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GAZİANTEP ISLAM SCIENCE AND TECHNOLOGY UNIVERSITY FACULTY OF MEDICINE

Experimental and Applied Medical Science

Volume 3, Issue 1

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Contact information:

Gaziantep Islam Science and Technology University, Faculty of Medicine
Beştepe neighbouhood, Street number 192090 6/1, Zip Code 27010 Şahinbey/Gaziantep
Tel: +90 342 909 7500
E-mail: eams@gibtu.edu.tr

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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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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 Medical Science 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 journal receives manuscripts for consideration to be publishing in the form of research articles, reviews, letter to editor, brief notification, 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.

Ethical Principles and Publication Policy

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.

Experimental and Applied Medical Science strictly adheres to the principles set forth by "Helsinki Declaration" whose web address is indicated below. https://www.gibtu.edu.tr/Medya/Birim/Dosya/20210525133548_b192cec0.pdf

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*

Etik İlkeler ve Yayın Politikası

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.

Experimental and Applied Medical Science internet adresi aşağıda yer alan "Helsinki Deklarasyonu" ile belirlenen ilkelere sıkı sıkıya bağlıdır. https://www.gibtu.edu.tr/Medya/Birim/Dosya/20210525133548_b192cec0.pdf

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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All researchers should have contributed to the article directly either academically or scientifically. Authors should have contributed either one or a few of planning, performing, writing or reviewing of manuscript. All authors should approve the final version. It is the authors'

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. Değerlendirmeve 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.

Derginin tüm masrafları Gaziantep İslam Bilim ve Teknoloji Üniversitesi Tıp Fakültesi tarafından karşılanmaktadır. Reklam vermeyi düşünen kişi veya kurumlar yayın ofisi ile iletişime geçmelidir. Reklam görselleri sadece Baş Editör'ün onayı ile yayınlanabilir.

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.

Statements or opinions expressed in the manuscripts reflect the views of the author(s) and not the opinions of the Medical Faculty of Gaziantep Islam Science and Technology University, editors, editorial board, and/or publisher; the editors, editorial board, and publisher disclaim any responsibility or liability for such materials.

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.

Bütün çalışmalar ve editör kurulu ile yazışmalar çevrimiçi olarak, https://dergipark.org.tr/tr/pub/eams adresi üzerinde yayın ofisine gönderilmelidir.

İnternet adresi aşağıda belirtilmiş olan, ilk olarak 13/12/1951 tarih ve 5846 sayılıKanun ile Resmi Gazete'de yayımlanan, sonraları üzerinde değişiklikler yapılmış veya yeni kısımlar eklenmiş olan Fikir ve Sanat Eserleri Kanunu'na göre; yayına kabul edilen makalelerin her türlü yayın hakkı dergiyi yayınlayan kuruma aittir. Ancak makalelerdeki düşünce ve önerilertamamen yazarların sorumluluğundadır.

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Author Guidelines

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. Pages should 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 görüşünedayanacaktır.

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.
Papers describing animal experiments must define species, strain, sex, age, supplier and number of animals used. An ethical statement concerning the use of animals, or the details of ethical approvals, consent and recruitment of human subjects should be clearly stated. *Results* and *Discussion* can be broken down into subsections for improving the comprehensibility. The Results should not repeat methodological details and should avoid the discussion of the data.
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

All dimensions and measurements must be specified in the metric system.

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.

Kaynaklar “Vancouver” tarzında yazılmalıdır. Kaynaklar yazılırken, “Times New Roman” 10 punto kullanılmalıdır. Birden çok yazar virgülle ayrılmalıdır. Hem makale hem de kitap referanslarında, eğer üçten çok yazar varsa, 3. Yazardan sonra virgül ve “et al.” ifadesi kullanılmalıdır. Kitapta bir bölümden referans yapılıyorsa ve sadece bu bölüme ait çok sayıda yazar varsa, kitabın başlığı ve bölümü belirtilip, bölüm yazarlarının ilk üçü yazılıp veardından sonraki yazarlar için “et al.” ifadesi eklenmelidir.

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.
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.

asit fenetil ester (KAFE) ve miyokardiyal iskemi reperfüzyon (Mİ/R) hasarı. İnönü Üniversitesi Sağlık Bilimleri Dergisi 2012; 1: 10-5.

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.

Submit illustrations as separate files, only as TIFF or EPS files, with a minimum resolution of 300dpi.

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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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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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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Sayısal veri tablolarının her biri, sayılarla sırayla numaralandırılmış bir başlık ile birlikte ve metinde Tablo 1, Tablo 2, vb. olarak atıfta bulunulmuş halde, ayrı sayfalarda çift aralıkla hazırlanmalıdır. Her tablonun kısa ama açıklayıcı bir başlığı olmalıdır. Tablo dipnotlarında açıklayıcı hususlara yer verilmelidir.

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ozlem.akay@gibtu.edu.tr

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Gaziantep Unıversıty, Faculty of Medıcıne
ilkaydogan@gantep.edu.tr

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 takeonly 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.

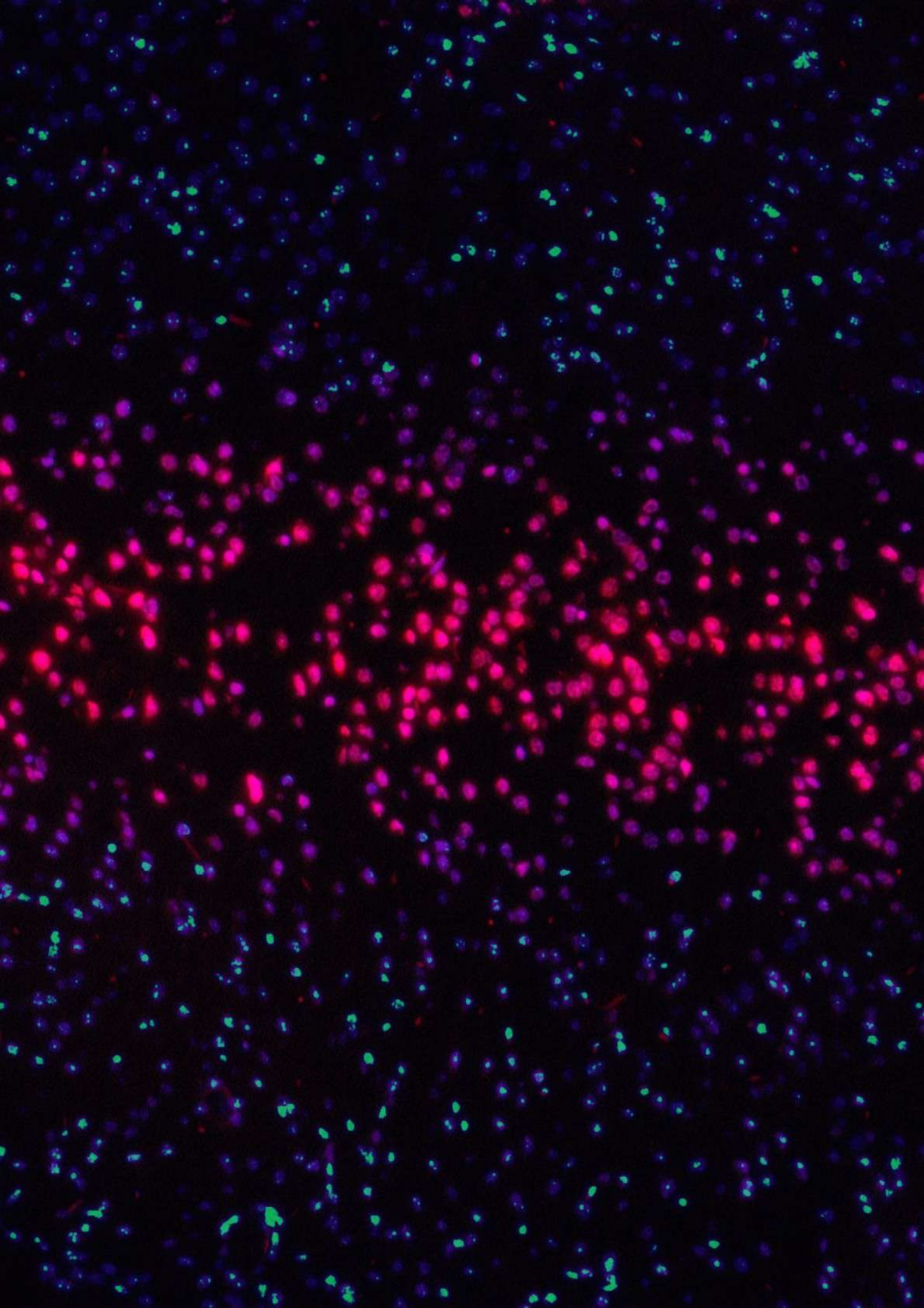
Nowadays, academic studies are accelerating, multiplying and diversifying. The need for channels where scientific studies, opinions and ideas can be freely expressed and easily shared with experts, researchers or postgraduate students who are still in the learning phase is increasing day by day. "Experimental 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 reviewed evaluation 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 improvetheir 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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Neutrophil-to-Lymphocyte Ratio and Insulin Resistance Relationship in Obese Individuals with Normal and Impaired Glucose Tolerance

Eyyüp Murat EFENDİOĞLU[1*], Mustafa ARAZ[2]

[1]*Gaziantep University, Faculty of Medicine, Department of Internal Medicine, Division of Geriatric Medicine, Gaziantep, Turkey.*
[2]*Gaziantep University, Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Gaziantep, Turkey.*

Abstract

Neutrophil-to-lymphocyte ratio (NLR) may be a predictor of glucose intolerance in obese individuals and may be useful in the early detection of glucose intolerance. In this study, we aimed to investigate the relationship between NLR and insulin resistance (IR) in obese individuals with normal and impaired glucose tolerance. Seventy-three obese patients and 27 healthy controls were included in this study. The participants' sociodemographic characteristics, anthropometric measurements (height, body weight, and waist circumference), fasting plasma glucose, oral glucose tolerance test (OGTT), insulin, HbA1c, total cholesterol, triglyceride, high-density lipoprotein (HDL), thyroid-stimulating hormone (TSH), complete blood count, and C-reactive protein (CRP) results were obtained from the files. Homeostasis Model Assessment Insulin Resistance (HOMA-IR) values were calculated. The mean age of the 100 patients was 36.4±10.5 years, and 59.0% were female. There was a statistically significant positive correlation between the HOMA-IR and the BMI (r = 0.457, p = 0.000), HbA1c (r = 0.359, p = 0.000), CRP (r = 0.444, p = 0.000), and waist circumference (r = 0.478, p = 0.000). There was no statistically significant difference between the obese and healthy control groups in terms of NLR. However, there was a significant difference in NLR, CRP, and neutrophil counts between the high HOMA-IR and normal HOMA-IR groups. In our study, neutrophil counts and CRP were determined to be higher among obese individuals than among healthy individuals. The NLR was increased significantly among patients with IR.

***Key words**: Obesity, Insulin resistance, Neutrophil-to-lymphocyte ratio.*

[*]**Corresponding Author:** Eyyüp Murat Efendioğlu, E-mail: eefendioglu@gmail.com. ORCID ID: 0000-0002-3257-7352.

