ISSN 2757-847X | e-ISSN 2718-0964

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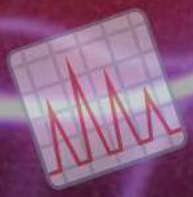
Experimental and Applied Medical Science



Official Journal of Gaziantep Islam Science and Technology University, Faculty of Medicine

August 2022 , Volume 3, Issue 2

GAZİANTEP İSLAM BİLİM VE TEKNOLOJİ ÜNİVERSİTESİ TIP FAKÜLTESİ

GAZİANTEP ISLAM SCIENCE AND TECHNOLOGY UNIVERSITY FACULTY OF MEDICINE

Experimental and Applied Medical Science

Volume 3, Issue 2

Official Journal of Gaziantep Islam Science and Technology University, Faculty of Medicine

ISSN: 2757-847X

e-ISSN: 2718-0964

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Dizinler/Indexing

Türkiye Atıf Dizini, Türk Medline, Google Scholar, Europub, Scilit, ASOS indeks, Advanced Science Index, Academic Resource Index, Eurasian Scientific Journal Index, Crossref, General Impact Factor

Publishing date: 22.08.2022
Yayın tarihi: 22.08.2022

Owner/İmtiyaz Sahibi

On behalf of the Medical Faculty of Gaziantep Islam Science and Technology University
Gaziantep İslam Bilim ve Teknoloji Üniversitesi Tıp Fakültesi adına

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Clerk of Editorial Office/Sorumlu Yazı İşleri Müdürü

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Aim

Experimental and Applied MedicalScience aims at being a current and easily accessible academic publication in which striking research results that will improve the quality of life and are unique from every field of medical sciences are presented.

Scope

Experimental and Applied MedicalScience is an open-access, internationally double-blind peer reviewed academic medical journal and published in English four times a year, under the auspices of Medical Faculty of Gaziantep Islam Science and Technology University. The journalreceives manuscripts for consideration to be publishing in the form of research articles, reviews, letter to editor, briefnotification, summary notification etc.which could have been presented from within the country or abroad and including experimental animal studies related to the pathogenesis of diseases, pharmacological, clinical, epidemiological and deontological studies, also studies in the fields of improving public health, health services or health insurance. During evaluation or publication no charge is demanded from authors.

The journal is published every 3 months (March, July, September and December) with 4 issues per year. The literary language of the journal is English. Abstract part of the manuscript only should also be submitted in Turkish.

Amaç

Experimental and Applied Medical Science, yaşam kalitesini arttıracak çarpıcı araştırma sonuçlarının sunulduğu, tıp bilimlerinin her alanında benzersiz, güncel ve kolay erişilebilir bir akademik yayın olmayı hedeflemektedir.

Kapsam

Experimental and Applied Medical Science, Gaziantep İslam Bilim ve Teknoloji Üniversitesi Tıp Fakültesi himayesinde yılda dört kez İngilizce olarak yayınlanan açık erişimli, uluslararası çift kör hakemli bir akademik tıp dergisidir. Dergi, yurt içinden veya yurt dışından, hastalık patogenezleri ile ilişkili deneysel hayvan çalışmalarını, klinik, farmakolojik, epidemiyolojik, deontolojik çalışmalar ile beraber halk sağlığının geliştirilmesi amacı taşıyan ve sağlık hizmetleri veya sağlık sigortaları konularında araştırma makalelerini, derlemeleri, vaka sunumlarını, kısa bildirimleri, özet bildirimleri vs. yayınlamak için değerlendirmeye kabul etmektedir.

Değerlendirme veya yayın sırasında yazarlardan herhangi bir ücret talep edilmez.

Dergi 3 ayda bir (Mart, Temmuz, Eylül ve Aralık) yılda 4 sayı olarak yayımlanır. Derginin yazı dili İngilizcedir. Makalenin sadece özet kısmı Türkçe olarak da gönderilmelidir.

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Manuscripts are only considered for publication provided that they are original, not under consideration simultaneously by another journal, and have not been previously published. Direct quotations, tables, or illustrations that have extracted from any copyrighted material must be accompanied by written authority for their use from the copyright owners. All manuscripts are subject to review by the editors and referees. Deserving to be publishing is based on significance, and originality of the material. If anymanuscript is considered to deserve publishing, it may be subject to editorialrevisions to aid clarity and understanding without changing the data presented.

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Editorial Board declares that all reported or submitted studies conducted with "human beings" should be in accordance with those principles.

Manuscripts presenting data obtainedfrom a study design conducted with human participants must contain affirmation statements in the *Material and Methods* section indicating approval of the study by the institutional ethical review committee and "informed consent" was obtained from each participant. Also all manuscripts reporting experiments in which laboratory animals have been used should include an affirmation statement in the *Material and*

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Makaleler, orijinal/özgün olmaları, eş zamanlı olarak başka bir dergi tarafından incelenmemeleri ve daha önce yayınlanmamış olmaları koşuluyla yayına kabul edilebilmesi için değerlendirmeye alınır. Telif hakkıyla korunan herhangi bir materyalden alınan doğrudan alıntılar, tablolar veya resimler, kullanımları için telif hakkı sahiplerinden alınan yazılı izinle birlikte sunulmalıdır. Tüm yazılar editörler ve hakemler tarafından incelemeye tabidir. Yayınlanmaya hak kazanılması, materyalin önemine ve özgünlüğüne bağlıdır. Herhangi bir makalenin yayınlanmayı hak ettiği düşünülürse, sunulan veriler değiştirilmeden netlik ve anlayışa yardımcı olmak için editör revizyonlarına tabi tutulabilir.

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Editör Kurulu, "insan" ile yapılan tüm raporlanan veya sunulan çalışmaların bu ilkelere uygun olması gerektiğini beyaneder. İnsan katılımcılarla yürütülen bir çalışma tasarımından elde edilen verileri sunan makaleler, *Gereç ve Yöntemler* bölümünde çalışmanın kurumsal etik inceleme komitesi tarafından onaylandığını ve her katılımcıdan "bilgilendirilmiş onam" alındığını belirten onay ifadeleri kullanmalıdır. Ayrıca laboratuvar hayvanlarının kullanıldığı deneyleri bildiren tüm yazılar, *Gereç ve Yöntemler*

Methods section validating that all animals have received human care in compliance with the "Guide for the Care and Use of Laboratory Animals" whose web address is below and reveal approval by the institutional ethical review board. https://www.gibtu.edu.tr/Medya/Birim/Dosya/20210818130308_dca61056.pdf

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Processing and publication processes are free of charge. Any fee can not be requested from the authors during the evaluation and publication process. All manuscripts must be submitted via the online submission system, which is available at https://dergipark.org.tr/tr/pub/eams. The journal guidelines, technical information, and the required forms are available on the journal's web page.

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bölümünde, internet adresi aşağıda belirtilmiş olan "Laboratuvar Hayvanlarının Bakımı ve Kullanımı Kılavuzu"na uygun olarak tüm hayvanların insanî bir bakım aldığını doğrulayan bir beyan ile kurumsal etik inceleme kurulunun onayını içermelidir.

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Çalışma sürecine katkı sağlayan ticari bir ilişki veya çalışmaya maddi destek sağlayan bir kurum varsa; yazarlar ticari ürün, ilaç, aracılık eden şirket ile ticari bir ilişkilerinin olmadığını veya varsa ne tür bir ilişkisi (danışmanlık veya başka bir anlaşma) olduğunu beyan etmelidir.

Değerlendirme ve yayınlama süreçleri ücretsizdir. Değerlendirme ve yayın sürecinin hiçbir aşamasında yazarlardan ücret talep edilmez. Tüm yazılar https://dergipark.org.tr/tr/pub/eams adresinde bulunan çevrimiçi başvurusistemi üzerinden gönderilmelidir. Dergi ile ilgili kullanım kılavuzları, teknik bilgiler ve gerekli formlar derginin internet sayfasında yer almaktadır.

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Tüm araştırmacılar, makaleye doğrudan akademik veya bilimsel olarak katkıda bulunmuş olmalıdır. Yazarlar, makalenin planlanması, uygulanması, yazılması veya gözden geçirilmesi aşamalarından birine veya birkaçına katkıda bulunmuş olmalıdır. Tüm yazarlar nihai versiyonu onaylamalıdır. Bilimsel kriterlere uygun bir makale

responsibility to prepare a manuscript that meets scientific criterias.

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All manuscripts involving a research study must be evaluated in terms of biostatistics and it must be presented altogether with appropriate study design, analysis and results. ***p*** values must be given clearly in the manuscripts. Other than research articles, reviews, case reports, letters to the editor, etc. should also be original and up to date, and the references and, if any, their biostatistical parts should be clear, understandable and satisfactory.

The publication language of the journal is English. In addition, the abstract part of the article must be uploaded in both Turkish and English. Manuscripts should be evaluated by a linguist before being sent to the journal.

All manuscripts and ecorrespondence with the editorial board must be sent to the editorial office, at https://dergipark.org.tr/tr/pub/eams.

According to the Law on Intellectual and Artistic Works, which was first published in the Official Gazette with the law number 5846 on 13/12/1951, whose web address is below, and on which subsequently various changes have been made or novel parts added in time, all kinds of publication rights of the articles accepted for publication belong to the institution that

hazırlamak yazarların sorumluluğundadır.

Dergide yayınlanan yazılarda ifade edilenler veya görüşler, Gaziantep İslam Bilim ve Teknoloji Üniversitesi Tıp Fakültesi, editörler, yayın kurulu ve/veya yayıncının görüşlerini değil, yazar(lar)ın görüşlerini yansıtır; editörler, yayın kurulu ve yayıncı bu tür materyaller için herhangi bir sorumluluk veya yükümlülük kabul etmez.

Araştırma çalışması içeren tüm yazılar biyoistatistiksel açıdan değerlendirilmeli ve uygun çalışma düzeni, verilerin analizi ve sonuçları ile birlikte sunulmalıdır. ***p*** değerleri yazılarda açık olarak verilmelidir. Araştırma makaleleri dışında derlemeler, olgu sunumları, editöre mektuplar vb. de orijinal/özgün ve güncel olmalı ve kaynaklar ile eğer varsa biyoistatistiksel kısımlar açık, anlaşılır ve tatminkâr şekilde açıklanmış olmalıdır.

Derginin yayın dili İngilizce'dir. Ayrıca makalenin özet kısmı hem Türkçe hem de İngilizce olarak yüklenmelidir. Yazılar dergiye gönderilmeden önce bir dilbilimci/konunun uzmanı tarafından değerlendirilmelidir.

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Submission of a paper will be taken into consideration provided that it has not previously been published and not being considered at that moment for publication elsewhere. Decision as to publication of papers submitted to the ***Experimental and Applied Medical Science*** will be based on the opinion of the Editorial Board as to the significance and originality of the work.

Manuscripts should be prepared electronically by using “office word” or any other text-processing package compatible with that, formatted for A4 size, double-spaced throughout, and using a “Times New Roman” 12-point font. Articles must be written in English. Abstracts must be written in both Turkish and English. Text should flush left, and not be justified. Words should not be hyphenated. Pagesshould be numbered sequentially.

There should be a separate title page with:

a) The title
b) The authors' names
c) The laboratory of origin, with complete address of each author
d) A running title
e) Corresponding author and e-mail
f) Conflict of interest
g) Acknowledgements

The main body of full-length paper should be divided into:

1. Abstract
2. Introduction
3. Material and Methods
4. Results
5. Discussion

Yazım Kuralları

Bir çalışmanın dergimize gönderilmesi bu çalışmanın daha önce yayınlanmamış veya başka bir akademik dergide şu anda yayınlanmak üzere değerlendirilmiyor olması koşulu ile mümkündür.***Experimental and Applied Medical Science***’a gönderilen her türlü çalışmanın yayınlanmasına ilişkin karar, Yayın Kurulu'nun çalışmanın önemi ve özgünlüğü konusundaki görüşüne dayanacaktır.

Çalışmalar, ya “office word” programı ile ya da bu program ile uyumlu uygun bir metin işleme programı kullanılarak, A4 boyutunda hazırlanmalı, baştan sona çift aralıklı ve “Times New Roman” tarzında 12 punto yazı tipi kullanılarak elektronik ortamda yazılmalıdır. Makaleler İngilizce yazılmalıdır. Özetler hem Türkçe hem de İngilizce olarak yazılmalıdır. Metin iki yana yaslandırılmamalı, sadece sola yaslanmamalıdır. Kelimeler kısa çizgi ile hecelenmemelidir. Sayfalar sırayla numaralandırılmalıdır.

Aşağıdakileri içeren ayrı bir başlık sayfası olmalıdır:

a) Başlık
b) Yazarların isimleri
c) Her yazarın tam adresi ile birlikte çalıştıkları laboratuarlar
d) Kısa başlık
e) İletişimdeki yazar ve iletişim bilgileri
f) Çıkar çatışması beyanı
g) Bilgilendirme

Tam uzunluktaki kağıdın ana gövdesi şu bölümlere ayrılmalıdır:

1. Özet
2. Giriş

6. Conclusion
7. Conflict of interest
8. Acknowledgement
9. References

In general, there are no a maximum specific word length laid down as a condition for any manuscript. The general principle is that a manuscript should be as long as necessary to communicate the scientific message clearly and effectively at the most, but should be as short as possible to avoid undue repetition or redundancy with a complete presentation of the information.
In the *Materials and Methods* section, the source of all compounds, equipment or software should be identified by the full name of the supplier, city, state/country. The chemical names of any drug should precede the trade name.
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The results of statistical tests should be incorporated in the body of the text, typically in the *Results* section, rather than in figure legends. Adequate description of statistical analysis should be provided. Statistical measures of variation in the text, illustrations and tables, should be identified.

3. Gereç ve Yöntemler
4. Sonuçlar
5. Tartışma
6. Bağlam
7. Çıkar çatışması
8. Bilgilendirme
9. Kaynaklar

Genel olarak, herhangi çalışma için şart koşulan belirli bir kelime sayısı/metin uzunluğu yoktur. Genel ilke; bir makalenin bilimsel mesajı açık ve etkili bir şekilde iletmek için gerektiği kadar uzunolabileceği, ancak gereksiz tekrar veya fazlalık olmadan bilgilerin eksiksiz birsunumunu elde etmek için mümkün olduğunca kısa olması gerektiğidir.
Gereçler ve Yöntemler bölümünde, tüm bileşiklerin, malzemelerin veya yazılımların kaynağı, tedarikçinin tam adı, şehir, eyalet/ülke ile tanımlanmalıdır. Herhangi bir ilacın kimyasal isimleri ticari isminden önce gelmelidir.
Hayvan deneylerini açıklayan makaleler,tür, soy, cinsiyet, yaş, tedarikçi ve kullanılan hayvan sayısını açıkça tanımlamalıdır. Hayvanların kullanımına ilişkin bir etik beyan veya insan deneklerin etik kurul onayları, bilgilendirilmiş onamları ve çalışmaya dâhil edilmelerine ilişkin ayrıntılar açıkça belirtilmelidir. *Sonuçlar ve Tartışma* bölümleri, anlaşılırlığı artırmak için alt bölümlere ayrılabilir. Sonuçlar, metodolojik ayrıntıları tekrarlamamalı ve verilerin tartışılmasından kaçınmalıdır.
İstatistiksel testlerin sonuçları, şekillerin altındaki açıklama kısımlarından ziyade metnin gövdesine, tipik olarak Sonuçlar bölümüne dâhil edilmelidir. İstatistiksel analizin yeterli bir şekilde açıklaması sağlanmalıdır. Metinde, resimlerde ve

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All subscripts, superscripts, Greek letters and unusual characters must be clearly identified.

