydalı özelliklere sahiptir.

Yöntem: Bu çalışmada çeşitli bitkilerin uçucu yağlarından tanımlanmış bazı kimyasal maddelerin (verbenon, propiyonik asit, laktik asit, 1-butanol, 2-butanol ve sitronellol) *Ae. aegypti* larva ve yumurtalarına karşı ovisidal ve larvisidal aktiviteleri 24 gözenekli plakada araştırılmıştır.

Bulgular: Verbenon, propiyonik asit ve laktik asidin sivrisinek larvaları üzerinde doza bağlı etki gösterdiği gözlenmiştir. Verbenon, 29.369 ppm LC_{50} değeri ile larvalara karşı en etkili madde olarak belirlenmiştir.

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of 29.369 ppm. Propionic acid and lactic acid were highly effective with LC_{50} values ranging between 40.45-47.63 ppm. 1-butanol, 2-butanol and citronellol did not cause any mortality. This study is among the few studies that have assessed the ovicidal effects of essential oils on mosquito eggs. After 120 hours of exposure, verbenone, propionic acid and 1-butanol were observed to present a dose dependent effect on mosquito eggs. Verbenone also emerged as the most effective against eggs with an LC_{50} value of 11.877 ppm. At 200 and 100 ppm <25% hatched but afterwards a drastic increase in egg hatching was observed with decreased with concentration.

Conclusion: EOs have promising potential as a safe and effective larvicidal and ovicidal agent for controlling *Aedes* mosquito populations. Such studies pave the way for developing new mosquito control formulations utilizing these effective essential oils.

Key Words: *Aedes*, verbenone, citronellol, essential oil, vector control

Propiyonik asit ve laktik asit, 40.45-47.63 ppm arasında değişen LC_{50} değerleri ile oldukça etkili olmuştur. 1-bütanol, 2-bütanol ve sitronellol herhangi bir etki göstermemiştir. Bu çalışmada ayrıca uçucu yağların sivrisinek yumurtaları üzerindeki ovisidal etkileri de değerlendirilmiştir. Verbenon, propiyonik asit ve 1-bütanolün 120 saatlik değerlendirme süreci sonunda sivrisinek yumurtaları üzerinde doza bağlı bir etki gösterdiği gözlemlenmiştir. Verbenon 11.877 ppm LC_{50} değeri ile yumurtalara karşı en etkili madde olarak ortaya çıkmıştır. 200 ve 100 ppm'de <%25 yumurtadan çıkma oranı gözlemlenmiş ancak daha düşük konsantrasyonlarda yumurta açılma oranında artış ortaya çıkmıştır.

Sonuç: Esansiyel yağlarda bulunan bazı etken maddeler *Aedes* sivrisinek popülasyonlarının kontrolünde larvisidal ve ovisidal olarak güvenli ve etkili bir mücadele yöntemi olma potansiyeline sahiptir. Bu tür çalışmalar sivrisinek mücadelesinde esansiyel yağlar kullanılarak yeni formülasyonların geliştirilmesi yolunu açacaktır.

Anahtar Kelimeler: *Aedes*, verbenon, sitronellol, esansiyel yağ, vektör mücadelesi

INTRODUCTION

Aedes aegypti (L., 1762) (Diptera: Culicidae), commonly known as the yellow fever mosquito, poses a significant threat to public health due to its role as a vector for numerous viral diseases including dengue fever, Chikungunya, Zika fever, and yellow fever. This mosquito thrives in densely populated urban areas, where it preferentially feeds on humans, even when other mammals are available (1,2). *Ae. aegypti* exhibits primarily diurnal biting behavior that is often inconspicuous with peak activity occurring around sunrise and sunset. However, they can also bite at night in well-lit areas (3,4). This mosquito exhibits a cosmo-tropical distribution in tropical regions year-round, its reach. Additionally, its geographic range extends beyond its African origins and now encompass more temperate areas during the summer months (5). Human activities, particularly international travel and trade have significantly facilitated this global spread. Historically, maritime transportation played a major role in establishing *Ae. aegypti* populations across continents (6–8).

Mosquito control programs rely heavily on monitoring *Ae. aegypti* populations to assess their prevalence, dispersal patterns, and abundance. This involves inspecting potential breeding sites, employing traps for both adult mosquitoes and eggs (ovitraps), and eliminating potential breeding sites or applying larvicides (chemicals that kill mosquito larvae) in these areas and applying insecticides targeting adult mosquitoes (adulticides) can be used (9,10). Common chemical adulticides including organophosphates (like malathion) and pyrethroids (like permethrin)

can be applied through various methods, such as indoor residual spraying, bed net impregnation, or aerial spraying (11-13). The effectiveness of chemical insecticides is often short-lived, as mosquitoes can develop resistance with continuous use, and many are associated with potential health and environmental concerns (14). Biocontrol agents like bacteria (*Bacillus thuringiensis israelensis*) or Insect growth regulators (IGRs) which kill or disrupt mosquito's development cycle can also be used to control mosquitoes as they pose minimal risk to humans, and other beneficial organisms (15,16) .

Essential oils (EOs) are a class of concentrated, aromatic, and volatile liquids extracted from various plant parts (flowers, leaves, stems, bark, and fruits) using established techniques (hydro distillation, steam distillation, dry distillation) or more ecologically friendly methods (supercritical fluid extraction, microwave-assisted extraction, ultrasound-assisted extraction) (17,18). These EOs are primarily composed of terpenoids and phenylpropanoids and have a range of beneficial properties, including antioxidant, antibacterial, and insecticidal activities (19). Consequently, EOs have potential applications in various fields, including pharmaceuticals, food science, and agriculture. Notably, their insecticidal properties have been explored against a diverse array of pests (17,20,21).

This study primarily investigated the ovicidal and larvicidal activity of some chemicals identified in essential oils of plants against *Aedes aegypti*.

MATERIAL and METHOD

Aedes aegypti mosquitoes were reared in cages within an insectary maintained at a temperature of 28-30°C and 70-80% relative humidity, with a 12-hour photoperiod in Vector Control Laboratory at Aydın Adnan Menderes University, Türkiye. To facilitate blood feeding, females were presented with defibrinated sheep blood via an artificial feeder (4). Eggs were hatched in plastic containers, and the larvae were subsequently fed crushed fish scale (Tetramin®) for larvicidal experiments. Eggs were air dried for 2 days before use in ovicidal experiments.

Commercially available chemicals (purity > 85%) found in plant essential oils were obtained from Sigma Aldrich (Table 1, Figure 1). Stock solutions of 200, 100, 50, and 25 ppm (µg/mL) were prepared by dissolving these chemicals in distilled water for use in the bioassays.

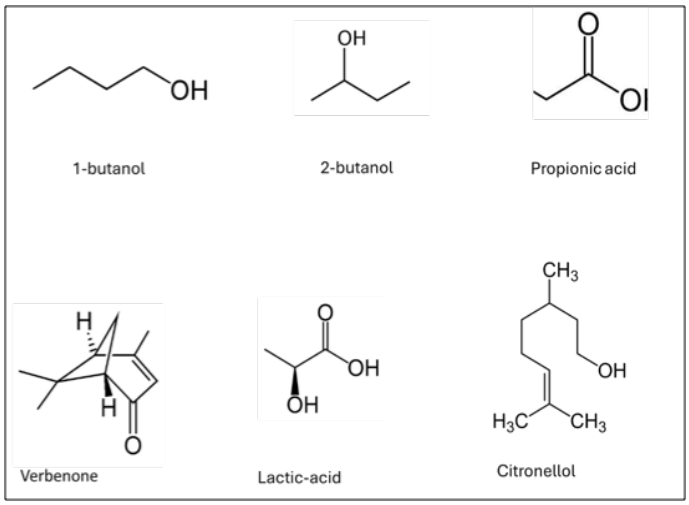

Figure 1. Chemical structures of verbenone, propionic acid, lactic acid, 1-butanol, 2-butanol and citronellol

Table 1. Information and lethal values of the selected chemicals from plant-derived essential oils

Chemicals	Plant source	Compound type	Company (purity)	Larvicidal		Ovicidal	
				LC_{50} (µg/mL) (95% CL)	Chi-square X^2	LC_{50} (µg/mL) (95% CL)	Chi-square X^2
Citronellol	Rose, geranium and lemongrass (22,23)	terpenoid	≥95% (Sigma)	ND	ND	138.037 (101.190-243.685)	2.462
Verbenone	Rosemary (41)	terpene	≥93% (Sigma)	29.369 (27.718-31.662)	0.769	11.877 (0.911-24.481)	2.194
Propionic acid	Rhynhosia bedd-domei (42)	carboxylic acid	≥99.5% (Sigma)	40.454 (22.800-55.619)	15.330	30.576 (15.294-43.212)	3.924
1-butanol	Jack fruit, Japanese ginger, maize, musk melon (24,25)	alcohol	≥99.9% (Sigma)	ND	ND	80.634 (25.312-56.627)	12.666
2-butanol	Truffle (43)	alcohol	≥99.8% (Sigma)	ND	ND	85.535 (7.246-45.751)	12.894
Lactic acid	Sugar plant wastes (44)	carboxylic acid	≥85% (Sigma)	47.631 (41.648-53.451)	4.627	99. 416 (68.171-190.042)	0.314
Mortality (%) ±SD	Negative control	0.0 ±0.0	0.0 ±0.0	-		-	
	Positive control	100±0.0	100±0.0	-		-	

LC values are expressed in ppm (µg/mL) and they are considered significantly different when 95% CL fail to overlap. Values are means ± S.D. Negative control: distilled water. Positive control: Vectobac® 12AS 0.19 ml/L for larvicidal. ND: Not determined.

Larvicidal bioassay

Ten third instar mosquito larvae were dispensed into wells of 24-well plates (Sigma, Corning Costar Multiple Well Plates, CLS3524) and exposed to varying concentrations of chemicals (200, 100, 75, 50, 25 ppm), while a negative control group received only distilled water and a positive control the commercial *Bti* (0.19 ml/l), (*Bacillus thuringiensis var. israelensis*), (VectoBac 12AS, Valent Biosciences, USA). Each well contained a total volume of 1 ml and 10 larvae per well. Required concentrations of the compounds were dissolved in distilled water. The plates were incubated at 24°C, and larval mortality was assessed after 48 hours. Larvicidal activity was determined by calculating the LC_{50} which represents the concentration (in µg/ml) that induced 50% mortality within 48 hours. Larvae were considered dead if they remained immobile after probing. Each treatment had six replicates and the entire experiment was repeated three times (26). Experiments were done under laboratory conditions.

Ovicidal bioassay

Ten intact and healthy mosquito eggs were dispensed into wells of 24-well plates (Sigma, Corning Costar Multiple Well Plates, CLS3524) using a fine brush and exposed to varying concentrations of chemicals (200, 100, 75, 50, 25 ppm), while a negative control group received only distilled water. Required concentrations of the compounds were dissolved in distilled water. Each well contained a total volume of 1 ml and 10 eggs per well. The plates were incubated at 24°C, and egg hatching rates was assessed after 120 hours. Ovicidal activity was determined by calculating the LC_{50} which represents the concentration (in µg/ml) that induced 50% mortality. Eggs were considered dead if there is no eggshell fracture with no rising of the egg buster and there was no L1 stage in the well. Each treatment was replicated six times, and the entire experiment was repeated three times (26). Experiments were done under laboratory conditions.

Statistical analyses

LC values and their 95% confidence interval of the compounds against eggs (ovicidal) and larvae (larvicidal) was evaluated using probit analysis. Data from control groups with less than 5% mortality were excluded from the analysis. To compare differences on the effects of the compound, a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test ($p < 0.05$) was employed. Prior to ANOVA, the data underwent arcsine transformation. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Larvicidal bioassay

The larvicidal effects of the chemicals found in various plant derived EOs against *Ae. aegypti* larval mortality is given in Figure 1. After 48 hours of exposure, verbenone, propionic acid and lactic acid were observed to present a dose dependent effect on mosquito larvae. At 200, 100 and ppm these compounds caused 100% mortality but afterwards a drastic drop in efficacy was observed with decreased with concentration. The LC_{50} value of Verbenone, propionic acid and lactic acid against *Ae. aegypti*were determined as Table 1. 1-butanol, 2-butanol and citronellol did not cause any mortality. Analysis of variance results showed that there were significant differences between the chemical compounds ($F(5, 150) = 366$; $p<0.0001$), tested concentrations ($F(4, 150) = 593.3$; $p<0.0001$) and their interaction ($F(20, 150) = 142.4$; $p<0.0001$) (Figure 2).

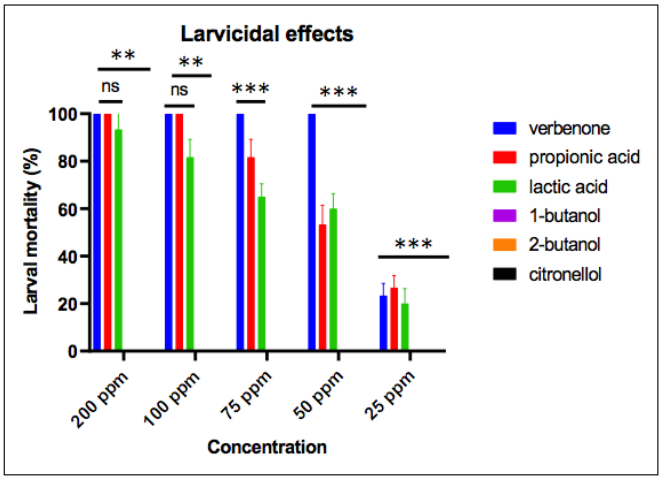

Figure 2. Larvicidal effects of different doses of verbenone, propionic acid, lactic acid, 1-butanol, 2-butanol and citronellol against 3rd instar *Aedes aegypti* larvae

Ovicidal bioassay

The ovicidal effects of the chemicals found in various plant derived EOs against *Ae. aegypti* larval mortality is given in Figure 3. After 120 hours of exposure, verbenone, propionic acid and 1- butanol were observed to present a dose dependent effect on mosquito eggs. At 200 and 100 ppm <25% hatched but afterwards a drastic increase in egg hatching was observed with decreased with concentration.

The LC_{50} value of the chemicals against *Ae. aegypti* eggs were determined as Table 1. Analysis of variance results showed that there were significant differences between the chemical compounds ($F(6, 175) = 25.56$; $p<0.0001$), tested concentrations ($F(4, 175) = 35.01$; $p<0.0001$) and their interaction ($F(24, 175) = 2.369$; $p=0.0007$) (Figure 3).

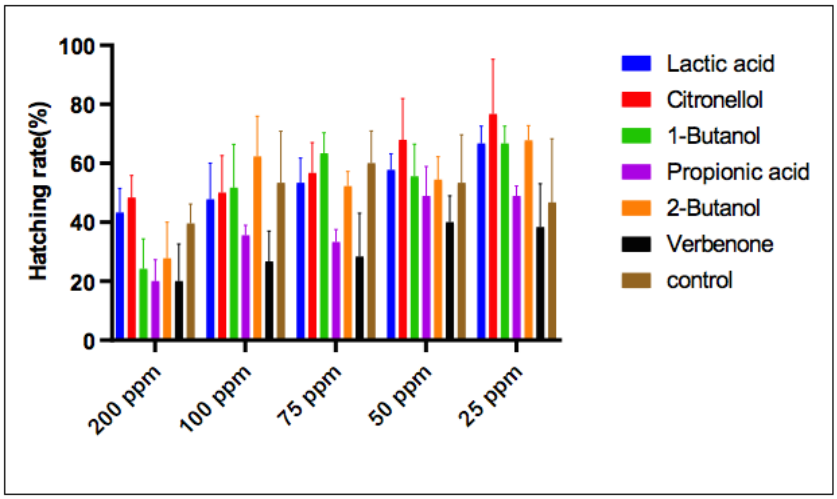

Figure 3. The hatching rate of the mosquitoes after treatment with chemicals

DISCUSSION

Essential oils (EOs) possess complex chemical compositions and are characterized by a diversity of distinct compounds (phytochemical diversity) with different biological properties. Some EOs and their components can kill insects or disrupt their growth at various stages of their life cycle (17,27). This study assessed the effects of six chemicals found in various plant derived EOs against *Ae. aegypti* eggs hatching and larval survival. Results showed that the chemicals tested showed varying effects. After 24 hours of exposure, verbenone, propionic acid and lactic acid were observed to present a dose dependent effect on mosquito larvae. Verbenone emerged as the most effective against larvae with an LC_{50} value of 29.369 ppm. Propionic acid and lactic acid were highly effective with LC_{50} values ranging between 40.45-47.63 ppm. 1-butanol, 2-butanol and citronellol did not cause any mortality.

Numerous studies have explored the potential of essential oils and their components for mosquito larvae control, these studies have shown that commercially available oils like turmeric (*Curcuma longa*), sweet orange (*Citrus sinensis*), lemongrass oil, cinnamon bark oil and lemon (*Citrus limon*) are particularly effective against different mosquito species belonging to the *Culex*, *Aedes*, and *Anopheles* genera (28–33). For instance, (34) investigated the potential of four essential oils (lemon, lavender, peppermint, and neem) as larvicides against *Ae. aegypti* and revealed high larvicidal activity for lemon, peppermint, and

lavender oils, with lemon oil exhibiting the strongest effect (LC_{50} = 10.676 ppm). Conversely, neem oil displayed the lowest larvicidal potency (LC_{50} = 38.058 ppm). Manimaran et al. (28) evaluated the larvicidal and knockdown effects of 25 essential oils against *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti*. Eight oils (calamus, cinnamon, citronella, clove, eucalyptus, lemon, mentha, and orange) demonstrated complete larvicidal activity at a concentration of 1000 ppm and complete knockdown effect at 10% concentration. Such studies pave the way for developing new mosquito control formulations utilizing these effective essential oils. Selvi et al. (45) evaluated the activities of some naturally growing medicinal plants (*Salvia verticillata, Leucanthemum vulgare, Inula vulgaris* and *Matricaria chamomilla*) in Türkiye. Plant extracts had high larvicidal activity on *Ae. albopictus* larvae. Similarly, Usta et al. (46) evaluated oviposition deterrent, ovicidal and skin repellent activities of *Salvia verticillate* and *Matricaria chamomilla* against *Aedes albopictus* and stated that these plants have potential in this sense.