Introduction

Obesity is an epidemic worldwide and increases the risk for many diseases, particularly insulin resistance (IR) and type 2 diabetes mellitus (T2DM). Obesity is considered a state of chronic and low- grade inflammation as well as increase doxidative stress (1). The relationship between chronic low-grade inflammation, IR, and other obesity-associated metabolic disturbances has become increasingly recognized (2). Insulin resistance is common in patientswith chronic inflammatory diseases characterized by unbalanced secretion of proinflammatory and anti-inflammatory cytokines. Measurement of IR provides an early and strong prediction of T2DM (3). The precise molecular effect leading to IR is not yet understood, but several studies have shown the relationship between systemic inflammation and IR (4, 5). Recent studies have shown that combined indices derived from whole blood counts, such as neutrophil-to-lymphocyte ratio (NLR), are good indicators of systemic inflammation and immune status and are widely used in clinical diagnosis and prognosis evaluation. Generally, both acute and chronic inflammation cause elevated NLR through relative neutrophilia and lymphopenia. Neutrophil-to-lymphocyte ratio is a dynamic state and influenced by inflammatory cytokinesand endocrine effects of the hypothalamic-pituitary axis (6, 7).

Previous studies indicate that an elevated NLR is associated with cardiovascular diseases (acute coronary syndrome outcomes, heart failure, and atrial fibrillation) (8-10). Also, NLR has been recently defined as a novel potential inflammation marker in many cancers and inflammatory diseases (11-13).

In a recent study, it was stated that NLR could be used as a marker for obese individuals with high IR, but the lack of measurement regarding other inflammatory markers such as C-reactive protein and demographic data (eg, waist circumference) stood out as important limitations in that study (14).

Neutrophil-to-lymphocyte ratio may be a predictor of glucose intolerance in obese individuals and may be useful in the early detection of glucose intolerance. In this study, we aimed to evaluate the relationship between NLR and IR in obese individuals with normal and impaired glucose tolerance.

Materials and Methods

All procedures performed in studies involving human participants were in accordance with the ethical standards ofthe institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Approval for the study was granted by the Gaziantep University Clinical Research Ethics Committee with decision number 2015/330. A total of 100 outpatients between the ages of 18-65 who applied to our Endocrinology and Metabolism Outpatient Clinics of the Department of Internal Medicine were included in this study from December 2014 to December 2015 and the files of the patients were scanned retrospectively. A body-mass index (BMI) of 18.5 to 25 kg/m^2 was considered normal, and a BMI of 30 kg/m^2 and higher was considered obese.

The participants were divided into 3 groups:

Group 1: Obese patients with normal glucose tolerance (NGT) (BMI $\geq$30 kg/m^2, n=43).

Group 2: Obese patients with impaired glucose tolerance (IGT) (BMI ≥30 kg/m^2, n=30).

Group 3: Healthy control group with impaired fasting glucose (normal BMI and NGT, n=27).

Major exclusion criteria were infectious conditions, malignancies, inflammatory rheumatic diseases, pregnancy, chronic kidney disease, acute and chronic liver disease. The participants' sociodemographic characteristics, anthropometric measurements (height, body weight, and waist circumference), fasting plasma glucose, OGTT, insulin, HbA1c, total cholesterol, triglyceride, HDL, TSH, complete blood count, and CRP results were obtained from the files.

Neutrophil-to-lymphocyte ratio was calculated as the simple ratio between the absolute neutrophil and lymphocyte count, which were both obtained from the same automated blood sample. Fasting insulin was measured by enzyme-linked immunosorbent assay, and IR was calculated using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR). Homeostatic Model Assessment for Insulin Resistance was calculated using the equation: HOMA- IR=Fasting insulin (μU/mL) x Fasting glucose (mg/dL) /405 and a HOMA-IR value of ≥2.5 was considered IR.

Statistical analysis

The normality of distribution of continuous variables was tested by the Kolmogorov Smirnov test. The independent samples t-test was used to compare two normally distributed independent groups, Mann–Whitney U-test to compare two non-normally distributed independent groups, and Kruskall Wallis test was used to compare three non-normally distributed independent groups. Relationships between categorical variables were tested with chi-square analysis, and relationships between numerical variables were tested with Spearman's rank correlation coefficient. SPSS for Windows version 22.0 package program was used for statistical analysis and $p<0.05$ was considered statistically significant.

Results

The mean age of the 100 patients was 36.4 ± 10.5 years, and 59.0% were female. Participants' socio-demographic characteristics, anthropometric measurement results, and laboratory analysis results are shown in Table 1. CRP and neutrophil counts were significantly higher in the IGT and NGT obese groups compared to the healthy control group ($p = <0.001$ and $p = 0.007$, respectively). NLR was found to be higher in individuals with high HOMA-IR compared to those with normal HOMA-IR ($p = 0.050$) (Table 2). There was a statistically significant positive correlation between the HOMA-IR and the BMI ($r = 0.457$, $p = 0.000$), HbA1c ($r = 0.359$, $p = 0.000$), CRP ($r = 0.444$, $p = 0.000$), and waist circumference ($r = 0.478$, $p = 0.000$) (Table 3).

Table 1: Participants' socio-demographic characteristics, anthropometric measurement results, and laboratory analysis results.

	IGT obese (n=30)	NGT obese (n=43)	Healthy control (n=27)	p	Total (n=100)
Gender					
Female	17 (56.7%)	26 (60.5%)	16 (59.3%)	0.948	59 (59.0%)
Male	13 (43.3%)	17 (39.5%)	11 (40.7%)		41 (41.0%)
Age(years) [#]	41.5±9.3*	34.3±11.8	34.1±7.3	0.005*	36.4±10.5
BMI (kg/m²)[#]	37.3±6.5*	34.4±4.4*	24.0±2.7*	<0.001*	32.4±7.1
Waist circumference (cm) [#]	121.1±11.9*	114.7±15.4*	84.9±12.3*	<0.001*	108.5±20.0
Insulin (mU/ml) [†]	13.6	9.9	6.0*	<0.001*	9.6
HbA1c (%)[†]	6.0*	5.5	5.4	0.001*	5.6
HOMA-IR[†]	3.4	2.2	1.3*	<0.001*	2.2
Total cholesterol (mg/dl) [†]	209	197	200	0.662	199
Triglyceride (mg/dl) [†]	157	151	117	0.082	146
HDL (mg/dl) [†]	49	45*	53*	0.007*	48
TSH (uIU/mL) [†]	2.3	2.5	1.5*	<0.001*	2.1
CRP (mg/dl) [†]	4.6	4.1	1.2*	<0.001*	2.9
Neutrophil (10^{3}/µL) [†]	4745*	4310	3710*	0.007*	4245
Lymphocyte (10^{3}/µL) [†]	2645	2500	2200	0.076	2495
NLR[†]	1.78	1.53	1.65	0.880	1.63

*$p \leq 0.05$. [#]Data are presented as mean±SD; [†]Data are presented as median; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; BMI, body mass index; HOMA-IR, the homeostasis model assessment- estimated insulin resistance; CRP, C-reactive protein**;** NLR, neutrophil-to-lymphocyte ratio.

Table 2: Comparison of the participants according to HOMA-IR values.

	HOMA-IR <2.5 (n=56)	HOMA-IR ≥2.5 (n=44)	*p*	Total (n=100)
Gender				
Female	36 (64.3%)	23 (52.3%)	0.306	59 (59.0%)
Male	20 (35.7%)	21 (47.7%)		41 (41.0%)
Age(years) #	34.3±10.2	39.1±10.3	0.024*	36.4±10.5
BMI (kg/m²)#	30.0±6.3	35.5±7.0	<0.001*	32.4±7.1
Waist circumference (cm) #	100.1±19.0	118.2±17.0	<0.001*	108.5±20.0
Insulin (mU/ml) †	6.7	16.9	<0.001*	9.6
HbA1c (%)†	5.4	5.8	<0.001*	5.6
Total cholesterol (mg/dl) †	196	209	0.158	199
Triglyceride (mg/dl) †	124	174	0.002*	146
HDL (mg/dl) †	50	45	0.045*	48
TSH (uIU/mL) †	1.9	2.3	<0.001*	2.1
CRP (mg/dl) †	1.9	4.5	<0.001*	2.9
Neutrophil ($10^3/\mu L$) †	3930	4765	0.005*	4245
Lymphocyte ($10^3/\mu L$) †	2505	2495	0.721	2495
NLR†	1.51	1.84	0.050*	1.63

*p≤0.05. #Data are presented as mean±SD; †Data are presented as median; BMI, body mass index; HOMA-IR, the homeostasis model assessment-estimated insulin resistance; CRP, C-reactive protein**;** NLR, neutrophil-to-lymphocyte ratio.

Table 3: Correlation analysis results between categorical variables.

		Age	BMI	Waist circumference	Insulin	HbA1c	CRP	NLR	HOMA-IR
Age	r		0.302	0.401	0.102	0.364	0.082	-0.064	0.148
	p		0.002*	0.000*	0.313	0.000*	0.415	0.529	0.141
BMI	r	0.302		0.847	0.420	0.339	0.588	-0.012	0.457
	p	0.002*		0.000*	0.000*	0.001*	0.000*	0.903	0.000*
Waist circumference	r	0.401	0.847		0.459	0.279	0.519	0.007	0.478
	p	0.000*	0.000*		0.000*	0.005*	0.000*	0.944	0.000*
Insulin	r	0.102	0.420	0.459		0.322	0.394	0.133	0.980
	p	0.313	0.000*	0.000*		0.001*	0.000*	0.188	0.000
HbA1c	r	0.364	0.339	0.279	0.322		0.428	-0.037	0.359
	p	0.000*	0.001*	0.005*	0.001*		0.000*	0.718	0.000
CRP	r	0.082	0.588	0.519	0.394	0.428		0.148	0.444
	p	0.415	0.000*	0.000*	0.000*	0.000*		0.142	0.000
NLR	r	-0.064	-0.012	0.007	0.133	-0.037	0.148		0.126
	p	0.529	0.903	0.944	0.188	0.718	0.142		0.212
HOMA-IR	r	0.148	0.457	0.478	0.980	0.359	0.444	0.126	
	p	0.141	0.000*	0.000*	0.000*	0.000*	0.000*	0.212	

r: Spearman rank correlation coefficient. *Significant at 0.01 level. BMI, body mass index; CRP, C-reactive protein (mg/dl); NLR, neutrophil-to-lymphocyte ratio; HOMA-IR, the homeostasis model assessment-estimated insulin resistance.

Discussion

Our study has shown that IR, BMI, CRP, neutrophil, and waist circumference were significantly higher in the obese patients with IGT and NGT compared to the healthy control group. There was no statistically significant difference for the NLR between groups. However, we observed that both NLR and neutrophil count increased as the IR level increased.

Experimental studies have demonstrated a link between chronic inflammation and IR through mechanisms that include obesity and atherosclerosis (15, 16). Obesity is also associated with inflammation. A previous study revealed that obesity, defined by BMI and waist circumference, was associated with inflammation (17).

Studies evaluating the relationship between NLR and obesity have shown that other markers such as CRP are more closely associated with obesity, and NLR tends to increase in obese patients (18, 19, 20). Previous studies illustrated that NLR could be a potential surrogate marker of systemic inflammation with its ability to predict CRP levels (21, 22). However, some studies opposed these observations. A study conducted in Turkey has reported that NLR was similar in obese and non- obese individuals (14). Also in a study, NLR was not found to be a good indicator of inflammation, while hs-CRP andleukocytes were more useful biomarkers to indicate inflammation in non-diabeticpatients with obesity (23).

In one study, NLR was found to be higher in individuals with IGT, newly diagnosed with diabetes by OGTT, and previously diagnosed with diabetes compared to individuals with NGT (24). Additionally, a study showed that NLR is a risk factor for IR with DM (25).

Imtiaz et al. demonstrated that systemic inflammation measured by NLR is closely associated with prevalent chronic conditions such as hypertension and diabetes (13). Another study has demonstrated the association of NLR with glucose intolerance and IR severity (26).

According to our results, we observed that HOMA-IR and HbA1c values were higher among the obese patient group with IGT than the obese patient group with NGT, while the NLR was similar between thetwo groups.