In the text, abbreviations should be used consistently. Abbreviations should be defined on first use.

References should be designed in “Vancouver” style. While writing references, “Times New Roman” 10 point font should be used. Multiple authors should be separated by a comma. If there are more than three authors, after the 3rd author, "et al." should be inserted with a comma, for both article and book references. If reference is made from a chapter in a book and there are many authors belonging only to this chapter, the title and chapter of the book are indicated, the first three of the chapter authors are written, and "et al." statement is added for subsequent authors.

Example:

1. Perell KL, Nelson A, Goldman RL, et al. Fall risk assessment measures: an analytic review. The journals of gerontology Series A, Biological sciences and medical sciences. 2001;56(12):M761-6.
2. Ha H, Han C, Kim B. Can Obesity Cause Depression? A Pseudo-panel Analysis. Journal of preventive medicine and public health = Yebang Uihakhoe chi. 2017;50(4):262-7.
3. Çekmen MB, Turgut M, Türköz Y, etal. Nitrik Oksit (NO) ve Nitrik Oksit Sentaz (NOS)'ınFizyolojik ve Patolojik Özellikleri. Türkiye Klinikleri Journal of Pediatrics. 2001;10(4):226-35.
4. Parlakpınar H, Örüm MH, Acet A. Kafeik

tablolarda istatistiksel varyasyon ölçütleri tanımlanmalıdır.

Tüm boyutlar ve ölçüler metrik sistemde belirtilmelidir.

Tüm alt simgeler, üst simgeler, Yunan harfleri ve olağandışı karakterler açıkça tanımlanmalıdır.

Metinde kısaltmalar tutarlı bir şekilde kullanılmalıdır. Kısaltmalar ilk kullanımda tanımlanmalıdır.

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Örnek:

1. Perell KL, Nelson A, Goldman RL, et al. Fall risk assessment measures: an analytic review. The journals of gerontology Series A, Biological sciences and medical sciences. 2001;56(12):M761-6.
2. Ha H, Han C, Kim B. Can Obesity Cause Depression? A Pseudo-panel Analysis. Journal of preventive medicine and public health = Yebang Uihakhoe chi. 2017;50(4):262-7.
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5. Yıldırım AB. The effects of maternal hypothyroidism on the immunoreactivity of cytochrome p450 aromatase in the postnatal rat testes. 2015; Doctoral thesis.

6. https://hsgm.saglik.gov.tr/depo/birimler/kanserdb/istatistik/Trkiye_Kanser_statistikleri_2016.pdf (Last access date: 21.09.2020).

7. Kuran O, İstanbul, Filiz Kitabevi. Sistematik Anatomi. 1983 p. 76-9.

8. Abbas AK, Andrew H Lichtman, Shiv Pillai. Cellular and Molecular Immunology. 6th ed. Philadelphia: Saunders Elsevier; 2007 p. 121-56.

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Tables of numerical data should each be typed with double spacing on separate pages numbered in sequence in numerals, provided with a heading, and referred to in the text, as Table 1, Table 2, etc. Each table should have a brief but descriptive heading. Explanatory matter should be included in footnotes to the table.

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Disclosure of conflict of interest and

4. Parlakpınar H, Örüm MH, Acet A. Kafeik asit fenetil ester (KAFE) ve miyokardiyal iskemi reperfüzyon (Mİ/R) hasarı. İnönü Üniversitesi Sağlık Bilimleri Dergisi 2012; 1: 10-5.

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financial support is required at the time of

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8. Abbas AK, Andrew H Lichtman, Shiv Pillai. Cellular and Molecular Immunology. 6th ed. Philadelphia: Saunders Elsevier; 2007 p. 121-56.

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Başvuru sırasında çıkar çatışmasının ve mali

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destek konularının açıklanması elzemdir. Yazarlar, makalelerinin yayımlanma tarihinden önce ortaya çıkabilecek ek çıkar çatışmalarını veya bulunan mali destekleri dergiye bildirmekle yükümlüdür. Tüm yazarlar, makalelerinin konusuyla doğrudan ilgili olsun ya da olmasın, tüm olası çıkar çatışmalarını ve mali desteği bireysel olarak açıklamalıdır.

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The Chancellor's Message

Dear Students and Academicians,

Islam has placed a huge emphasis on medicine since the beginning. According to the Islamic opinion, obeying certain medicinal recommendations is indispensable for a Muslim for both his and all society's good. Recently, the world has lived through unfortunate memories because of the pandemic. That is the neither the first nor the last threat for humanity. Hadiths narrated by Islamic scholars were even able to shed light on how to be at war with contagious diseases, epidemics or pandemics many centuries ago. Our beloved prophet, beloved servant of Allah (C.C.), Hz. Muhammed said that "If you hear of a plague somewhere, do not enter into there. If the plague occurs in your place, do not leave there", narrated by famous Islamic scholar Buhârî. This most fundamental principle for the fight against epidemics still remains valid today.

All advices regarding the medicine internalised from verses of the Quran, hadiths and the life of Hz. Muhammed are actually a set of principles, named as "Tıbb-ı Nebevî". Tıbb-ı Nebevî means medicinal principles and remarks of our prophet, Hz. Muhammed. It acts as a guideline for Muslims in certain major medical entities, such as general medicine, preventive medicine and treatment approaches. Hadith mentioned above obviously points out certain principles of preventive medicine. Besides, there are others, for instance, in a verse of the Quran, Allah (C.C) Almighty orders that mothers should breastfeed their babies for two years. Today, scientists announce a number of research studies revealing the benefits of breast milk and they suggest that a baby should be breastfed for two years provided that the baby should take only breast milk, not any other food supplement, during the first six months of the life.

We can find out lots of medicinal principles mentioned in the Quran or hadiths narrated by Islamic scholars. Also, Islamic world has managed to train honoured medical scientists during ages. One of famous medical scholars of his period was Ibn Sînâ who is well known with his genuine perspective through the medicine and adapting to orders of the Quran and medicinal principles of "Tıbb-ı Nebevî", really worth mentioning here. He wrote more than 100 books in the fields of medicine and philosophy and these were utilised in Europe as reference books until 18th century.

I believe in that Gaziantep Islam Science and Technology University Medical Faculty will be inspired by this great medicinal and cultural richness and will take its place in the modern medical world. I wish great success to the Medical Faculty Journal "Experimental and Applied Medical Science".

Wish you all the best

Prof. Dr. Mehmet Nihat Hatipoğlu
Chancellor of Gaziantep Islam Science and Technology University

Chief Editor's Message

Dear Readership,

While struggles continue at full speed to start education and training in our Medical Faculty which was brought to our country within the newly formed Gaziantep Islamic Science and Technology University, it has been just a kind of more than one year since our academic journal, the Experimental and Applied Medical Science in which we wholeheartedly believe will make a significant contribution to our academic community, sprouted. We are very happy to deliver the fifth issue of our academic magazine to our readership in print, as well as in electronic form.

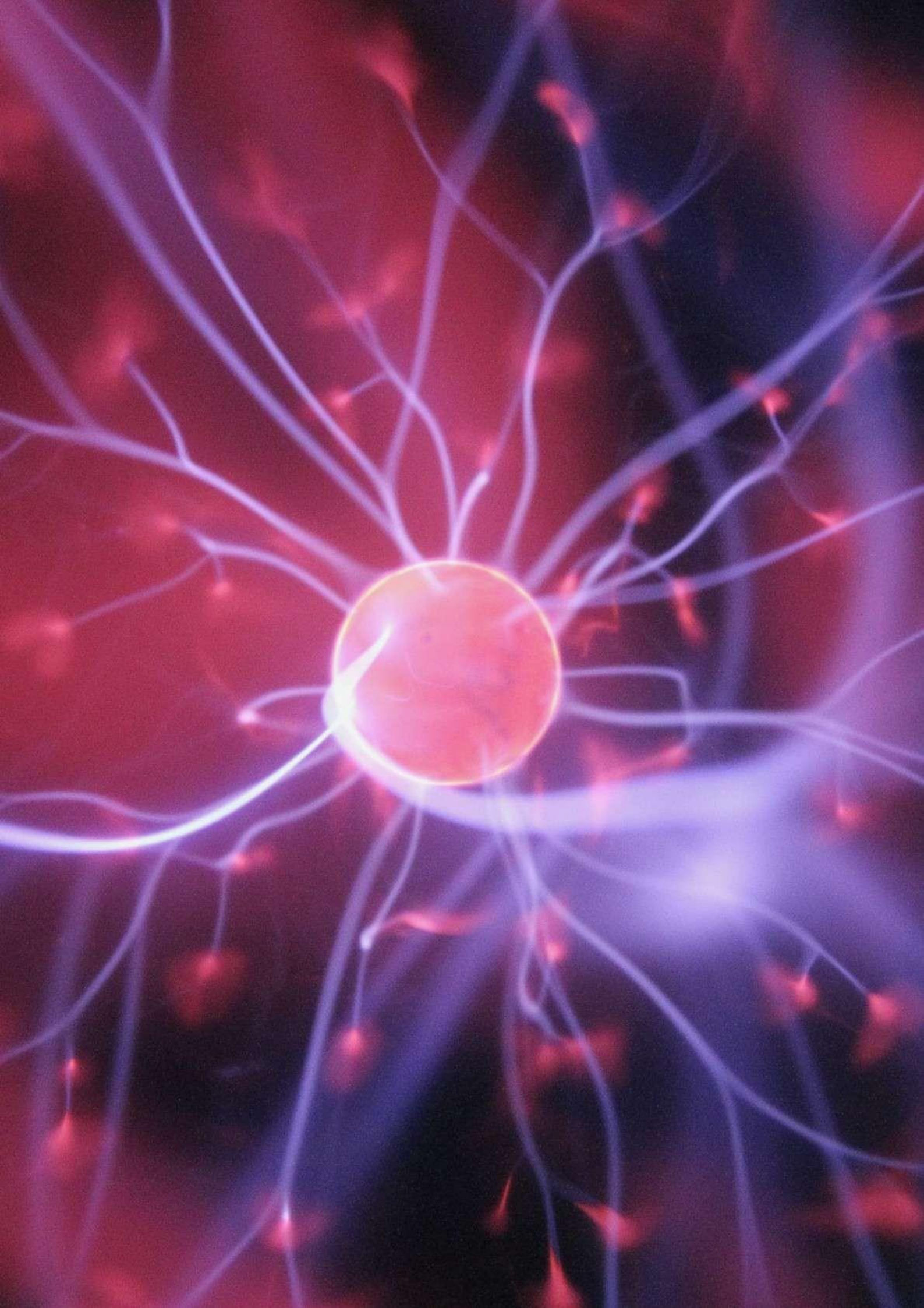
Nowadays, academic studies are accelerating, multiplying and diversifying. The need for channels where scientific studies, opinions and ideas can be freely expressed and easily shared with experts, researchers or postgraduate students who are still in the learning phase is increasing day by day. "Experimental and Applied Medical Science" has adopted it as a principle from the first day to bring together original and up-to-date studies, stimulating scientific views and ideas from every field of medicine that will potentially increase the quality of life with its readers both from home and abroad. With this fifth issue of our journal, we will continue to publish in English 4 (four) times a year, more than thirty manuscripts, in different types, research articles, case reports, reviews, etc. will have already been published and met with our readers. Recently, researchers have begun to understand the importance of having their studies published in international double-blind peer-reviewed journals. Since the first day of its publication, "Experimental and Applied Medical Science" has subjected the manuscripts received to an international double-blind peer review edevaluation process. For this reason, we aim not only to evaluate the manuscripts submitted with an aspect in which we decide whether the manuscript deserves to be publishing or not, but also to help researchers improve their educational or academic lives by providing on the spot feedback.

We are also happy that "Experimental and Applied Medical Science" which is only at the beginning of the road, has come a long way in a short time. In its a little more than 1 (one) year academic publication life, it has already started to be followed in nearly ten national or international indexes.

I would like to express my gratitude to our editorial and publishing boards, the esteemed academics who chose "Experimental and Applied Medical Science" for their manuscripts to have been submitted, all our readers, and our Rectorate for their unwavering support. I wish "Experimental and Applied Medical Science" the best success in its publication life.

Best Regards…

Chief Editor
Hamit Yıldız, Assoc. Prof.
Gaziantep University, Faculty of Medicine, Department of Internal Medicine



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Use of Routine Laboratory Tests in Diagnosis of Crimean-Congo Hemorrhagic Fever in the Emergency Department

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Abstract

Crimean-Congo hemorrhagic fever (CCHF) is a disease effecting multiple organ systems by microvascular damage and deterioration of hemostasis. Even though the main diagnosis relies on reverse transcriptase-polymerase chain reaction (rt-PCR), it is also known that thrombocytopenia, and/or leukopenia, elevated levels of alanine aminotranferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase and creatine kinase may be determined. In this study, our aim was to analyse the patients who were hospitalized with suspected CCHF and consequently undergone PCR testing. PCR (+) and PCR (-) patients were compared according to their laboratory results obtained in the emergency department (ED). In a 2-year period, a total of 150 (female/male: 47/103) patients of any age hospitalized with the suspicion of CCHF were involved into the study. The patients were divided into 2 groups according to their rt-PCR results as PCR (+) (and PCR (-) patients. Two groups were compared according to the laboratory results obtained in the ED. The most common complaint on admission was weakness (n=111, 74%) followed by fever (n=95, 63.3%) and headache (n=16, 10.7%). Ribavirin therapy was administered to 62 patients (41.3%). In 62 patients, PCR test was positive (41.3%). When PCR (+) and PCR (-) groups were compared according to the laboratory results obtained in the ED, number of patients with high AST/ALT, thrombocytopenia, low fibrinogen and aPTT levels were significantly higher in PCR (+) group. The diagnosis of CCHF is a challenging issue which requires high suspicion, particularly in the endemic regions. High AST/ALT, thrombocytopenia, low fibrinogen and aPTT levels determined in the ED should raise the suspect for the possibility of PCR positivity.

Key words: *Crimean-Congo hemorrhagic fever, Diagnosis, Emergency department.*

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Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a serious disease caused by the CCHF virus of the Bunyaviridae family (1). Transmission of the virus to humans occurs through tick bites, squashing an infected tick without gloves, exposure to blood and tissues of infected animals or contact with the blood of an infected person during the acute phase of the disease (2,3). Commonly known reservoires of the virus are cattle, sheep, goats, hedgehogs and hares. Numerous species of ticks can carry the virus; however, very few of them have been implicated as vectors. Hyalomma spp. is the most important tick vector since the virus is commonly isolated from it and its geographic distribution coincides with that of the disease (2).

The exact diagnosis of the disease is based on molecular biology tests. CCHF may be documented by PCR detection of the virus genome during the first daysafter the onset of illness, and then by serological testing for IgM antibodies as from the 2nd week afterinfection (3).

Similar to other viral hemorrhagic fevers, the disease may be divided into 3 clinical phases. The pre-hemorrhagic phase is characterised by non-spesific symptoms. In this phase, high fever of 39-41° C which lasts for 4-5 days can be determined. Also, myalgia, headache, retro-orbital pain and sometimes a stiff neck can be present. It can also be associated with gastrointestinal symptoms then occur withnausea and/or vomiting and more rarely diarrhea. The pre-hemorrhagic phase may last up to one week. Mucous tissue bleeding emerges in the hemorrhagic pahse. Besides epistaxis,hematemesis, more rarely melena, hemoptysis and hematuria, skin manifestations such as ecchymosis/purpura may be observed. Third phase is the convalescence phase. It starts 10–20 days after the onset of the first clinical symptoms and lasts for 10 days. Fatigue, tachycardia with labile blood pressure, temporary alopecia and memory impairment may be observed (3).