While research on larvicidal effects of EOs is extensive, ovicidal effects remain less explored. This study demonstrated that after 120 hours of exposure, verbenone, propionic acid and 1- butanol were observed to present a dose dependent effect on mosquito eggs. At 200 and 100 ppm <25% hatched but afterwards a drastic increase in egg hatching was observed with decreased with concentration. Few studies have assessed the ovicidal effects of essential oils on mosquito eggs. Muturi et al. (35) investigated the chemical composition and ovicidal activity of essential oils from garlic and asafoetida on *Cx. pipiens* and *Cx. restuans*. They identified ten and twelve distinct compounds in garlic and asafoetida essential oils, respectively with Allyl disulfide found as the most prevalent compound in garlic oil. These EOs exhibited significant impact on egg hatching as majority of the *Culex* egg rafts exposed to either garlic or asafoetida oil failed to hatch. In another study (36), *Cinnamomum verum* EO and its main compound, trans-cinnamaldehyde, showed strong ovicidal activity against mosquito eggs at high concentrations (30,000 ppm). EOs have promising potential as a safe and effective ovicidal agent for controlling *Aedes* mosquito populations. Research shows promise for using extracts from various plants to kill mosquito eggs (37). Studies have demonstrated the ovicidal activity of crude extracts from plants such as *Andrographis paniculata* (Lamiales: Acanthaceae), *Cassia accidentalis* (Fabales: Fabaceae), *Euphorbia hirta* (Malpighiales: Euphorbiaceae), *Eclipta alba* (Asterales: Asteraceae), *Cardiospermum halicacabum* (Sapindales: Sapindaceae), *Aegle marmelos* (Sapindales: Rutaceae), *Andrographis lineata* (Lamiales: Acanthaceae), *Cocculus hirsutus* (Ranunculales: Menispermaceae), *Tagetes erecta* (Asterales: Asteraceae), *Melothria maderaspatana* (Cucurbitales: Cucurbitaceae) and *Acalypha alnifolia* against various mosquito species, including *An. stephensi*, *An. subpictus*, *Ae. aegypti*, and *Cx. quinquefasciatus* (38–40). Further research is needed to optimize their use and develop practical control strategies.

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

Determination of biofilm formation capacity of *Metschnikowia reukaufii* strain

Metschnikowia reukaufii suşunun biyofilm oluşturma kapasitesinin belirlenmesi

Tuba BÜYÜKSIRIT BEDİR[1] (ID)

ABSTRACT

Objective: Biofilm is a community of microorganisms attached to biotic or abiotic surfaces. Microorganisms that form biofilms cause much more serious medical and industrial problems than their planktonic forms. In this sense, the ability of microorganisms to form biofilms must be known and precautions must be taken. The aim of this study is to create stress in the *Metschnikowia reukaufii* MN622824 strain, whose inhibitory substance production increases under stress conditions, by trying different environmental conditions and to determine its effect on biofilm formation. In addition, to observe its effect on biofilm formation on stainless steel (SS) surfaces, which are frequently used in food machines.

Methods: Biofilm formation was compared with the tube method and the microtitration plate method. For this purpose, 5 different media compositions containing 5% glucose, 10% glucose, 5% NaCl, 10% NaCl, containing no glucose and NaCl (sodium chloride), and 3 different incubation periods consisting of 3, 5 and 7 days were tested. Additionally, scanning electron microscopy imaging was performed to examine the adhesion of biofilm to stainless steel coupons.

ÖZET

Amaç: Biyofilm, biyotik veya abiyotik yüzeylere tutunan mikroorganizmalardan oluşan bir topluluktur. Biyofilm oluşturan mikroorganizmalar, planktonik formlarına göre çok daha ciddi tıbbi ve endüstriyel sorunlara neden olmaktadır. Bu nedenle mikroorganizmaların biyofilm oluşturma yeteneklerinin bilinmesi ve önlem alınması gerekmektedir. Bu çalışmanın amacı stres koşullarında inhibitör madde üretimi artan *Metschnikowia reukaufii* MN622824 suşunun, farklı çevre koşullarını deneyerek stres oluşturmak ve bunun biyofilm oluşumuna etkisini belirlemektir. Ayrıca, gıda makinelerinde sıklıkla kullanılan paslanmaz çelik (SS) yüzeylerde biyofilm oluşumuna etkisini gözlemlemektir.

Yöntem: Biyofilm oluşumu tüp yöntemi ve mikrotitrasyon plak yöntemi ile karşılaştırılmıştır. Bunun için glikoz ve NaCl (sodyum klorür) içermeyen, %5 glikoz, %10 glikoz, %5 NaCl, %10 NaCl içeren beş farklı ortam kompozisyonu ve 3, 5 ve 7 günlük periyotlardan oluşan üç farklı inkübasyon süresi denenmiştir. Ayrıca, biyofilmin paslanmaz çelik kuponlara yapışmasını incelemek için taramalı elektron mikroskobu görüntülemesi yapılmıştır.

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Results: In the tube method, the highest biofilm formation was observed after three days of incubation in tubes with 5% glucose added. No biofilm was formed in the tubes that were kept for seven days or 10% NaCl was added. In the microtitration plate method the strong biofilm formation (1.3022) was obtained after three days of incubation in a medium containing 5% glucose. In the control group without glucose and NaCl, moderate biofilm formation were observed on the 3rd day (0.7385) and the 5th day (0.6882). Biofilm formation was evaluated as absent or weak in media containing 10% glucose or NaCl. As glucose and NaCl concentrations increased, biofilm formation decreased. Reticular bond structure showing biofilm formation were imaged with a scanning electron microscope in the control group on days 3-5-7, 5% NaCl and 5% glucose concentration on the 3rd day, and 5% glucose concentration on the 5th day.

Conclusion: Biofilm formation in food factories poses a significant health problem. It was observed that increasing NaCl, glucose concentration and incubation time negatively affected the biofilm formation of the *Metschnikowia reukaufii* strain. Biofilm formation decreases or disappears in yeasts exposed to stress conditions. It is thought that the data obtained will help in biofilm control.

Key Words: *Metschnikowia reukaufii*, biofilm, glucose, sodyum chloride, biocontrol

Bulgular: Tüp yönteminde en yüksek biyofilm oluşumu %5 glikoz ilaveli tüplerde üç günlük inkübasyon sonrasında gözlenmiştir. Yedi gün bekletilen veya %10 NaCl eklenen tüplerde biyofilm oluşmamıştır. Mikrotitrasyon plak yönteminde, %5 glikoz içeren bir ortamda üç günlük inkübasyonun ardından güçlü biyofilm oluşumu (1.3022) elde edilmiştir. Glikoz ve NaCl bulunmayan kontrol grubunda 3. gün (0.7385) ve 5. gün (0.6882) orta derecede biyofilm oluşumu gözlenmiştir. %10 glikoz veya NaCl içeren ortamlarda biyofilm oluşumu yok veya zayıf olarak değerlendirilmiştir. Glikoz ve NaCl konsantrasyonları arttıkça biyofilm oluşumu azalmıştır. Biyofilm oluşumunu gösteren retiküler bağ yapısı taramalı elektron mikroskobu ile kontrol grubunda 3., 5. ve 7. günlerde, %5 NaCl ve %5 glukoz konsantrasyonunda 3. günde, %5 glukoz konsantrasyonunda ise 5. günde görüntülenmiştir.

Sonuç: Gıda fabrikalarında biyofilm oluşumu önemli sağlık sorunu oluşturmaktadır. Artan NaCl, glikoz konsantrasyonu ve inkübasyon süresinin *Metschnikowia reukaufii* suşunun biyofilm oluşumunu olumsuz etkilediği gözlenmiştir. Stres koşullarına maruz kalan mayalarda biyofilm oluşumu azalmakta veya ortadan kalkmaktadır. Elde edilen verilerin biyofilm kontrolünde yardımcı olacağı düşünülmektedir.

Anahtar Kelimeler: *Metschnikowia reukaufii*, biyofilm, glikoz, sodyum klorür, biyokontrol

INTRODUCTION

The complex matrix containing EPS (exopolysaccharide or extracellular polymeric substance), proteins, eDNA (extracellular DNA), various enzymes and the microorganism itself secreted by bacteria, yeast, mold, algae and protozoa is called biofilm (1). Biofilm can be found attached to a surface or embedded in an extracellular matrix (2). This biofilm matrix makes them resistant to harsh conditions and resistant to antibacterial drugs (3,4). Additionally, this resistance makes infections difficult to treat and causes a wide variety of chronic diseases (5). At the same time, biofilm formation protects microorganisms against various harsh environments (ultraviolet radiation, extreme temperature, extreme pH, high salinity, high pressure, poor nutrients, antibiotics, etc.), increases microbial competitiveness in environments, and is also used in some cellular functions (6,7). In particular, biofilms

formed by pathogenic microorganisms (8) and decaying microorganisms are inappropriate sources of microbial contamination. Such microbial cells are likely to contaminate raw materials and food during processing, leading to food spoilage and economic losses for producers (9). They are also major obstacles in the food industry and healthcare industry, as their ability to form biofilms protects them from ordinary cleaning procedures and allows them to persist in the environment. This persistence results in increased microbial load in the food processing environment and the final food product; this leads to spoilage and shortened shelf life, as well as increased risks from infectious disease outbreaks from food sources (10). Therefore, biofilm formation must be prevented at the initial stage or the resulting biofilm structure must be eliminated in the food industry. As biofilm-related infections become common, knowing the various aspects and functionality of biofilm formation will facilitate the implementation of methods to prevent these structures and will help determine the measures to be used to combat these infections (11).

Yeasts form biofilms by adhering to abiotic surfaces such as wood, stainless steel, glass and plastic polymers. Many yeast species such as *Saccharomyces cerevisiae*, *Candida albicans* and *Cryptococcus neoformans* are known to form biofilms. Some strategies, such as good hygiene practices, including regular cleaning and disinfection of surfaces, can help reduce biofilm formation. Using antimicrobials and alternative methods to eliminate yeast biofilms can also help ensure food safety (12).

Metschnikowia reukaufii (Ascomycota, Saccharomycetales) are yeasts commonly found in flowers and flower nectar, environments with high sucrose concentrations (400 g/l). It is found predominantly in the nectar of plants such as *Helleborous foetidus* (13,14). Although the ecological function of this yeast is relatively unknown, many studies relate it to nectar sugar composition, synthesis of volatile compounds and even increased temperature of nectars, all factors that can influence the behavior of pollinators (15,16).

The objectives of this study were to compare the biofilm formation ability of the *Metschnikowia reukaufii* MN622824 strain, which is not pathogenic but increases the production of inhibitory substances under stress conditions, using the tube method and the microtitration plate method, and its biofilm formation capacity was evaluated. Its effect on stainless steel (SS) surfaces, which are frequently used in the food industry, was determined by observing the scanning electron microscope (SEM).

MATERIAL and METHOD

Strains and growth conditions

In this study, *M. reukaufii* (Genbank Accession Number: MN622824, http:// www.ncbi.nlm.nih.gov/blast)) strain isolated from flower at Süleyman Demirel University, Faculty of Engineering, Department of Food Engineering was used. *M. reukaufii* strains were activated in Malt Extract broth (Merck, Germany) adjusted to pH 5.5. The inoculated tubes were incubated at 30°C for 24 hours. Then, the absorbance was adjusted to 0.5 at 570 nm on the spectro-photometer for each culture.

Testing different glucose and NaCl concentrations

Malt Extract broth was used as a medium to observe the effect of different stress conditions on biofilm formation. After adjusting the pH value of the media, different concentrations of glucose and NaCl were added. Five different media compositions including without glucose and NaCl, 5% glucose, 10% glucose, 5% NaCl, 10% NaCl and three different incubation periods as 3, 5 and 7 days were applied.

Biofilm analysis with tube method

Biofilm formation was examined by adding different amounts of glucose and NaCl to Malt Extract broth, whose pH value was adjusted to 5.5. Yeast strains growing in these media were discharged from the tubes after 3-5-7 days of incubation. The evacuated tubes were washed with sterile phosphate

buffer saline (pH 7.3) and dried. Then, 10 mL of the solution prepared with distilled water containing 0.1% crystal violet was added to the tubes and incubated for 10 minutes. After incubation, the crystal violet solution in the tubes was drained and the excess dye was washed with ionized water. After this process, the tubes were left to dry. After the tubes dried, values of absent/weak (1), medium (2) and strong (3) were given according to the density of the crystal violet solution adhering to the tube (17).

Biofilm analysis with microtitration plate method

Biofilm formation of *M. reukaufii* yeast strain by the microtitration plate method was performed as previously described in Zhang et al. (18) with a few modifications. Overnight culture was diluted into Malt Extract broth prepared with different specifications (without glucuse and NaCl, %5 glucose, %10 glucose, %5 NaCl, %10 NaCl) at a ratio of 1:100. Diluted cultures were added to a 96-well plate, 100 µL per well. It was then incubated at 30°C for 3-5-7 days. Additionally, negative control wells contained Malt Extract broth only. After incubation, total cell-mass was measured as absorbance at 630 nm by spectro-photometer.

After the measurement, the plates were emptied, the wells were washed with sterile water and then the drying process was carried out. Following the drying process, 125 µL of 0.1% crystal violet solution was added to each well and incubated for 20 minutes at room temperature. It is known that the dye bound to adhered cells in the wells can be resolubilized and measured in optical density with a spectro-photometer. With this method, the unbound crystal violet solution was washed with distilled water and dried. 100 µL of 95% ethanol was transferred to each well to dissolve and release the bound dye. The dye solution dissolved in ethanol in each well was transferred to a clean plate, respectively. Finally, absorbances at 492 nm were measured with a spectro-photometer. The results obtained were calculated with the formula B= A492/A630 and the degrees of biofilm formation were determined: No biofilm producer ($B<0.1$), Weak biofilm producer ($0.1\leq B\leq 0.5$), Moderate biofilm producer ($0.5\leq B\leq 1$), Strong biofilm producer ($B\geq 1$).

Scanning electron microscope analyzes

The yeast strains were then tested to form biofilms on SS surfaces in food machines frequently used in the fermentation industry. SS coupons with biofilm formation were examined with SEM and biofilm structures were visualized. For this purpose, steel coupons were cleaned with 70% ethanol for 10 minutes. Then, it was washed with sterile distilled water and dried at 60 ºC for 2 hours. The dried coupons were placed in heat-resistant glass containers, covered first with aluminum foil and then with their own lids, and sterilized in an autoclave at 121°C for 15 minutes. Steel coupons were placed in 24-well microplates. 2 mL of the media in different compositions including without glucose and NaCl, 5% glucose, 10% glucose, 5% NaCl, 10% NaCl was added to each well. Then, the absorbance of the freshly prepared cultures was adjusted by incubating them at 30°C for 24 hours using Malt Extract broth. For each different concentration, it was incubated at 30°C for 3, 5 and 7 days. After incubation, the steel coupons removed from the plates were washed three times with 10 mL of sterile distilled water to remove non-adherent cells.

Steel coupons were prepared to be examined with SEM as described previously Kaya et al. (19). For this purpose, the coupons were fixed in 2.5% glutaraldehyde (prepared with 0.1 M phosphate-buffered saline (PBS), pH 7.4) overnight at 4°C. They were washed twice with 0.1 M PBS. Then, it was fixed in 1% osmium tetroxide (Electron Microscopy Sciences, Hatfield, Pa) for 1 hour and then washed with distilled water. For dehydration, the samples were passed through ethyl alcohol series for 15 minutes (30%, 50%, 70%, 90%, and 96%), and in the final stage, they were treated with absolute alcohol for 30 minutes and dried. Steel coupons are placed on aluminum stabs and plated with gold. It was examined with the FEI / Quanta 450 FEG brand SEM within the Hitit University Scientific Technical Application and Research Center.

RESULTS

Tube method

To observe the effect of different stress conditions on biofilm formation, different concentrations of glucose and NaCl were added after adjusting the pH value of the media. Biofilm formation was compared with the tube method in five different media compositions including without glucose and NaCl, 5% glucose, 10% glucose, 5% NaCl, 10% NaCl and 3 different incubation periods as 3, 5 and 7 days. The results obtained are shown in Figure 1.

According to the tube method results, the highest biofilm formation was observed after three days of incubation in tubes with 5% glucose added. No biofilm formation was observed in the tubes incubated for seven days. Additionally, biofilm formation was prevented in tubes to which 10% NaCl was added.

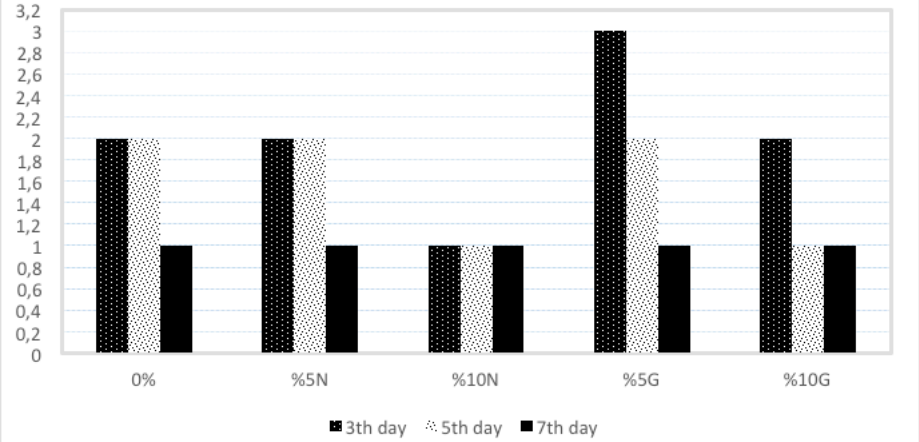

Figure 1. Biofilm formation of *M. reukaufii* strain by tube method

0%: without glucose and NaCl, %5N: 5% NaCl, %10N: 10% NaCl, %5G: 5% glucose, %10G: 10% glucose

Microtitration plate method

In detecting biofilm formation with the tube method, visual qualitative analysis is performed according to the density on the bottom and/or walls of the tubes (17). Since grading this staining in the tubes may vary from person to person, biofilm formation should be tested with the microtitration plate method as well as the tube method. The microtitration plate method is a quantitative method that is based on the optical density of the crystal violet solution added to the biofilm matrix and gives numerical results by measuring spectrophotometrically (18). To observe biofilm formation under different conditions, absorbances were measured with a spectrophotometer first at 630 nm and after fixation at 492 nm. The obtained absorbance values were calculated with the formula and the results are given in Table 1.

According to the microtitration plate method results, moderate biofilm formation was observed on day 3 and day 5 in a glucose and NaCl-free environment, while no biofilm formation was observed on day 7. Biofilm formation was observed on the 3rd day in the medium supplemented with 5% NaCl, but no biofilm was formed as the incubation period was prolonged. While *M. reukaufii* strain produced the most biofilm in the medium supplemented with 5% glucose, no biofilm was observed in the medium supplemented with 10% glucose. As the amount of added glucose increased, biofilm formation decreased. Comparable results according to biofilm formation are shown in Figure 2.