The present study had several limitations. This preliminary study involved a retrospective analysis of a small population from a single institution. Despite these limitations, this study has some strengths. We included participants without comorbidities that could affect inflammatory parameters, thereby ensuring more homogeneous groups of participants. In addition, combining anthropometric measurements and laboratory measurements was one of the strengths of our study.

In conclusion, neutrophil counts and CRP were determined to be higher among obese individuals than among healthy individuals in our study. Also, NLR, neutrophil counts, and CRP were found to be significantly higher in individuals with IR compared to individuals without IR.

Studies with prospective designs and multiple NLR measurements may provide more robust evidence to demonstrate the role of NLR as a marker of subclinical inflammation and a predictor of prediabetes risk.

Conflict of interest: The authors report no actual or potential conflicts of interest. The authors confirm independence from the sponsors, the content of the article has not been influenced by the sponsors.

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Comprehensive Analysis of Basal Ganglia Densities in Acute Middle Cerebral Artery Ischemia on Computed Tomography

Erdal KOMUT[1*], Seval KOMUT[2]

[1]*Hitit University, Department of Radiology, Faculty of Medicine, Çorum, Turkey.*
[2]*Hitit University, Department of Emergency Medicine, Faculty of Medicine, Çorum, Turkey.*

Abstract

To present a new objective radiological criterion that can detect early cerebral ischemia byanalyzing the density changes in the basal ganglia, which are well-characterized anatomical structures in non-contrast brain tomography of patients with acute ischemic stroke confirmed by diffusion-weighted magnetic resonance imaging. Patients who underwent brain tomography and diffusion-weighted imaging due to a suspected acute ischemic stroke with normal tomography findings were included in the study. Ischemic cases were included in the acute ischemic stroke group, and those that were not diagnosed with ischemia were included in the control group. The densities of the thalamus and basal ganglia were measured in all patients. In the control group, the left basal ganglia and thalamus densities were higher compared to the right side. In the acute ischemic stroke group, the densities of the lentiform and caudate nucleus were significantly higher on the non-ischemic side than on the ischemic side. The acute ischemic stroke group had a lower symmetrical agreement in terms of the densities of the basal ganglia and thalamus compared to the control group. In acute ischemic cases, density changes in the basal ganglia and thalamus are promising indicators that can be used in radiological diagnosis in the early period and at the time of non-contrast brain tomography.

Key words: *Stroke, Brain tomography, Emergency medicine.*

[*]**Corresponding Author:** Erdal Komut, E-mail: erdalkomut@hotmail.com. ORCID ID: 0000-0003-2656-0420.

Introduction

Acute ischemic stroke (AIS) is one of the most important causes of morbidity and mortality in developed countries (1). In the diagnostic management of AIS, non-contrast brain computed tomography (CT) maintains its place as the basic and first-line radiological method (2). Brain computed tomography is a fast imaging method that can be accessed in many centers and directly affects diagnosis. The first imaging method used in patients with suspected AIS is non-contrast brain CT, which is extremely successful in preventing hemorrhagic cerebrovascular events (CVEs), including very small brainstem hemorrhages. However, the success of CT in the diagnosis of AIS in the hyperacute period is much lower than that of hemorrhagic processes. Therefore, diffusion-weighted magnetic resonance imaging (DW-MRI), which allows for the diagnosis of acute infarction within minutes, is considered to be the most sensitive and specific radiological modality in the diagnosis of AIS (3, 4). In addition, in the early ischemic CVE process, in which significant ischemic changes are not yet visible on non-contrast brain CT, findings that may offer an idea about the presence or localization of ischemia in the irrigation areas of the middle cerebral artery (MCA) have been previouslydescribed (5, 6). These findings include thehyperdense MCA sign, obscured lentiform nucleus sign, and insular ribbon sign (7). However, these findings are subjective and require a high level of practitioner experience to achieve an accuratediagnosis.

This study aimed to present a new objective radiological criterion that can detect early cerebral ischemia by analyzing density changes in the basal ganglia, which are anatomical structures that can be well characterized on CT, in patients with ischemic CVE confirmed by DW-MRI.

Materials and Methods

Local ethics committee approval was obtained for the study (decision no: 359, date 25/11/2020). This retrospective study involved the examination of the images obtained from the hospital radiological picture archiving and communication system (PACS) belonging to patients older than 18 years, who underwent non-contrast brain CT and DW-MRI imaging after being admitted to the emergency department with a suspicion of acute ischemic CVE between August 1, 2020
and December 30, 2020. The radiological images were analyzed by a radiologist with ten years of neuroradiology experience. The first brain CT images of the patients following their presentation were evaluated. The patients with significant signs of ischemia on brain CT were excluded from the study. In patients without significant ischemia findings on CT, the bilateral thalamus, lentiform nucleus and caudate nucleus densities were measured and recorded in Hounsfield units (HU). Then, the DW-MRI images of these patients at the time of the same admission were analyzed. The patients with no signs of acute ischemia on DW-MRI were included in the control group, and thosewith ischemia in the right or left unilateral MCA irrigation area on the DW-MRI images were evaluated as the AIS group.

Brain CT Technique

The brain CT images were acquired using a 128-slice, Optima CT660 CT device (General Electric Medical Systems, Milwaukee, WI, USA). During imaging,the patients were in a supine position. Sedation or contrast agent injection was

not used for the examinations. The routine brain CT axial images were obtained according to following parameters: slice thickness, 5 mm; interval, 5 mm; kV, 120; mA, 150-200; pitch, 0.531; rotation time, 0.5 s; collimation, 40 mm; matrix, 512; FOV, 20 cm.

DW-MRI Technique

For the routine examination protocol, the b-value was taken as 1000 s/mm^2 and the slice thickness as 5 mm.

Density Measurement Technique

In axial CT sections, at the lateral ventricular level, thalamus density (TD), lentiform nucleus density (LND), and caudate nucleus density (CND) were measured for both sides using the same size regions of interest. Figure 1 presents the measurements of density values in the basal ganglia and thalamus in a patient without AIS. Figure 2 shows the images obtained from a patient with acute ischemia detected on DW-MRI and apparent diffusion coefficient (ADC) despite the absence of a significant ischemia finding in the first non-contrast brain CT image taken in the same plane due to the suspicion of AIS. Basal ganglia density measurements made on the CT images of the same patient are given in Figure 3.

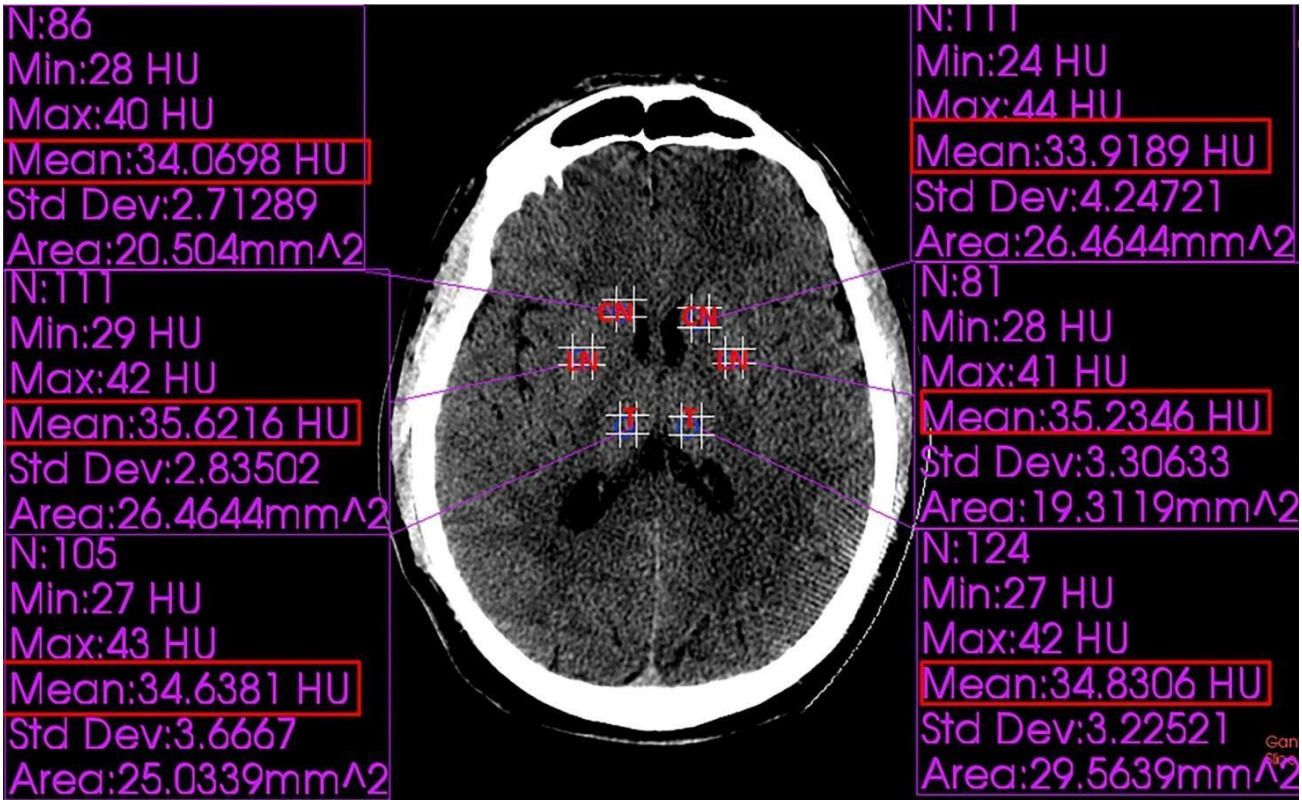

Figure 1: Measurement of density values in the basal ganglia and thalamus in a patient without ischemic CVE.

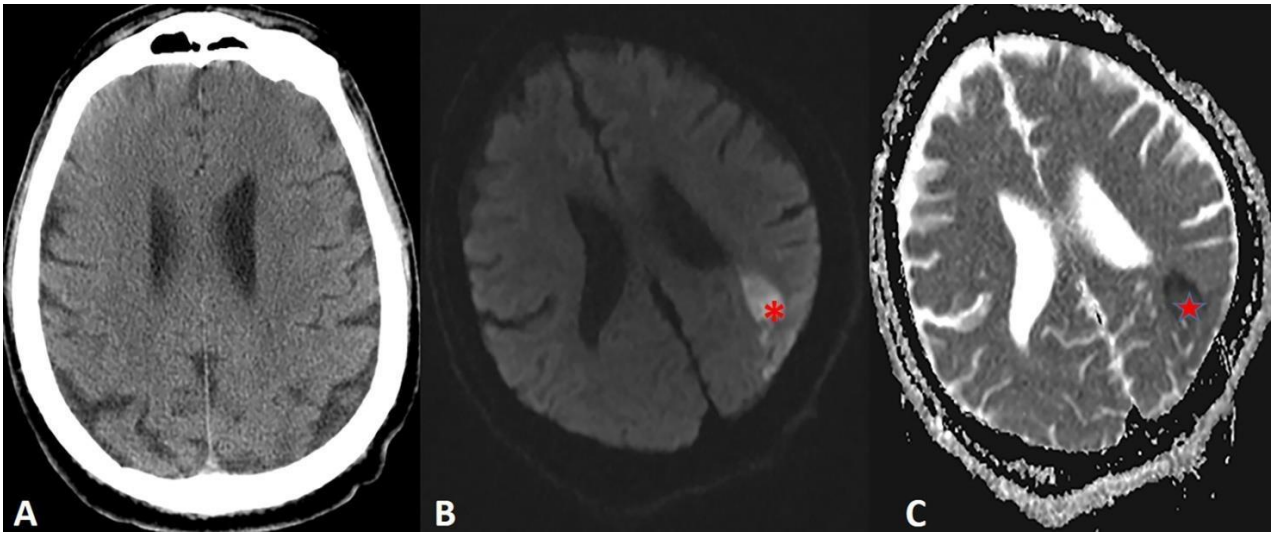

Figure 2: In the patient with suspected AIS (A); No significant ischemia findings on CT, (B); Parenchymal hyperintensity due to ischemia is shown in DWI (asterisk), (C); Signal loss supporting ischemia is shown on ADC images (star).