Infection with the CCHF virus results in endothelial dysfunction and capillary leaking of red blood cells and plasma into tissue. Endothelial damage leads to activation of the coagulation cascadeand thrombocytopenia, which increases bleeding (4). Thus, patients with CCHF disease have leukopenia, thrombocytopenia, and elevated AST, ALT, LDH and CPK levels. Additionally, higher AST and ALT levels are associated with severe cases (5).

In treatment, a nucleosid analog Ribavirin is used since it has been shown to inhibit CCHF virus replication. If administered in the early phase, Ribavirin descreases the mortality (1). It is essential to detect the disease in the early phase and initiate the treatment with ribavirin and supportive measures (6).

It was previously reported that the predictors of CCHF in the Emergency Department (ED) were epistaxis and elevated K+, WBC and AST levels (2). In this study, we aimed to determine the patients who will experience the severe disease by analysing findings in the ED.

Materials and Methods

After obtaining ethical approval from the Local Ethics Comitee, (Decision no: 373, date 23/12/2020), we retrospectively collected the medical data of the patients hospitalized in Hitit University Hospital with suspected CCHF. A total of 150 (female/male: 47/103) patients of any age

hospitalized with the suspicion of CCHF between January 1st 2019 and December 31st 2020 were involved into the study. Blood samples of the patients were sent to an advanced center for RT-PCR test. CCHF virus RNA in the blood samples through rt-PCR evaluation were considered confirmed CCHF cases.

Patients were divided into two groups as PCR (+) (group 1) and PCR (-) (group 2) according to the test results. Demographical features, complaints on admission, vital signs (temperature, blood pressure, pulse rate) and physical examination findings (according to the systems) of the patients were investigated. Besides, laboratory findings of the patients such as complete blood count (CBC), blood biochemistry test, and coagulation panel were investigated and compared according to the groups.

Statistical analyses were performed with SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Descriptive data were presented as mean ± standard deviation (SD). The ShapiroWilk test was used to analyze normal distribution assumption of the quantitative outcomes. To compare two groups, Student t test was used for normally distributed data and Mann-Whitney U test was used for non-normal data. $p<0.05$ was accepted as statistically significant in comparisons.

Results

Into the study, a total of 150 patients were included. Of these patients, 103 (68.7%) were male. Mean age of male patients was 47.88 ± 17.37 (min-max: 18-89 years). Number of female patients was 47 and mean age of female patients was 45.43±8.8 (min-max: 18-75 years). Ninety-six patients lived in rural areas (64%) and 54 patients lived in the city center (36%). When complaints on admission to the ED were investigated, 111 had weakness (74%), 95 had fever (63.3%), 16 had headache (10.7%), 12 had diarrhea (8%), 4 had abdominal pain (2.7%), and 3 had syncope (2%). General characteristics of the patients are listed in Table 1.

Table 1: General characteristics of the groups.

	n (%)	**n PCR (+)**	**n PCR (-)**	***p***
Symptoms				
Weakness	111 (73.5%)	52	59	0.021
Fever	95 (62.9%)	35	60	0.142
Headache	16 (10.6%)	10	6	0.069
Diarrhea	12 (7.9%)	6	6	0.525
Abdominal pain	4 (2.6%)	1	3	0.501
Syncope	3 (% 2)	3	0	0.037
Method of Treatment				
Ribavirin Therapy	62 (41.1%)	40	22	0.0
Outcome				
Discharge	149 (98.7%)	62	87	0.4

In 62 patients, PCR test was positive (41.3%). Ribavirin treatment was performed to 62 patients (41.3%). Mean length of stay in the hospital was 5.13±3.15 days (min-max: 1- 21 days). When two groups (PCR (+) and PCR (-) were compared, number of patients with high AST/ALT, thrombocytopenia, low fibrinogen and aPTT levels were significantly higher in PCR (+) when compared to the number of PCR (-) patients. Comparison of PCR (+) and (-)

groups in terms of laboratory results are summarized in Table 2.

Table 2: Comparison of laboratory findings of the groups.

Laboratory Finding	n (%)	PCR (+)	PCR (-)	*p*
AST elevation	84 (55.6%)	50	34	0.015*
ALT elevation	63 (41.7%)	40	23	0.019*
CK elevation	48 (31.8%)	25	23	0.067
CRP elevation	107 (70.9%)	42	65	0.589
Fibrinogen decrease	6 (4%)	4	2	0.008*
INR elevation	2 (1.3%)	0	2	0.232
aPTT elevation	132 (87.4%)	59	73	0.023*
PLT decrease	122 (80.8%)	56	66	0.018*
WBC decrease	90 (59.6%)	52	38	0.0

Discussion

Our results revealed that high AST/ALT, thrombocytopenia, low fibrinogen and aPTT levels are more common in PCR (+) group when compared to the PCR (-) patients group.

In our study, majority of the patients were male adults. In a study with 21,680 patients who presented to the ED due to tick bite, it was reported that 60.2% of the patients were male (5). In another study by Tekin et al., it was reported that number of male patients were higher than females in both CCHF survivors and non-survivors (7). This finding may not be surprising since nearly 90% of the cases occur in farmers, slaughterhouse workers or butchers (8). These jobs, which are risky in terms of the disease are usually performed by men. Additionally, when the endemic regions are considered (Africa, Eastern Europe, the Middle East, and Asia), men tend to pariticipate into the social life and expose to ticks and their hosts more often than females.

Another finding in our study was that majority of the patients were middle-aged individuals living in rural areas. It well-known that the disease is common in the rural areas of the region and in the actively working age group (9). However, it must be kept in mind that individuals living in the city center who often visit rural areas are also under the risk of tick exposure (5). Although age and gender do not have any significance on survival, as mentioned above, male individuals in the active-working age are under greater risk of the disease (7).

Signs and symptoms of CCHF are well-documented in the literature. However, none of these signs and symptoms are specific to the disease, hence the disease may be misdiagnosed easily, particularly in the prehemorrhagic stage. Symptoms may include fevers, chills, myalgias, dizziness, headache, mood swings, soreness, photophobia, sore throat, neck pain and stiffness, lymphadenopathy, and gastrointestinal symptoms, including nausea, vomiting, abdominal pain, and diarrhea Patients may also present with bleeding from various sites of the body as an initial complaint (such as epistaxis, petechiae, ecchymosis, melena, gingival bleeding, hematemesis, hematuria, and hematoma) (9,10). In our study, compatible with the literature, fever was the most common symptom followed by weakness, diarrhea, headache, abdominal pain and syncope. Clinicians, particularly those who work in endemic regions, must be aware of non-specific signs and symptoms of CCHF to prevent delays in treatment initiation.

In our study ribavirin therapy was given to 62 patients. Ribavirin is a synthetic guanosine analogue, which directly inhibits replication of the viral genome (11). The usefullness of ribavirin is enhanced when initiated in the early (pre-haemorrhagic) phase. At this stage, the number of the virus increases rapidly in the abdominal cavity. At the other hand, the virus has not yet systemically spread to other organs. So, ribavirin is most effecctive in this phase thanks to its high gastrointestinal tract absorbability property (12). One reason for low ribavirin therapy percentage in our study may be the delays in the diagnosis of the disease. The patients may totally recover with supportive thearpy before the results of PCR test is obtained.

Another possible reason may be the controversies on efficacy of ribavirin therapy. Studies with a higher level of evidence are rare and suggest a lack of efficacy for ribavirin. Superiority of ribavirin therapy over supportive care in terms of mortality, length of hospital stay, need for transfusion and platelet countrecovery time has not been clearly proved (3).

As mentioned above, diagnosis of the disease with laboratory tests in the early phase is crucial for patients (12). In many facilities, the definite diagnosis of CCHF relies on Reverse-transcription PCR (RT-PCR) testing. The viral load detected in RT-PCR also correlates with the disease severity. The percentage of correct results may be increased by usin Real-time PCR test. However, there is still a need for a rapid diagnostic tool which could be performed with low technical requirements as a point-of-care test to facilitate early therapeutic intervention and appropriate infection control precautions to minimize the potential for nosocomial spread (10).

Crimean-Congo hemorrhagic fever causes thrombocytopenia in almost all cases. Other prominent laboratory findings are decreased prothrombin time (PT), increased activated partial thromboplastin time (aPTT) and hypofibrinogenemia. Cytopenia due to macrophage activation syndrome, elevated LDH and CK levels may also be observed (3). It was also reported that higher AST and ALT levels might help predicting the severe cases (13). In a study, clinical features and laboratory findings of PCR (+) and PCR (-) patients were compared and elevated AST and K levels along with lower PLT and WBC levels were obtained in PCR (+) group (2). In our study, predictors of CCHF Disease in terms of laboratory findings in the ED were high AST/ALT, thrombocytopenia, low fibrinogen and aPTT levels. Studies on a rapid and simple test for CCHF diagnosis such as prognostic capacity of the mean platelet volume-to-platelet count ratio (MPVPCR) and the red cell distribution width-to-platelet count ratio (RDWPCR) for the systemic inflammatory response in patients with CCHF still continues [7]. Despite promising results, CCHF continues to be a challenging issue for Emergency Medicine and Infectious Diseases specialists.

Conclusion

Crimean-Congo hemorrhagic fever disease has been reported in 30 countries in Africa, Asia, Eastern Europe, and the Middle East (14). Although numerous species of ticks can carry the virus the most important tick vector is the Hyalomma spp. (15). The disease should be suspected in patients with unexplained bleeding (epistaxis, petechiae, ecchymosis, melena, gingival bleeding, hematemesis, hematuria, hematoma…) and compatible laboratory

findings (thrombocytopenia, leukopenia, and increased levels of aminotransferases) in endimic regions. Ribavirin and supportive therapy are the mainstays of treatment and early initiation of therapy is related with good outcome in CCHF. The main diagnosis of the disease relies on rt-PCR testing, however, according to our results, high AST/ALT, thrombocytopenia, low fibrinogen and aPTT levels may help predicting ill patients on admission to the ED. Thus, treatment may be initiated in the early stage of the disease and morbidity/mortality may be reduced.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

Authors contributed equally to the study. No funding was received.

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Experimental and Applied Medical Science 3, 2: 318 - 334, 2022. DOI 10.46871/eams.2022.37

SIMILARITIES AMONG COUNTRIES DURING THE COVID-19 PANDEMIC

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Abstract

On Jan 30, 2020, The World Health Organization (WHO) declared the current novel coronavirus disease 2019 (COVID-19) epidemic a Public Health Emergency of International Concern. The new type of coronavirus (2019-nCoV) is a new virus among viruses under the name. The novel coronavirus disease 2019 (COVID-19) pandemic has spread from China to 25 countries. This study aims to identify the countries that seem similar to each other by examining their situations during the COVID-19 process. For this purpose, cluster analysis was performed for 30 countries considering the total cases per million, total deaths per million, population over the age of 65, Gross Domestic Product (GDP) per capita, and hospital beds per 100k obtained from the Humanitarian Data Exchange (HDX) website for the dates of 15 May 2020 and 23 January 2021. Partition coefficient, partition entropy, modified partition coefficient, silhouette, fuzzy silhouette, and Xie and Beni index were used to determine the optimal number of clusters the optimal number of clusters was found to be 4. Thus, the countries were grouped into 4 clusters for both datasets. According to the results of the analysis, the similarities among the countries were evaluated by comparing their figures for both dates during the pandemic.

Keywords: *COVID-19, countries, clustering, similarities*

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Introduction

At the beginning of December 2019, the COVID-19 virus that slipped from animals to humans in Wuhan city, China, caused an outbreak of respiratory illness and spread significantly into other countries in Asia. Then, to other continents such as the Pacific region, North America, Europe, and even Africa (1). The World Health Organization (WHO) defined the SARS-CoV-2 virus (initially known as 2019-nCoV) outbreak as a severe global threat.

Similar to other viral respiratory infections, SARS-CoV-2 or COVID-19 can be transmitted through the respiratory tract. It mainly causes respiratory tract infections and develops severe pneumonia in infected patients who may require intensive care. Severe disease may result in death due to progressive respiratory failure (2).

The number of COVID-2019 cases is rising around the world. Everyone is susceptible to this virus, but the elderly and those with underlying diseases are more at risk of adverse outcomes. Current knowledge has shown that the death rate is high in people with chronic underlying diseases. Therefore, special attention should be paid to the elderly and immunocompromised patients. Infections might progress rapidly in these groups and timely clinical decisions are needed (3).

This virus, which kills thousands of people and affects tens of thousands of people, causes people to fear and panic. While a new one is added to the deaths caused by the Coronavirus every day, most people are investigating what it can do to protect it from the virus. Although there is no cure for coronavirus yet, there are points to consider and some precautions that everyone can take. The heaviest losses in the virus spread from China all over the world are in Europe. Life has almost

stopped all over the world due to the virus. While the new type of coronavirus (COVID-19) epidemic took 42 thousand lives worldwide, the total number of cases exceeded 860 thousand. Countries around the world struggling with the coronavirus have been struggling with the spread of the outbreak by taking some measures to prevent the spread of the outbreak. The COVID-19 Government Measures were implemented by governments worldwide in response to the Coronavirus pandemic. The researched information available falls into five categories: Social distancing, Movement restrictions, Public health measures, Social and economic measures, Lockdowns. Each category is broken down into several types of measures (ACAPS Government Measures Dataset). The timeline illustrates (Figure 1) when the first case of COVID-19 was reported in each affected country based on when the country first appeared on The Center for Systems Science and Engineering (CSSE) dashboard (on top) relative to when it first appeared in a WHO situation report (on bottom). The countries listed in blue were reported by CSSE before the WHO, and those listed in red were reported after the WHO. While the new type of coronavirus (COVID-19) epidemic, which led to the worldwide public health crisis, faced severe human and material losses, primarily in Europe and North America, countries in different parts of the world have come a long way in combating the outbreak in their way.

There are many studies on COVID-19. Some of them are as follows. Yang et al. (2020) used a purely data-driven statistical method to estimate the case fatality rate (CFR) in the early phase of the COVID-19 outbreak. Daily numbers of laboratory-confirmed COVID-19 cases and deaths were collected from January 10 to February

3, 2020, and divided into three clusters: Wuhan city, other cities of Hubei province, and other provinces of mainland China.