Table 1. Biofilm formation of *M. reukaufii* strain by microtitration plate method

	0%	%5N	%10N	%5G	%10G
3th day	0.739±0.25	0.627±0.07	0.240±0.05	1.302±0.15	0.255±0.01
5th day	0.688±0.31	0.510±0.05	0.396±0.06	0.603±0.19	0.124±0.01
7th day	0.275±0.05	0.374±0.02	0.414±0.02	0.658±0.14	0.118±0.01

Data are expressed as means ± standard deviation

0%: without glucose and NaCl, %5N: 5% NaCl, %10N: 10% NaCl, %5G: 5% glucose, %10G: 10% glucose

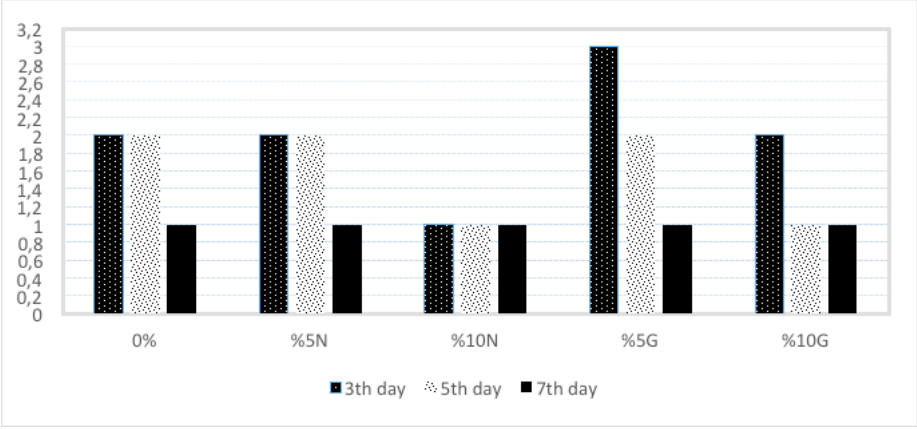

Figure 2. Biofilm formation of *M. reukaufii* strain by microtitration plate method

0%: without glucose and NaCl, %5N: 5% NaCl, %10N: 10% NaCl, %5G: 5% glucose, %10G: 10% glucose

Scanning electron microscopy analysis

The effect of incubation time on the adhesion of *M. reukaufii* strain grown in Malt Extract Broth in different environments (without glucose and NaCl, 5% glucose, 10% glucose, 5% NaCl, 10% NaCl) to SS surfaces was investigated. Biofilm conditions and SEM images formed on steel coupons are given in Figure 3.

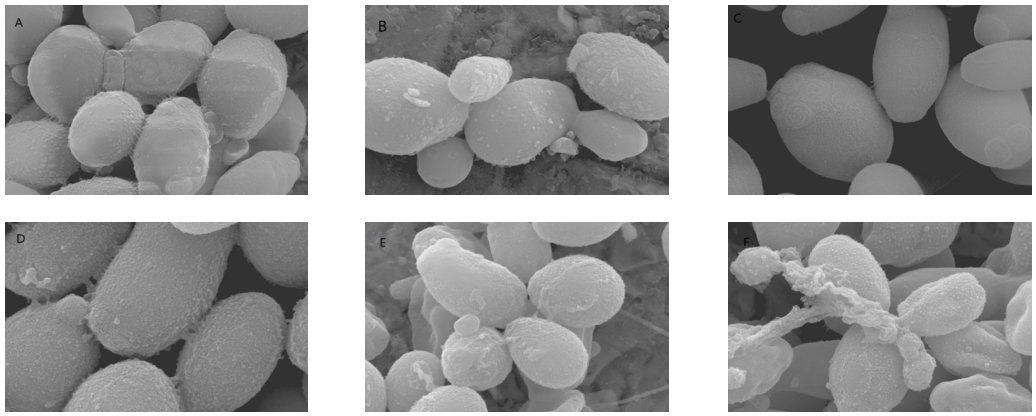

Figure 3. SEM images of biofilm formation of M. reukaufii strain on SS coupons

(A) 0%-3th day, (B) 0%-5th day, (C) 0%-7th day, (D) 5% NaCl-3th day, (E) 5% glucose 3th day, (F) 5% glucose 5th day

DISCUSSION

The majority of yeast species operate between water activity values of 0.9-1.0. It has been stated that the optimum water activity value for the growth of *S. cerevisiae* is between 0.975 and 0.999, and when this value falls below 0.94, the yeast cannot develop. It is also known that the ethanol produced by yeast causes water stress in the cell by reducing the water activity value. In this case, the hydrogen bonds in the hydrated cell components interact and the enzyme and membrane structure in the cell is disrupted (20). In light of this information, as the amount of NaCl and glucose increased, water activity decreased, which prevented biofilm formation.

The effect of glycerin concentration, also known as sugar alcohol, on biofilm formation of *S. cerevisiae* strain was investigated. It was observed that by adding glycerin to the liquid medium, the water activity of the medium decreased and the yeast could not grow. According to this information, it is thought that as the concentration of glycerin increases to 5%, the metabolic activities of the yeast slow down and they cannot form biofilms. It is also known that environmental factors are very important for the development of microorganisms and biofilm production (21). In another study, biofilm formation of the *A. hydrophila* strain was investigated at different glucose concentrations using the microtitration plate method. Biofilm formation decreased with the addition of glucose to the medium. While the addition of 0.05% glucose did not significantly reduce biofilm formation compared to the control (0% glucose), biofilm formation was significantly inhibited at 0.25% glucose concentration (22).

In a study, biofilm formation of *S. cerevisiae* strains was investigated at different NaCl concentrations. The highest biofilm formation was observed when the pH of the medium was 5.0 and in the tube without NaCl addition. It was determined that biofilm formation decreased as the NaCl ratio increased, and biofilm did not form when the ratio reached 10% (21). It has been reported that high NaCl concentration prevents cells from adhering to surfaces (23). Similarly, it was determined that biofilm formation of *Salmonella* enterica strains was restricted by the increase in the amount of NaCl (24). Increasing the NaCl concentration in the environment causes a decrease in cell hydrophobicity. Therefore, the presence of different amounts of NaCl in the environment changes cell surface properties and biofilm distribution. It has been reported that high NaCl ratio inhibits cell adhesion (25). It is thought that yeast cell adhesion is inhibited by the increase in NaCl concentration, as a result of which the cells cannot attract each other and form biofilms. Giaouris et al. (23) showed that high sodium chloride concentration (10.5% NaCl; aw 0.95) prevented cells from adhering to the plate. Mizan et al. (26) reported that Vibrio parahaemolyticus produced the best biofilm at 2% NaCl concentration. It has been observed that when this rate reaches 5%, it produces the least. Studies show that increasing NaCl ratio at the same temperature disrupts the structure of the cell and prevents biofilm formation.

It is known that increasing the NaCl concentration in the environment disrupts the ion balance in the cell. High amounts of NaCl intake causes an increase in the amount of Na+ and Cl ions in the cell. This situation causes low water potential and ion toxicity (27). Although the sensitivity of the microorganism to NaCl increased at higher osmotic pressures, the harmful effect of salts on the development of this microorganism was determined to be due to the specific ion effect rather than the osmotic effect (28). In the light of this information, it is understood that as the amount of NaCl ions in the cell increases, yeast activity is affected and biofilm formation decreases. It has been observed that the growth of yeast slows down as the NaCl ratio increases in the medium (29). Since the high amount of NaCl in the medium prolongs the development time of the yeast, it is thought that after 24 hours of incubation, the yeast cannot grow much in the medium and cannot reach sufficient cell density for biofilm formation.

Compared to this study, this study supports the conclusion that as the NaCl concentration increases in the medium, the yeast cannot complete its development and the level of biofilm formation decreases. According to the study, it was observed that microorganism cells in the biofilm were under more osmotic stress than planktonic cells and that high osmotic potential prevented biofilm formation (30).

According to the SEM analysis, the reticular bond structure indicating biofilm formation was visualized in the SS coupons of the control group incubated for 3-5-7 days. However, biofilm formation gradually decreased in coupons incubated for 5 and 7 days. This showed that 3 days of incubation period was sufficient for the *M. reukaufii* strain to form a biofilm. This can be explained by the fact that the cells lose or decrease their viability after 3 days. Similarly, biofilms were observed on day 3 in the tube with 5% glucose addition. No biofilm was observed on SS coupons in environments containing NaCl, except for the tube containing 5% NaCl (3 days incubation). As in the tube and microtitration plate method, no biofilm formation was observed on SS coupons in media prepared at 10% NaCl concentrations. This showed that NaCl concentration prevented the growth of yeasts. Betts et al. (29) concluded in a study that the *S. cerevisiae* strain could not grow at 8% NaCl, whereas the maximum NaCl rate suitable for the growth of the yeast was 4.8%. It is stated that S. cerevisiae needs high water activity (0.65) to carry out its metabolic activities. This yeast needs water to ferment. It has been noted that environments containing high sugar can impose osmotic stress to negatively affect cell physiology. It is thought that a decrease in the amount of biofilm formation is observed because the metabolic activities of the yeast are limited by reducing the water activity of the environment (31). Microorganisms and biofilm formation are affected by changes in environmental conditions. The ability of *Salmonella enteritidis* to form biofilms on SS surfaces was examined at different temperatures (5, 20 and 37°C), pH (4.5, 5.5, 6.5 and 7.4) and water activity values (0.5, 1.5, 5.5 and 10.5% NaCl) (23). It has been reported that maximum biofilm formation is reached in 6 days at 20°C. It has been reported that biofilm formation after seven days of incubation at 20°C was not dependent on pH change and that high sodium chloride concentration (10.5% NaCl, aw 5 ± 0.94) clearly inhibited the adhesion of cells to the coupons.

In conclusion; microorganisms produce a gel-like layer defined as biofilm in order to be more resistant to adverse conditions. In the food industry, heat treatments to extend the shelf life of foods and chemicals used to clean the materials in the process do not affect the biofilm formed by microorganisms. For this reason, biofilm formation in food factories poses a health problem. It was observed that increasing NaCl, glucose concentration and incubation time negatively affected biofilm formation. It is very important for food factories that biofilm formation disappears under these stress conditions. The data obtained as a result of this study is intended to shed light on practices to prevent biofilm formation.

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
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Türk Hijyen ve Deneysel Biyoloji Dergisi

Intraarticular injectional evaluation of dextrose prolotherapy; an experimental study in rat knee osteoarthritis model

Dekstroz proloterapisinin eklem içi enjeksiyonla değerlendirilmesi; sıçan diz osteoartrit modelinde deneysel bir çalışma

Altay SAVALAN[1] (ID)

ABSTRACT

Objective: Osteoarthritis (OA) of the knee with a prevalence of 365 million is the most common joint disorder in the world and is frequently encountered in the older population. However, the pathogenesis of osteoarthritis remains unclear, and yet it is not possible to effectively prevent the progression of OA. Therefore, it is of great importance to find more appropriate and effective treatment modalities for osteoarthritis. Although Hypertonic Dextrose Prolotherapy (HDP) appears to be a promising interventional treatment for knee OA, the dosage and immediate effects of this application require preliminary clinical as well as clinical studies.

Methods: In this study, to provide new information in the treatment of OA, animal models of OA were developed and used for assesment of different concentration of Dextrose prolotherapy. In accordance with this purpose and before starting the model development and treatment, the volume optimization of injectable solution in knee joint performed using trypan blue dye.

Results: According to the results, injection of more than 50 µl has the capacity of leakage out of the knee joint. Animal models of OA were developed by intra-articular

ÖZET

Amaç: Diz osteoartriti (OA) 365 milyon prevalansı ile dünyada en sık görülen eklem hastalığıdır ve sıklıkla yaşlı popülasyonda görülmektedir. Ancak osteoartritin patogenezi belirsizliğini korumasına rağmen OA'nın ilerlemesini etkili bir şekilde önlemek mümkün değildir. Bu nedenle osteoartritte daha uygun ve etkili tedavi yöntemlerinin bulunması büyük önem taşımaktadır. Hipertonik Dekstroz Proloterapisi (HDP), diz OA'sı için umut verici bir girişimsel tedavi gibi görünse de bu uygulamanın dozajı ve anlık etkileri, klinik çalışmaların yanı sıra ön klinik çalışmalar da gerektirmektedir.

Yöntem: Bu çalışmada, OA tedavisinde bilimsel destek sunmak amacıyla, OA hayvan modelleri geliştirilmiş ve bu modeller farklı konsantrasyonlarda dekstroz proloterapisi değerlendirilmesinde kullanılmıştır. Bu amaç doğrultusunda model geliştirmeye ve tedaviye başlamadan önce tripan mavisi boya kullanılarak diz eklemine enjekte edilebilir solüsyonun hacim optimizasyonu gerçekleştirildi.

Bulgular: Sonuçlara göre 50 µl'den fazla enjeksiyonun diz eklemi dışına sızma kapasitesi vardır. OA'nın hayvan modelleri, 50 µl'lik nihai hacime

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injection of 1 mg freshly prepared single dose Sodium Monoiodoacetate (MIA) in a final volume of 50 µl. Then, for the first time, the effect of different concentrations of Dextrose prolotherapy on the treatment of OA was investigated on these rat knee OA models.

Conclusion: After 28 days of follow-up, all applied concentration of Dextrose (5%, 10%, 15% and 25%) demonstrated the capacity to significantly (p<0.05) decrease hind paw weight distribution in a dose-independence manner. In conclusion, dextrose prolotherapy appears to be a safe treatment method for effective treatment, recovery and pain control in knee osteoarthritis. Future studies may also demonstrate the synergistic effect of dextrose prolotherapy with other therapeutic methods.

Key Words: Dextrose Prolotherapy (DP), Knee Osteoarthritis (OA), Sodium Monoiodoacetate (MIA), Hypertonic Dextrose Prolotherapy (HDP)

sahip ve 1 mg taze hazırlanmış tek doz Sodyum Monoiyodoasetat (MIA) içeren solusyonun eklem içi enjeksiyonu yoluyla geliştirildi. Daha sonra ilk kez farklı konsantrasyonlarda dekstroz proloterapisinin OA tedavisine etkisi bu sıçan deney hayvanı diz OA modelleri üzerinde araştırıldı.

Sonuç: 28 günlük takipten sonra, uygulanan tüm Dekstroz konsantrasyonları (%5, %10, %15 ve %25), dozdan bağımsız bir şekilde arka pençe ağırlık dağılımını önemli ölçüde (p<0.05) azaltma kapasitesini gösterdi. Sonuç olarak dekstroz proloterapi, diz osteoartritinde etkili tedavi, iyileşme ve ağrı kontrolü için güvenli bir tedavi yöntemi olarak görünmektedir. Gelecekte yapılacak çalışmalar dekstroz proloterapinin diğer terapötik yöntemlerle sinerjik etkisini de gösterebilir.

Anahtar Kelimeler: Dekstroz Proloterapisi (DP), Diz Osteoartriti (OA), Sodyum Monoiyodoasetat (MIA), Hipertonik Dekstroz Proloterapisi (HDP)

INTRODUCTION

Knee Osteoarthritis (OA) is the most common chronic, progressive, and disabling joint disease, often resulting in a poor quality of life. According to clinical and radiographic assessments, the prevalence of OA is 50% over the age of 60 and over 80% at the age of 75 (1,2). The main clinical symptom of OA is pain, which is one of the leading causes of disability in OA (3,4).Current treatments of OA mainly include analgesic agents and viscosupplementation. However, these treatment and supplementations only reduce OA symptoms such as joint pain. Furthermore, some studies suggest that these drugs are not sufficiently beneficial and may cause adverse drug reactions (5,6).

Osteoarthritis has often been described as a non-inflammatory, and simply a degenerative joint disease that predominantly caused by mechanical factors and genetic predisposition. However, there is increasing evidence that shows inflammation is high in OA and the pathogenesis of this disease is much more complex than just a degenerative process that may contribute to the progression of this disease (7,8).

Prolotherapy is one of the promising options for the treatment of painful musculoskeletal conditions such as knee OA, particularly when other standard treatments have proved to be ineffective (9). Intra-articular or extra-articular applications of hypertonic dextrose (a natural form of glucose normally found in the body) as prolotherapy agent is an effective injectional therapy with few adverse effects, that can be used in treating many chronic musculoskeletal problems, including osteoarthritis (OA) (10–12). Initially, Dextrose prolotherapy was thought to

contribute to the treatment of OA by inducing inflammatory pathways. Studies in this direction reveal that the inflammatory environment created after dextrose prolotherapy is very short-term and transient. The study conducted by Jensen and colleagues, shows that although the inflammatory effect of Dextrose prolotherapy is seen initially (at 6 and 24 hours), this effect is not seen after 72 hours. More importantly, when the inflammatory effects were compared, Dextrose prolotherapy and saline (control group) produced the same level of inflammatory effect. This study suggests that the inflammatory effect is more likely to be due to damage to the injection site (13). Dextrose itself works through repairing injured musculoskeletal tissue by stimulating the body's natural healing mechanisms. Dextrose prolotherapy stimulates different growth factors such as platelet derived GF, ILGF and transforming growth factor ß, which in turn results with the expression of type I and III collagens. According to recent studies, adequate supply of glucose to chondrocytes during inflamatory conditions and matrix degradation can interrupt the detrimental inflammatory cycle and induce synthesis of hyaluronan, thereby promoting cartilage repair (14,15). Despite its long history and widespread use as a form of a single or complementary therapy, there are still disparities over Dextrose optimal effective concentrations. In the light of the abovementioned information, in this study it was aimed to analyse different concentrations of Dextrose to determine the optimal effective concentrations of this cheap, easy-to-use and harmless prolotherapy factor for the treatment or relieving pain of knee OA (Figure 1).

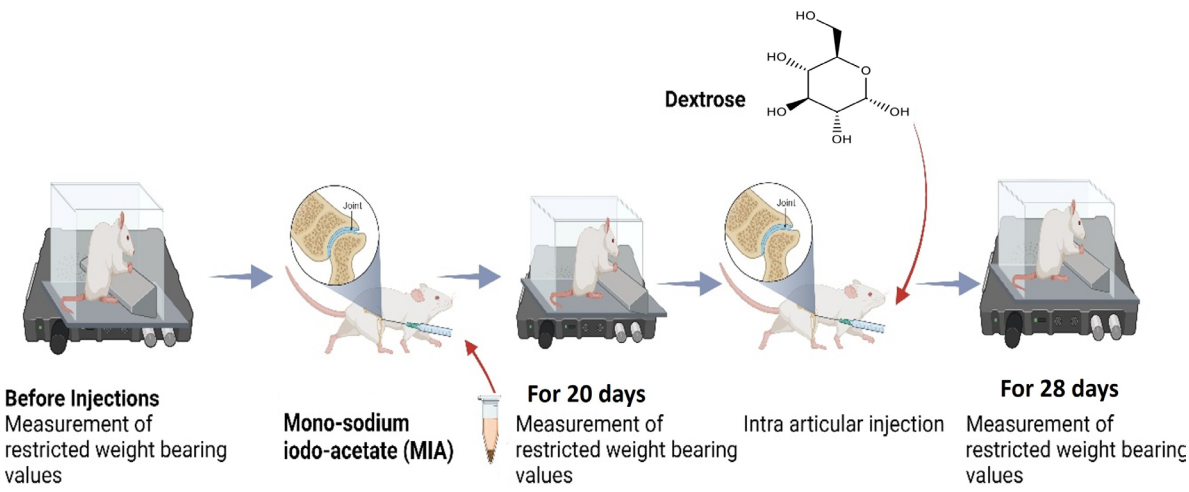

Figure 1. Flowchart of the in vivo experiment. Created by biorender.com

MATERIAL and METHOD

Creating the OA Model

In this study, 40 male 10-12 weeks Wistar rats (200 ± 30 g) were supplied by Koç University- Animal Facility and kept under animal breeding conditions. The experimental protocol was approved by the Local Ethics Committee for Animal Experimentation of Koc University (2018-31). Animals were fed with standard rat chow and provided with pure water. They were kept in individually ventilated cages under 22 °C room temperature with air filter.