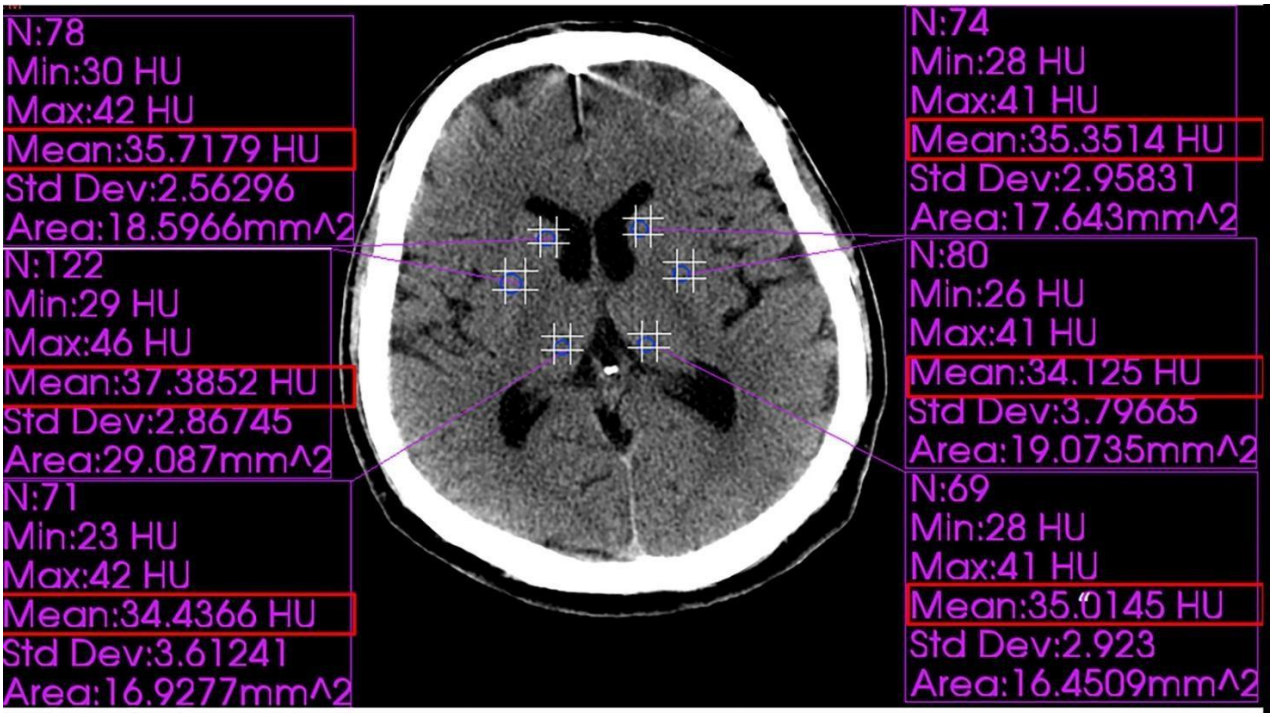

Figure 3: Measurement of the basal ganglia and thalamus densities at the lateral ventricular level on the CT images of the patient whose CT, DW-MRI and ADC images are given in Figure 2. The patient has an ischemic area on the left and the left LND is 3.2 HU, as being lower than the contralateral measurement.

Statistical Analysis

In this study, statistical analyses were performed using SPSS (Version 22.0, SPSS Inc, Chicago, IL, USA, License: Hitit University) software package. Descriptive statistics were presented with mean ± standard deviation and median (min-max) values in accordance with the data distribution. Categorical data were presented as numbers and percentages (%). The distribution of normality was analyzed using the Kolmogorov-Smirnov test. In the comparison of numerical variables between two independent groups, the

independent-samples t-test (Student's t-test) was used for normally distributed data. For continuous variables, the paired t-test was used for data showing a normal distribution in dependent two-group comparisons, and the Wilcoxon signed-rank test was conducted for data that did not show a normal distribution. Relationships between categorical variables were investigated using the chi- square test. The agreement between the measurements was evaluated with the intraclass correlation coefficient (ICC). In addition, the Bland-Altman plots were constructed with 95% limits of agreement to visually show the agreement between the two measurements. $p < 0.05$ was taken as the statistical significance level.

Results

There were a total of 104 patients in the sample. Fifty-three (51%) of the patients were in the control group and 51 (49%) were in the AIS group. Fifty-one (49%) of the participants were male and 53 (51%) were female. The mean age was 71.07±9.04 (59-95) years. The mean age of the patients in the control group was 70.96±9.94 years and that of the patients in the AIS group was 71.18±8.10 years, indicating no statistically significant difference (p=0.905). In the control group, 47.2% (n=25) of the patients were male and 52.8% (n=28) were female. In the AIS group, 51% (n=26) of the patients were men and 49% (n=25) were women. The gender ratios were statistically similar between the two groups (p=0.698).

In the control group, the left TD, LND and CND measurements of the patients were statistically significantly different from the right-side measurements (p=0.001, p<0.001, and p<0.001, respectively, Table 1). Figure 4 presents the distribution of the right and left measurements of the TD, LND and CND parameters in the control group.

Table 1: Comparison of the TD, LND and CND values between the right and left sides in the control group and between the ischemic and non-ischemic sides in the AIS group.

	Control group			**AIS group**		
	Right (n=53)	**Left (n=53)**	***p* value**	**Ischemic (n=51)**	**Non-ischemic (n=51)**	***p* value**
	Mean±SD	**Mean±SD**		**Mean±SD**	**Mean±SD**	
TD	33.74±2.62	33.96±2.59	**0.001****	36.64±2.54	37.03±2.46	0.179*
LND	34.87±3.05	35.13±2.98	**<0.001****	36.75±2.98	38.92±2.77	**<0.001****
CND	34.84±3.00	35.15±3.03	**<0.001****	37.01±3.04	37.78±3.45	**0.014****

TD, Thalamus density; LND, Lentiform nucleus density; CND, Caudate nucleus density.
*Paired t-test.
**Wilcoxon signed-rank test.

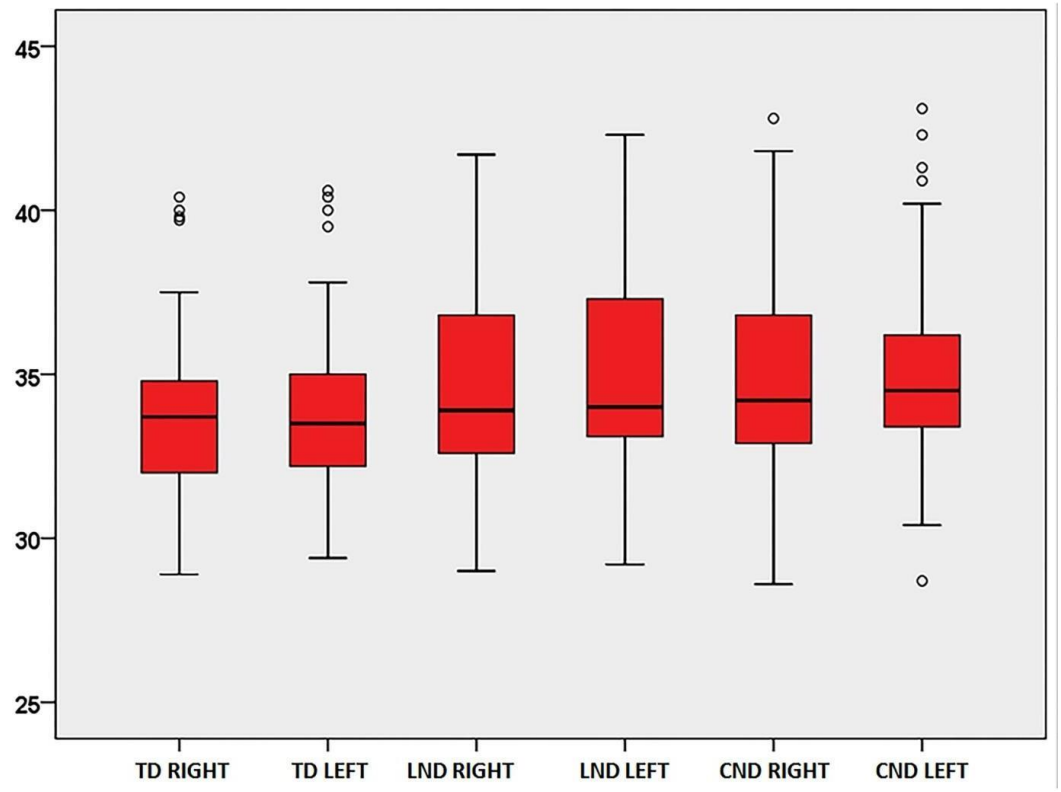

Figure 4: Distribution of the right and left measurements of the TD, LND and CND parameters in the control group.

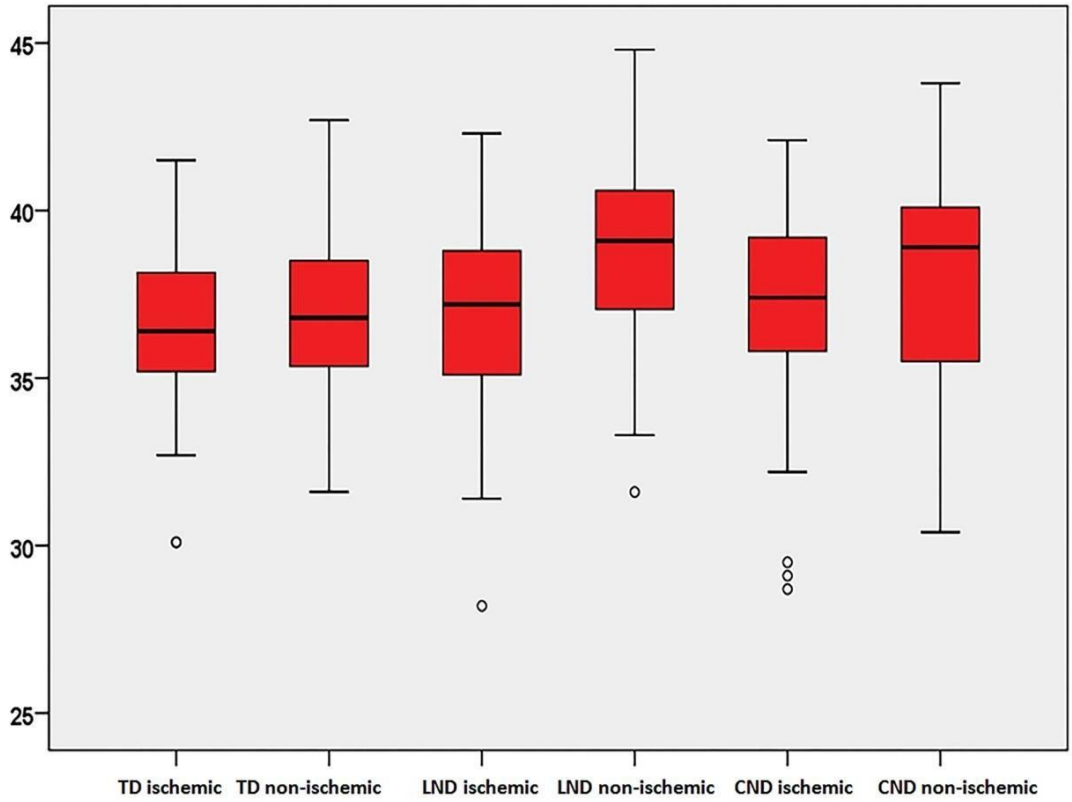

Figure 5: Distribution of the ischemia and non-ischemic side measurements of the TD, LND and CND parameters in the AIS group.