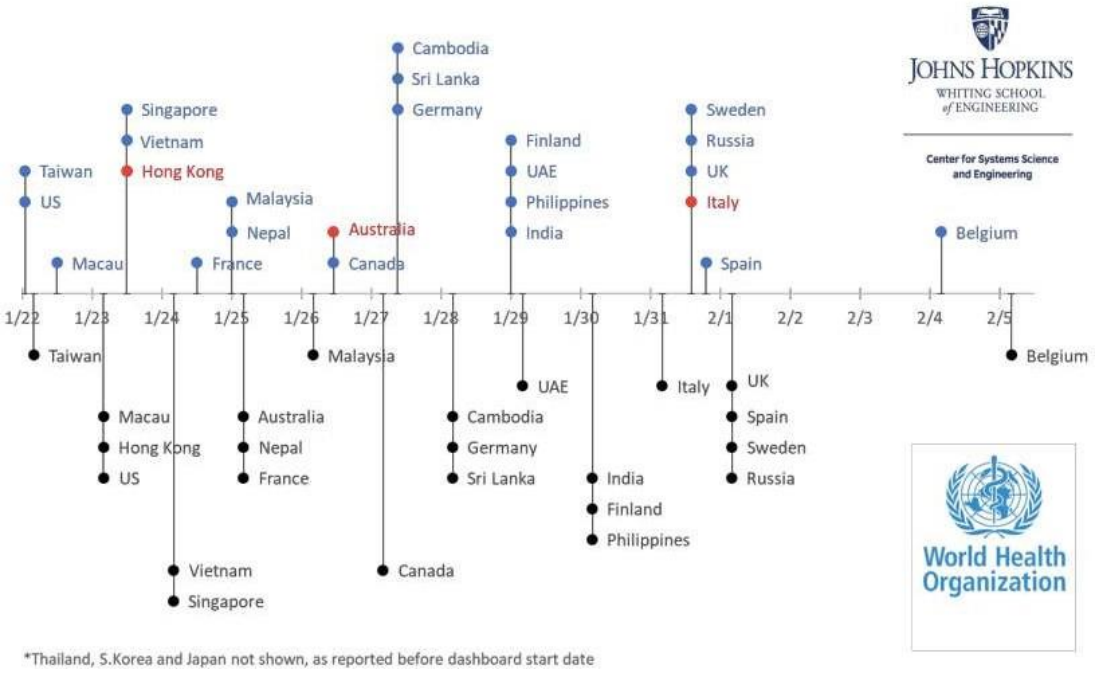

Figure 1. Comparison of COVID-19 case reporting between JHU CSSE, the WHO, and CCDC (4).

The simple linear regression model was applied to estimate the CFR from each cluster (5). Emami et al. (2020) examined all relevant articles that reported clinical characteristics and epidemiological information of hospitalized COVID-19 patients. The data of 76993 patients presented in 10 articles were included in their study. According to the meta-analysis, the pooled prevalence of hypertension, cardiovascular disease, smoking history, and diabetes in people infected with SARS-CoV-2 were estimated as 16.37% (95%CI: 10.15%-23.65%), 12.11% (95%CI 4.40%-22.75%), 7.63% (95%CI 3.83%-12.43%) and 7.87% (95%CI 6.57%-9.28%),

respectively.[3] Fontanet et al. (2020) conducted a retrospective closed cohort study among pupils, their parents, and siblings, as well as teachers and non-teaching staff of a high school located in Oise Between 30 March and 4 April 2020. Participants completed a questionnaire that covered the history of fever and/or respiratory symptoms since 13 January 2020 and had blood tested for the presence of anti-SARS-CoV-2 antibodies. The infection attack rate (IAR) was defined as the proportion of participants with confirmed SARS-CoV-2 infection based on antibody detection. Blood samples from two blood donor centers collected between 23 and 27 March 2020 in the Oise department were also tested for the presence of anti-SARS-CoV-2 antibodies (6). Chang et al. (2020) performed a systematic review in PubMed and Embase to find relevant case series. Because some reports were published in Chinese journals, the journals and publications of the Chinese Medical Association related to COVID-19 were completely reviewed. A random-effects model was used to pool clinical data in the meta-analysis (7). Rodriguez-Morales et al. (2020) performed a systematic literature review with meta-analysis, using three databases to assess clinical, laboratory, imaging features, and outcomes of COVID-19 confirmed cases. Observational studies and also case reports were included and analyzed separately. They performed a random-effects model meta-analysis to calculate pooled prevalences and 95% confidence intervals (95%CI) (8). Roosa et al. (2020) used phenomenological models that have been validated during previous outbreaks to generate and assess short-term forecasts of the cumulative number of confirmed reported cases in Hubei province, the epicenter of the epidemic, and for the overall trajectory

in China, excluding the province of Hubei. They collected daily reported cumulative confirmed cases for the 2019-nCoV outbreak for each Chinese province from the National Health Commission of China. Here, they provided 5, 10, and 15-day forecasts for five consecutive days, February 5th through February 9th, with quantified uncertainty based on a generalized logistic growth model, the Richards growth model, and a sub-epidemic wave model (9). Jia et al. (2020) collected information on COVID-19 clusters in Qingdao City. The epidemiological characteristics and clinical manifestations were analyzed. Eleven clusters of COVID-19 were reported in Qingdao City between January 29, and February 23, 2020, involving 44 confirmed cases, which accounted for 73.33% of all confirmed cases. From January 19 to February 2, 2020, the cases mainly concentrated in the district that had many designated hospitals. Patients aged 20-59 y old accounted for the largest proportion (68.18%) of cases; the male-to-female sex ratio was 0.52:1. Three cases were infected from exposure to confirmed cases. The average incubation period was 6.28 d. The median number of cases per cluster was 4, and the median duration time was 6 d. The median cumulative number of exposed persons was 53 (10). Gupta and Shankar (2020) gave statistical estimates of the infected population by using counts of fatalities and previously estimated parameters for the progress of the disease. The doubling time, τ, is a crucial unknown input parameter that affects these estimates, and may differ strongly from one geographical location to another. They suggested a method for estimating epidemiological parameters for COVID-19 in different locations within a few days, so adding to the information required for gauging the success of public health interventions (11). Liu et

al. (2020) presented a timely and novel methodology that combines disease estimates from mechanistic models with digital traces, via interpretable machine-learning methodologies, to reliably forecast COVID-19 activity in Chinese provinces in real-time. Specifically, their method can produce stable and accurate forecasts 2 days ahead of the current time and uses as inputs (a) official health reports from the Chinese Center Disease for Control and Prevention (China CDC), (b) COVID-19-related internet search activity from Baidu, (c) news media activity reported by Media Cloud, and (d) daily forecasts of COVID-19 activity from GLEAM, an agent-based mechanistic model (12). Jung et al. (2020) studied statistically estimated the confirmed case fatality risk (cCFR) and the basic reproduction number-the average number of secondary cases generated by a single primary case in a naïve population by using the exponential growth rate of the incidence. They modeled epidemic growth either from a single index case with illness onset on 8 December 2019 (Scenario 1), or using the growth rate fitted along with the other parameters (Scenario 2) based on data from 20 exported cases reported by 24 January 2020 (13). Anastassopoulou et al. (2020) provided estimates of the main epidemiological parameters based on the publicly available epidemiological data for Hubei, China from January 11 to February 10, 2020. They provided an estimation of the case fatality and case recovery ratios, along with their 90% confidence intervals as the outbreak evolves. Based on a Susceptible-Infectious-Recovered-Dead (SIDR) model, they provided estimations of the basic reproduction number (R0), and the per-day infection mortality and recovery rates (14). Randhawa et al. (2020) identified an intrinsic COVID-19 virus genomic signature and used it

together with a machine learning-based alignment-free approach for an ultra-fast, scalable, and highly accurate classification of whole COVID-19 virus genomes. The proposed method combines supervised machine learning with digital signal processing (MLDSP) for genome analyses, augmented by a decision tree approach to the machine learning component, and a Spearman's rank correlation coefficient analysis for result validation (15). Ghosal et al. (2020) aimed at tracing a trend related to death counts expected at the 5th and 6th week of the COVID-19 in India. A validated database was used to procure global and Indian data related to coronavirus and related outcomes. Multiple regression and linear regression analyses were used interchangeably. Since the week 6 death count data was not correlated significantly with any of the chosen inputs, an auto-regression technique was employed to improve the predictive ability of the regression model (16). Vaishya et al. (2020) aimed to review the role of Artificial Intelligence (AI) as a decisive technology to analyze, prepare us for the prevention and fight against COVID-19 (Coronavirus) and other pandemics. A rapid review of the literature was done on the database of Pubmed, Scopus, and Google Scholar using the keyword of COVID-19 and AI. Collected the latest information regarding AI for COVID-19, then analyzed the same to identify its possible application for this disease (17). Altındağ (2021) determined the effects of fear during the Covid-19 epidemic period on job satisfaction and job performance of bank employees with a proposed model (18).

The aim of this study is to determine the countries that are similar to each other in the process by examining the states of the countries in the COVID-19 process.

Methods

Fuzzy k-medoids algorithm

Fuzzy clustering analysis is a method that is formed by extending cluster analysis with fuzzy logic and is quite common in terms of application. Fuzzy clustering algorithms have two main stages. The first of these; is to find a suitable function to determine each sample membership degree of each cluster, and the second is to obtain a method that calculates cluster centers. In the fuzzy k-medoids algorithm, which is the fuzzy version of the k-medoids algorithm, observations are evaluated according to the degree of membership that expresses their level of belonging to the clusters. The fuzzy k-medoids method is based on minimizing the objective function given below.

$$P(i,j) = \sum_{i=1}^{n} \sum_{j=1}^{k} u_{ij}^{m} d_{ij}^{2}$$

$$u_{ij} = \frac{1}{\sum_{q=1}^{k} \left(\frac{d_{ij}}{d_{qi}}\right)^{2/(m-1)}}$$

Where, m is the fuzzifier parameter, u_{ij}, represents the association degree of membership of the ith object to the jth cluster, and $\sum_{j=1}^{k} u_{ij} = 1$. d_{ij}^{2}, is a dissimilarity measure between the *j*th cluster center and the *i*th object (19-20-21).

Although different methods are used in distance measurement, Euclidean distance measurement is generally taken into consideration. The processing steps of the fuzzy k-medoids algorithm are as follows;

Step 1: Initialize the membership function u_{ij} with random values between 0 and 1.

Step 2: Calculate k fuzzy cluster centers. These centers represent the object selected from the dataset.

Step 3: Compute the objective function.

Step 4: Continue the previous steps until the distance between the cluster centers and the observations is minimal.

Step 5: Calculate final membership values for the observations and generate clustering results.

Data and Results

The present study aims to identify countries that show similarities in terms of coping with the COVID-19 during the pandemic and to evaluate the situations of similar countries. Therefore, cluster analysis was conducted by taking into account the total cases per million, total deaths per million, population over the age of 65, Gross Domestic Product (GDP) per capita, and hospital beds per 100k obtained from the Humanitarian Data Exchange (HDX) website for 30 countries for the dates of 15 May 2020 and 23 January 2021 (22). These countries are Sweden, Singapore, Italy, United Kingdom, Belgium, Spain, United States, Switzerland, Canada, Qatar, Turkey, Vietnam, Philippines, Nepal, Malaysia, Cambodia, India, China, Iran, Sri Lanka, South Korea, Japan, France, Germany, Finland, Russia, Belgium, Norway, Australia, and Austria. The dates were selected randomly. The analysis was carried out using the R statistical software.

Since the observations that take enormous value within the data set can change the center point and average of the cluster to be included in the analysis, clustering is performed by using the observation positioned closest to the middle point in the cluster, instead of calculating the average of the observations in the cluster. The k-medoids algorithm is less sensitive to outliers compared to k-means clustering, as it relies on the most centrally positioned object in a cluster. In clustering problems, the use of the k-medoids algorithm is recommended in terms of the relative ease of use, computing performance, and use in large data sets (23). If clusters do not differ distinctly from each other, or if some objects are unstable in cluster

membership, it would be useful to prefer fuzzy clustering methods instead of classical clustering methods. For this reason, cluster analysis was performed with the fuzzy k-medoids method. A total of six clustering validation indices were widely used to choose the optimal number of clusters. The indices include partition coefficient (PC), partition entropy (PE), modified partition coefficient (MPC), silhouette (SIL), fuzzy silhouette (SIL.F), and Xie and Beni index (XB). It should be noted that the optimal number of clusters was observed at the maximum value of each of these indices except for PE, where the optimal number of clusters was found at its minimum value (24-25). The cluster validity indices obtained for both dates examined are given in Table 1 and Table 2, respectively.

Table 1. The cluster validity indices according to the number of clusters for the date of 15 May 2020

The number of clusters	PC	PE	MPC	SIL	SIL.F	XB
2	**0.84**	**0.26**	**0.69**	0.45	0.50	0.36
3	0.79	0.38	0.68	0.49	0.57	0.43
4	0.77	0.45	**0.69**	**0.53**	**0.64**	0.78
5	0.72	0.56	0.65	0.44	0.53	**1.75**
6	0.63	0.76	0.56	0.19	0.36	1.27

Table 2. The cluster validity indices according to the number of clusters for the date of 23 January 2021

The number of clusters	PC	PE	MPC	SIL	SIL.F	XB
2	0.70	0.46	0.40	0.44	0.49	**1.37**
3	**0.84**	**0.32**	0.75	0.56	0.65	0.37
4	0.83	0.35	**0.77**	**0.63**	**0.66**	0.32
5	0.78	0.46	0.72	0.50	0.54	0.52
6	0.75	0.54	0.70	0.44	0.47	0.75

As seen in Table 1, the clustering validation index values of the PC (0.84) and PE (0.26) suggested 2 clusters while the index values of the SIL (0.53) and SIL.F (0.64) suggested 4 clusters. On the other hand, the clustering validation index value of the MPC (0.69) suggested 2 or 4 clusters. Moreover, the clustering validation index of XB (1.75) suggested 5 clusters. In the study, k=4 was selected as the optimum number of clusters since it was suggested by the majority of indices. Also, since it was envisaged that the number of clusters for the study would be 4, the number of appropriate clusters was taken as 4. According to this result, Singapore, the United States, Norway, Switzerland, Canada, Portugal, and Qatar are in Cluster 1. Cluster 2 includes Sweden, Italy, the United Kingdom, France, Belgium, and Spain. Cluster 3 includes Turkey, Vietnam, Philippines, Nepal, Malaysia, Cambodia, India, China, Iran, and Sri Lanka. Cluster 4 includes South Korea, Japan, Germany, Finland, Russia, Australia, and Austria.

Table 2 reveals that 3 clusters were suggested according to the index values of the PC (0.84) and PE (0.32), while the number of clusters suggested was 4

according to the index values of the MPC (0.77), SIL (0.63), and SIL.F (0.66) values. On the other hand, the index value of XB (1.37) suggested 2 clusters. Similarly, since it was suggested by the majority of indices and it was thought that the number of clusters for the study would be 4, k=4 was selected as the optimum number of clusters. Accordingly, Australia, Canada, Norway, Qatar, Singapore, and Finland are in Cluster 1. Cluster 2 includes Sweden, Italy, the United Kingdom, France, Belgium, Spain, Portugal, Switzerland, and United States. Cluster 3 includes Turkey, Vietnam, Philippines, Nepal, Malaysia, Cambodia, India, China, Iran, and Sri Lanka. Cluster 4 includes South Korea, Japan, Germany, Russia, and Austria. Table 3 presents the countries in the clusters obtained by conducting cluster analysis using the data for the dates of 15 May 2020 and 23 January 2021.

The countries that best manage the COVID-19 crisis on a global scale are included in Cluster 4. These countries have tried to prevent the spread of the virus in society by isolation and social distancing measures they have implemented since the early days of the pandemic. Schools, businesses, public institutions, and borders of these countries were closed. Firstly, they tested the people who traveled abroad and those who were in contact with them. Secondly, everyone who showed symptoms of the disease was tested. Then, they began to conduct widespread tests for the people living in urban areas where the outbreak could spread. The countries in Cluster 3 rank second in terms of their performance during the pandemic. If these countries continue to take measures, they will manage the process well. The countries that have been worst affected by the pandemic are those in Cluster 1. In these countries, the virus has been on the stage of spreading across the

country. Moreover, they delayed acting against it. To manage the process, they need to hire more people, train them, and increase their capacity. If sufficient precautions are not taken, the number of cases will continue to increase.

Table 3. Countries in the clusters were obtained by conducting cluster analysis using data for the dates of 15 May 2020 and 23 January 2021.