Single intra-articular injection of mono-sodium iodoacetate (MIA), an inhibitor of glycolysis, into the rat knee femorotibial joint causes cartilage damage and degeneration, similar to the signs of human

OA (1,2,16). To create an animal model of OA, the Restricted Weight Bearing values in the left and right legs of rats were measured with the incapacitance device (Figure 1a) and their suitability for the study was checked by the % hind left leg weight (17). Both legs of the animals to be included in the study should have equal or very close weight bearing power and the hind left leg weight value should be 50%. During the experiment, rats were allowed to get used to the apparatus and were obtained five times and the average were considered as a data. For this purpose, the rats were placed in the chambers of the apparatus (made of angular plexiglass). Thus, the animals placed their hind left and right legs on two separate pressure plates as shown in the figure (Figure 1a). A video on the formation of the OA model, available on the Jove website, was used to visually be trained and perform the in vivo experiment (18,19). Then, evaluation of the volume and optimization of the point of intraarticular injection was assessed applying Intraarticular injection of water soluble blue stain (Trypan Blue) solution.

The limited weight bearing value was calculated by incorporating the following formula into the microsoft exel-2010 program (Formula 1).

$$\text{Hind left leg weight \%} = \left[\frac{\text{Hind left leg weight}}{(\text{Hind left leg weight} + \text{Hind right leg weight})}\right] X\ 100$$

Formula 1. Formula for measuring the limited weight bearing value of the left leg (the leg to be administered physiological serum or MIA)

For induction of MIA-induced osteoarthritis, rats were anesthetized with isoflurane and positioned on their backs.After shaving and disinfection of knee area, the knee was positioned at a 90° angle to reveal the white patellar tendon below the patella. Pressing the patellar tendon with the fingertip 1 mg of freshly prepared MIA (experimental) and blank (control) solution in 50 µL sterile saline was injected vertically (5 mm) into the joint cavity in the junction of the gap and the lateral patellar tendon of male wistar rats using 26 G needle. It was important to not felt a resistance when the needle was in the articular space (19). The pain related behavior were tested at 2, 5, 8, 12, 16, and 20 days after injection.

Animals with a left leg weight bearing value in the range of 42 ± 3% were considered as OA developed and suitable for the study, and rats below (advanced OA) and above (no OA or initial OA) this value were removed from the study with the least painful method within the framework of ethical rules.

OA Model Treatment

From 40 animals with MIA injected as OA model in left leg and saline injected as control in right legs, those with a limited restricted weight bearing values for left leg closest to 42% were selected and randomly assigned to four different treatment groups consisting of six animals each, for therapeutic administration in left legs. Dextrose injections were performed intraarticularly into the knee joint (lateral) as a single dose under anesthesia (3% isoflorane).

Post-treatment Pain Assessment (posterior left leg limited weighted pressure value)

After the treatment, the limited weight bearing value was measured five times a day at different intervals in all animals and the average was calculated. The results obtained were then compared with the pre-treatment results and the recovery rates of the different groups calculated. The recovery curves were compared by evaluating the differences within and then between the groups.

This study was approved by the Koç University Local Ethics Committee for Animal Experimentation (Date: 07.11.2018 and Number: 2018-31).

RESULTS

Creating the OA Model

Rats are one of the mostly used animal models in the preclinical studies for both investigating the pathophysiology and developing treatments for joint disorders namely, osteoarthritis, rheumatoid arthritis, etc (20). The injection volume of solutions differs

in wide ranges, from 20 to 200 µL in intraarticular injection experiments that was carried out with rat knees. Large differences in applied volume between the samples can certainly effect the outcomes of the studies. In addition excess amount of injection will end up with leakage from the articular (21). Therefore, not only the site of injection, but also the volume of injectible solution are important in knee joint injections. According to our study injection over 50 µl will pave the way for the leakage of blue stain out side of the joint into the leg (figure 2b). Therefore freshly prepared MIA (OA model leg), saline (control leg) and dextrose (treatmemt) solution were injected into the joint cavity in the junction of the gap and the lateral patellar tandons of animals in 50 µl final solutions using 26 G needle.

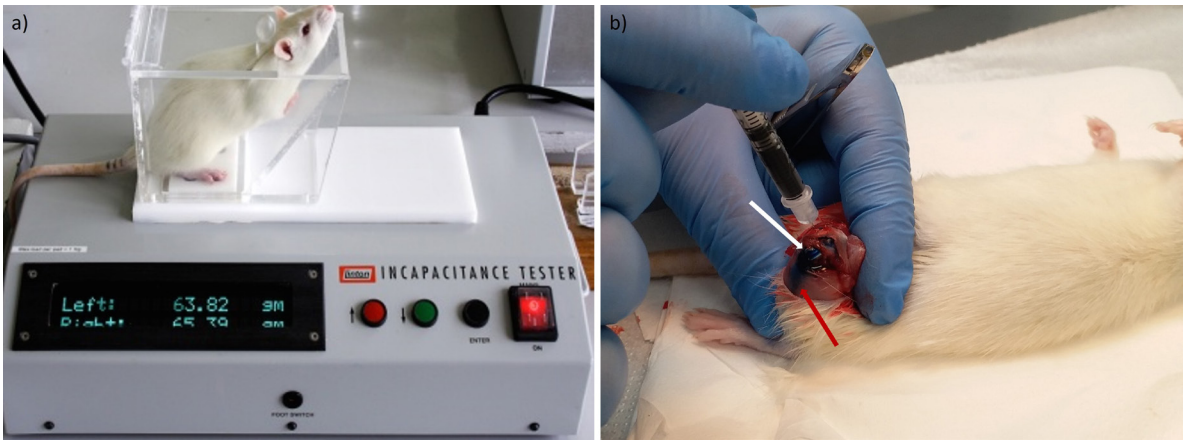

Figure 2. a) Position of the rat's legs pressed against the plate of the incapacitance device. b) injection of water-slouble blue stain material into rat knee joint. red arrow shows the stain leaking in the leg through the ankle and white arrow indicated the stainin in the injection site of the joint

According to Formula 1, a healthy Wistar rat should have equal or very close weight-bearing power of both legs around 50% (17). However, this value was expected to decrease by about 8% from the normal value for the leg that developed model OA due to MIA injection and thus to be around 42%. Animals with a left leg weight bearing value in the range of 42 ± 3% were considered as animals with osteoarthritis and suitable for the study. The left and right leg values measured for each rat with incapacitance meter and calculated according to Formula 1. Each formula result was summed and averaged. Standard deviation data prepared from Excel were added below these values. For 24 Wistar male rats, graphs were obtained from each average formula value (Figure 3).

In general, pattern formation was observed. As can be seen from the sharp decrease in the values, a response occurred immediately after the first injection in the majority of the animals. Although there was a recovery period in animals afterwards, the model maintained its success. Response results were generally in the range of 42 ± 3 (Figure 3).

OA Treatment

The t-test and two-way analysis of ANOVA (Graphpad Prism 9 Software) were used for statistical evaluation after Dextrose treatment. As seen in Figure 2, statistically significant ($p<0.05$) improvement was observed in 24 animals after Dextrose prolotherapy treatment compared to MIA injection (Figure 4a). On the other hand, when compared according to the Dextrose ratios injected at different rates (5%, 10%, 15% and 25%); although improvement (increase in weight bearing capacity) was observed after injection of each Dextrose ratio, no statistical significant difference was observed between the groups (Figure 4b).

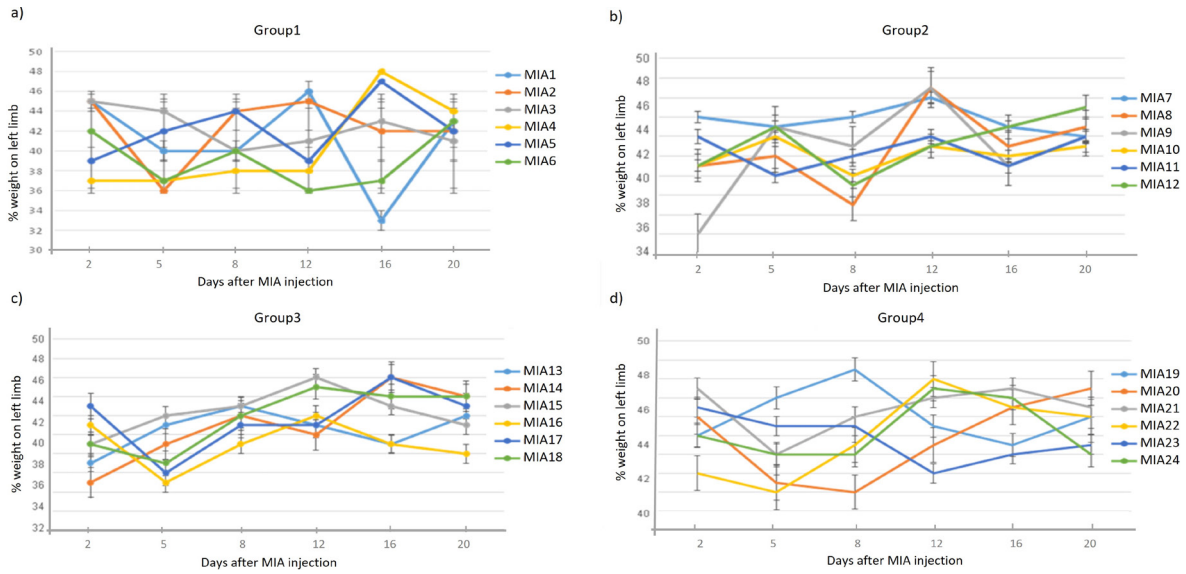

Figure 3. Effect of MIA injection into the rat left knee in different groups (1-4). Left leg weight bearing percentages of four different groups (a-d) at different days after MIA injection

Figure 4. a) Statistical analysis showing the effect of Dextrose injection on the Left leg weight bearing capacity of animals (24 rats) with OA after Dextrose injection $p<0.05$ b) Statistical analysis showing the effect of Dextrose injection at different ratios (5%, 10%, 15%, 25%) on the left leg weight bearing capacity of animals (6 rats in each ratio) with OA after Dextrose injection

Incapacitance measurements of Dextrose-treated animals (0.5 ml/1 dose) were performed starting from day 2 for four weeks. According to the left leg weight bearing measurments, the improvement mostly starts from day 14 after treatment (Figure 5a-d). When leg weights at the end of MIA injection were compared with those of at the end of Dextrose treatment, some, but not complete, recovery was observed.

In the left legs measurements several days after Dextrose injection, the rats put less weight on their left legs in a stable state may be due to the damage to the injection site (13). Although, the recovery starts several days after Dextrose injection, the rate of recovery increases after two weeks. The effects of dextrose percentages on treatment from day 2 are given in Figures 6 (a-d).

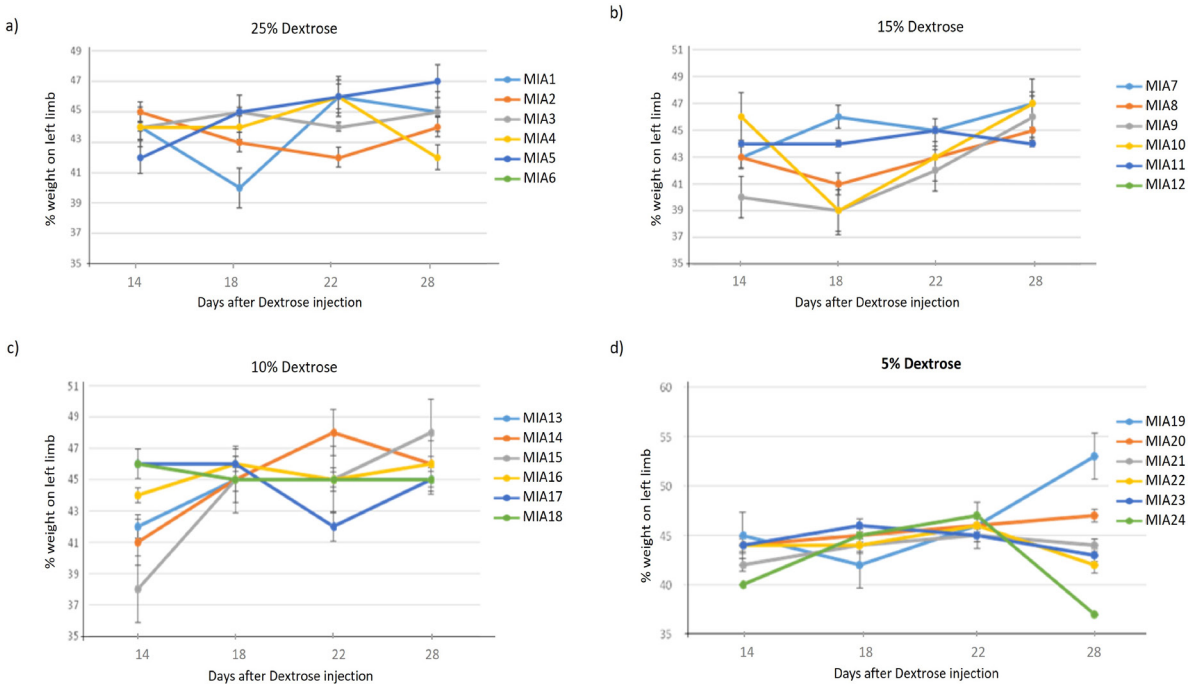

Figure 5. Left leg weight bearing percentages after a)25%, b)15%, c)10% and d)5% Dextrose injection

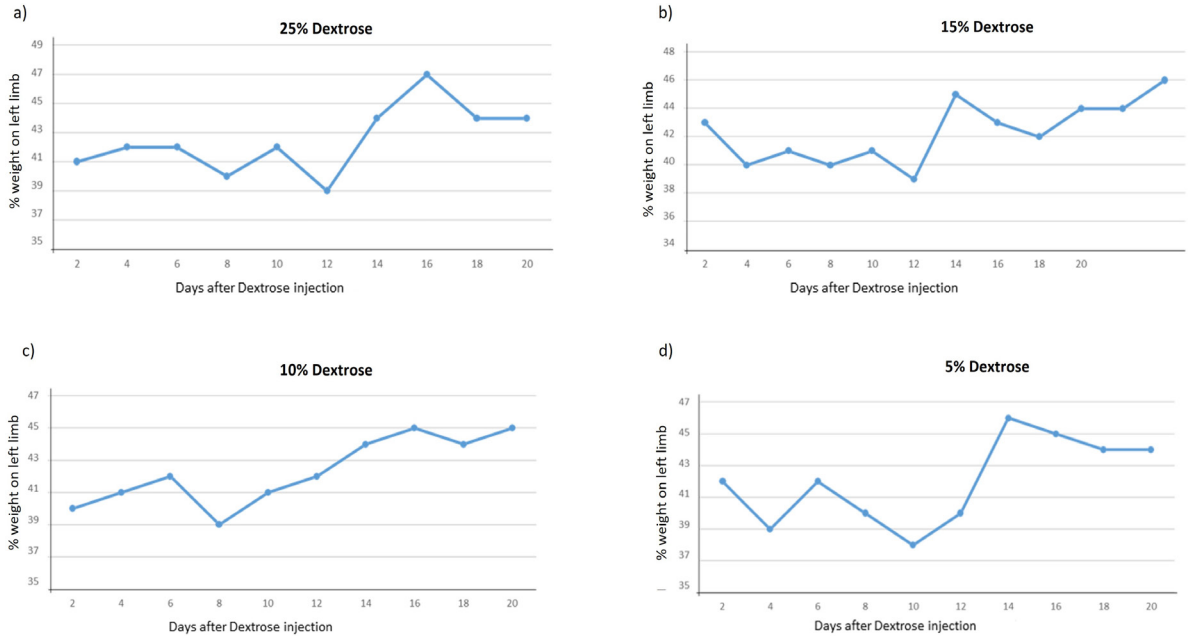

Figure 6. Graph of arithmetic mean values of incapacitance measurements before and after a)25%, b)15%, c)10% and d)5% Dextrose injection

DISCUSSION

Knee osteoarthrit remains as one the most common degenerative disease and pain associated with this disease still continues to remain as a major unmet medical need. Therefore, effective therapeutic options to treat OA pain are still very much warranted.

In vitro studies on the dose of Dextrose to be administered have shown that lower doses are effective in the treatment of OA, while higher doses trigger the inflammatory environment (22).

In *in vivo* studies on dextrose prolotherapy injections demonstrated early inflamatory response overall, similar to that of saline injections or needlestick procedures which was resolved by 72 h ostinjection (13). Prolotherapy injections create an inflammatory response, but this response is variable and overall, not uniformly different from that caused by saline injections or needlestick procedures (13). Although short term therapeutic effects of dextrose prolotherapy were more abundant, there are some studies that show improvements with dextrose prolotherapy treatment in the long term up to two years (23).

Our current in vivo study on rat demonstrated that injection of more than 50 µl has the capacity of leakage out of the knee joint and pave the way for fake results. Our study showed no significance diffirence in the therapeutic effects of different concentration of dextrose on developed rat animal OA model. The current study adds two important findings. Considering the post-treatment results in our in vivo study, in general, it can be suggested that Dextrose treatment was statistically and significantly effective four weeks after injection at all doses of Dextrose. However, no significant difference in pain reduction efficiency between Dextrose doses was observed. Therefore, dextrose at any concentration between 5% to 25% can be used as treatment strategy in OA patients alone or in combination with other therapeutic techniques. However, generalisability of this statement requires further studies in combination with other therapetic techniques and clinical studies

In conclusion; Dextrose prolotherapy is one of the most inexpensive and safe therapeutic methods for effective treatment, recovery and pain control of knee osteoarthritis. In experiments for which rat is used in developing and treatment of animal Knee OA model, application of 50 µl injecable solutions seams to be the optimal effective volume. It also comes out that no significant difference presence in the effeciency of applied concentration of Dextrose alone. However, there is a need for further studies related to synergistic effect of prolotherapy together with other therapeutic methods.