The TD measurement values on the ischemic and non-ischemic sides of the patients in the AIS group were not statistically significantly different (p=0.179, Table 1). However, the LND and CND measurements of these patients were statistically significantly higher in the non-ischemic side compared to the ischemic side (p < 0.001 and p=0.014, respectively, Table 1). Figure 5 shows the distribution of the measurements of the TD, LND and CND parameters among the patients with and without ischemia in the AIS group. According to the ICC values, there was an excellent agreement between the TD, LND and CND measurements on the right and left sides of the 53 patients in the control group [0.971 (0.946-0.984), 0.986 (0.961-0.994), and 0.969 (0.936-0.984), respectively, Table 2]. For the 51 patients in the AIS group, the ICC values revealed a moderate agreement between the TD, LND and CND measurements of the ischemic and non-ischemic sides [0.656 (0.469-0.788), 0.511 (0.042-0.753), and 0.642 (0.447-0.779), respectively, Table 3].

Table 2: Analysis of agreement in the TD, LND and CND measurements between the left and right sides for the control group.

Control group	**ICC (95% CI)**	***p* value**
TD right-left	0.971 (0.946-0.984)	**<0.001**
LND right-left	0.986 (0.961-0.994)	**<0.001**
CND right-left	0.969 (0.936-0.984)	**<0.001**

TD, Thalamus density; LND, Lentiform nucleus density; CND, Caudate nucleus density; ICC indicates intraclass correlation cofficient; 95% CI, Confidence interval.

Table 3: Analysis of agreement in the TD, LND and CND measurements the ischemic and non-ischemic sides for the AIS group.

AIS group	**ICC (95% CI)**	***p* value**
TD ischemic- non-ischemic	0.656 (0.469-0.788)	**<0.001**
LND ischemic-non-ischemic	0.511 (0.042-0.753)	**<0.001**
CND ischemic-non-ischemic	0.642 (0.447-0.779)	**<0.001**

TD, Thalamus density; LND, Lentiform nucleus density; CND, Caudate nucleus density; ICC indicates intraclass correlation cofficient; 95% CI, Confidence interval.

Figures 6 and 7 show the Bland-Altman plots that were constructed with 95% limits of agreement to visually demonstrate the agreement between the TD, LND and CND measurements in the control and AIS groups.

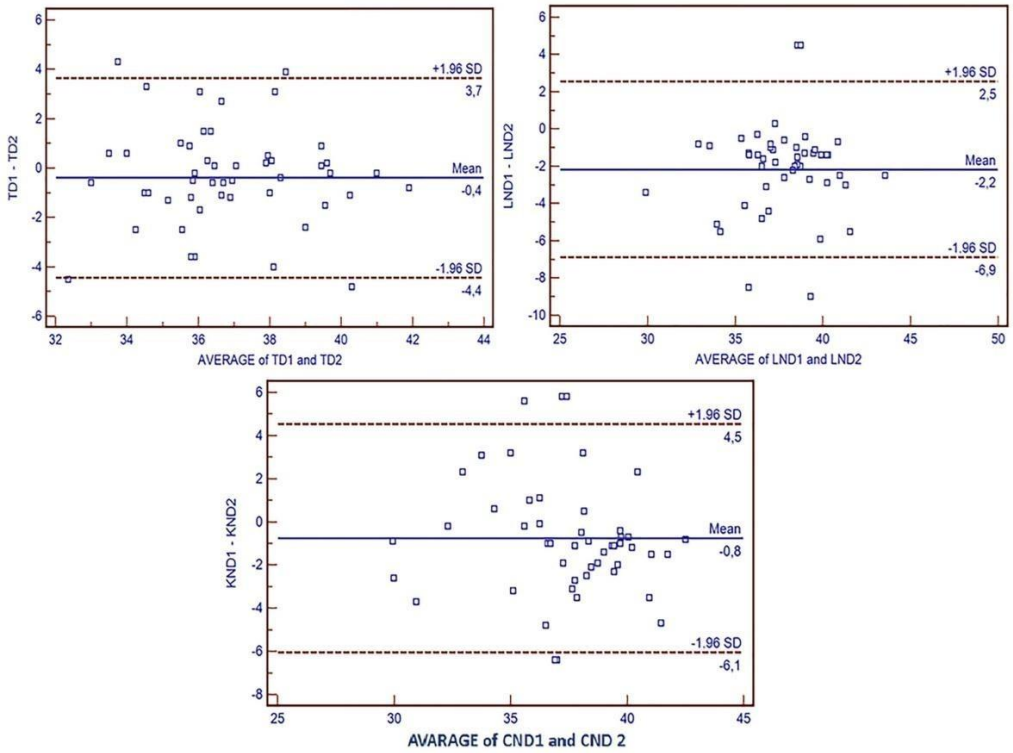

Figure 6: Bland-Altman plots constructed with 95% limits of agreement to visually demonstrate the agreement between the TD, LND and CND measurements in the control group.

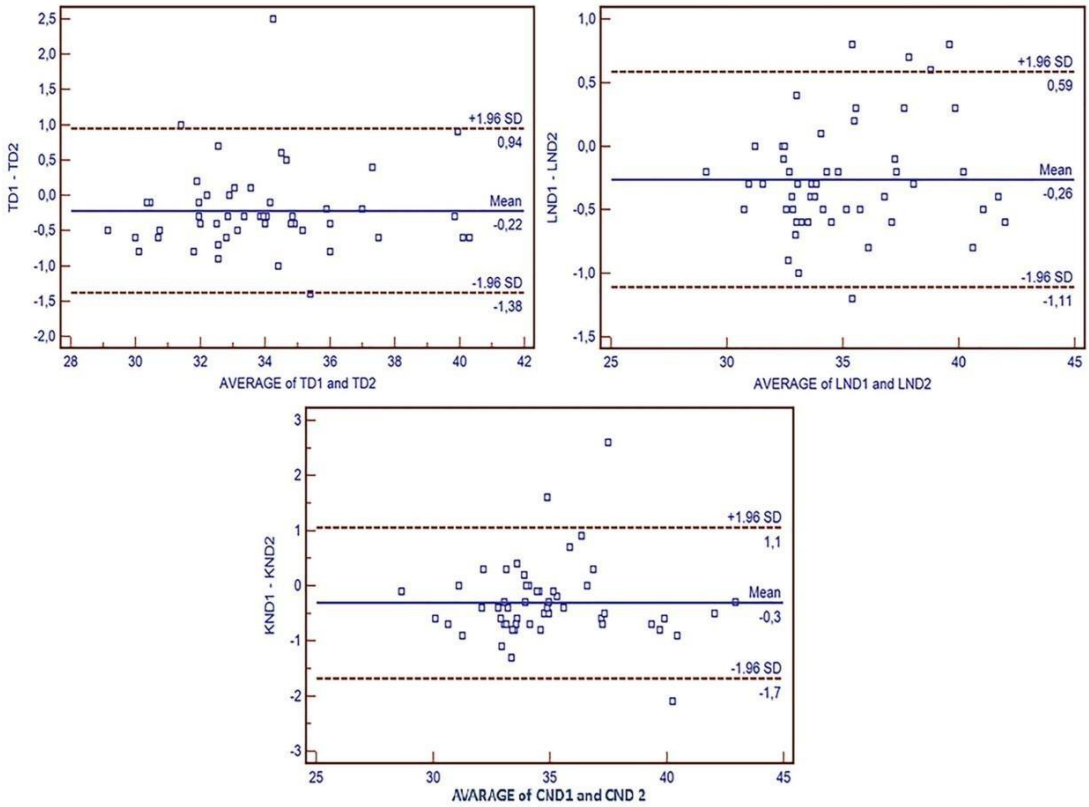

Figure 7: Bland-Altman plots constructed with 95% limits of agreement to visually demonstrate the agreement between the TD, LND and CND measurements in the AIS group.

Discussion

Acute ischemic stroke is the most common cause of mortality following cardiovascular diseases and cancer (8, 9). If AIS can be recognized within the first three hours, thrombolytic therapy can be performed and extremely successful clinical results can be achieved (9). Although there are high- sensitivity neurological examination findings for this important clinical situation, most are not specific (10). The first radiological tool used in the diagnostic management of these cases is non-contrast brain CT, which can successfully exclude possible intracranial hemorrhage and tumor presence (11, 12). Today, the CT device has taken its place in almost all centers with emergency departments (9). Typical and distinct ischemia findings in brain CT often occur after the first six hours, and some radiological markers that may be significant in terms of ischemia can sometimes be identified during this period (9). The hyperdense MCA sign, hypoattenuation in LN, insular ribbon sign, loss of gray-white matter distinction, and obscured sulcus are among the main early markers (6, 13). DW-MRI is a highly sensitive radiological modality that can reveal acute ischemia within minutes or hours (14). However, MRI is still not as common as CT in many centers. It is also clear that a high level of experience is required for the recognition of early MRI ischemia markers that can be easily identified in CT. Therefore, after the first CT examination, which is considered to be normal in the presence of AIS suspicion, the patient is frequently referred for a DW-MRI examination in the same center if equipped or a different center. This situation not only causes delays in diagnosis but also leads to difficulties in terms of cost effectiveness. In our study, we investigated the efficacy of an objective method that can provide ease of application, different from the subjective markers previously described, in patients with suspected AIS, which is a common disease that warrants an early diagnosis.

The basal ganglia and the thalamus are symmetrical deep gray matter elements located at the base of the forebrain andhave wide and complex relationships with the cerebral cortex and other brain structures (15). The thalamus, CN and LN are structures that can be well characterized in the axial plane in cross- sectional radiological images. The arterial supply of the basal ganglia is mainly provided by the anterior cerebral artery (ACA) and the lenticulostriate arteries originating from MCA (16). Therefore, it should be known that changes secondary to ischemia may occur in these structures in occlusive diseases of ACA and MCA. Thus, in our study, we also examined the effect of MCA with a large cerebralirrigation area on the density differences ofarterial supply loss in ischemic events and investigated its contribution to clinical practice.

In our study, the patient group with MCA ischemia was examined. Lateral lenticulostriate arteries originating from the MCA supply the lentiform nucleus. Therefore; density changes in LND are the most important variable for our study. However, the caudate nucleus is supplied by the medial lenticulostriate arteries. However, ACA, like MCA, originates from the internal carotid artery. Therefore, it can be thought that it may also be affected in proximal MCA ischemia. The thalamus is supplied by arteries originating from the posterior circulation. Therefore, we do not expect significant statistical

results in TD variability. However, in our study; TD variability, which are important and easily identifiable anatomical structures, was also evaluated.