Cluster	**1**	**2**	**3**	**4**
Clustering according to data on 15 May 2020	Singapore **United States** Norway **Switzerland** Canada **Portugal** Qatar	Sweden Italy United Kingdom France Belgium Spain	Turkey Vietnam Philippines Nepal Malaysia Cambodia India China Iran Sri Lanka	South Korea Japan Germany **Finland** Russia **Australia** Austria.
Clustering according to data on 23 January 2021	**Australia** Canada Norway Qatar Singapore **Finland**	Sweden Italy United Kingdom France Belgium Spain **Portugal** **Switzerland** **United States**	Turkey Vietnam Philippines Nepal Malaysia Cambodia India China Iran Sri Lanka	South Korea Japan Germany Russia Austria

As can be seen in Table 3, which shows the results of the analysis conducted

using the data for 15 May 2020, the United States, Switzerland, and Portugal are in Cluster 1. However, these countries were included in Cluster 2 according to the results of the analysis using data for 23 January 2021. These countries have begun to manage the process well, albeit partially. Finland and Australia are in Cluster 4 according to the analysis performed using the data for 15 May 2020. However, these countries were found to be in Cluster 1 according to the analysis conducted using the data for 23 January 2021. In this case, it can be interpreted that these countries could not manage the process well.

Discussion and Conclusions

The coronavirus, which appeared in Wuhan, China in December 2019, has caused thousands of people to die. With each passing day, the number of both those who died and those who got the virus is increasing. While several countries have taken strong measures to prevent the spread of the virus during the pandemic others have managed the process poorly. In the present study, the situations of the countries during the COVID-19 pandemic were examined and evaluated. In this context, two datasets were created for 30 countries by taking into account the data (total cases per million, total deaths per million, population over the age of 65, Gross Domestic Product (GDP) per capita, and hospital beds per 100k) obtained from the Humanitarian Data Exchange (HDX) website for the dates of 15 May 2020 and 23 January 2021. The fuzzy k-medoids clustering algorithm was applied to the obtained datasets. Six indices (partition coefficient, partition entropy, modified partition coefficient, silhouette, fuzzy silhouette, and Xie and Beni index) were used to determine the optimal number of clusters. According to these index values, the optimal number of clusters was found to be 4. Thus, the

countries were grouped into 4 clusters for both datasets. While Cluster 4 included the countries that have managed the COVID-19 pandemic well, Cluster 1 included the countries that have been worst affected. United States, Switzerland, and Portugal were in Cluster 1 according to the analysis of the data for the date of 15 May 2020, while these countries were in Cluster 2 according to the results of the analysis using the data for 23 January 2021. It can be interpreted that these countries have begun to manage the process well, albeit partially. Moreover, Finland and Australia were included in Cluster 4 according to the analysis of the data for 15 May 2020, while these countries were in Cluster 1 according to the analysis of the data obtained for 23 January 2021. It is suggested that these countries need to take more serious measures by reviewing their decisions taken in this process. Countries in Cluster 1 need to take serious measures and implement them as soon as possible, while countries in other clusters should continue to apply their measures without compromise.

Besides, the vaccines developed by some companies against COVID-19 have begun to be shot by countries considering the order of priority. It is thought that these vaccines will bring an end to the pandemic.

Since the data about the numbers of vaccines have not been found for some countries included in this study yet, the variable of the number of vaccines was not involved in the dataset for the date of 23 January 2021.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

No institution has given financial support to the study. All researchers contributed equally to the study.

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INVESTIGATION OF THE EFFECT OF CHLOROQUINE ON ADRIAMYCIN-INDUCED KIDNEY DAMAGE

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Abstract

Although Adriamycin (ADR) is an important anticancer drug used in chemotherapy, it causes nephrotoxicity. The inflammation pathway has an important role in ADR-induced nephrotoxicity. Chloroquine (CLQ), which is used as an antimalarial drug, is used in many diseases. Also, CLQ is known as an anti-inflammatory. In this study, we aimed to investigate the effect of CLQ against nephrotoxicity caused by ADR through the inflammatory pathway. Groups were formed as follows; Control (n = 8), CLQ (n = 8) 50 mg / kg intraperitoneally (i.p.) per day, ADR (n = 8) 2 mg / kg i.p. every 3 days, ADR + CLQ (n = 8) 2mg / kg / i.p. ADR + 50 mg / kg / i.p. CLQ. The experiment took a total of 30 days. At the end of the experiment, kidney tissues were taken from the rats under anesthesia. After fixation in the removed kidney tissues, the tissues were embedded in paraffin by histological methods. Sections were taken from kidney tissues. Renal tissue histopathology and Tumor necrosis factor-alpha (TNF-α) and Nuclear factor-κB p65 (NF-κB p65) immunoreactivities were evaluated. When the kidney tissue was examined, it was seen that damage was caused by ADR. In addition, it was observed that TNF-α and NF-κB p65 immunoreactivities in the kidney significantly increased in the ADR group ($p < 0.05$). Damage and inflammatory markers were found to be decreased in the ADR + CLQ group ($p < 0.05$). Chemotherapeutically administered ADR appears to cause nephrotoxicity. CLQ administered was found to reduce this toxicity. As a result, we showed that the damage caused by ADR-induced nephrotoxicity decreased with the application of CLQ through the TNF-α and NF-κB p65 inflammation pathway.

Keywords: *Adriamycin, Chloroquine, Inflammation, Kidney*

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Introduction

Chronic kidney disease is known as an important health problem in the world. Kidney disease is usually associated with loss of kidney function (1). Among the various factors that contribute to kidney disease, many factors such as inflammatory processes and oxidative stress play a role (2). ADR, also known as doxorubicin, is an important anthracycline antibiotic and is used in cancer treatment. In addition, the use of ADR causes toxicity in the heart and kidney (3, 4). ADR ensures the removal of histone proteins from the chromatin and prevents DNA replication by inhibiting the topoisomerase II enzyme (5). This cytotoxicity of ADR occurs due to ADR-induced toxicity, free radical formation, and oxidative stress (6). Kidney damage caused by ADR includes events such as free radical formation, oxidative damage and inflammation, which leads to tissue damage (7). After ADR is taken into the cell, it causes cell damage and apoptosis due to the breaking of the DNA chain, inhibition of macromolecule biosynthesis, and formation of hydroxyl radicals. ADR enables the generation of reactive oxygen species (ROS) (8). Depending on the increase in oxidative damage, a significant degree of ADR-induced nephrotoxicity is seen in inflammation, and the increased inflammation may lead to fibrosis in the kidney (9, 10).

TNF-α is a cytotoxic factor, plays a key role in the pathogenesis of fibrosis, is involved in many inflammatory responses that can induce the release of many cytokines, and act as a chemotactic molecule to take up neutrophils and monocytes (11). TNF-α triggers the activation of Ikappa-B (IκB) kinase (IKK) / NF-κB and mitogen-activated protein kinase (MAPK) / AP-1 pathways, which are necessary for the expression of proinflammatory cytokines and induction of many biological substances (12). NF-κB is known as the transcription factor widely expressed in various cells and tissues and can respond rapidly to various inflammatory stimuli (13). One of the subunits of NFkB is p65 (14). Activated NF-kB (ie free NF-kB p50 / p65) leads to the release of pro-inflammatory cytokines such as TNF-α (15).

Expression of NF-κB / TNF-α is found in almost all mammalian cells, and this is activated by a number of stimuli. The NF-κB / TNF-α pathway plays an important role in the normal physiological regulatory network of immune and inflammatory responses (16, 17).

Antimalarial drugs (eg Chloroquine) have been developed primarily to treat malaria; However, they are beneficial in many

dermatological, immunological, rheumatological, and severe infectious diseases and are known as an anti-inflammatory (18).

The effect of CLQ on the NF-κB p65 / TNF-α pathway, which plays an important role in inflammation in ADR-induced kidney damage, is unknown. In light of all the above-mentioned information, we aimed to determine the effects of CLQ on NF-κB p65/ TNF-α in ADR-induced kidney damage.

Materials and methods

In this study, 8-week-old 150-200 gr adult male 32 Wistar albino type rats produced in Erciyes University Hakan Çetinsaya Experimental Research Application and Research Center (DEKAM) were used. The rats kept in the cages were kept at 21 ° C and 12 hours in a light / dark environment in the normal order of the day and their water and nutrient needs were met. Experimental groups were formed by weighing the subjects and bringing them together so that their weights were close to each other.

Experimental procedure

Rats were divided into four groups as follows;

1. Control group (n = 8) untreated rats,

2. CLQ (Chloroquine (N4 -(7-Chloro-4-quinolinyl)-N1 ,N1 -dimethyl1,4-pentanediamine diphosphate salt) Sıgma Aldrich C6628) group (n = 8) group for 30 days intraperitoneally (i.p.) 50 mg / kg CLQ (CLQ was dissolved in saline)(19),

3. ADR group (Koçak Farma) (n=8) 2 mg / kg ADR intraperitoneally (i.p.) every three days for 30 days (20),

4. ADR + CLQ group (n = 8) every three days i.p. 2 mg / kg ADR and for 30 days 50 mg / kg/i.p. of CLQ was administered.

At the end of the study rats were decapitated after intraperitoneal ketamine (Pfizer) (75mg/kg)+xylazine (Rompun, Bayer) (10mg/kg). After the kidney tissues were taken from the rats, they were put in formaldehyde for histopathological and immunohistochemical examination.

Histopathological evaluation

Histopathological evaluation of the kidney tissue was made using routine histological methods. Kidney tissues were fixed in 10% formalin solution for 24-48 hours, dehydrated with alcohol, purged with xylene, and embedded in paraffin and cut into 5 μm thick sections. Hematoxylin-eosin (H&E) staining was performed to evaluate histopathological changes in tissue samples (21). Photos were taken under a light microscope (Olympus BX53;

Olympus, Tokyo, Japan) and analyzed by the study group.

Immunohistochemistry

The immunohistochemistry method was used to determine the immunoreactivity of TNF-α and NF-κB p65 antibodies in kidney tissues. Avidin biotin peroxidase method was used to determine the difference in TNF-α (bs-2081R, Bioss) and NF-κB p65 (bs-0465R, Bioss) expression. Paraffin sections were deparaffinized with xylene. For antigen recovery, 0.01M 10% citrate buffer was applied at 600w for 7 minutes in the microwave and then allowed to cool for 10 minutes at room temperature. Parts washed with phosphate buffer (PBS) were treated with 3% hydrogen peroxide (H_2O_2) for 12 minutes to prevent endogenous peroxidase activity. It was washed 3 times with PBS again for 5 minutes. The staining kit (TA-125-HDX, Thermo Fisher Scientific, Waltham, MA, USA) was used for the next steps. After washing again 3 times in PBS, the ultra v block solution was added to the tissues and kept in the tank for 10 minutes. TNF-α and NF-κB p65 antibodies were then added to the tissues and incubated at 4 ° C overnight. After rewashing, the peroxidase present in the kit and displaying diaminobenzidine (DAB) (TA-060-HDX, Thermo Fisher Scientific, Waltham, MA, USA) was treated with the peroxidase substrate for 1.5 minutes to make its immunoreactivity visible (21). Photos were taken under a light microscope (Olympus BX53; Olympus, Tokyo, Japan) and analyzed by the study group. To evaluate antibody expressions, each group was scored in 30 different areas by 3 histologists according to the staining intensity. These scores are summarized as follows;

0: no staining, 1: little staining, 2: moderate staining, 3: intense staining.

Statistical analysis

Kolmogorov - Smirnov test was used to determine the normal distribution of the data. One-way analysis of variance and posthoc Tukey test were used to determine differences between groups. Results are presented as mean ± Standard deviation (SD). Graph pad Prism 8.0 software was used for statistical analysis. $P < 0.05$. It was considered statistically significant.

Results

Histopathology results

The image of Hematoxylin & eosin in kidney tissue is given in figure 1. Healthy

kidney tissue is seen in the control group. When looking at the ADR group, tubular damage, glomerular degeneration, and hemorrhagic areas are seen. When we looked at the ADR + CLQ group, it was seen that these damages did not exist.

Immunohistochemistry results

TNF-α and NF-κB p65 immunoreactivity results and immunohistochemistry staining images are shown in Table 1 and Figure 2. TNF-α and NF-κB p65 immunoreactivity increased significantly in the ADR group compared to the control group ($p < 0.05$). A statistically significant decrease in TNF-α and NFk-B p65 immunoreactivity was observed in the ADR + CLQ group compared to the ADR group ($p < 0.05$).

Discussion

ADR is a promising drug for cancer patients, which is used in many cancer treatments. However, side effects are seen in patients. One of these side effects is nephrotoxicity. ADR is a chemotherapeutic that causes kidney damage in particular. ADR shows its harmful effects on cells by inhibiting transcription and replication (22). ADR-induced nephrotoxicity is one of the best-known models of chemotherapy-induced kidney injury. ADR causes functional and morphological changes in the kidney (9). CLQ, which is used in malaria and pulmonary hypertension, is also known as an autophagy inhibitor (23). Autophagy plays a role in the pathophysiology of many diseases. CLQ, an important autophagy inhibitor, and its derivative, hydroxychloroquine, are important drugs (24). CLQ was originally used and discovered in the treatment of inflammatory diseases. CLQ is known to have anti-inflammatory properties (18). Recently, it has also been used as an anti-inflammatory in the treatment of COVID-19 (25). In the present study, we also induced nephrotoxicity with ADR. In the present study, ADR was applied chronically. We have shown that there is an effect between the kidney damage that occurs and the increased inflammation. When the effect of ADR, which we applied chronically, was examined at the end of the experiment, we observed that similar to other studies, it had negative effects such as tubular epithelial shedding, glomerular damage, and hemorrhage in the kidney (26). We observed that these apparent damages decreased in the ADR + CLQ group. Oxidative stress and inflammation play an important role in the formation of these damages (9).

The inflammatory response has a complex mechanism. The exact mechanisms underlying the effect of ADR on inflammation are unclear. Studies have shown that there may be an interaction between inflammation and nuclear factor kappa B (NF-KB) (27). It has been proven that NF-κB, an important transcriptional activator, regulates the expression of inflammatory factors (28). As a result of increased phosphorylation of NF-kB, it has been stated that the inflammatory response is significantly increased (29). TNF-α is known as a proinflammatory cytokine. It is produced by many cells in the kidney as a result of ADR application (30). TNF cytokine family members are the best inducers of NF-κB. Cytokines belonging to the TNF family primarily stimulate genes that regulate inflammation through the NF-kB pathway. It contains a transactivation site to initiate p65 transcription from NF-kB proteins (16). Two pathways are involved in the stimulation of NF-kB, the canonical and noncanonical pathways. The most important target of these is canonical p65 (31). NF-kB is stimulated by cytokines such as TNF-α (32). ADR administration promotes inflammation and increases the level of NF-κB p65. Reactive oxygen species increase in the body with the application of ADR. This leads to oxidative stress and leads to cell damage (33). In the present study, NF-κB p65 and TNF-α immunoreactivity increased significantly with the application of ADR. Activation of this pathway also increases TNF-α expression. In previous studies, it has been reported that ADR increases NF-κB p65 and TNF-α expressions in the kidney (34-36). In another study, it was reported that inflammation increased due to the increase of oxidative stress by ADR and accordingly, proinflammatory cytokines increased (10). In our study, an increase in NF-kB p65 and TNF-α immunoreactivity was observed. In the ADR + CLQ group, it is seen that inflammatory cytokines are decreased. Some studies say that with the activation of autophagy, kidney damage is reduced (37). There are also studies showing that increased autophagy will cause kidney damage (38). In another study, it was found that CLQ application increased lipid peroxidation in kidney tissue and damage occurred in tubules. They think this is due to the long-term use of CLQ (23, 39). They showed that the autophagy activator has a destructive role in kidney stone formation, while the autophagy inhibitor CLQ inhibits the adhesion of crystals to kidney epithelial cells. They also showed that CLQ attenuated renal cell damage and oxidative damage (40). The autophagy inhibitor CLQ used in the current study appears to reduce this damage through inflammation.