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ETHICS COMMITTEE APPROVAL

* This study was approved by the Koç University Local Ethics Committee for Animal Experimentation (Date: 07.11.2018 and Number: 2018-31).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

Evaluation of metabolic syndrome and its biochemical parameters in individuals with gouty arthritis and asymptomatic hyperuricemia

Gut artriti ve asemptomatik hiperürisemisi olan bireylerde metabolik sendrom ve biyokimyasal parametrelerin değerlendirilmesi

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ABSTRACT

Objective: Hyperuricemia, which occurs as a result of high uric acid levels, the end product of purine metabolism, is related with asymptomatic hyperuricemia (ASH) when it does not show symptoms and gouty arthritis (GOUT) when urate crystals occur. Hyperuricemia may interact with metabolic syndrome components. Therefore, the aim of the study was to assess the relationship of GOUT and ASH with metabolic syndrome.

Methods: The study was conducted with 145 individuals diagnosed with GOUT and ASH. Data on general characteristics, biochemical test results (serum fasting insulin, fasting blood glucose, uric acid, and blood lipids (such as low-density lipoprotein, total cholesterol, triglyceride, and high-density lipoprotein)), anthropometric (waist and hip circumferences, body weight and height) and blood pressure (systolic and diastolic) measurement results were collected. Bioelectrical Impedance Analysis was used to measure body composition. The National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) diagnostic criteria were used for the detection of

ÖZET

Amaç: Pürin metabolizmasının son ürünü olan ürik asit düzeyinin yüksek olması sonucu ortaya çıkan hiperürisemi, semptom göstermediğinde asemptomatik hiperürisemi (ASH) ve ürat kristalleri oluştuğunda gut artriti (GUT) ile ilişkilidir. Hiperürisemi, metabolik sendrom bileşenleriyle etkileşime girebilir. Bu nedenle çalışmanın amacı, GUT ve ASH'nin metabolik sendromla ilişkisini değerlendirmektir.

Yöntem: Bu çalışma, GUT ve ASH tanısı alan 145 birey ile gerçekleştirilmiştir. Genel özellikler, biyokimyasal test sonuçları [serum açlık insülini, açlık kan şekeri, ürik asit ve kan lipitleri (düşük yoğunluklu lipoprotein, toplam kolesterol, trigliserit ve yüksek yoğunluklu lipoprotein gibi)], antropometrik (bel ve kalça çevresi, vücut ağırlığı ve boy uzunluğu) ve kan basıncı (sistolik ve diyastolik) ölçüm sonuçları ile ilgili veriler toplanmıştır. Vücut kompozisyonunu ölçmek için Biyoelektrik İmpedans Analizi kullanılmıştır. Metabolik sendromun saptanmasında Ulusal Kolesterol Eğitim Programı Erişkin Tedavi Paneli III (NCEP-ATP III) tanı kriterleri kullanılmıştır. Hastalardan yazılı

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metabolic syndrome. Written informed consent form was obtained from patients. Patients who were not volunteers, who were pregnant or lactating, diagnosed with cancer, chronic renal failure, chronic liver failure, and individuals using diuretic drugs were not included in the research. In the analyses of all hypothesis tests, $p<0.05$ was accepted significant.

Results: The metabolic syndrome frequency was significantly higher in the GOUT group than in the ASH group ($p<0.05$). The difference between body mass index (BMI) values according to gender and groups was statistically significant among women ($p<0.05$). Mean body fat percentage values were significantly higher in women in both GOUT and ASH groups ($p<0.05$).

Conclusion: The frequency of metabolic syndrome was significantly higher in the GOUT group. In addition, BMI and body fat percentage values were significantly higher in women. Abdominal obesity and possible hyperinsulinemia may cause more serious problems in the presence of hyperuricemia. Therefore, multiple parameters (various biochemical and anthropometric measurements) should be evaluated together.

Key Words: Uric acid, asymptomatic hyperuricemia, gout, metabolic syndrome

bilgilendirilmiş onam formu alınmıştır. Gönüllü olmayan, hamile veya emziren, kanser tanısı almış, kronik böbrek yetmezliği ve kronik karaciğer yetmezliği olan ve diüretik ilaç kullanan kişiler araştırmaya dahil edilmemiştir. Tüm hipotez testlerinin analizlerinde $p<0.05$ anlamlı olarak kabul edilmiştir.

Bulgular: GUT grubunda metabolik sendrom prevalansı ASH grubuna göre anlamlı derecede yüksekti ($p<0.05$). Cinsiyet ve gruplara göre beden kütle indeksi (BKİ) değerleri arasındaki fark kadınlar arasında istatistiksel olarak anlamlı idi ($p<0.05$). Ortalama vücut yağ yüzdesi değerleri hem GUT hem de ASH grubundaki kadınlarda anlamlı derecede yüksek olarak bulunmuştur ($p<0.05$).

Sonuç: GUT grubunda metabolik sendrom prevalansı anlamlı derecede yüksekti. Ayrıca, kadınlarda BKİ ve vücut yağ yüzdesi değerleri anlamlı derecede yüksekti. Abdominal obezite ve olası hiperinsülinemi, hiperürisemi varlığında daha ciddi sorunlara neden olabilir. Bu nedenle, birden fazla parametrenin (çeşitli biyokimyasal ve antropometrik ölçümler) birlikte değerlendirilmesi gerekmektedir.

Anahtar Kelimeler: Ürik asit, asemptomatik hiperürisemi, gut, metabolik sendrom

INTRODUCTION

Gout is a type of arthritis that occurs when uric acid levels are irregular. It is a systemic disease that results in the accumulation of monosodium urate crystals in tissues. Elevated serum uric acid levels above a certain threshold cause the formation of uric acid crystals. The clinically gout is seen as acute gouty arthritis, asymptomatic hyperuricemia, chronic tophaceous gout, and intercritical period. Furthermore, many people with hyperuricemia may not develop gout, so uric acid crystals may not form (1). Uric acid, the metabolic end product of purine metabolism, has recently been related with many chronic diseases, including metabolic syndrome (2).

There are numerous primary and secondary reasons of hyperuricemia. Modifiable factors that can cause hyperuricemia are: high purine diet, obesity, uncontrolled hypertension, alcohol consumption, hypertriglyceridemia, some drugs (such as thiazides and low-dose aspirin), and insufficient urine output (<1400 ml/day). Chronic hyperuricemia is dangerous for health because it causes urate crystal accumulation, which can cause gout, urolithiasis, and uric acid nephropathy. Asymptomatic hyperuricemia is an elevated uric acid level (women: >6 mg/dL or men: >7 mg/dL). However, there are no symptoms associated with urate crystal formation. Detailed

health and nutritional history, physical examination, and laboratory findings are important in the treatment of asymptomatic hyperuricemia (3). The comorbidities of gout vary according to the affected system. Some of these are: stroke, hypertension, coronary heart disease, heart failure, atherosclerosis, nephrolithiasis, Alzheimer's and Parkinson's diseases, osteoporosis, chronic kidney disease, osteoarthritis, and diabetes. When evaluated in general, metabolic syndrome, which includes different metabolic components together, has an important place in gout (4). According to the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) criteria, metabolic syndrome prevalence was reported as 62.8% in gout patients and 25.4% in those without gout (5). Metabolic syndrome is a chronic low-grade inflammation status related with genetic or environmental factors. Insulin resistance or high blood glucose, visceral adiposity (high waist circumference), atherogenic dyslipidemia (especially declined high-density lipoprotein (HDL) and enhanced triglyceride (TG)), and increased blood pressure are the most important factors that cause metabolic syndrome (6). A significant correlation was found among metabolic parameters (TG, waist circumference, HDL-cholesterol, and blood pressure) and serum uric acid level. Especially, high triglyceride level was shown as the most effective parameter on serum uric acid level (7). Hyperuricemia is common in individuals with metabolic syndrome. It was suggested that there may be an increase in uric acid absorption secondary to hyperinsulinemia (8).

The purpose of this research was to examine the association of gouty arthritis and asymptomatic hyperuricemia with metabolic syndrome.

MATERIAL and METHOD

Patients

The study included volunteers diagnosed with gouty arthritis (GOUT) and asymptomatic hyperuricemia (ASH) and presented to the Internal Medicine Outpatient Clinic within 5 months. The study included 145 individuals (52 men and 93 women) in 18-65 years old.

After the individuals were briefed about the research, a written informed consent form was obtained. Patients who were not volunteers, who were pregnant or lactating, diagnosed with cancer, chronic renal failure, chronic liver failure, and individuals using diuretic drugs were not included in the research.

In order to assess the socio-demographic features (age, gender, and educational status etc.) and general health information (such as smoking and alcohol use status) of the people, a questionnaire form with multiple-choice and open-ended items was administered by the researchers through face-to-face interviews.

Among the patients presented or consulted to the Internal Medicine Outpatient Clinic, persons with ASH or GOUT based on their biochemical and clinical findings were included in the study.

Biochemical Indicators

The serum fasting insulin, fasting blood glucose, uric acid, and blood lipids (such as low-density lipoprotein (LDL), total cholesterol, TG, and HDL) values were recorded from patient files. In the evaluation of biochemical indicators, reference ranges of the Private Lokman Hekim Ankara Hospital Laboratory were exerted. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) amounts were counted for the detection of insulin resistance. A HOMA-IR of ≥2.7 was defined as insulin resistance (9).

Measurement of Blood Pressure

Blood pressures were measured with a manual sphygmomanometer by a nurse assigned by hospital. Blood pressures (systolic and diastolic) were recorded by receiving the mean of two consecutive measurements while at rest. Hypertension is described as systolic blood pressure-140 mm Hg and greater and/or diastolic blood pressure-90 mm

Hg and greater and/or receiving anti-hypertensive treatment (10).

Anthropometric Measurements and Body Composition

Waist-hip circumferences, body weight, height, and body composition measurements were taken. Bioelectrical Impedance Analysis (BIA) was used for body weight measurement, while the patients were in thin clothes, without socks and shoes. Height was measured with a Seca stadiometer. While taking the height measurements of the individuals, they were in the Frankfort plane (eye and auricle are at the same level) and their feet were together. Body weights and heights were calculated using the body mass index (BMI) formula: [BMI (kg/m^2) = Body Weight (kg) / Height (m^2)], and the outcomes were classified in accordance with the World Health Organization (WHO) classification. Accordingly, <18.50 kg/m^2 was evaluated as underweight, 18.50-24.99 kg/m2-normal, 25.00-29.99 kg/m^2-overweight, and ≥30.00 kg/m^2-obese (11).

Waist circumference measurement was made by measuring the circumference through the midpoint between the lowest rib and the crista iliaca with an inflexible tape measure. According to the WHO classification, waist circumference values were evaluated as <94 cm-normal, ≥94 cm-risk, and ≥102 cm-high risk in men; <80 cm-normal, ≥80 cm-risk, and ≥88 cm-high risk in women (12).

The hip circumference was measured from the highest point, parallel to the ground, and standing on the side of the individual with an inflexible tape measure. Waist/hip ratio was calculated with this formula: [Waist/Hip Ratio = Waist Circumference (cm) / Hip Circumference (cm)]. The outcomes were assessed according to the WHO classification. Accordingly, waist/hip ratio was determined as <0.90 normal and ≥0.90 risk in men; <0.85 normal and ≥0.85 risk in women (12).

Detection of Body Composition

The body composition of individuals (body fat percentage etc.) was assessed using BIA. The individuals were asked not to eat at least two hours before the measurement, not to drink much water before the measurement, not to drink tea/coffee 4 hours before the measurement, not to perform heavy physical activity, and not to consume alcohol 24 hours before. Body fat percentage was classified as ≤5% lean, 6-24% normal, and ≥25% risk in men; ≤8% lean, 9-31% normal, and ≥32% risk in women (13).

Metabolic Syndrome Diagnostic Criteria

The NCEP-ATP III diagnostic criteria were used for the detection of metabolic syndrome (Table 1) (14).

Informed Consent

The authors declare that they obtained a written informed consent from the patients and/or volunteers included in the article and that this report does not contain any personal information that could lead to their identification.

Table 1. NCEP-ATP III diagnostic criteria for metabolic syndrome (14)

• Abdominal obesity (waist circumference: >102 cm-men, >88 cm-women)
• Hypertriglyceridemia (≥150 mg/dL for TG)
• Low HDL (HDL: <40 mg/dL-men, <50 mg/dL-women)
• Hypertension (≥130/85 mm Hg for blood pressure)
• Hyperglycemia (≥110 mg/dL for fasting blood glucose)

*Exhibiting at least three of the parameters is necessary for metabolic syndrome.

NCEP-ATP III: National Cholesterol Education Program-Adult Treatment Panel III, TG: triglyceride, HDL: high-density lipoprotein.

Statistical Analysis

Number (n) and percentage (%) were used for categorical variables. Mean, standard deviation (SD), minimum, and maximum values were used for continuous variables. Whether the data were normally distributed was assessed with the Kolmogorov-Smirnov Test and histograms. In the assessment of categorical variables, the Pearson's Chi-square (x^2) Test was performed when the assumptions were met; the Fisher's Exact (x^2) Test was performed when the number of samples in the crosstab was insufficient and the assumption could not be met. Statistical Package for Social Sciences 22.0 was applied in the statistical assessment of the data. In the analyses of all hypothesis tests, $p<0.05$ was accepted significant.

This study was approved by the Baskent University Non-Interventional Clinical Research Ethics Committee (Date: 12.04.2016 and Number: 94603339-604.01.02/12438).

RESULTS

Basic Information of the Individuals

In the study, 63.4% (n=92) of the individuals were women and 36.6% (n=53) were men. The mean age of all individuals was 37.2±12.8 years; 34.0±12.7 years for the ASH group and 41.0±12.0 years for the GOUT group. In the ASH group, 15.0% were housewife, 7.5% were civil servant, 17.5% were worker, 13.8% were self-employed, 1.3% were retired, and 45.0% were in other professions. In the GOUT group, 21.5% were housewife, 18.5% were civil servant, 1.5% were worker, 21.5% were self-employed, 15.4% were retired, and 21.5% were in other professions (Data not shown).

In the ASH group, 71.3% were non-smokers, 10.0% had been smokers before and quit smoking, and 18.8% were current smokers; 50.8% of the GOUT group were non-smokers, 1.5% had been smokers before and quit smoking, and 47.7% were current smokers. In the ASH group, 11.3% consumed alcohol, whereas 26.2% of the GOUT group consumed alcohol. It was observed that 88.8% of the ASH group and 73.8% of the GOUT group did not consume alcohol (Data not shown).

Metabolic Syndrome Frequency

The metabolic syndrome frequency was statistically significantly higher than the ASH group. The metabolic syndrome frequency of the individuals participating in the study is shown in Table 2.

The distribution of metabolic syndrome frequency by age and the groups (ASH and GOUT) is shown in Table 3. The highest metabolic syndrome frequency was 47.6% in individuals aged 20-29 for ASH group and 41.4% in individuals aged 40-49 for GOUT group (Table 3).

Table 2. Comparation of metabolic syndrome in accordance with ASH and GOUT groups and gender

Metabolic Syndrome		ASH (n= 80)		GOUT (n= 65)		Total (n= 145)		
		n	%	n	%	n	%	*p*
Presence of metabolic syndrome	Women	15	71.4	13	44.8	28	56.0	0.061
	Men	6	28.6	16	55.2	22	44.0	
	Total	21	100.0	29	100.0	50	100.0	
Absence of metabolic syndrome	Women	51	86.4	13	36.1	64	67.4	<0.001*
	Men	8	13.6	23	63.9	31	32.6	
	Total	59	100.0	36	100.0	95	100.0	

ASH: asymptomatic hyperuricemia, GOUT: gouty arthritis.

Table 3. Metabolic syndrome frequency according to ASH and GOUT and age groups

			Metabolic Syndrome	
Disease Group			**n**	**%**
ASH	**Age (year)**	20-29	10	47.6
		30-39	4	19.0
		40-49	2	9.6
		50-59	4	19.0
		60-65	1	4.8
GOUT	**Age (year)**	20-29	5	17.2
		30-39	4	13.8
		40-49	12	41.4
		50-59	7	24.2
		60-65	1	3.4

ASH: asymptomatic hyperuricemia, GOUT: gouty arthritis.

Distribution of Anthropometric Measurements of ASH and GOUT Groups

In the ASH group, 42.9% of men and 42.4% of women were obese, 57.1% of men and 28.8% of women were overweight, and 0.0% of men and 28.8% of women were normal. In the GOUT group, 73.1% of women were obese, 19.2% were overweight, and 7.7% had normal BMI; these values were 56.4%, 41.0%, and 0.0% in men, respectively. The difference among BMI values according to gender and groups was statistically important among women (p=0.021) (Table 4).

While the mean waist circumference of the men was 106.3±20.29 cm in the ASH group, it was 104.1±17.26 cm in the GOUT group. It was 90.3±21.85 cm in women in the ASH group and 95.0±12.50 cm in women in the GOUT group. There was no signification among the mean values of waist circumference between the groups (p=0.691 for men, p=0.302 for women) (Data not shown). In the ASH group, 50.0% of the men and 45.5% of the women were in the high-risk group. In the GOUT group, 56.5% of men and 65.4% of women were in the high-risk group. There was no significant differentiation between the groups by waist circumference classification (p=0.105) (Table 4).

Mean hip circumference of men was 109.2±11.20 cm in the ASH group and 110.0±11.67 cm in the GOUT group; it was 103.6±12.24 cm in women in the ASH group and 107.8±10.51 cm in women in the GOUT group. There was no statistical signification among the mean hip circumference values between the groups (p=0.832 for men, p=0.126 for women) (Data not shown).

Mean waist/hip ratio was 0.97±0.10 for men and 0.84±0.10 for women in the ASH group. The mean waist/hip ratio was 0.93±0.09 for men and 0.87±0.06 for women in the GOUT group. There was no statistical signification among the mean values of waist/hip ratio between the groups (p>0.05) (p=0.215 for men, p=0.103 for women). According to the mean waist/hip ratio classification, in the ASH group, 21.4% of the men were in the normal group and 78.6% of the men in the risk group, while 48.5% of the women were in the normal group and 51.5% in the risk group. In the GOUT group, 28.2% of the men were in the normal group and 71.8% in the risk group; 30.8% of the women were in the normal group and 69.2% were in the risk group. There was no

significative difference among waist/hip ratio values according to gender and groups (p=0.123) (Table 4).

The mean body fat percentage (%) was 29.9±4.42 for men and 35.4±6.77 for women in the ASH group. In the GOUT group, mean body fat percentage (%) for men was 30.7±7.80 and 39.2±4.22 for women. In both GOUT and ASH groups, mean body fat percentage values were higher in women and the difference among them was statistically important (p=0.008) (Data not shown). In terms of the body fat percentages, 92.9% of men and 72.7% of women in the ASH group were in the risky class, while 92.3% of both gender in the GOUT group were in the risky class. There was a significant differentiation between the groups by gender (p=0.040) (Table 4).