Contrary to what was expected in our study, the left side TD, LND and CND values of the patients in the control group were statistically significantly higher than their right side measurements. Although the basal ganglia and the thalamus are known to be symmetrical anatomical structures, this situation may be a result of variability in vascularization. Considering that the mean age of the patients in the control group was 70.96 ± 9.94 years, we believe that potential carotid systemstenosis or asymmetric atherosclerotic changes may have led to individual density differences. Nevertheless, for the 53 patients in this group the TD, LND and CND values had an excellent agreement between the right and left side measurements while there was a moderate agreement between the ischemic and non- ischemic side measurements of TD, LND and CND among the 51 patients in the AIS group. This indicates that in patients without occlusive acute cerebrovascular pathologies, the difference in density caused by other factors affecting arterial supply is much less than in the patient group with AIS. Supporting this, while the highest mean density among TD, LND and CND was found to be 0.31 in our control group (CND mean right-left density difference), the mean density difference in the patient group reached 2.17 (LN mean ischemic-non-ischemic side density difference). However, it was an expected result that TD did not show a significant difference in density between the ischemic and non-ischemic sides. In light of these findings, we can state that there was a significant decrease in the LND and CND values on the ischemic side. This was atrial study investigating the use of basal ganglion density differences in routine non-contrast brain CT examinations on anatomical structures that can be easily demonstrated as an objective and quantitative marker of acute ischemia, regardless of the experience of the practitioner. Although the data obtained indicated that especially LND and CND significantly decreased on the ischemic side in the patients with AIS, the study also had important limitations. As discussed above, TD, LND and KND are in different densities compared to their symmetry in the control group. The reason for this can be considered as atherosclerosis, which is the most common and basic pathological process associated with cardiovascular system diseases (17). In asymptomatic and apparently healthy individuals, atherosclerosis occurs earliest and most commonly in the peripheral arteries and carotid system. Asymptomatic carotid arterial stenosis is a common vascular pathology associated with atherosclerosis, and its incidence increases in parallel to age (18, 19).

It can be easily stated that this vascular disease, which occurs in an asymptomatic patient group, will adversely affect arterial supply to cerebral structures. Therefore, the density analysis of twin intracranial structures that show symmetry would not offer reliable results beyond doubt without performing a carotid Doppler examination and excluding the possibility of carotid artery stenosis. In future similar studies, creating patient groups from individuals that are known not to have carotid system stenosis may provide more accurate results. It should also be noted that diagnostic algorithms for a common public health problem such as AIS have been

created over numerous studies conducted with large patient groups over many years. Our study was conducted over a short period of time with a limited number of patients, and therefore we consider that more objective results can be revealed by future studies with larger patient groups.

Conclusion

In light of the data revealed by our study, in acute ischemic cases, density changes in the basal ganglia and thalamus are promising for use in radiological diagnosis in the early period at the time of non-contrast brain tomography, which can also eliminate the need for DW-MRI. Similar studies to be conducted with larger and selected patient groups can present further data that will show that this method can make serious contributions to clinical practice in future.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

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In Vitro Investigation of the Effects of Caffeic Asid Phenethyl Ester Contribution to Chemotherapeutic Drug Treatment on the Neuroblastoma Cell Line (SH SY- 5Y) on the Inflammatory Process

Çiğdem KARACA[1*], Hilal SUSAM ŞEN[2], Fatma FIRAT[3], Esra ASLAN[3]

[1]*Gaziantep Islam Science and Technology University, Faculty of Medicine, Department of Histology & Embryology, Gaziantep, Turkey.*
[2]*Afyonkarahisar Health Science University, Faculty of Medicine, Department of Pediatrics, Afyonkarahisar, Turkey.*
[3]*Afyonkarahisar Health Science University, Faculty of Medicine, Department of Histology & Embryology, Afyonkarahisar, Turkey.*

Abstract

Neuroblastoma is a sympathetic nervous system tumor with a 5-year survival rate of less than 50% in high-risk patients that are unresponsive to conventional multimodal treatments. Although chemotherapy, radiotherapy, and surgery are used according to the stages in the treatment protocols, the complications and toxic effects of these treatments are also among the causes that increase mortality. Topotecan is an agent that is actively used in the treatment of neuroblastoma, but its use is limited due to its toxic effects. For these reasons, neuroblastoma is a disease that needs new treatment approaches and agents to reduce the complications of existing treatments. Caffeic Acid Phenethyl Ester (CAPE) is a natural compound that is known for its anti-inflammatory, immunomodulatory, antioxidant, and anticancer properties and is particularly preferred because of its selective effects on cancer cells. Regulation of the inflammatory microenvironment during cancer treatment is an important factor in the treatment plan. According to this information, we aimed to compare the effects of Topotecan and CAPE treatments on the inflammatory process in the SHSY-5Y neuroblastoma cell line with IL-1α, IL-6, IL-18, and VEGF antibodies on the apoptoticprocess using the TUNEL method. After the cells have been cultured, four groups were formed. Topotecan and CAPE were given separately to the 1st and 2nd groups, Topotecanand CAPE were given together to the 3rd group for 24 hours. A control group was formed by not giving any substance. Then, staining was done with ICC and TUNEL methods. In the results, IL-1α, IL-6, IL-18, and VEGF staining intensities were found to be increased in the CAPE and Topotecan applied groups, especially in the CAPE+Topotecan co-administered

[*]**Corresponding Author:** Çiğdem Karaca, E-mail: drc_karaca@hotmail.com. ORCID ID: 0000-0003-2106-2422.

group at the end of 24 hours. In the evaluation made with TUNEL staining, although the most apoptotic cells were seen in the CAPE applied group, an increase in apoptosis was also detected in the groups that were administered Topotecan and CAPE+Topotecan together The results of our study showed that CAPE and Topotecan triggered apoptosis by exacerbating inflammation in SH-SY5Y neuroblastoma cells, more apoptotic cells in the CAPE group were more cytotoxic than Topotecan, and when used together, CAPE had a synergistic effect with Topotecan.

Key words: *Apoptosis, CAPE, Mediators of inflammation, SHSY-5Y, Topotecan.*

Introduction

Neuroblastoma (NB) is a pediatric sympathetic nervous system tumour arising from neural crest cells. It can also be found in sympathetic nerve ganglia located throughout the sympathetic nervous system in the neck, chest, abdomen, and pelvis (1). The molecular basis of NB is not fully understood. The heterogeneity of subspecies of NB variations creates difficulties in the identification and classification of the disease (2). Tumors with heterogeneity are rare, but very aggressive and often with a severe prognosis. The clinical and biological features of NB also differ according to the primary tumour sites (3). According to the World Cancer Report, more than 15% of child deaths from cancer are composed of patients with NB (4-6). Pediatric cancers are treated with a combination of therapies (surgery, radiation, chemotherapy and targeted therapy) selected according to the type and stage of the cancer (7). Although clinical diagnosis and treatment for NB are continually improving, the 5-year survival rate for children with high-risk NB remains less than 50% (8, 9). Low-risk NBs are mainly seen in children younger than 12 months, and surgical treatment is usually sufficient. In other cases, chemotherapy is used as the first-line treatment.

Chemotherapy, radiation therapy and stem cell transplantation can be used for high-risk NB treatment. A 5-year survival rate approaching 70% has been achieved with different and combined treatment approaches (10). But current treatment for high-risk patients includes intensive and toxic chemotherapy. In stage 4 patients, chemotherapy causes more treatment-related deaths than surgery, accounting for 15.5% of all deaths. An important side effect of chemotherapy in patients with NB is the development of secondary myelodysplastic syndromes and transformed acute myeloid leukaemia (11). For these reasons, the pursuit of new treatments in NB continues. Topotecan is a semi-synthetic derivative of camptothecin and is an antineoplastic agent used in the current treatment of NB, as it activates the topoisomerase I-inhibitor (12). It triggers cell death by creating DNA breaks (13). It causes haematological toxicities, especially severe myelosuppression (14). New and supportive treatment agents are needed in the treatment of NB because of its cytotoxic effects in other tissues and many undesirable side effects.

Caffeic acid phenethyl ester (CAPE), a natural phenol in honeybee propolis, has been reported to have anti-inflammatory, antifungal, antimicrobial,

immunomodulatory, antimutagenic and antioxidant properties (15-19). As an antioxidant agent, CAPE completely blocks the production of reactive oxygen species in human neutrophils and xanthine/xanthine oxidase systems at a concentration of 10 μM and demonstrates adequate antioxidant capacity (20). CAPE has been shown to increase cancer cell death by apoptotic mechanisms in many cancer cell lines (21-24). Macroscopic studies have also been conducted and it has been suggested that treatment with CAPE causes a significant dose-dependent reduction in tumour growth by assessing volume and weight of tumour in the xenograft model (25). On the other hand, in a study conducted in Burkitt lymphoma cells, it has been stated that CAPE causes cell death with pyknotic nuclei devoid of nuclear or nucleosomal fragmentation, which shows the characteristic features of necrosis rather than apoptosis, and it has been emphasised that CAPE, which is an anti-inflammatory agent, causes necrotic cell death in cancer cells (26). CAPE has also been shown to be an effective agent in preventing metastases (27). It has also been suggested that CAPE has a specific antiproliferative effect on cancer cells, and also exerts homoeostatic control in multicellular organisms for cell proliferation, differentiation, wound healing, and regulation of adaptive responses of differentiated cells (28).

Cytokines are cellular regulatory proteins that are classified as chemokines, interferons, interleukins, lymphokines, and are involved in these pathophysiological mechanisms by directing the inflammatory response. Cytokines that increase non-specific immunity and inflammation are known as proinflammatory cytokines. IL- 1, IL-5, IL-6, IL-8, TNF-α, IFN-ɣ are proinflammatory cytokines. IL-18 is a cytokine formerly known as interferon (IFN) gamma inducing factor (IGIF). Although it is similar to the IL-1 family in structure and function, it is also considered as a new member of the IL-1 family (29-31). The inflammatory micro-environment of a tumour is a complex network of multiple cells, cytokines, enzymes, and signalling pathways. Inflammatory cytokines are the most effective targets for modulating the inflammatory micro environment during cancer treatment. Inflammation is a regulatory system with both pro- and anti-tumour properties. As part of the immune response, inflammation can activate immune cells and induce the production of inflammatory proteins such as cytokines and enzymes to inhibit thegrowth of tumours. Therefore, appropriately manipulating inflammation isconsidered a promising strategy for cancer therapy (32, 33).

VEGF (Vascular Endothelial Growth Factor) is a highly specific mitogen for vascular endothelial cells. Expression of VEGF is increased by activated oncogenes and various cytokines in response to hypoxia. It is known that VEGF accelerates tumour development by inducing endothelial cell proliferation, promoting cell migration and inhibiting apoptosis and is a metastatic marker (34, 35). In addition, it has been emphasised in recent studies that the increase in VEGF expression in SHSY-5Y cells is not associated with tumour progression and metastasis (36, 37).

SH-SY5Y was first subcloned in 1978 from the SK-N-SH cell line isolated from abone marrow biopsy of a four-year-old girlwith NB in 1973. It has a morphologically similar phenotype to neuroblast cells in vitro and is used in neurodegenerative

disease studies and NB modelling due to its neuronal cell-like characteristics (38, 39).

In this study, it has been aimed to evaluate the IL-1α, IL-6, IL-18 and VEGF immunoreactivities of Topotecan andCAPE in the SH-SY5Y NB cell line and toobserve their effects on the inflammation and angiogenesis process, and on theapoptotic process with the TUNEL method.

Material and Method

Cell Passage

In our study, commercially purchased human SH-SY5Y NB cell line was used. Cells were incubated in RPMI medium containing 10% (v/v) heat-inactivated fetal calf serum (FCS) and 5 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin in an atmosphere of 37 °C, containing 5% CO2 and 95% air. The cells were placed in a chamber slide (150-mm flask) containing 12 ml of medium in 75 cm² flasks and incubated for 2 days. Cells with 80% confluency were removed by adding 0.25% trypsin. Trypsin activity was terminated by adding a medium twice the volume of trypsin to the resulting suspension. The suspension was taken into a 15 ml Falcon tube and centrifuged at 400xg and 25 °C for 5 minutes to obtain cell debris (pellet). After removing the supernatant, 2400 µl of fresh medium was placed on the pellet and mixed with a pipette. Cell culture was performed in a12-well (2 pcs.) chamber slide with 200 µl suspension in each well. The wells were divided into CAPE (5 µM, 24h), Topotecan (2 µM, 24h), CAPE (5 µM)+Topotecan(2 µM) and Control groups, and drugs were applied at the given doses. The procedures were repeated 3 times.