Limitation of this study; Since the study was not conducted with financial support, many more parameters could not be looked at. Therefore, it is important in that it provides the basis for future studies. In new large-scale studies, it will shed light on both histological and biochemical parameters.

Conclusion

The results of the present study indicate that ADR-induced nephrotoxicity occurs. As a result of this nephrotoxicity, damage occurs in the kidney tissue. The importance of the NF-κB p65 / TNF-α pathway is remarkable in our study, which shows that especially inflammation plays an important role in the injury. In addition, we see that CLQ, which we use as an anti-inflammatory, reduces inflammation and associated kidney damage. It has been shown that CLQ affects this effect through the NF-κB p65 / TNF-α pathway and inhibits this pathway. With the studies to be conducted, demonstrating the importance of the inflammatory pathway in reducing the side effects of different chemotherapeutic agents used in cancer treatment and determining anti-inflammatory agents against them will play an important role in reducing these damages. It will also play an important role in determining this controversial and research-open feature of CLQ.

Acknowledgements

Ethical approval: The approval of Erciyes University Experimental Animals Ethics Committee (Decision number: 19/205 and Date: 06.11.2019) was obtained.

Conflict of interest: There is no conflict of interest between the authors.

Financial support: No financial support received.

Peer-review: Externally and internally peerreviewed.

Author Contributions

ATA and EK designed the study, and EK, ATA, TC and DK performed the experiment. EK and ATA contributed in analyzing the data. EK wrote the manuscript.

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Figure Legends

Figure 1. Hematoxylin and eosin (H&E) staining image of the groups. A. Control group, B. CLQ group, C. ADR group, D. ADR + CLQ group. Yellow arrow: Hemorrhagic area, arrowhead: glomerular degeneration and black arrow: Tubular damage, Image magnification x200.

Abbreviations: ADR: Adriamycin, CLQ: Chloroquine

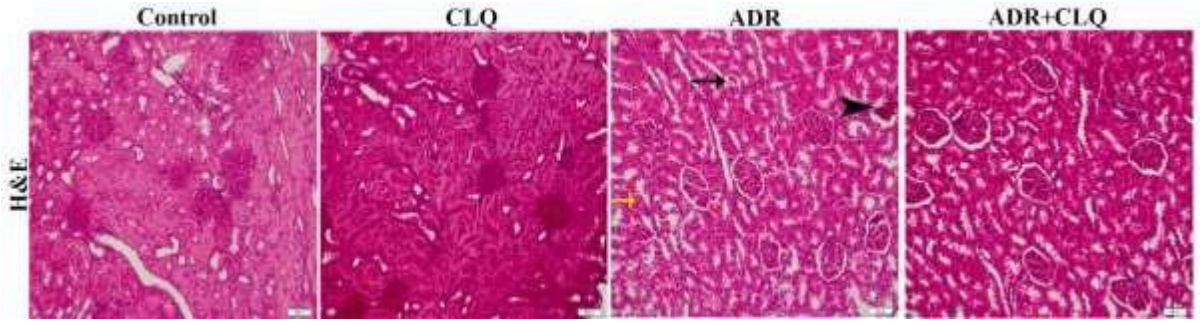

Figure 2. TNF-α and NF-κB p65 immunohistochemistry staining images in kidney groups. Image magnification X200.

Abbreviations: ADR: Adriamycin, CLQ: Chloroquine, TNF-α: Tumor necrosis factor-α, NFk-B p65: Nuclear Factor kappa B

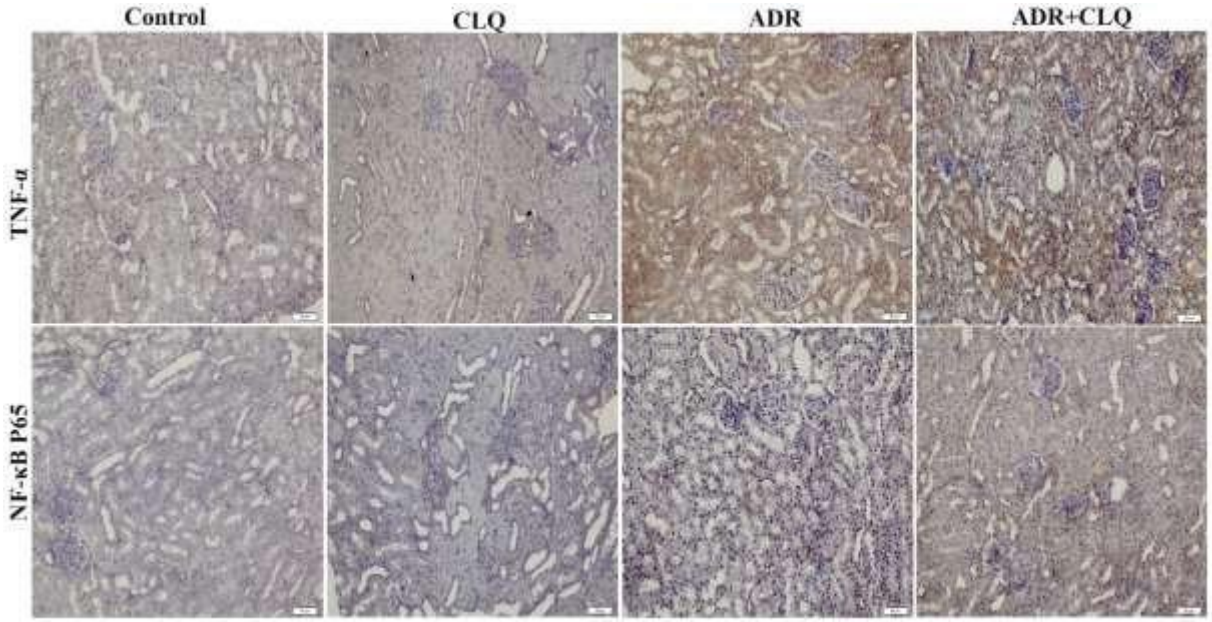

Table 1. TNF-α and NF-κB p65 immunohistochemistry results between groups

Gruplar	**Kontrol**	**CLQ**	**ADR**	**ADR+CLQ**	***p***
TNF-α immunoreactivity	0.33±0.47[a]	0.23±0.43[a]	2.70±0.46[b]	1.26±0.52[c]	0.0001
NF-κB p65 immunoreactivity	0.20±0.40	0.16±0.37	2.80±0.40	1.36±0.55	0.0001

Data are expressed as mean ± standard deviation. There is no significant difference between groups containing the same letter. p <0.05 was considered significant.

Abbreviations: ADR: Adriamycin, CLQ: Chloroquine, TNF-α: Tumor necrosis factor-α, NF-κB p65: Nuclear Factor kappa B P65

Experimental and Applied Medical Science, 3, 2: 345-353, 2022. DOI 10.46871/eams.2022.39

ANALYSIS OF THIOLE/DISULFIDE HOMEOSTASIS IN PATIENTS WITH DECUBITUS ULCER

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ABSTRACT

Aim: *There is a growing body of literature that recognises the importance of decubitus ulcer. Decubitus ulcer is tissue necrosis and ulceration that inevitably occurs in tissues exposed to pressure for a long time. Reoxygenation of ischemic tissue results in the formation of reactive oxygen metabolites, during ischemia/reperfusion. Thiols are antioxidant substances belonging to the mercaptan group. This study aims to contribute to this growing area of research by exploring thiol/disulfide levels, a new oxidative stress parameter, in patients with decubitus ulcer.*

Materials and Method: *This study consists of two groups, one is the control group and the other is the patient group. While the control group consists of 50 healthy individuals, the patient group consists of 50 patients with decubitus ulcer. Native thiol and total thiol levels were measured by spectrophotometric method. Data management and analysis were performed using SPSS software (version 22). Statistical significance level was accepted as $p<0.05$.*

Results: *While the average native thiol, total thiol level, reduced thiol ratio, and thiol oxidation reduction ratio levels in the patient group were lower than the control group; disulfide and oxidized thiol levels were higher than the control group ($p<0.05$).*

Conclusion: *Total thiols, native (free) thiols and disulfide levels were significantly changed in the patient group compared to healthy individuals. The most striking result to emerge from the data is that plasma dynamic thiol/disulfide levels alter and shift to the disulfide side in patients with decubitus ulcers.*

Keywords: *Thiol/disulfide homeostasis, decubitus ulcer, oxidative stress, tissue damage*

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Introduction

Since Jean Martin Charcot described it in 19th century, a considerable amount of research has grown up around the theme of pressure ulcers. Decubitus ulcers, also called pressure sores, are tissue necrosis and ulceration of the skin and/or soft tissue due to prolonged or strong pressure (1). Blood flow stops in the area of tissue exposed to a pressure above the capillary pressure (more than 30 mmHg). This results tissue hypoxia, necrosis and ulceration (2). Decubitus ulcers usually occur in areas where there are bony prominences. The severity of these injuries is evaluated in four stages. Stage I is the mildest of the decubitus ulcers. The epidermis is intact but there is non-blanchable hyperemia. The dermis is exposed in Stage II. However subcutaneous fat tissues is not affected. In Stage III, there is a full-thickness skin loss, but fascia, muscle, tendon, ligaments, cartilages and bony tissues are intact. In stage IV, complete loss of skin and serious damage occurs in subcutaneous structures such as skin, muscles and tendons (2), (3), (4).

Oxidative stress is defined as the deterioration in molecular and cellular functions as a result of the disruption of the balance between the formation of free radicals or reactive oxygen species and the antioxidant system. Reactive oxygen species are constantly formed in the human body and are removed by antioxidant defense systems. Antioxidants can act by scavenging reactive oxygen species, preventing their formation or repairing the damage they cause (5). In severe lesions such as skin ulcers or burn wounds, lipid peroxides are markedly increased. Lipid peroxidation is caused by the deformation of cell membrane phospholipids by oxidizing radicals. It has been reported that free radicals have a role in ischemic and inflammatory diseases and make wound healing difficult (6).

Thiols are a class of organic sulfur derivatives (mercaptans) containing a sulfhydryl group (-SH) in their active site (7) and are very important buffers that can interact with almost all physiological oxidants (8). Thiols, which are important antioxidants, have critical roles in the non-enzymatic destruction of reactive oxygen molecules and in preventing the formation of oxidative stress (9). It has been reported that thiols in plasma play physiological roles as free radical scavengers and act as antioxidants (10).

While oxidative products such as reactive oxygen species are reduced by donating their unpaired electrons to thiol-containing compounds; thiol groups are oxidized (11), (12). Thiols enter into oxidation reactions and

form disulfide bonds with oxidant molecules (9), (11). This bond is called an SS-bond or disulfide bridge. The disulfide bonds formed can be reduced back to thiol groups. Thus, dynamic thiol/disulfide homeostasis is achieved (12) (Figure 1). Thiol/disulfide homeostasis is required for detoxification (9). Thiol/disulfide homeostasis parameters are native thiol, total thiol, native thiol/total thiol ratios (antioxidant parameters) and disulfide, disulfide/native thiol, and disulfide/total thiol ratios (oxidant parameters) (9), (10). Native thiol contains only reduced thiols; total thiol includes both reduced and oxidized thiols (9).

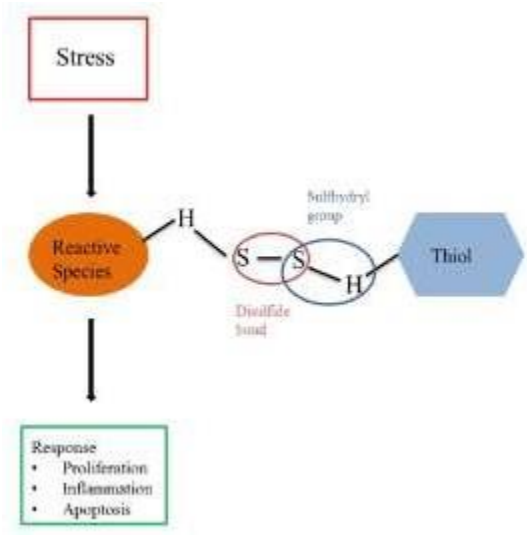

Figure 1. Thiol-disulfide homeostasis (13).

Measurement of plasma total thiols and assessment of thiol/disulfide homeostasis have been reported to be good indicators of excess free radical formation in humans (10). Systemically, oxidative stress can be assessed by measuring thiols (R-SH, sulfhydryl compounds) (13). In this study, it was aimed to investigate the blood levels of thiol and disulfide, which is a marker of total oxidant status, and the thiol/disulfide ratio in patients with decubitus ulcer hospitalized in the intensive care unit.

Material and Method

50 patients with decubitus ulcer and 50 healthy control cases hospitalized in Gaziantep University Medical Faculty Internal Diseases Intensive Care Unit in 2019 were included in the study. Gaziantep University Faculty of Medicine Ethics Committee approval was obtained for this study. For the evaluation of thiol/disulfide homeostasis, 10 ml venous blood samples were taken from each individual in the patient and control groups. The collected blood was transported to the laboratory in the first 30 minutes and centrifuged at 1500 rpm for 10 minutes and the plasma was separated. The obtained samples were stored in capped eppendorf tubes at -80°C until biochemical analysis.

Laboratory Method

Thiol/disulfide homeostasis parameters (Thiol, disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol),

which is one of the oxidative stress markers, were measured with a method developed by Erel and Neşelioğlu (12). Native thiol level (NTL) and total thiol level (TTL) were determined and their levels were measured. Half of the difference between the results obtained by subtracting the native thiol (-SH) amount from the total thiol (-SH+- S-S-) content shows the disulfide (-S-S-) level. In addition, thiol oxidation reduction ratio (-SH) × 100/ (-SS-), oxidized thiol ratio ((-S-S) x 100/ (-SH+-S-S-)) and reduced thiol ratio [-SH × 100/ (-SH+) -S-S-)] was calculated using these parameters.

Statistical analysis

Excel 2019 and SPSS 22.0 (Statistical Package for Social Sciences) statistics software were used to evaluate all the data obtained from the study. Normality analysis of variable and continuous data was evaluated with the Kolmogrov Smirnov test. The Student's T test was used to compare normally distributed data, while the Mann Whitney U test was used to compare data that did not show normal distribution. Normally distributed data were given as mean ± standard deviation. Data that did not show normal distribution were given as median (minimum/maximum). Statistical significance level was defined as $p < 0.05$.

Results

In this study, we compared thiol and disulfide levels and thiol/disulfide ratios which are indicators of total oxidant status, in patients with decubitus ulcer in the intensive care unit and healthy volunteers. Fifty people, 16 (32%) male and 34 (68%) female, were included in the healthy control group. Fifty patients, 21 (42%) male and 29 (58%) female, were included in the decubitus ulcer developed patient group. The mean age of the control group was 58.22 ± 9.10; the mean age of patients with decubitus ulcer was 55.96 ± 11.63 (Table 1). There was no statistical difference between the groups in terms of age and gender variables ($p>0.05$).

The oxidant and antioxidant parameters we examined in our study are given in Table 2. We observed that the levels of total thiol level, native thiol level, reduced thiol ratio, and thiol oxidation reduction ratio were significantly lower in patients with decubitus ulcer compared to the control group ($p<0.05$). On the other hand, disulfide and oxidized thiol levels were higher in the decubitus ulcer group compared to the control group ($p<0.05$).