Table 4. Distribution of anthropometric measurements of ASH and GOUT groups by gender

	Men				Women							
Anthropometric Measurements	**ASH**		**GOUT**		**ASH**		**GOUT**		**Total**		**Men**	**Women**
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	***p***	***p***
BMI (kg/m²)												
Underweight	-	-	1	2.6	-	-	-	-	1	0.7		
Normal	-	-	-	-	19	28.8	2	7.7	21	14.5		
Overweight	8	57.1	16	41.0	19	28.8	5	19.2	48	33.1	0.533	**0.021***
Obese	6	42.9	22	56.4	28	42.4	19	73.1	75	51.7		
Waist circumference (cm)												
Normal	2	14.3	9	23.1	25	37.9	4	15.4	40	27.6		
Risk	5	35.7	8	20.4	11	16.6	5	19.2	29	20.0	0.542	0.105
High risk	7	50.0	22	56.5	30	45.5	17	65.4	76	52.4		
Waist/hip ratio												
Normal	3	21.4	11	28.2	32	48.5	8	30.8	54	37.2	0.735	0.123
Risk	11	78.6	28	71.8	34	51.5	18	69.2	91	62.8		
Body fat percentage (%)												
Normal	1	7.1	3	7.7	18	27.3	2	7.7	24	16.6	1.000	**0.040***
Risk	13	92.9	36	92.3	48	72.7	24	92.3	121	83.4		

ASH: asymptomatic hyperuricemia, GOUT: gouty arthritis, BMI: body mass index.

Metabolic Syndrome Frequency according to ASH and GOUT Groups (Presence of Insulin Resistance and Dyslipidemia)

In Table 5, the metabolic syndrome frequency was evaluated by groups, the presence of dyslipidemia, and insulin resistance of the individuals participating in the study. While 71.4% of women and 28.6% of men in the ASH group had metabolic syndrome, 86.4% of women and 13.6% of men did not have metabolic syndrome. In the GOUT group, there was metabolic syndrome in 44.8% of women and 55.2% of men; metabolic syndrome was not found in 36.1% of women and 63.9% of men. Insulin resistance was 72.7% in men with metabolic syndrome, while it was 57.1% in women. The differentiation between the groups was not important (p=0.061) (Table 5).

In terms of dyslipidemia status, 40.9% of men with metabolic syndrome had total cholesterol ≥200 mg/dL, 68.2% LDL ≥100 mg/dL, 18.2% HDL <35 mg/dL, and 36.4% TG ≥150 mg/dL. In women with metabolic syndrome, 28.6% had total cholesterol ≥200 mg/dL, 53.6% had LDL ≥100 mg/dL, 25.0% had HDL <35 mg/dL, and 28.6% had TG ≥150 mg/dL. However, no statistical significance was found among these values (p=0.361 for total cholesterol, p=0.295 for LDL, p=0.561 for HDL, p=0.558 for TG) (Table 5).

Table 5. Metabolic syndrome frequency according to ASH and GOUT groups, presence of insulin resistance and dyslipidemia

	Metabolic Syndrome									
	Yes (n= 50)				No (n= 95)					
Hospital Reference Values	Men (n= 22)		Women (n= 28)		Men (n= 31)		Women (n= 64)			
	%	n	%	n	%	n	%	n	p^1	p^2
ASH	28.6	6	71.4	15	13.6	8	86.4	51	0.061	0.644
GOUT	55.2	16	44.8	13	63.9	23	36.1	13		
Insulin resistance										
HOMA-IR <2.7	27.3	6	42.9	12	32.3	10	67.2	43	0.352	**<0.001***
HOMA-IR ≥2.7	72.7	16	57.1	16	67.7	21	32.8	21		
Dyslipidemia										
Total cholesterol <200 mg/dL	59.1	13	71.4	20	51.6	16	82.8	53	0.361	**0.010***
Total cholesterol ≥200 mg/dL	40.9	9	28.6	8	48.4	15	17.2	11		
LDL <100 mg/dL	31.8	7	46.4	13	29.0	9	53.1	34	0.295	**0.027***
LDL ≥100 mg/dL	68.2	15	53.6	15	71.0	22	46.9	30		
HDL <35 mg/dL	18.2	4	25.0	7	6.5	2	-	-	0.561	**0.010***
HDL 35-55 mg/dL	59.1	13	64.3	18	83.8	26	59.4	38		
HDL >55 mg/dL	22.7	5	10.7	3	9.7	3	40.6	26		
TG <150 mg/dL	63.6	14	71.4	20	83.9	26	100	64	0.558	**0.030***
TG ≥150 mg/dL	36.4	8	28.6	8	16.1	5	-	-		

p^1: Men and women with metabolic syndrome.

p^2: Evaluation of the metabolic syndrome frequency by men and women without metabolic syndrome.

ASH: asymptomatic hyperuricemia, GOUT: gouty arthritis, HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, LDL: low-density lipoprotein, HDL: high-density lipoprotein, TG: triglyceride.

DISCUSSION

The current study provides insights into the relationship of ASH and GOUT with metabolic syndrome. We used the biochemical, blood pressure, anthropometric, and body composition measurements to assess the all participants with ASH, GOUT, and metabolic syndrome. Hyperuricemia, abdominal obesity, low HDL, hypertension, hypertriglyceridemia, and hyperglycemia were the parameters used in this research to determine the association of ASH and GOUT with metabolic syndrome. In both gout and asymptomatic hyperuricemia, metabolic syndrome components may be affected. Thus, factors that may cause hyperuricemia and metabolic syndrome components should be evaluated together.

The mean of waist circumference, one of the indicators of abdominal obesity, was 106.3±20.29 cm in men and 90.3±21.85 cm in women in the ASH group in this study and it was concluded that both values were higher than the reference values (12). In another study conducted with 83 patients with ASH, a significant relationship was found among hyperuricemia with glucose intolerance, abdominal obesity, and hypertriglyceridemia, which are components of metabolic syndrome, excluding hypertension and HDL (15). Therefore, blood lipid and glucose controls, and certain anthropometric measurements (such as waist and hip circumference) should be routinely performed in treatment approaches in hyperuricemia.

In the cross-sectional study, there were 174 gout patients, 48.3% women and 51.7% men. The metabolic syndrome prevalence was 54.6% and no significant difference was found between women (59.5%) and men (50.0%) (16). In this study, 44.8% of women and 55.2% of men in the GOUT group had metabolic syndrome. Unlike the previous study (16), the metabolic syndrome frequency was greater in men with gout in this study.

In this study, the men/women ratio was 3/2 among gout patients. In the research carried out by Jung et al. (17) in Korean gout patients and investigating the metabolic syndrome prevalence, the men/women ratio was 10.6/1, which was higher than our study. Furthermore, the mean age of gout patients was 51.28±15.07 years in the same study (17) and it was 41.0±12.68 years in our study, which was lower. The variation in the prevalence of gout according to age may be due to the socio-demographic features of the persons participating in the study.

According to a cross-sectional study of 9,206 Chinese individuals, when compared with BMI and waist circumference, waist/height ratio was accepted as the independent variable in the estimation of the presence of hyperuricemia (18). In a study conducted in Taiwanese men, a significant and linear relationship was found between waist/height ratio and gout. In addition, it was emphasized that waist/height ratio is a better anthropometric measurement in defining gout when compared to BMI, waist/hip ratio, and waist circumference (19). In this study, it was noted that all of the men (100.0%) and 71.2% of the women in the ASH group, 97.4% of the men and 92.3% of the women were overweight and obese in the GOUT group. In both groups, it was detected that men were more in the overweight and obese classes. In addition, it was found that 50.0% and more of men in both groups (ASH and GOUT) were in the high-risk group in terms of waist circumference measurements. Therefore, the evaluation of different anthropometric measurements together rather than a single anthropometric measurement and the fact that alcohol consumption is higher in men in nutritional habits should not be ignored in treatment protocols.

In terms of body fat percentages, 92.9% of men and 72.7% of women in the ASH group; 92.3% of both men and women in the GOUT group were in the risk group in this study. In a study examining the relationship between body fat distribution and uric acid metabolism, visceral fat accumulation elevated the hyperuricemia risk in elderly and middle-aged individuals, independent of neck circumference, BMI, and waist circumference (20). In a study of Vietnamese men with primary gout (n= 107) and healthy (n= 107) Vietnamese men, mean total body and trunk fat

masses in gout patients were found to be significantly higher than control group. Therewithal, it was stated that there was a strong and positive correlation between BMI and total body fat mass; a strong and positive correlation between trunk fat mass and waist circumference in gout patients. In addition, in accordance with the original and revised NCEP-ATP III criteria, the metabolic syndrome prevalence was significantly greater in gout patients (21). The balance of body fat composition and fat distribution can be shown among the factors included in the pathogenesis of systemic and metabolic diseases.

Gout patients in this study, 18.5% were in the age range of 20-29, 24.6% were 30-39, and 30.8% were 40-49 and it was specified that the gout frequency increased with age. On the contrary, according to the results of a cross-sectional study, a significant and negative relation was detected between age and uric acid concentrations in 653 individuals with gout (22).

In a study of 348 men gout patients, the dyslipidemia prevalence was significantly greater in those with excess fat (body fat percentage ≥25.0%) compared to those with normal body fat percentage (70.1%, 54.0%, respectively) (23). In a study of 41 primary gout patients to examine the metabolic syndrome prevalence and its constituents, 21 gout patients (51.0%) exhibited three or more of the metabolic syndrome components. Dyslipidemia was determined as one of the most common metabolic syndrome criteria with a rate of 73.17% (30/41) (24). In this study, when the components of dyslipidemia were examined, 40.9% of men with metabolic syndrome had total cholesterol ≥200 mg/dL, 68.2% had LDL ≥100 mg/dL, 18.2% had HDL <35 mg/dL, and 36.4% had TG ≥150 mg/dL. In women with metabolic syndrome, these values were determined as 28.6%, 53.6%, 25.0%, and 28.6%, respectively. The higher HDL levels in men than in women can be demonstrated by the fact that men are physically more active. The metabolic syndrome prevalence in gout patients was significantly greater (43.6%) than the Korean control group. In comparison to the control group, gout patients had more metabolic syndrome components. At the same time, BMI and HDL were stated as important factors in the emergence of metabolic syndrome in gout patients (25). In order to prevent cardiometabolic complications that may occur in gout patients with metabolic syndrome, metabolic syndrome components should also be targeted in the treatment.

It was noticed that individuals with insulin resistance had a substantially greater rate of metabolic syndrome. The mean serum uric acid level was greater in those with insulin resistance than in those without insulin resistance, but this differentiation was not important. Similarly, the mean serum uric acid level was greater in those with metabolic syndrome in comparison to those without metabolic syndrome, but it was not statistically important. It was revealed that there was no relationship among serum uric acid level with metabolic syndrome and insulin resistance (26). In the study in which 46 patients (men) with primary gout were classified according to the presence of metabolic syndrome, gout patients had significantly greater HOMA-IR levels. Furthermore, those with metabolic syndrome had significantly greater uric acid levels. In addition, gout patients with metatarsophalangeal (big toe joint) joint erosion had significantly higher insulin resistance values (27). In another study, the metabolic syndrome prevalence in gout patients was found to be 30.1% according to ATP III criteria and 50.6% according to WHO Asia-Pacific criteria. In addition, the mean HOMA-IR value in gout patients was determined as 2.63±1.36 and it was observed that it was significantly greater than the control group (28). In this study, insulin resistance was found in more than half of men and women with metabolic syndrome. Since there are common factors that can cause hyperinsulinemia and hyperuricemia, care should be taken when questioning a nutritional history.

The individuals in both (ASH-GOUT) groups were selected by the determined criteria and data were collected through the questionnaire in face-

to-face interviews, which increased the originality of the data. These were the strengths of the study.

The limitations of the study incorporated the smaller sample size and it was a single centre study. We recommend that further population-based studies (epidemiological, meta-analysis etc.) and also multicenter studies should be carried out in future to evaluate the association of gouty arthritis and asymptomatic hyperuricemia with metabolic syndrome.

In conclusion, while gout is seen more with increasing age, asymptomatic hyperuricemia can also occur at younger ages. As there is an increase in blood uric acid levels without symptoms in asymptomatic hyperuricemia, urate crystals formed in gout may adversely affect the prognosis and treatment approaches of the disease. In both gout and asymptomatic hyperuricemia, metabolic syndrome components (waist circumference, HDL, TG, blood pressure, and blood glucose) may be affected. Therefore, factors that may cause hyperuricemia and hyperinsulinemia should be evaluated together. Furthermore, the development of metabolic syndrome can be prevented by evaluating various anthropometric measurements, biochemical tests, and nutrition and health history together in gout and asymptomatic hyperuricemia treatment.

ETHICS COMMITTEE APPROVAL

* This study was approved by the Baskent University Non-Interventional Clinical Research Ethics Committee (Date: 12.04.2016 and Number: 94603339-604.01.02/12438).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Türk Hijyen ve Deneysel Biyoloji Dergisi

Evaluation of cultivable aerobic vaginal microbiota of repeat breeder and healthy pregnant dairy cows

Tekrarlayan kızgınlık yaşayan ve sağlıklı gebe süt ineklerinin kültürü yapılabilir aerobik vajinal mikrobiyotasının değerlendirilmesi

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ABSTRACT

Objective: Vaginal microbiota on bovine fertility has been minimally explored, and data on this are limited, despite the fact that vagina itself is a first barrier against ascending pathogens in the reproductive tract and interacts with host mucosa. Contamination of the female genital tract or dysbiosis of the vaginal flora have risks for the development of reproductive problems. Subclinical endometritis is one of the many variables that might lead to repeat breeder syndrome, and its significance has been neglected. In this study, we aimed to examine cultivable aerobic vaginal microbiota of repeat breeder (RB) cows and healthy pregranant (HP) cows at 60th day following artificial insemination.

Methods: A total of 45 Holstein breed dairy cows aged between 3 and 8 years including 20 repeat breeder (RB) cows (Group I = RBG), alongside 25 healthy pregnant (HP) cows (Group II=HPG) that conceived within one or two inseminations were used as study groups. The vaginal swab samples collected from RBG and HPG were incubated at 37 °C for 24 hours in fluid thioglycollate. On the next day, part of the initial culture was inoculated onto blood agar and EMB agar examined for

ÖZET

Amaç: Vajinal mikrobiyotanın sığır fertilitesi üzerindeki etkisi çok az araştırılmıştır ve bu konuda sınırlı veri mevcuttur. Vajinanın kendisi üreme yolunda yukarı doğru çıkan patojenlere karşı ilk bariyer olup konak mukozası ile etkileşime girer. Dişi genital yolun kontaminasyonu veya vajinal floranın disbiyozu, üreme problemlerinin gelişmesi için risk taşır. Subklinik endometrit, tekrarlayan kızgınlık sendromuna yol açabilecek birçok değişkenden biridir ve önemi göz ardı edilmiştir. Bu çalışmada, yapay tohumlamadan sonraki 60. günde tekrarlayan kızgınlık (RB) inekleri ve sağlıklı gebe (HP) ineklerin kültürü yapılabilir aerobik vajinal mikrobiyotasını incelemeyi amaçladık.

Yöntem: Çalışma grupları olarak, 3 ila 8 yaşları arasında 20 tekrarlayan kızgınlık (RB) ineği (Grup I = RBG) ve bir veya iki tohumlama içinde gebe kalan 25 sağlıklı gebe (HP) ineği (Grup II=HPG) olmak üzere toplam 45 Holstein cinsi süt ineği kullanıldı. RBG ve HPG'den alınan vajinal sürüntü örnekleri, sıvı tiyoglikolat içinde 37 °C'de 24 saat inkübe edildi. Ertesi gün, başlangıç kültürünün bir kısmı kanlı agar ve EMB agar üzerine ekilerek kültürlenebilir vajinal mikrobiyota

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the cultivable vaginal microbiota. The identification of bacteria was done with an automated system.

Results: A total of 26 species of 16 genera distributed among 4 phyla including Proteobacteria, Firmicutes, Actinobacteria, Bacteroiodetes were identified. The relative abundance of Proteobacteria in HP group comparing with the RB group was associated with an increase in progesterone. HP group presented relatively greater richness with 21 species followed by the RB group with 17 identified species. HP group possessed a more diverse microbiota in comparison to those suffering from subclinical endometritis. The relative abundance of *Escherichia* genus and *Escherichia coli* in RB group might have induced supressing the release of luteinizing hormone.

Conclusion: As a conclusion, the diversity of the vaginal microbiota and numerically abundance of some genus and species had favourable/unfavorable effects on reproductive fertility. The consortium of vaginal microbiota should be explored entirely in further studies since vagina is the first entrance of pathogens via ascending route in the reproductive tracts.

Key Words: Repeat breeder, pregnant, cows, vaginal microbiota

için incelendi. Bakterilerin tanımlanması otomatize bir sistem ile gerçekleştirildi.

Bulgular: Proteobacteria, Firmicutes, Actinobacteria ve Bacteroidetes dahil olmak üzere 4 şubeye dağılan 16 cinse ait toplam 26 tür tespit edilmiştir. HP grubunda Proteobacteria'nın relatif bolluğunun RB grubuna kıyasla progesteron artışı ile ilişkili olduğu bulunmuştur. HP grubu, 21 tür ile nispeten daha büyük bir zenginlik göstermiş, bunu 17 tür ile RB grubu takip etmiştir. HP grubu, subklinik endometritis yaşayanlara kıyasla daha çeşitli bir mikrobiyotaya sahipti. RB grubunda *Escherichia* cinsi ve *Escherichia coli*'nin relatif bolluğu, luteinize edici hormonun salınımını baskılamış olabilir.

Sonuç: Sonuç olarak, vajinal mikrobiyotanın çeşitliliği ve bazı cins ve türlerin sayısal bolluğu üreme fertilitesi üzerinde olumlu/olumsuz etkilere sahiptir. Vajina, üreme yolunda patojenlerin yukarı doğru girişi için ilk giriş noktası olduğundan vajinal mikrobiyota konsorsiyumu gelecekteki çalışmalarda ayrıntılı araştırılmalıdır.

Anahtar Kelimeler: Tekrarlayan kızgınlık, gebe, inekler, vajinal mikrobiyota

INTRODUCTION

The prospective origin of the reproductive tract microbiota is diverse. Microorganisms can enter the reproductive tract from other anatomical sites. In particularly, weakened physical cervical barrier at birth allows the introduction of bacteria into the genital system either from the vagina or from the environment via the vagina and also from the feces and animal skin to the genital tract (1). Besides this, negative abdominal pressure after parturition which causes a craniad sinking of the anus and subsequently of the vagina with increased craniad angulation of the dorsal portion of the vulva in predisposed animals. Air sucking in severely affected animals additionally triggers a reflux of urine from the vestibule to the vagina which results in urine accumulation in the lower part of the vagina (pneumovagina) (2). The cattle rumen typical flora such as *Porphyromonas*, *Fusobacterium*, and *Bacteroides* (3) were excreted in feces and then because of enviromental contamination uterus infections increased, subsequently occurence of metritis (4). Moreover, vaginal flora and uterus flora shares the same pathogens as previously described (5). Plausible pathogenicity of bacterial categories isolated from the lumen of the uterus are divided into three categories: Uterine pathogens, *Trueperella*, *Escherichia coli*, *Prevotella* spp., *Bacteroides* spp.,

Fusobacterium spp.; Potential pathogens, *Bacillus licheniformis, Enterecoccus faecalis, Pasteurella* spp., *Staphylococcus aureus, Peptostreptecoccus* spp., *Nonhaemolytic streptecocci*; Oportunistic contaminants, *Clostridium perfringens, Klebsiella pneumonia, Micrococcus* spp., *Proteus* spp., *Aspergillus* spp., *Streptecoccus* spp., *Haemolytic streptecocci* (6). The ascension pathway of bacteria into the uterus is likewise plausible from vagina. Additionally, all of these bacteria are the result of fecal contamination of bedding, the environment, and fur (7).