Immunocytochemical Staining Protocol

Cells forming the control group and treated with CAPE, Topotekan and CAPE+Topotekan groups were cultured in the 12-well chamber slide. The medium was removed with a sterile pipette at the end of the 24th hour and 4% paraformaldehyde was used for fixation of cells. After washing with PBS, they were kept on ice for 15 minutes in 0.1% Triton-X100 solution. The cells were incubated with 3% H_2O_2 for endogenous peroxidase inactivation. After washing with PBS three times for 5 min, it was treated with blocking solution for 10 minutes. Cells were incubated with primary antibodies VEGF (1/250, ab76055- abcam), IL-1α (1/250, ab9614-abcam), Il-6 (1/250, ab233706, abcam), IL-18 (1/250, ab191152-abcam) for 1 hour at room temperature. Then washed 3 times with PBS. After treatment with secondary antibody, it was colored using DAB Substrate. Mayer's hematoxylin was used for counter-staining and covered with a water-based sealer.

Cells on stained slides were counted under light microscopy with Image Analysis Program (NIS elements, Japan). 500 different cells were counted at x20 objective magnification. While scoring, thearea with the highest score was determinedby scoring the staining degree. Scoring wasdone by a semi-quantitative method (H- Score). Stained cells were evaluated in terms of percentage and staining intensity was taken as a second criteria. There is no, slight, moderate and severe staining were assessed as 0,1,2 and 3, respectively.

Tunel Staining Protocol

After removing the media in the wells with a sterile pipette, applications were made according to the ApoTag Plus PeroxidaseIn Situ Apoptosis Detection Kit (S7101)

protocol. After washing the wells with PBS, they were fixed with 4% paraformaldehyde for 30 minutes. Then, they were permeabilised with Triton X 100 for 15 minutes on ice and washed with distilled water. After the endogenous preoxidase blockade was applied for 5 minutes at room temperature with 3% hydrogen peroxide (H_2 O_2), they were washed again with PBS and incubated for at least 10 minutes by applying 75 µL of Equilibration Buffer. 55 µL of TdT ENZYME was dropped, Stop Wash Buffer was applied, and then they were incubated with Anti-Digoxigenin Conjugate for 30 minutes at room temperature in a humid environment. DAB substrate was applied and waited for 3 to 6 minutes, and they were treated with water-based blocking solution by counterstaining with Mayer's Hemotoxylin. Evaluation of apoptosis in SHSY-5Y cells was calculated using the Apoptotic index (AI) formula, considering cells with brown staining nuclei as TUNEL positive.

AI = Number of TUNEL Positive Cells x 100 / Total number of cells (40).

Statistical Analysis

The data obtained as a result of the Apoptotic Index and H-Score evaluation were prepared as mean ± standard error. Shapiro Wilk test was used to evaluate the normality of the data. T test was applied in dependent groups. Statistical comparisons were made using one-way ANOVA followed by post-hoc test (Tukey). Comparisons where P-values are less than 0.05 were considered statistically significant. Analyses were made in the SPSS 20.0 package program.

Results

VEGF Staining Intensities

The most intense staining was seen in the CAPE+Topotecan applied group. There was no significant difference in staining between the groups in which CAPE and Topotecan were given separately (p= 0.928). In the CAPE+Topotecan applied group, the staining intensities were increased compared to the CAPE and Topotecan administered group (p=0.011, p=0.025). There was also a significant increase in staining between the groups administered CAPE+Topotecan, CAPEand Topotecan and the Control group(p<0.001, p<0.001, p<0.001) (Figure 1)
(Graphic 1).

IL-1α Staining Intensities

The most intense IL-1α staining was seen in the CAPE+Topotecan applied group. There was no significant difference in staining between the groups administered CAPE and CAPE+Topotecan and Topotecan (p=0.177, p= 0.294). In the CAPE+Topotecan administered group, the intensity of staining increased compared to the Topotecan administered group (p=0.012). There was also a significant increase in staining between the groups administered CAPE+Topotecan, CAPEand Topotecan and the Control group (p<0.001, p<0.001, p<0.001) (Figure 1)
(Graphic 1).

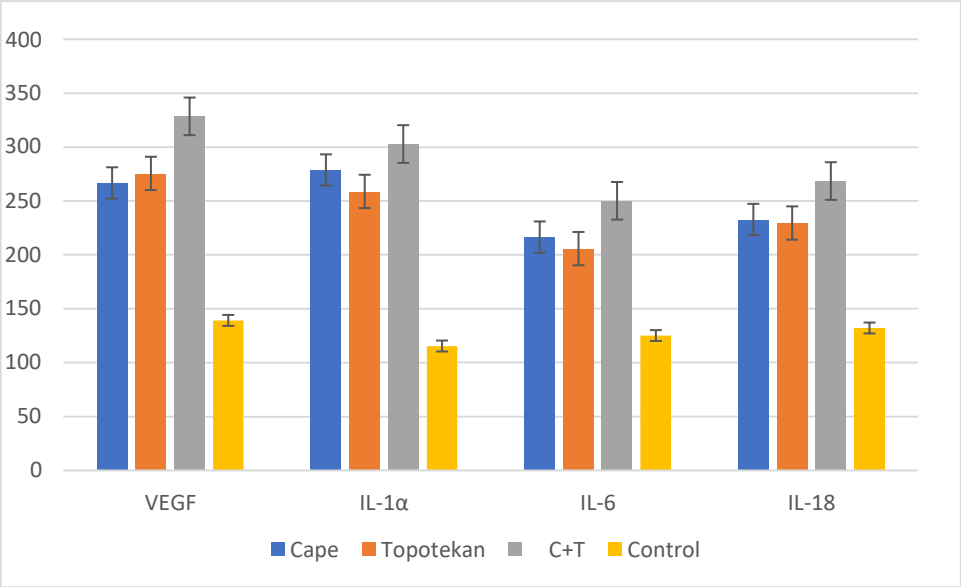
Il-6 Staining Intensities

The most intense IL-6 staining was seen in the CAPE+Topotecan applied group. There was no significant difference in staining between the groups administered CAPE and Topotecan (p= 0.570). In the CAPE+Topotecan administered group, the intensity of staining increased compared to the Topotecan and CAPE administered group (= 0.002, p=0.013). There was also a significant increase in staining between the groups administered CAPE+Topotecan, CAPE and Topotecan and the Control

group ($p<0.001$, $p<0.001$, $p<0.001$) (Figure 1) (Graphic 1).

Il-18 Staining Intensities

While the staining intensity of IL-18 was increased in the CAPE+Topotecan applied group compared to the CAPE and Topotecan applied groups, this increase was not considered statistically significant ($p= 0.117$, $p=0.083$). There was also no significant difference in staining between the groups administered CAPE and Topotecan ($p= 0.995$). There was also a significant increase in staining between the groups administered CAPE+Topotecan, CAPE and Topotecan and the Control group ($p<0.001$, $p<0.001$, $p<0.001$) (Figure 1) (Graphic 1).



Graphic 1: H-Score evaluations of staining intensity with VEGF, IL-1α, IL-6, IL-18 antibodies after CAPE, Topotecan and CAPE+Topotecan application for 24 hours.

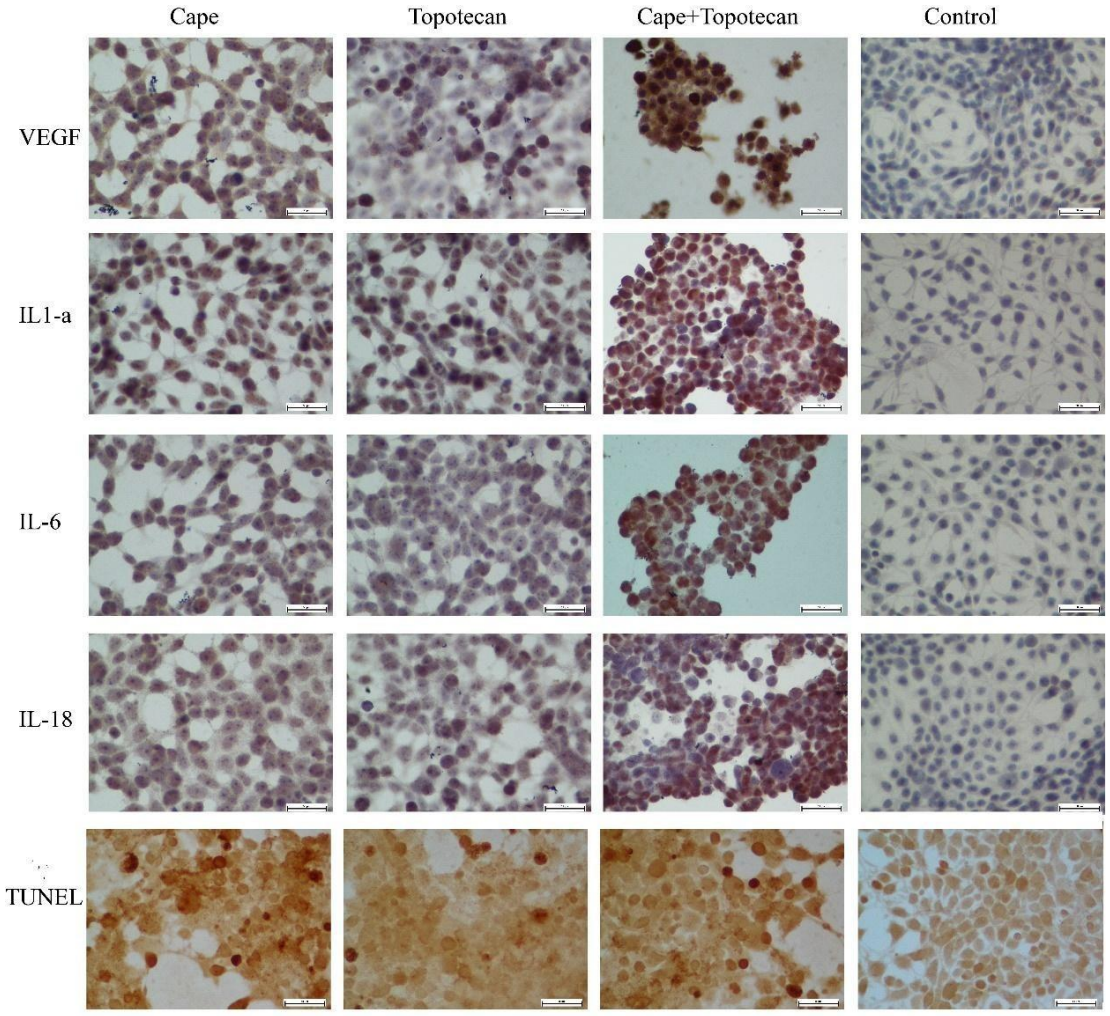
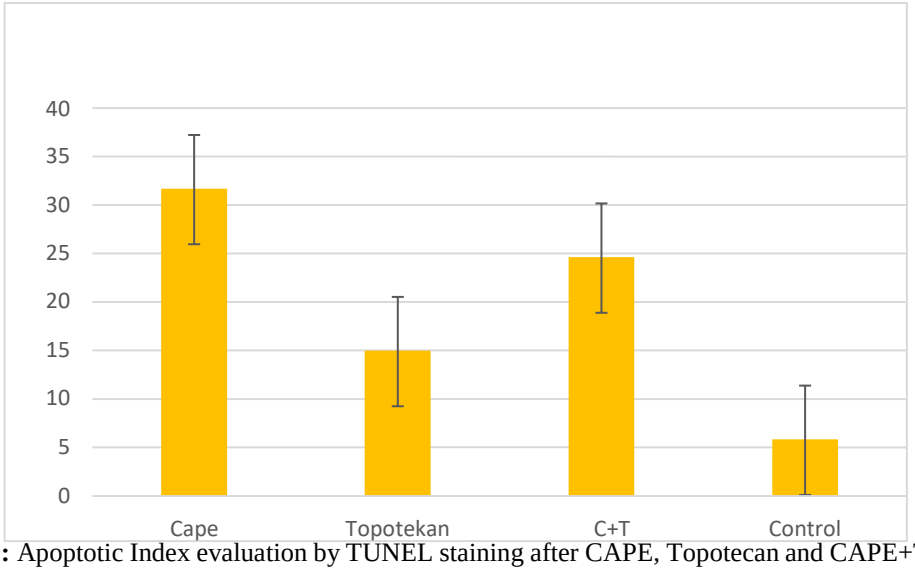


Figure 1: ICC staining and TUNEL staining with VEGF, IL-1α, IL-6, IL-18 antibodies after CAPE, Topotecan and CAPE+Topotecan application for 24 hours. 20X objective, scale bar 50μm.