Table 1. Demographic characteristics of the decubitus ulcer patient group and the healthy control group.

	AGE (years)	**GENDER n (%)**	**P value**
Decubitus ulcer patient group	55.96 ± 11.63	Male 21 (%42) Female 29 (%58)	>0.05
Healthy control group	58.22 ± 9.10	Male 16 (%32) Female 34 (%68)	>0.05

Table 2. Oxidant and antioxidant levels of the groups.

	Decubitus ulcer patient group (n=50)	**Healthy control group (n=50)**	**P value**
TTL (µmol/L)	214.97 ± 75.95	423.62 ± 70.31	<0.05
NTL (µmol/L)	115.16 ± 54.11	307.14 ± 57.74	<0.05
DISULPHIDE (µmol/L)	59.9(2.10/130.60)*	48.15 (7.70/129.80)*	<0.05
RTR (%)	53.49 ± 16.75	72.88 ± 11.28	<0.05
OTR (µmol/L)	25.65 (12.80/99.20)*	13.45(2.90/31.50)*	<0.05
TORR (%)	297.08 ± 197.13	477.48 ± 197.40	<0.05

TTL: total thiol level, NTL: native thiol level, RTR: reduced thiol ratio, OTR: oxidized thiol level, and TORR: thiol oxidation reduction ratio. *Values are presented as median (minimum/maximum).

Discussion

In this study, we investigated the thiol/disulfide homeostasis parameters, which are among the oxidant-antioxidant parameters, in patients with decubitus ulcer hospitalized in the intensive care unit. Ischemia develops when prolonged and/or strong mechanical pressure is applied to the body surface. As a result, cellular changes that can lead to tissue necrosis and ulceration occur (14). Prolonged pressure causes occlusion of blood vessels, ischemia, decline of nutrients and accumulation of metabolites. All these conditions cause tissue damage. Ischemia/reperfusion injury has an important place in the pathogenesis of decubitus ulcer. The duration of ischemia and the frequency of ischemia/reperfusion injury cycles increase tissue damage. Changes in the patient's body position eliminate the mechanical force on the skin. This causes tissue reperfusion (15). During ischemia/reperfusion, reoxygenation of ischemic tissue triggers the formation of reactive oxygen metabolites that have deleterious effects on cellular functions (14), (16) and may exacerbate the damage caused by ischemia (15).

Oxidative stress occurs as a result of the imbalance between reactive oxygen species and antioxidant molecules (the imbalance in favor of prooxidants). Overproduction of reactive oxidative species causes deterioration in the structure of proteins and lipids. Breakage in DNA structures and oxidation in cell membrane proteins and lipids occur (11). There is evidence that oxidative stress leads to harmful biochemical reactions and contributes to many diseases (17). Studies have shown that oxidative stress develops in patients with pressure ulcers (18), increased malondialdehyde levels and myeloperoxidase activity and decreased glutathione level (14), (16).

In physiological conditions, proteins targeted by reactive oxygen species are thiols. Thiols are mercaptans containing sulfhydryl residues and are the main molecules that coordinate antioxidant protective mechanisms (9). Thiol synthesis can be performed in all eukaryotic living cells. As the synthesized thiol groups are used for antioxidant purposes in the cell, their levels in the blood decrease (19). Measurement of total thiol in plasma and evaluation of thiol/disulfide homeostasis is an important indicator of free radical formation (10), (12). The deterioration of thiol-disulfide homeostasis as a result of the increase in oxidative molecules can be considered as a harbinger of possible diseases (11). Thiol/disulfide homeostasis has important

roles in antioxidant defense, detoxification, signal transduction, apoptosis, regulation of enzymatic activity and cellular signal transduction mechanisms. It has been reported that thiol/disulfide homeostasis is disrupted (the balance shifts towards disulfide) in the pathogenesis of various diseases such as cancer, diabetes mellitus, cardiovascular diseases, chronic renal failure, and rheumatoid arthritis. Systemic oxidative stress can be demonstrated by a decrease in free thiol (native thiols) and total thiol levels and an increase in disulfide levels (13). Erel and Neşelioğlu (12) showed that the reduced thiol concentration increased, the native thiol concentration decreased, and the disulfide values increased under conditions of increasing oxidant concentrations.

In this study, we drew attention for the first time to thiol/disulfide homeostasis in patients with decubitus ulcer in the intensive care unit and showed that this balance was impaired in patients with decubitus ulcer. Thiol is an antioxidant molecule that is abundant in the organism. Thiol-disulfide balance is an important parameter that shows the disorder in the oxidant-antioxidant balance. Our results showed that thiol levels were lower and disulfide levels were higher in patients with decubitus ulcer. This situation reveals the presence of oxidative process in patients with decubitus ulcer. It can be said that disulfide bond formation from the thiol group increases due to the reactive oxygen species formed and the thiol-disulfide balance shifts to the disulfide side. Dynamic thiol/disulfide homeostasis is only one of many oxidant-antioxidant systems in our body and does not show the total antioxidant level of the body (19). The inability to evaluate other inflammatory markers and oxidative stress parameters and the fact that they were not compared with thiol/disulfide balance parameters are among the limitedness of our study.

Conclusion

We showed that thiol/disulfide homeostasis, which is one of the oxidative stress parameters, is altered in patients with decubitus ulcer. With this study, he drew attention to this subject for the first time and gave information about the subject. However, more in-depth and comprehensive research is needed.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgement

No financial support was received from any institution for this research. All researchers contributed equally to the study.

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Experimental and Applied Medical Science, 3, 2: 354-364, 2022. DOI 10.46871/eams.2022.40

THE DIAGNOSTIC VALUE OF IMMATURE GRANULOCYTE PERCENTAGE IN ACUTE APPENDICITIS PATIENTS

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Experimental and Applied Medical Science, 3, 2: 358-370, 2022. DOI 10.46871/eams.2022.40

Abstract

Studies have suggested that immature granulocyte percentage (IG%) levels may be an early marker of inflammation. In this study, it was tried to investigate whether there is a diagnostic value of IG% in appendicitis patients. This study was conducted retrospectively between the dates of 01.01.2018 and 01.05.2019 at Mersin University Faculty of Medicine, Department of Emergency Medicine. Patients over 18 years of age who were admitted to the emergency department and operated with the diagnosis of acute appendicitis were included in the study. The patients were classified into three groups as normal (control group), simple and complicated appendicitis. A total of 353 patients with a pre-diagnosis of appendicitis, 194 (55%) male, were included in the study. The mean age of the patients was calculated as 35.1 ± 14.0. The leukocyte parameter was found to be significant in distinguishing the disease; It was determined that the values of the patient group were higher than the values of the control group. (p values, respectively; 0.022 and 0.038). For CRP parameter; those with inflamed appendix were found to be lower than those with perforated appendicitis (p <0.001). When the neutrophil percentage parameter is examined; it was determined that patients with perforated appendicitis were higher than those with inflamed appendicitis (p <0.001). The immature granulocyte percentage (IG%) was found to have low power to distinguish disease and complications. For immature granulocyte percentage (IG%); AUC (Area under curve) was 0.505 and cut value was 0.2 while p value was 0.9128 in differentiating the disease. In distinguishing complications; AUC was 0.649 and cut value was 0.4 while p value was 0.0510. In our study, it was found that immature granulocyte percentage was statistically insignificant in distinguishing disease and complication. It was determined that the leukocyte value was significant in distinguishing the disease, and the CRP and percentage of neutrophils were statistically significant in distinguishing complications.

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1. Introduction

Acute appendicitis is a disease with inflammation of the appendix vermiformis and is the most common surgical cause of acute abdomen among patients with abdominal pain presenting to emergency services (1).

The lifetime risk of appendicitis is 7% worldwide, 8.6% for men and 6.7% for women. Appendectomy rates are 12% in men and 26% in women. It is thought that this rate is lower in Asian and African societies due to fiber diet (2).

The clinical diagnosis of acute appendicitis is often difficult because symptoms may be atypical due to other concomitant medical conditions (3). The main clinical decision in the diagnosis of a patient with suspected appendicitis is whether to have surgery. Ideally, the goal is to quickly treat all cases of appendicitis without unnecessary surgical intervention.

Anamnesis, physical examination, laboratory tests and imaging methods are used in the diagnosis and management of acute appendicitis. The tests used for diagnosis and determination of clinical severity are leukocyte count, C-reactive protein (CRP) level, procalcitonin, and total bilirubin (4-11). However, in the results obtained in the studies, we still do not have an easily accessible, reliable test that can be used to diagnose acute appendicitis and determine its severity. Immature granulocytes (IG) are cells released from the bone marrow into the blood during the early stages of the inflammatory process. In previous studies, it was found that the percentage of immature granulocytes was found to be high in peripheral blood samples of patients with septic disease (12-16.)

The cornerstone in the management of patients with acute appendicitis is to make an early diagnosis before serious complications develop and to find the balance between surgery and non-surgical recovery. In this study, our aim is to determine whether the percentage of IG can be used in clinical decision making in the diagnosis of acute appendicitis and determining its complications.

2. Materials and Methods

In our study, patients aged 18 years and older who applied to Mersin University Faculty of Medicine, Department of Emergency Medicine between 01.01.2018 and 01.05.2019 and were operated with the diagnosis of acute appendicitis were included. Before starting the study, approval was obtained with the decision of the Mersin University Clinical Research Ethics Committee dated 13/05/2020 and numbered 2020/358. The study was carried out retrospectively by scanning the hospital electronic medical information system. Demographic data, leukocyte count, CRP levels, immature granulocyte percentages and Neutrophil percentages of all patients were recorded. The patients were divided into three groups according to their histopathological findings: Control group; normal appendix, Appendicitis group; uncomplicated appendicitis (acute appendicitis), Complicated appendicitis group; complicated appendicitis (perforated, plastron).

2.1. Exclusion Criteria

• Having heart failure

• Presence of hematological disease

• Having cancer

• Presence of chronic infection

• Having liver disease

• Presence of vascular disease

• Unable to access file information

• Having an infection or inflammatory disease

For the determination of leukocyte, neutrophil percentage and immature granulocyte percentage, after blood was drawn into the tube with EDTA, the measurement was made using the electrical impedance method on the (Beckman Coulter LH 780) analyzer and the ratios were calculated. Serum CRP level was measured by turbidimetric method (Roche Cobas C 501). The normal reference values of the parameters in our study were as follows: Leukocyte (4500-10000/mm3), Neutrophil percentage (42.2%-75.2%), Immature granulocyte percentage (0-0.6), Serum CRP (0-5 mg/dL).

2.2. Statistical Analysis

Normality controls of continuous measurements were tested with the Shapiro Wilk test. Analysis of variance was used for group comparisons. Levene test was used for homogeneity of variances. One Way ANOVA was used for the differences between the group means in those whose homogeneity of variances condition was met, and the Welch test was used when the condition was not met. For pairwise comparisons, Bonferroni test was used when variances were homogeneous and Games Howell test was used when homogeneity is not supplied. Pearson correlation coefficient was used to control the relationship between continuous variables. Number and percentage values were given as descriptive statistics. $p<0.05$ was taken as statistical significance.

3. Results

A total of 387 patients over the age of 18 who applied to Emergency Department between 01.01.2018 and 01.05.2019 were operated with a preliminary diagnosis of acute appendicitis, 34 patients were excluded from the study. A total of 353 appendiceal patients, 194 (55%) male and 159 (45%) female, were included in the study. Of the appendicitis patients included in the study, 50 of patients (14.2%) were in control group, 287 (81.3%)non-complicated appendicitis and 16 (4.5%) complicated appendicitis patients (Figure 1).

The mean age of the patients included in the study was calculated as 35.1 ± 14.0 years. The mean age of men was calculated as 33.7 ± 13.2, and the mean age of women as 36.9 ± 14.9, statistically significant difference was found between the mean age of the sexes (p=0.034).

For the leukocyte parameter; differences between groups were found to be significant (p=0.027). When the differences were examined, it was observed that the leukocyte values of only those with normal appendix were lower than those with non-complicated appendicitis (p=0.035). When the CRP parameter was examined, the differences between the groups were found to be significant (p=0.004). When the differences were examined, it was determined that the CRP values of those with complicated appendicitis were much higher than the CRP values of the control group and those with non-complicated

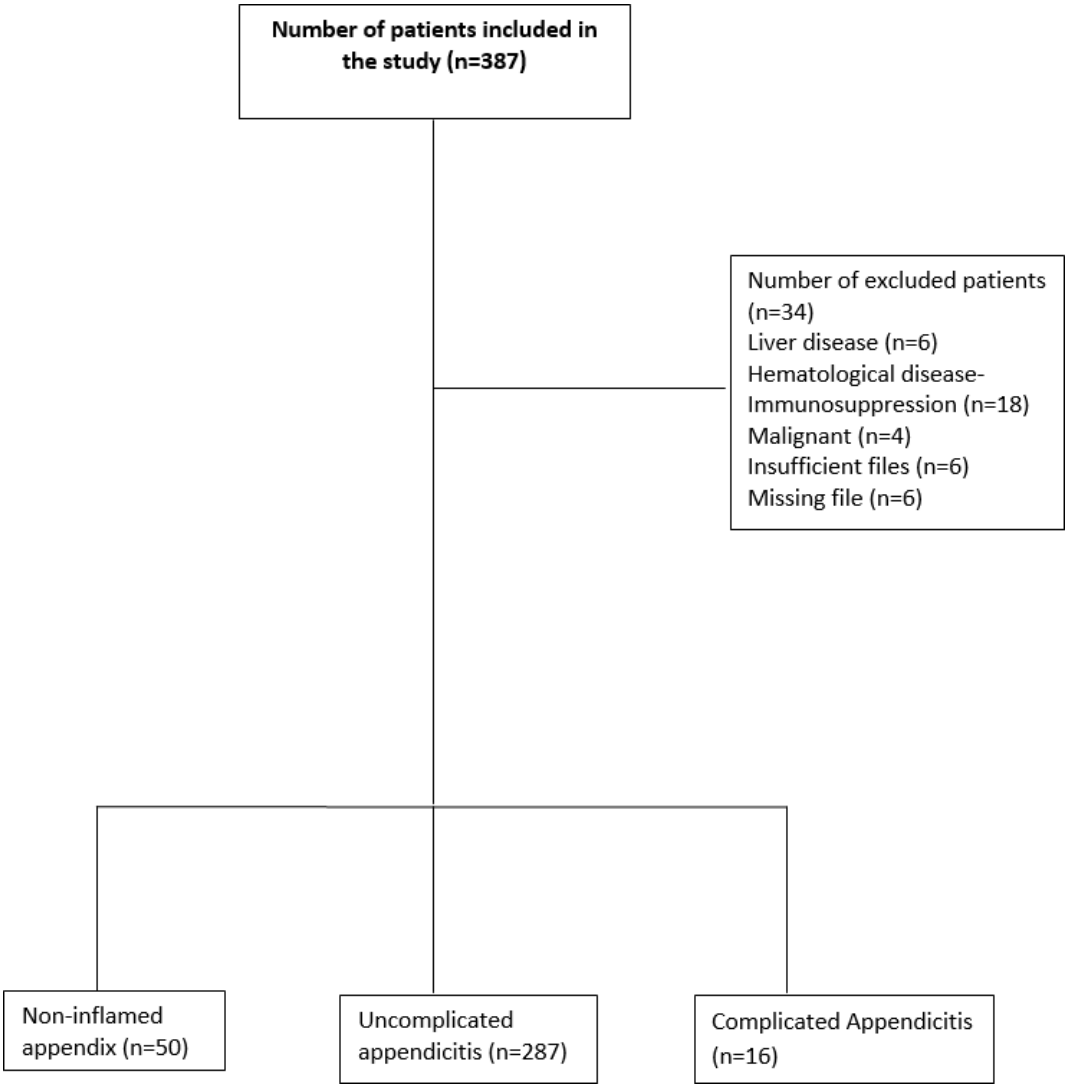


Figure 1. Flow chart of the patients included in the study

appendicitis (p values; 0.038 and 0.013, respectively). For the neutrophil percentage parameter, the differences between the groups were significant (p=0.003), and when the differences were analyzed, it was determined that the mean of the complicated group was higher than the control group and the non-complicated group (p values; 0.002 and 0.014, respectively). (Table 1).