In cattle breeding, reproductive efficiency is the most important criterion of productivity. Reproductive yield is reduced due to genetic, nutritional, hormonal or infectious problems. (8-10). In these reproductive problems, reproductive canal infections affect fertility and cause economic loss (10). Changes in vaginal microflora may lead to infertility in cattle (11). Since the host microorganisms of the vaginal flora are in permanent interaction with the mucosa they are the first protective barrier against the pathogens and sperm carried by the ascending route in the genital tract (11). On the other hand, presence of specific bacterial populations in the vagina has risks for the development of reproductive problems (11,12). Contamination of the female genital tract or dysbiosis of the vaginal flora leads to infections of genital tract, abortion, premature parturition (10,11). Repeat breeding syndrome is another major infertility problems of herds (13). Animals exhibiting regular estrus cycle and normal heat signs but failed to conceive after three or more inseminations are named as repeat breeders (13). Subclinical endometritis is one of the many variables that might lead to repeat breeder syndrome, and its significance shouldn't be overlooked.

Although studies on genital canal flora in cattle generally focus on uterus. Considering the significance of vaginal flora being the first entrance of microorganisms to genital canal, this study aimed to compare the vaginal cultivable aerobic bacterial flora of repeat breeder cows and healthy pregnant cows in order to discuss the effects on reproduction in the light of literatures.

MATERIAL and METHOD

Study groups

A total of 45 Holstein breed dairy cows aged between 3 and 8 years including 20 repeat breeder (RB) cows (Group I = RBG) that have undergone three or more inseminations without conception and showed no genital pathology, alongside 25 healthy pregnant (HP) cows (Group II=HPG) that conceived within one or two inseminations were used as study groups

Sampling

The vaginal swab samples collected from RBG and HPG were collected on the 60th day following artificial insemination. Before vaginal swab sampling from cows in order to prevent contamination, the tail was lifted upwards, and the external genital area with vulval lips was cleaned with benzalkonium hydrochloride (Zefirolum®, Kimpa, Istanbul). Then, the area was dried with a sterile towel. Subsequently, with gloved hands, a sterile cotton swab on a polypropylene shaft was rotated for more than 10 seconds between the opened vulval lips (by the same individual each time), sampling was done from the posterior and dorsal aspects of the vagina. Swabs were transferred into sterile tubes containing thioglycolate broth as a transport media and transported to the laboratory at 4 °C and immediately processed for bacteriological examination.

Bacteriological examination

For bacteriological examination, all vaginal swab samples were incubated at 37 °C for 24 hours in fluid thiogly-collate (Becton-Dickonson BBL, 221196, USA). On the next day, part of the initial culture was inoculated onto blood agar (Becton-Dickonson BBL, 297876, USA) and EMB agar (Eosin-Methylenblue-Lactose-Saccharose) (Becton-Dickonson BBL, 221355, USA) and incubated at 37 °C for another 24 hours. According

to colony morphology and Gram color features, identified colonies were quantitatively assessed. For the identification of the bacteria, their direct cultures were performed using BBL Crystal (Becton-Dickinson, Sparks, USA) Gram-Positive and Gram-Negative ID system kits and its computer program.

According to the subparagraph k of the 8th article of the "Regulation on the Principles and Procedures of Animal Experiment Ethics Committees" published in the Official Gazette dated 15.02.2014 and numbered 28914, the collection of fecal or bedding samples and sample collection by swabbing are not subject to the approval of the Local Ethics Committee for Animal Experiments (HAYDEK).

RESULTS

Creating the OA Model

Frequency of 45 samples with bacterial isolation was determined to be 86.66% (39/45). All of the vaginal swab samples (100%) collected from RBG exhibited bacterial growth whereas 19 out of 25 vaginal swabs (76%) from HPG were found to be positive in terms of bacterial isolation.

A total of 26 species of 16 genera distributed among 4 phyla including Protobacteria, Firmicutes, Actinobacteria, Bacteriodetes were identified (Figure 1). Comparing the number of phyla distributed between two groups were as follows: Twenty-one Proteobacteria, three Actinobacteria were determined in RBG. One Bacteriodetes, 32 Protobacteria, two Actinobacteria were detected in HPG. Both groups shared the same number of bacteria (n=14) belonging to Firmicutes phylum (Figure 1). The most frequent species belonging to predominant genera for RBG and HPG were *Bacillus* (36.84%, n=14/38), *Sphingomonas* (15.78%, n=6/38), *Flavimonas* (n=13.15%, 5/38), *Esherichia* (7.89%, n=3/38) *Corynebacterium* (7.89%, n=3/38) genera, and *Bacillus* (31.25%, n=15/48), *Sphingomonas* (18.75, n=9/48), *Flavimonas* (14.5%, 7/48), *Actinobacter* (6.25, n=3/48) genera, respectively (Figure 2).

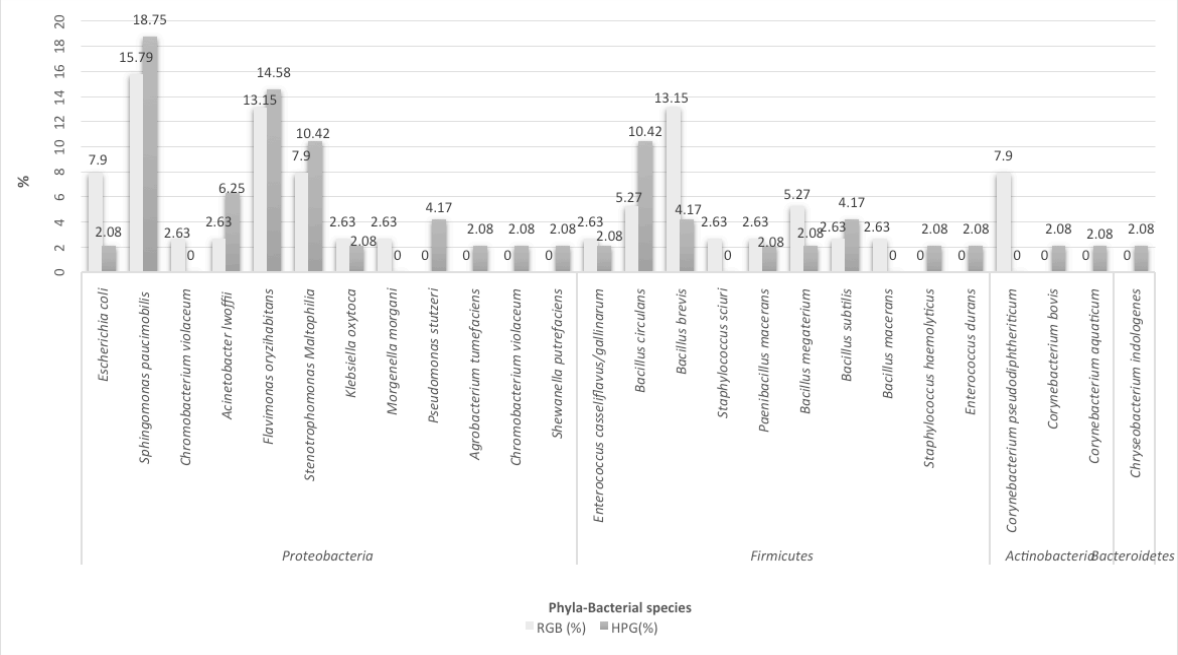

Figure 1. Distribution of species and phyla between Repeat Breeder Group (RBG) and Healthy Pregnant Group (HPG)

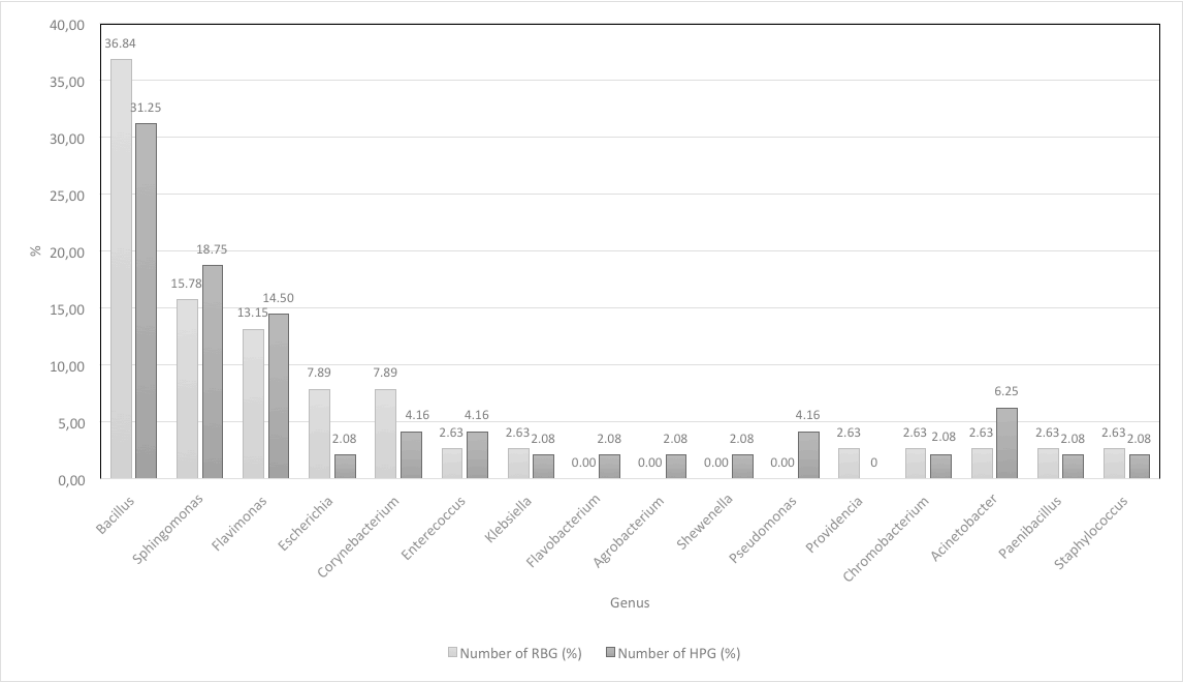

Figure 2. Distribution of genera between repeat breeder group (RBG) and healthy pregnant group (HPG)

In RBG, the most isolated species shared the first five places were Sphingomonas paucimobilis (15.79%), *Flavimonas oryzihabitans* (13.15%), *Bacillus brevis* (13.15%), *Escherichia coli* (7.90%), *Corynebacterium pseudodiphtheriticum* (7.90%), and *Stenotrophomonas maltophilia* (7.90%) while *Sphingomonas paucimobilis* (18.75%), *Flavimonas oryzihabitans* (14.58%), *Stenotrophomonas maltophilia* (10.42%), *Bacillus circulans* (10.42%), and *Acinetobacter iwoffii* (6.25%) was observed as the first five species (Figure 1). Regarding cows with RB syndrome Bacillus represented most heteregenous group, including 6 different species with a predominance of *Bacillus brevis* (13.15%) while HPG represented 5 different species (Figure 1).

HPG presented relatively numerically greater richness with 21 species followed by the RBG group with 17 identified species. Bacterial species common to both two groups were as follows: *E. coli*, *Enterococcus casseliflavus/gallinarum*, *Sphingomonas paucimobilis*, *Bacillus circulans*, *Bacillus brevis*, *Acinetobacter iwoffii*, *Flavimonas oryzihabitans*, *Paenibacillus macerans*, *Bacillus megaterium*, *Stenotrophomonas maltophilia*, *Bacillus subtilis*, *Klebsiella oxytoca* (Figure 1). The following were the bacterial species that were isolated in solely RBG: *Chromobacterium violaceum*, *Staphylococcus sciuri*, *Corynebacterium pseudodiphtheriticum*, *Bacillus macerans*, and *Morgenella morgani*. *Agrobacterium tumefaciens*, *Staphylococcus haemolyticus*, *Chryseobacterium indologenes*, *Chromobacterium violaceum*, *Corynebacterium bovis*, *Shewanella putrefaciens*, *Enterococcus durans*, *Corynebacterium aquaticum* were determined in solely HPG (Figure 1).

DISCUSSION

In postpartum dairy and beef cattle, colonization by microbial infections in the reproductive system can have a negative impact on reproduction. For

instance, uterine infections brought on by harmful bacteria found in dairy cows are common and have an impact on fertility through mediating anovulation and damaging developing oocytes in a way that hinders fertilization or obstructs natural development (14). Fertility disorders like "repeat breeder syndrome" (RBS) can be brought on by postpartum disease that progresses into a chronic subclinical infection (15). According to Bhat et al. (15), uterine bacterial colonization can result in inflammation, mucosal denudation, alterations in secretion, and embryonic mortality. It can also increase the number of services provided per conception, early culling rates, and days open (16). Infection takes place in the uterus of the cow right after calving. The cow is usually able to clear the infection within a few days after calving but if this does not happen then metritis can develop, but also other forms of uterine disease and inflammation such as subclinical endometritis will take place (17).

Reproductive diseases may arise as a result of the presence or absence of particular bacterial populations in the vagina (5,18,19). Because the microorganisms that live there constantly contact with the host mucosa, they serve as the first line of defense against sperm and ascending infections in the bovine reproductive tract. The vaginal microenvironment has recently been suggested to play a significant role in bovine fertility (20-22), despite the fact that research on the bacteria residing in the bovine reproductive tract has historically focused on pathogen populations that colonize the uterus during the postpartum period (12). The abnormalities in the vaginal communities in human females were demonstrated to have been linked to reproductive diseases like pelvic inflammatory illness, miscarriages, and preterm births by Digiulio et al. (23). Therefore, it is crucial to investigate the vaginal microbial ecology in order to comprehend the possible impact of a persistent and disrupted postpartum microbiota on mother health (23), as well as cow health.

Regarding the reproductive performance dairy cows affected by uterine diseases according to the results of existence of microorganisms in the genital canal environment was controversial. In the current study, we associated failed pregnancy outcomes with possible subclinical endometritis based on a rate of 100% bacterial isolation from the vaginal samples of RBG. Contrary to our assumption, Sens and Heusier (24) highlighted positive isolation of microorganisms was not one of the main evidence to subclinical endometritis. Moreover, Paiano et al. (25) demonstrated that 40% of genital channel samples from subclinic endometritis cows did not exhibit bacterial isolation. On the other hand, it was interpreted that the 76% bacterial isolation rate determined in HPG was too high to be underestimated. The positive bacterial microflora of genital canal was thought to support pregnancy in line with Sens and Heusier (24), and Gilbert and Santos (26).

Consistent with our findings, Firmicutes and Proteobacteria were reported the most commonly bacterial phyla found in the vagina of dairy cow by Laguardia-Nascimento et al. (27) and Nesengani et al. (28), but not for Bacteroidetes. The cause of repeat breeder syndrome are categorized into three main reasons comprising ovulation delay, uterine microbiota and luteal deficiency (29). The abundances of bacterial communities that fluctuate during different phases of the estrous cycle in cows are influenced by either high oestrogen or low progesterone levels (30). Research has shown that around days 10–12 after insemination, milk and plasma progesterone concentrations are lower in cows that do not conceive than in animals that conceive over the same period. The consistently low progesterone concentration throughout the estrous cycle is associated with pregnancy failure. Ault et al. (21) reported that the increase of Firmicutes in the vagina was attributed to the decrease in progesterone concentration. However, in this study, it was determined that the abundance of the Firmicutes phylum was equal in both the RBG and the HPG, in line with Moreno et al. (11).

However, the relative abundance of Proteobacteria

(n=32) in HPG comparing with the RBG was associated with an increase in progesterone in line with the declaration of Ault et al. (18). The results suggested that fluctuation of vaginal bacterial population is dependent on the circulating steroid hormones (31). In contrast to the findings of Ault et al (18), the relative abundance of Protobacteria phylum compared with Firmicutes in RBG in the current study was associated with repeat breeder syndrome (RBS) due to being the one of the three phyla not only involved in postpartum uterine infections but also commonly associated with bovine necrotic vulvovaginitis (5, 32). Parallel to Morena et al. (11), Protobacteria (N=21) was determined the most abundant pylum with following Firmicutes (n=14), and Actinobacteria (n=3).

Postpartum disease can evolve into chronic subclinical infection, causing fertility disorders such as the RBS (15). Furthermore, it has been reported that subclinical lesions and inflammation caused by microbiota alteration in the genital system canal can have negative effects during the transportation of spermatozoa, sometimes hindering the formation of fertilization (33). Within first two weeks of calving, up to 40% of animals having a kind of metritis and in 10-15% of these animals infection persists for at least another 3 weeks causing chronic uterine endometritis was a general agreement (1). Moreover, 30-35% of cows have subclinical endometritis between 4 and 9 weeks postpartum (34). Salasel et al. (35) reported that subclinical endometritis was one of the main reason of cows to become repeat breeder. Actinobacteria, Firmicutes and Proteobacteria phyla were reported as an intrauterine bacteriological findings in repeat breeder cows by Pothmann et al. (36). In our study, the same phyla emphasized by Pothmann et al. (36) were determined in both cultivable aerobic vaginal microbiota of RBG and HPG. In the current study, RBG presented numerically lower richness (38 identified species) whereas 48 bacterial species were identified among HPG in line with the Paiano et al.'s (25) results. In terms of the numerically variety of bacteria present in the genital canal, researches indicated that healthy cows possessed a more diverse microbiota in comparison to those suffering from subclinical and chronic endometritis (11, 25).

Sphingomonas paucimobilis, *Flavimonas oryzihabitans* dominance in both groups did not make sense regarding reproduction when we evaluated the first two most frequently isolated species for both groups. The most frequently isolated *Bacillus* genus with the rate of 36.84% in RBG were found to be concordent with a study conducted in Brazil which declared the most frequent genus as *Bacillus* in cows suffering from subclinical endometritis (25). However, Ballas et al. (37) demonstrated that *Bacillus* isolation rate from the genital channel samples of healthy cows was more than the cows suffering from endometritis. Whereas *Bacillus* genus with the rate of 31.25% in HPG was too high to ignore. The significant of the presence of *Bacillus macerans* and abundance of *B. brevis* (13.15%) in RBG when compared to HPG in the current study regarding pregnancy should be examined deeply in further studies.