The difference between the groups for the percentage of immature granulocytes was not significant. It can be said that only the leukocyte parameter is effective in separating normal and sick individuals (inflamed and complicated appendicitis) (p=0.0014, AUC=0.627). The cutoff point for the WBC parameter was calculated as 13.2. The positive predictive value (PPV) by cutoff point was 90.81 for WBC (Table 2). That means, 90.81% of what we call a acute appendicitis according to the WBC value is really acute appendicitis (without distinguishing between simple and complicated). However, values below the cut-off value determined for WBC were found to be low in exclusion of the disease. The other three laboratory values were not statistically significant in distinguishing sick individuals (Figure 2, Table 2).

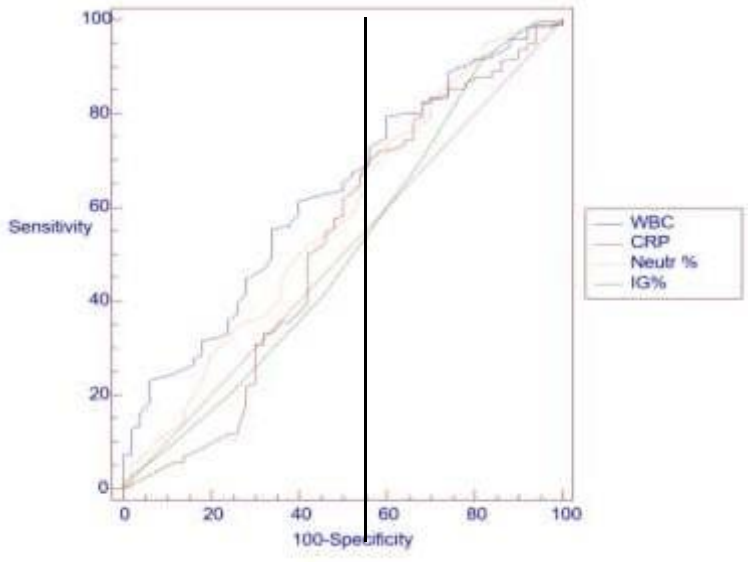
Table 1. Comparison of laboratory values between groups. WBC; white blood cells, CRP; C-reactive protein, Neutrophil %; neutrophil percentage, IG %; immature granulocytes percentage.

	Non-inflame Appendix (n=50)	**Un-complicated appendicitis (n=287)**	**Perfore Apandisit (n=16)**	**P**
Age	37,5 ±15.2	33,9 ± 13,2	48.4±18,3†	**0,007**
WBC	12,11 ± 3,61	13,97±5,7*	15,23±6,1	**0,027**
CRP	52,10±75,2	31,37±49,2	163.31±158,9*,†	**0.004**
Neutrophil %	73,18±12,4	75,82±9,6	82,37±7,4*	**0,003**
IG%	0,42±0,2	0,43±0,1	0,5±0,2	0,201

Table 2. Comparison of laboratory values of all appendicitis patients with normal apendix. WBC; white blood cells, CRP; C-reactive protein, Neutrophil %; neutrophil percentage, IG %; immature granulocytes percentage

	Cut-off	**AUC (p)**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**LR(+)**	**LR (-)**
WBC	>13,2	**0,627 (0,0014)**	55,45 (49,65-61,13)	66,00 (51,23-78,79)	**90.81** (85.69-94.55)	19.64 (13.93-26.47)	1,63 (1,3-2,0)	0,68 (0,4-1,0)
CRP	≤ 28	0,524 (0,5903)	70,30 (64,81-75,39)	44,00 (30,00-58,74)	88.38 (83.69-92.14)	19.64 (12.74-28.22)	1,26 (0,9-1,7)	0,68 (0,5-0,9)
NEU %	>71	0,575 (0,0719)	73,93 (68,60 - 78,78)	40,00 (26,41 - 54, 82)	88.19 (83.57-91.89)	20.20 (12.80-29.46)	1,23 (0,9-0,7)	0,65 (0,5-0,9)
Ig %	>0,2	0,505 (0,9128)	92,08 (88,44 - 94,86)	18,00 (8,59 - 31,44)	87.19 (83.02-90.65)	27.27 (13.33-45.53)	1,12 (0,6-2,0)	0,44 (0,3-0,7)

Figure 2. Comparative ROC analysis of disease discrimination characteristics of WBC(white blood cells) CRP(C-reactive protein), Neutr %(neutrophil percentage), IG%(immature granulocytes percentage)

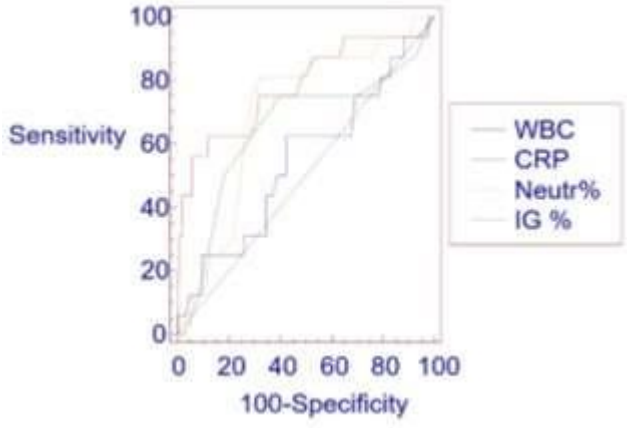


When Table 3 is examined, it is seen that the CRP and Neutrophil percentage parameters have a high statistical discrimination power. According to the table, non-complicated appendicitis patients are better differentiated by CRP values, while complicated appendicitis patients are better differentiated by Neutrophil parameter. For CRP; cut-off value was taken as 69 mg/L. It has been observed that the test's ability to distinguish patients with non-complicaed appendicitis is quite high; The selectivity value was calculated as 88.15 and the negative predictive value (NPV) as 97.68. This means: 88.15% of those with truly simple appendicitis have a CRP value of less than 69. In addition, 97.68% of individuals with a CRP below 69 actually have non-complicated appendicitis. Neutrophil % value, on the other hand, has a very high ability to distinguish complicated appendicitis. When the cut-off value of 81 for the neutrophil % value was taken, the selectivity in determining the complication was calculated as 88.15% and the negative predictive value as 98.48%; viz 98.48% of those with a neutrophil value above 81% have complicated appendicitis (Figure 3, Table 3).

Table 3. Comparison of laboratory values when patients were classified as simple and complicated. WBC; white blood cells, CRP; C-reactive protein, Neutrophil %; neutrophil percentage, IG ; immature granulocytes percentage

	Cut-off	**AUC (p)**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**LR (+)**	**LR (-)**
WBC	>14.4	0.546 (0.5430)	62.50 (35,47 - 84,71)	57.49 (51,55 - 63,28)	7.58 (3,70-13,49)	96,49 (92,52-98,69)	1,47 (1,0-2,2)	0,65 (0,3-1,2)
CRP	>69	**0.781 (0.0001)**	62.50 (35,47 - 84,71)	**88.15** (83,84- 91,65)	22.73 (11.49-37,85)	**97,68** (95,02-99,14)	5,28 (3,6-7,7)	0,43 (0,2-0,9)
NEU %	>81	**0.701 (0.0072)**	**81.25** (54,34 - 95,73)	67.60 (61,85 - 72,98)	12,26 (6,70-20,06)	**98,48** (95,61-99,67)	2,51 (2,0-3,2)	0,28 (0,1-0,8)
IG %	>0.4	0.649 (0.0510)	75.00 (47,63 - 92,58)	59.93 (54,01 - 65,65)	9,45 (4.98-15,93)	97,73 (94,28-99,36)	1,87 (1,4-2,5)	0,42 (0,2-1,0)

Figure 3. ROC analysis of four parameters by disease subgroups comparatively. WBC; white blood cells, CRP; C-reactive protein, Neutr %; neutrophil percentage, IG %; immature granulocytes percentage



4. Discussion

The lifetime risk of appendicitis is 8.6% in men and 6.7% in women. Appendectomy rates are 12% in men and 26% in women (2). The reason for the high rate in women is thought to be the inability of definitive diagnostic confirmation and the confusion of pelvic pathologies with appendicitis in women17. Similarly, in our study, the rate of negative appendectomy was found to be higher in female patients; It was determined that 73.7% of men had inflamed appendicitis, while 74% of women had normal appendix.

Clinical findings, laboratory tests and imaging methods are used together in the diagnosis of acute appendicitis (1-3). Many biomarkers have been used in the diagnosis of acute appendicitis for a cost-effective approach, to shorten the hospital stay of patients, and to detect complications early (4-11).

As demonstrated in studies, it has been observed that inflammatory markers are important in the diagnosis of acute appendicitis, but other factors that increase inflammation reduce the diagnostic value (1-3). In addition, it is stated in studies that laboratory findings should be evaluated together with clinical findings (1-3). However, supportive tests are still needed for accurate diagnosis and early detection of complications (1-3).

The leukocyte value is used as a diagnostic parameter in patients with acute appendicitis. High leukocyte values support the diagnosis. However, it should be kept in mind that it also increases in other inflammatory conditions and the diagnosis of acute appendicitis cannot be excluded in values below the cut-off value. In a meta-analysis study conducted by Andersson in 2004, the sensitivity of the white blood cell count (WBC) in the diagnosis of acute appendicitis was found to be 83% and the specificity 67%, and it was shown that the predictive values increased as the white blood cell count increased (3). In the same study, the positive likelihood ratio (LR+) of high WBC values in predicting perforation was found to be 7.263. In the study conducted by Suat Ulukent et al., the sensitivity for the WBC cutoff value of 8650 was 76%, the specificity was 94%, and the AUC was 0.911. However, there was no analysis for complicated appendicitis in this study()18.

In the study of Zuhoor K Al-gaithy, when normal appendix and appendicitis patients were compared, WBC sensitivity was 76.8%, specificity was 65.5%, and AUC was 0.701. When the complicated and normal group were compared, the AUC was 0.76, the sensitivity 76.6, the specificity 72.4, the positive predictive value was 97%, and the negative predictive value was 16.1%()19. In a study conducted by Dong Hyuk Shin et al., the sensitivity of the WBC value was 70.9%, the specificity 65.7%, and the AUC value was 0.687()20. In our study, the literature is supported, and it was found that a high WBC value has a high ability to distinguish sick individuals. According to the result of our study, when the cut-off value was 13.200/mm3 in distinguishing sick individuals, the AUC value was found to be 0.627, and the sensitivity was found to be 55.45% and the selectivity as 66%. The positive predictive value, which is statistically significant in distinguishing sick individuals, is 90.81%. However, in our study, it was revealed that WBC did not determine the risk of complications of the disease.

C-Reactive Protein (CRP) is an acute phase reactant that starts to rise at 8-12 hours of inflammation and peaks at 24-48 hours. In the study conducted by Ghimire, it was shown that there is a clinical correlation between the high CRP value and the probability of acute appendicitis()21. In the study conducted by Amalesh et al., it is stated that CRP has no place in the diagnosis; In this study, the sensitivity was 91%, while the specificity was 42%22. In a study conducted with 542 patients, the AUC value of CRP in acute appendicitis patients was 0.60 on the first day, 0.77 on the second day and 0.88 on the third day; in the case of perforation, the AUC value is 0.90 on the first day()23. According to a meta-analysis, the sensitivity of CRP was found to be 65-85%, and the specificity as 59-73%()24. In our study, on the other hand, CRP value was found to be an important marker in determining complications in correlation with the general studies. For CRP; Below 69 mg/L is indicative of uncomplicated appendicitis; The selectivity value was 88.15, the sensitivity value was 62.5%, the NPV value was 97.68, and the PPV was 22.7%.

The percentage of neutrophils has become a hemogram parameter that has started to be included in the statistics in the differential diagnosis of diseases in recent years. In a retrospective study conducted on 897 patients with suspected acute appendicitis, the sensitivity was 87.2% and the specificity was 26.1% when the Neutrophil % cut-off value was 74% and above. In the same study, the combined specificity of CRP, WBC and Neutrophil percentage values was found to be quite low as 6.1%()25. In the study conducted by Xharra et al., when the cut-off value of the neutrophil percentage was taken as 75% and above, the sensitivity of the neutrophil percentage alone was 79.1%, and the specificity was 61%. In the combination of WBC, CRP and neutrophil percentage, the sensitivity was 95.3% and the specificity was 91.9%()26. In the study conducted by Andersson et al., it was stated that the Neutrophil % value could be used as a tool in determining complications()27. In our study, it was observed that the % Neutrophil was not statistically significant in diagnosing, however, it was an important laboratory value in determining complications. When the cut-off value was taken as 81 for the neutrophil % value, the selectivity was calculated as 88.15% and the negative predictive value as 98.48%.

Immature granulocyte cells are a collection of precursor cells formed by promyelocyte, myolocyte, and metamyolocytes that are not normally found in peripheral blood()28. Studies show that immature granulocytes can be used as an early inflammation marker in the presence of inflammation()12-16. There are studies showing that immature granulocyte amounts can be significantly high in many inflammatory conditions such as liver abscess, infective complications after cardiac surgery, sepsis or acute abdomen, and therefore it can be used as an early diagnosis tool()12-16. Ayres et al. stated that they excluded the diagnosis of sepsis with a specificity of 90.9% and a sensitivity of 38.5% in patients with a cut-off value of 2% for IG%, and stated that IG% is a marker that can be used in early diagnosis in patients with sepsis()29. In their study, Park et al. found that the sensitivity of IG% for sepsis was 81.3% and the specificity was 91%()30. In the study conducted by Ünal Y. on 438 patients, the cut-off value for the percentage

of IG was 0.4, the sensitivity was 47%, and the specificity was 88.4%. When the cut-off value was 0.6 in patients with complicated appendicitis, the sensitivity was 94.4% and the specificity was 97.9%()12. In the study of Jae-Sang Park et al., the cut-off value was 0.3, the sensitivity was 68% and the specificity was 44%. In the same study, other inflammatory markers were found to be more significant than the percentage of immature granulocytes in the ROC curve analysis()31. In our study, when the sick and non-patient group was examined, when the cut-off value was 0.2 for IG%, the sensitivity was 92.08% and the selectivity was 18%. It was observed that it was not statisically significantand other parameters were significantin the comparative ROC analysis. When the simple and complicated groups were compared, the sensitivity was 75% and the selectivity was 59.93% for the cut-off value of 0.4.

The limitations of our study are that it is single-centered and the temporal relationship between clinical findings and laboratory values is not mentioned.

5. Conclusion

As a result, CRP, WBC and neutrophil percentage have been found to have diagnostic value in the evaluation of patients with suspected acute appendicitis. It has been revealed in our study that the percentage of immature granulocytes, which has been suggested as a new marker of inflammation in recent years, has no place in the diagnostic evaluation of acute appendicitis. When evaluated together with previous studies, it was concluded that there is still no reliable test in the management of acute appendicitis, and future studies are important.

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