The relative abundance of *Escherichia* genus and *Escherichia coli* with the rates of 7.89% and 7.90% in RBG was found to overlap the study reported the presence of *Escherichia* and *Truperalla* associated with clinical endometritis (38). The presence of numerous adhesion molecules in *E. coli*, particularly its adhesion to the genital region via the fimH gene, formation of biofilm with Type 1 pili, and its exotoxins, have been shown to increase the severity of uterine infections in studies (39). However, in our study, *E. coli* isolation in RBG was not attributed to clinical endometritis because no clinical signs observed in cows. However, the abundance of subclinical uterine colonization of *E. coli* was commented to be considerable due to decreasing the fertility by damaging uterus and inducing ovarian dysfunction by influencing to some degree the pulsatile secretion of luteinizing hormone, the lifespan of corpus luteum and delaying uterine inovulation (11,31).

Pascottini et al. (40) demonstrated that cows with subclinical endometritis had greater

relative abundance of *Corynebacterium* spp., and *Staphylococcus* than cows with clinical endometritis. Although the rate of 7.89% abundance was determined in RBG, and *Corynebacterium pseudodiphtheriticum* was determined in only RBG, the abundance of Corynebacterium genus was thought to be no effect on reproduction. Competible with our comment, Pothmann et al. (36) reported the abundance of Corynebacterium genus with the rate of 20.7% and when the researchers measure progestrone and eastridiol and combined with ultrasonographic asssessment of ovaries, the results indicated an ovarian activity was observed in 95% of the RB cows suffering from subclinical endometritis. In contrast to those, there were studies indicated the association of subclinical endometritis with Corynebacterium genus (41). The abundance of *Stenotrophomonas maltophilia* was not thought to be associated with reproduction problems due to the rate of bacteria in HPG was higher than RBG in the current study.

As a result, we thought that the bacterial diversity of the bovine vagina, the differences in vaginal microbial composition, and the dynamics of vaginal bacterial communities may be associated with reproductive failures and successes. This study identifies differences based on the phylum composition of the naturally occurring aerobic cultivable bacterial communities in the vagina of RB and HP cows. Furthermore, this study also contributes to the knowledge of the species-level local aerobic cultivable bacterial consortium in the vagina of RB and HP cows. The outcomes of the study provides fundamental information that serves as a resource in designing strategies, especially those aimed at enhancing reproductive success.

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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Coronavirus disease-19 risk factors

Koronavirüs hastalığı-19 risk faktörleri

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ABSTRACT

Coronavirus disease-19 (COVID-19) has spread around the world and been declared as a global pandemic by the World Health Organization. The disease has caused international social and economic impact, and high mortality. Quarantine and vaccination measures were used to control and prevent the disease. Several different types of vaccines have been developed against COVID-19, and novel drugs for COVID-19 are currently under development. Severity of disease can change depending on the predisposing conditions and risk factors. Many studies have been carried out to identify risk factors associated with the occurrence of the disease and severity of clinical manifestations, but different predisposing conditions and potential risk factors have been reported in these studies. Therefore, in this article, a review of studies was conducted to identify risk factors related to the COVID-19. The search was carried out in PubMed and publicly available preprints. Results of the literature review showed that age (>60 years old), male gender, Black and Hispanic race, obesity (body mass index > 30), underlying comorbidities such as chronic lung, kidney, liver, and heart diseases, diabetes, immunosuppression

ÖZET

Koronavirüs hastalığı (COVID-19) tüm dünyaya yayılmış ve Dünya Sağlık Örgütü tarafından küresel bir salgın olarak ilan edilmiştir. Hastalık uluslararası sosyal ve ekonomik etkiye ve yüksek ölüm oranına neden olmuştur. Hastalığı kontrol altına almak ve önlemek için karantina ve aşılama önlemleri kullanılmıştır. COVID-19'a karşı farklı aşı türleri geliştirilmiş olup tedavisi için de yeni ilaç geliştirme çalışmaları sürdürülmektedir. Hastalığın şiddeti predispozan koşullara ve risk faktörlerine bağlı olarak değişebilmektedir. Hastalığın ortaya çıkışı ve klinik belirtilerin şiddeti ile ilişkili risk faktörlerini belirlemek için birçok çalışma yapılmış ancak bu çalışmalarda farklı predispozan koşulların ve potansiyel risk faktörlerin ilişkili olduğu bildirilmiştir. Bu nedenle, bu makalede COVID-19 ile ilişkili risk faktörlerini belirlemek için yayınların incelemesi yapılmıştır. Yayın taraması, PubMed ve halka açık ön baskılarda gerçekleştirilmiştir. Literatür tarama sonuçları, yaş (>60 yaş), erkek cinsiyet, Siyah ve Hispanik ırk, obezite (vücut kütle indeksi >30), kronik akciğer, böbrek, karaciğer ve kalp hastalıkları, diyabet, immünsupresyon ve hipertansiyon gibi altta yatan komorbiditelerin, hastalığın oluşma riskini artıran ana

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and hypertension are the major factors increasing the risk of disease occurrence. It has been reported that the mortality rate due to SARS-CoV-2 infection was approximately three times higher in patients aged 41-60 years than in patients aged 18-40 years. Patients with active cancer are identified as at risk of severe forms of COVID-19. Severe outcomes of the disease are more common in pregnant women than non-pregnant women. Furthermore, smokers have a nearly two times higher risk of hospitalisation and have a six times higher risk of mortality than never-smokers. To establish a successful control program, it is important to determine the potential risk factors associated with the disease. Therefore, it is recommended to use risk assessment tools to develop effective public health strategies against pandemics.

Anahtar Kelimeler: COVID-19, SARS-CoV-2, pandemics, risk factors

faktörler olduğunu göstermiştir. Şiddetli akut solunum sendromu koronavirüs 2 (SARS-CoV-2) enfeksiyonuna bağlı ölüm oranının 41-60 yaş aralığındaki hastalarda, 18-40 yaş aralığındaki hastalardan yaklaşık üç kat daha fazla olduğu bildirilmiştir. Aktif kanseri olan hastalar, şiddetli COVID-19 formu riski altında olarak tanımlanmaktadır. Hamile kadınlarda, hamile olmayan kadınlara göre hastalığın şiddetli formu daha yaygındır. Ayrıca, sigara içenlerin hiç sigara içmeyenlere göre yaklaşık iki kat daha fazla hastaneye yatış riski ve altı kat daha yüksek ölüm riski bulunmaktadır. Başarılı bir kontrol programı oluşturmak için hastalık ile ilişkili potansiyel risk faktörlerinin belirlenmesi önemlidir. Bu nedenle, pandemilere karşı etkili halk sağlığı stratejilerini geliştirmek için risk değerlendirme araçlarının kullanılması önerilmektedir.

Key Words: COVID-19, SARS-CoV-2, pandemi, risk faktörleri

INTRODUCTION

The first case of Coronavirus disease-19 (COVID-19) was reported in China at the end of 2019, and then it has spread rapidly across countries due to the global connectivity and transportation worldwide. On 11 March 2020, it was declared as a pandemic. A novel coronavirus, the causative agent of the COVID-19 named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), belongs to the genus *Betacoronavirus* within the family *Coronaviridae* (1). The disease is characterised by fever, headache, fatigue, cough, myalgia, diarrhoea, and pneumonia. As of 18 March 2023, the total number of global confirmed cases and deaths were 760,360,956 and 6,873,477 deaths, respectively (2). The case fatality rate of disease ranged between 0.1% and 18.1% (3).

SARS-CoV-2 is mainly transmitted person to person via respiratory droplets and aerosols during sneezing and coughing. It has been reported that the proportion of asymptomatic COVID-19 cases could be as high as 81% (4). Asymptomatic carriers play role in the transmission of the disease. The severity of disease can change from person to person. Predisposing conditions and risk factors are associated with disease occurrence and severity of clinical manifestations (5).

Assessment of risk factors could be helpful for clinicians in identifying high risk patients. Furthermore, risk factors are important for developing effective health strategies against the next pandemic. Therefore, in this review available literature on risk factors related to COVID-19 was compiled (Table 1).

Table 1. The potential risk factors associated with severe outcomes of COVID-19

Strong associations	Weak associations	More studies needed to assess associations
Age (> 60 years old)	Chronic kidney disease	Ethnicity
Male gender	Smoking	Chronic liver disease
Chronic lung disease	Alcohol consumption	Allergy and asthma
Cardiovascular disease	Pregnancy	
Diabetes		
Cancer		
Obesity		

DEMOGRAPHIC FACTORS

Age and gender

The older age, especially >60 years, was frequently reported as a demographic factor associated with the severity of the disease (6,7). A study which was carried out in China found that mortality rates due to COVID-19 in patients ≤18 years, patients >18, ≤40 years, patients >40, ≤60 years, patients >60, ≤80 years and patients >80 years were 0.8%, 1.1%, 3.4%, 9.8% and 21.6%, respectively (8). Furthermore, a meta-analysis study reported that the risk of mortality and hospitalization due to SARS-CoV-2 infection increased per age year by 7.4% and 5.7%, respectively (9). Older age is associated with more comorbidities and weaker immune response (5). Furthermore, reduced levels of cells that express angiotensin-converting enzyme 2 (ACE-2) and lung progenitor cells in older adults may contribute to disease severity (10).

In terms of gender, males are more likely to susceptible to COVID-19 than females. The epidemiological studies reported that morbidity and mortality rates were higher among males than females (8,11,12). Furthermore, a meta-analysis study showed that male patients were required more intensive treatment unit admission than female patients (OR 2.84, 95% CI 2.06-3.92) (13). The differences in susceptibility to COVID-19 between males and females could be due to higher expression of ACE-2 in males, sex-based immunological differences, biology, genetics and behaviours (11).

Ethnicity

Ethnicity has been linked with higher SARS-CoV-2 infection rates (14). Studies which were carried out in the USA reported that Black and Latino patients had higher rates of hospital mortality than White patients (15,16). However, another study which was carried out in the USA found that ethnicity was not related to higher mortality in COVID-19 hospitalized patients (17). The ethnicity effect on the severity of the disease may be associated with social determinants of health such as employment, working conditions and housing (15).

PRE-EXISTING COMORBIDITIES

Chronic lung disease

Some early studies suggested that having a chronic lung disease is associated with high mortality and hospitalization rates due to SARS-CoV-2 infection (18, 19). A previous study which was carried out in China reported that rate of patients with chronic lung disease was highest in the critically ill group (20). However, a population cohort study reported that there was a lower risk of SARS-CoV-2 infection in patients who had COPD or interstitial lung disease (21). Severity of the disease in patients who had chronic lung disease could be explained by limited pulmonary reserve, restrictive ventilatory dysfunction and use of inhaled steroids (18,21).

Chronic liver disease

When compared to patients who had no pre-existing liver diseases, patients with pre-existing liver diseases have a higher susceptibility to SARS-CoV-2 infection (12). It has been reported that chronic liver diseases are at increased risk because of immunosuppression and metabolic dysfunction (22). A study which was carried out in the United States found that patients with chronic liver disease had a higher mortality rate (12%) than those without liver disease (4%) (23). On the contrary, another study found that having a chronic liver disease was not related to increased risk of mortality (OR 2.33) and severe outcomes of infection (OR 0.96) (24). Further studies are required to improve our knowledge about the impacts of pre-existing liver diseases on the severity of the disease.

Chronic kidney diseases (CKD)

Previous studies found that there was an increased risk of severe clinical manifestations in patients with CKD (24-26). A previous study identified adverse effects such as mechanical ventilation, intensive treatment and acute respiratory distress syndrome for the CKD patients with a RR of 2.63 (95% CI 1.33-5.17) (27). Studies reported that hospitalization rates varied between OR 1.38 (95% CI 1.19-1.60) and OR 3.9 (95% CI 2.4-6.3) in patients with CKD and COVID-19 (25,28). CKD patients with COVID-19 have a higher risk because of the attenuated immune system activation, which increases the susceptibility to infections (29).

Cardiovascular disease

Epidemiological results suggest that cardiovascular diseases are considered to be risk factors for increased disease mortality (24,30). The Centers for Disease Control and Prevention (CDC) (31) reported that hypertension and cardiovascular diseases increase the risk of severe outcomes. Hypertension is highly prevalent among older adults. Therefore, older adults have been reported to be at higher risk (24). It has been reported that there is a nearly 2.5-fold increase in the risk of severe outcomes for patients with hypertension (32,33).

The mechanism underlying the severe form of the disease in patients with cardiovascular diseases remains unclear. However, it has been suggested that high levels of ACE-2 expression on cardiomyocytes may play a role in virus mediated injury and viral susceptibility, and myocardial injury may lead to life-threatening arrhythmias and severity of the disease (30).

Antihypertensive drugs, such as angiotensin II receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors (ACEIs), are frequently used for treatment of cardiovascular diseases. Therefore, it has proposed to use ARB and ACEI drugs in patients with hypertension who are at higher risk (34). However, it has been reported that COVID-19 positivity rates in patients with ACEIs/ARBs users were not significantly different from non-users (35).

Allergy and asthma

Some of the epidemiological studies reported that allergy and asthma were associated with severe outcomes of the disease (36, 37). However, a study which was carried out in England reported that allergic rhinitis and asthma (aged less than 65) have protective roles against COVID-19. Further studies are needed to improve our knowledge about the impacts of allergy and asthma on the severity of clinical manifestations.

Diabetes

Severe and fatal cases were observed in diabetic patients compared to non-diabetic patients (6,19). Several studies reported that diabetic patients had an almost two-fold greater risk for hospital admission (38-40).

The association between severity of the disease and diabetes can be explained by the immunosuppressive effects of hyperglycaemia and higher expression of ACE-2 in diabetic patients (41).

The upregulation of ACE-2 causes endothelial cell activation and chronic inflammation, which lead to dysfunction of the alveolar-capillary barrier (42).

Cancer

Patients with active cancer are identified as at risk of COVID-19 severity. It has been reported that COVID-19 patients with hematologic cancers have the highest mortality rates among cancer patients (43). Mortality rate was 21% among COVID-19 patients who had cancer and 7.8% in non-cancer patients (44,45). A study which was carried out in the United Kingdom involving 16,749 hospitalized patients, reported a higher risk of mortality for cancer patients, OR 1.13 (95% CI 1.02-1.24) (46). Another study found that cancer patients who were diagnosed less than one year ago had a 2-fold increased risk of severe outcomes (47). The high mortality rate among cancer patients could be related with comorbidities, age and gender. Generally, cancer is common in older age with coexisting chronic diseases. Type of cancer, chemotherapy and duration may influence the severity of COVID-19 (47).

Pregnancy

The CDC reported that severe COVID-19 outcomes are more common in pregnant women than non-pregnant women (48). A systematic review of 17 studies and 84 neonates reported adverse outcomes of the disease in pregnant women were preterm birth (21.3%), foetal distress (10.7%), low birth weight (<2500 g, 5.3%), neonatal death (1.2%), neonatal asphyxia (1.2%) and stillbirth (1.2%) (49).

Although there is no evidence of vertical transmission, detection of SARS-CoV-2 in neonates has been reported (49-51). During the first trimester of pregnancy, high expression of ACE-2 in the placenta makes this trimester the most susceptible period for COVID-19 (52). Furthermore, it has been reported that intrauterine transmission of SARS-CoV-2 seems to occur in the last trimester of gestation (50). First and third trimesters of pregnancy are characterized as pro-inflammatory phases that are suitable for the virus activity; therefore, there is a higher risk for occurrence of the disease in the first and third trimesters than the second trimester (53). Further studies are required to determine whether the SARS-CoV-2 can cross the placenta and cause infection in foetal tissues.

LIFESTYLE

Obesity

Obesity is identified as a risk factor for hospitalization due to COVID-19 (54, 55). A study which was carried out in France found that severe obesity (body mass index (BMI) > 35) and obesity (BMI > 30) rates were 28.2% and 47.6% of the 124 COVID-19 patients, respectively (56). Furthermore, males who were obese had a higher risk og having severe COVID-19 (57). Obesity was also found to be a risk factor for respiratory diseases in humans, such as H1N1 influenza virus infection (58). Effects of the obesity in severity of the disease might be related to inflammation, which effects lung parenchyma and bronchi, and raised cytokines such as interleukin 6, which may cause impaired immune response (59).

Smoking

Smoking increases the risk of respiratory diseases. However, there are inconsistent reports on the relationship between smoking and severe outcomes of the disease. Some early studies suggested that there was no relation between smoking and severe illness. A population-based study in the United Kingdom found that there was no association between SARS-CoV-2 related mortality and smoking (60,61). It has been reported that nicotine may inhibit virus entry into cells by downregulating the ACE-2 receptors and have a pharmacological effect in COVID-19 (62).

On the contrary, a large, population-based study reported that the SARS-CoV-2 related mortality rate was increased among smokers (14,63). It has been reported that current smokers had a nearly two times (OR 1.80, 95% CI 1.26-2.29) higher risk of

hospitalisation and had a six times (OR 5.91, 95% CI 3.66-9.54) higher risk of mortality than never-smokers. Smoking is identified as at risk of COVID-19 severity because it damages the lungs and immune system, causing a decreased ability to fight the disease (64).

Alcohol consumption

Alcohol consumption can increase the risk of acute respiratory distress syndrome (ARDS) (OR 1.89; 95% CI 1.45-2.48) through alcohol-induced oxidative stress, interference of alveolar macrophage function and alveolar epithelium dysfunction (65). Chronic ethanol abuse increases the risk of developing ARDS because it damages the respiratory ciliated cells and immune system due to reduced T lymphocytes numbers and decreased natural killer cells function (66).

It has been reported that drinkers had a lower risk of COVID-19 compared to non-drinkers if they were drinking below the guideline levels (67). A study in the United Kingdom found that consumption of champagne, fortified wine, red wine and white wine had protective effect against the disease, but consumption of cider, spirits and beer increased the COVID-19 risk. They also did not find an association between COVID-19 mortality and subtypes of alcoholic beverages, amount and frequency (68). Alcoholic beverages have phenolic compounds, which have antioxidant properties. The concentration of phenolic compounds is highest in red wine, whereas it is lowest in spirits (69). Phenolic compounds have inhibitory effects against viruses such as influenza virus and other respiratory viruses (70). Therefore, it has been suggested that phenolic compounds have beneficial effects on SARS-CoV-2 infection (68).

CONCLUSION

In conclusion, SARS-CoV-2 induces mild to severe disease, and severity of disease can change from person to person depending on the predisposing conditions and risk factors. Severe outcomes of the disease are more common in elderly males (> 60 years old) with obesity and/or comorbidities (such as cardiovascular diseases, diabetes and cancer). Thus, additional care for this population may be required. An adequate, well-balanced diet and vaccination may be protective against the disease in this population. Furthermore, during pandemics, clinicians should pay more attention to the management of weight gain in patients with obesity.

The population aged 60 and over in the world is rising over the years, so the number of individuals susceptible to the severe form of the disease is increasing. Therefore, specific measures to support older people must be implemented during an outbreak, including access to medical services, social support, and essential supplies in quarantine. Furthermore, the vaccine needs to be made more accessible for older people.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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