TUNEL Staining

The most apoptotic cells were seen in the CAPE applied group. Apoptotic index was found to be increased in CAPE applied group compared to Topotecan and Control groups ($p=0.001$, $p<0.001$). Compared to the CAPE+Topotecan group, this increase was not considered significant ($p=0.120$). Apoptotic index in the topotecan administered group was found to be decreased compared to CAPE and CAPE+Topotecan groups ($p=0.001$, $p=0.032$) and increased compared to the control group ($p=0.041$). Apoptosis was increased in the CAPE+Topotecan applied group compared to the Control group and Topotecan applied group ($p=0.001$, $p=0.032$) (Figure 1) (Graphic 2).



Graphic 2: Apoptotic Index evaluation by TUNEL staining after CAPE, Topotecan and CAPE+Topotecan administration for 24 hours.

Discussion

NB is the most common extracranial solid tumour in childhood. While NB, which may arise from the sympathetic ganglia and/or adrenal medulla, may spontaneously transform or regress to a benign form within the first year, the 5-year survival rate of patients in the high-risk group is below 50% and the pursuit of new treatments is still ongoing. In this process, there is a need for agents that can specifically treat cancer cells while reducing the side effects of current treatments. Topotecan is a chemotherapeutic agent currently used in the treatment of NB. In our study, in addition to topotecan treatment, CAPE, which is known to have strong antioxidant effects and also has anti-inflammatory, antitumour, neuroprotective and immunostimulatory effects, was applied to SHSY-5Y NB cell lines. Guowen W. et al. reported that topotecan inhibited cell proliferation, cell migration and invasionin a time- and dose-dependent manner in the SHSY-5Y NB cell line (41). In another study, Beppu K. et al. reported that cell viability decreased and cell death increased in topotecan applied SHSY-5Y cells (21). In a study with CAPE, Tomiyama R. et al. stated that endoplasmic reticulum stress and autophagy increased in SHSY-5Y cells after CAPE application (42). In our study, in the TUNEL evaluation, apoptosis was found to be increased in SHSY-5Y cells treated with Topotecan, CAPE and CAPE+Topotecan compared to the control group. The most cell death was seen in the CAPE applied group, while cell death was found to be lower in the Topotecan applied group than in the CAPE and CAPE+Topotecan applied groups. It has been reported that CAPE is being effective by reducing the intercellular connection proteins such as β-catenin, claudin, and nectin, preventing the adhesion of cancer cells and weakening their intercellular connections (43-46). In our study, cell shedding was higher in the CAPE applied group compared to the other groups. This made us think that CAPE would have anticancer effects by both increasing cell death and weakening the adhesion strength and intercellular connections of cells.

VEGF-A is known as a key regulator of physiological and pathological

angiogenesis (47). It can be expressed by activated oncogenes and various cytokines in response to hypoxia. Deregulated VEGF expression contributes to the development of solid tumours by promoting tumour angiogenesis and to the ethiology of some diseases characterised by abnormal angiogenesis (48). Many studies have shown that VEGF-A plays an important role in the progression and metastasis of cancer cells. VEGF-A expression has been demonstrated in both human and NB cell lines (49-52). There are studies showing that VEGF expressions are decreased in SHSY-5Y cells treated with Topotecan and CAPE (21, 53). In our study, we observed an increase in VEGF staining intensity in SHSY-5Y cells in the Topotecan, CAPE and Topotecan+CAPE applied groups compared to the control group. Although this result we found contradicts the literature studies made with CAPE and Topotecan, it has been shown by recent literature studies that the increase in VEGF expression in SHSY-5Y cells is not associated with tumour progression and metastasis, and the roles of VEGF in NB tumourigenesis remain unclear (36, 37). However, increased VEGF expression has been evaluated in favour of increased inflammation in some studies (54-56). The fact that other inflammation markers were found to be increased in the treatment groups in our study supports this situation. Cytokines are molecules produced by immune system cells. They have an important role in the production and activity of immune system cells and blood cells. Although there are many different types, the most commonly used ones are interleukins, interferons and GM-CSF (Granulocytemacrophage colony-stimulating factor) (57). The importance of cytokines in cancer biology was understood at the end of the 1800s with the demonstration that some malignant tumours regressed after bacterial infections. Cytokines, while causing tumour regression by directly inhibiting growth on cancer cells, or acting by supporting other antitumour effects in the body, they can also be growth factors for malignant cells on the contrary, and provide therapeutic support by inhibitingthe effects of these cytokines (58). These bidirectional behaviours of cytokines change the approach in different tissues and different tumours and different antineoplastic drugs. In general, proinflammatory cytokines are mediators that are detected to be increased in the blood during cancer treatment. Tsavaris N. et al. found increased IFN-ɣ, IL-2, IL-6, GM-CSF cytokine levels in the peripheral blood of breast cancer patients they treated with taxane group antineoplastic drugs and stated that taxanes are also an effective treatment agent by increasing cytokines in patients with terminal breast cancer (59). Wood L. J. et al. administered rats an antineoplastic agent, etoposide, and observed that IL-6 serum levels increased, resulting in symptoms such as fatigue, weakness, loss of interest in social activities, and difficulty concentrating (60). However, in most studies, it has been stated that the increase in serum cytokines observed after the administration of many antineoplastic drugs, including topotecan, is responsible for chronic fatigue in patients (61). There are also studies showing that cytokines are decreased in thetumour micro-environment and tumour cells. In their study, Lasitiotaki I. et al.showed that IL-1β and IL-18 were increased in the plasmas of patients with lung cancer, while IL-6 and TNF-α were decreased in the tumour micro-

environment. As a result, they stated that tumour-induced immunosuppression occurred in the lung micro-environment and that this could provide new targets for cancer immunotherapy (62). Antineoplastic therapy promotes cell death by increasing cytokine expression and inflammation in the cancer micro-environment in most cancer cells. Tsang P. S. et al. reported that the NFkB pathway was activated in NB cells to which they applied Topotecan treatment, and as a result, inflammation in the cells increased (63). In our study, we observed an increase in staining in IL-6 and IL-18 compared to the control group, although it was more pronounced in IL-1α in Topotecan applied SHSY-5Y cells. This increase suggests that Topotecan triggers cell death by increasing inflammation in cancer cells.

CAPE is a well-known anti-inflammatory compound with antioxidant, antineoplastic and immunomodulatory properties. CAPE suppresses the inflammatory process by inhibiting cytokine and chemokine production, T-cell proliferation and lymphokine production, as well as reducing prostaglandin and leukotriene synthesis. It has also been suggested that the anti-inflammatory effect exhibited by CAPE is a result of inhibition of arachidonic acid release from the cell membrane (64, 65). CAPE is a compound with anticancer properties due to its cytotoxic effect directly against cancer cells by preventing chronic inflammation. Since it is selectively cytotoxic for cancer cells, it has been considered as an advantageous agent that can be used alongside antineoplastic agents. Studies in which CAPE acts by reducing inflammatory mediators in normal cell lines are frequently encountered in the literature. There is information that it results in a decrease in anti-inflammatory cytokines, especially in inflammatory disease models such as Alzheimer'sDisease and Helicobacter Pylori (66, 67). In cancer cell models, however, the effects of CAPE on cytotoxicity, cell death and metastasis have often been evaluated. There are also articles in the literature reporting that CAPE has an antiproliferative effect on cancer cell lines (68, 69). In contrast, CAPE has been reported to ameliorate brain atrophy after neurological injury, including ischaemia and epilepsy, by inhibiting neuronal apoptosis and astrocyte proliferation in animal models (70, 71). These findings reveal that CAPE is a potent bioactive compound and can initiate multiple molecular responses in treated cell/animal models (72). In our study, the proinflammatory cytokines IL-1α, IL-6 and IL-18 were found to be increased inSHSY-5Y cells to which we applied CAPE, which has a dominant anti- inflammatory feature, compared to the control group. This increase was greaterthan the group to which only topotecan was administered. The fact that the increase was higher in the CAPE+Topotecan applied group reveals the synergistic effect of these two molecules. In our study, we found that CAPE and Topotecan increased inflammation and cell death in SHSY-5Y cancer cell line, suggesting that theseagents trigger cell death by increasing inflammation in NB cells.

Conclusion

In our study, SHSY-5Y NB cell lines were immunostained with IL-1α, IL-6, IL-18 and VEGF antibodies by applying CAPE, Topotecan and CAPE+Topotecan for 24 hours, and TUNEL method was applied for

cell death evaluation. Inflammation and cell death were found to be increased in CAPE, Topotecan and CAPE+Topotecan applied groups. An abnormal VEGF increase was observed again after drug administration. This abnormal VEGF increase was considered as a pathway contributing to the inflammatory process rather than neovascularisation. Although the fact that CAPE causes an increase in inflammation mediators in the cancer cell line seems to be inverse to its general anti-inflammatory effect, it shows the selective effect of CAPE on neoplastic cells, which is a desired condition in cancer treatment and also shows that it is an agent that can be preferred in the treatment because it protects normal cells. In our study, it has been shown that CAPE and Topotecan increase inflammation and apoptotic cell death in the NB cell line. In this context, we believe that CAPE is an agent that can be used in addition to the others in the treatment of NB, and there is a need for in vivo and in vitro studies in this area.

Conflict of interest:

There is no conflict of interest between the authors.

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Review article

Monoclonal Antibodies: Production, Techniques, and Global Marketing

Hamit YILDIZ[1], Mehmet Tahir HÜSUNET[2], İbrahim Halil KENGER[3*]

[1]*Gaziantep University, Faculty of Medicine, Department of Internal Medicine, Gaziantep, Turkey.*
[2]*Çukurova University, Faculty of Science and Literature, Department of Biology, Adana, Turkey.*
[3]*Gaziantep Islam, Science, and Technology University, Faculty of Medicine, Department of Medical Genetics, Gaziantep, Turkey.*

Abstract

Monoclonal antibodies are becoming increasingly important for molecular immunology research. It has also become key components in a wide variety of clinical laboratory diagnostic tests. The wide applications of serum analytes in the detection and identification of cell markers and pathogenic agents have arisen in large part due to the excellent specificity ofthese unique reagents. Furthermore, continuous culture of hybridoma cells producing these antibodies offers the potential for an unlimited source of reagents. In essence, the continuous supply feature provides standardization of both reagent and assay technique, compared to thevery limited supply of polyclonal antibody reagents. Clearly, polyclonal and monoclonal antibodies have advantages and disadvantages in terms of production, cost, and general applications. As a result, monoclonal antibodies are produced only when necessary because their production is a time-consuming and laborious process, although highly rewarding. In this article, the production and application of monoclonal antibodies are illuminated to provide better understanding and formulate new ideas for clinicians and scientists alike.

Key words: *Monoclonal antibody, hybridoma, phage display*

***Corresponding Author:** İbrahim Halil Kenger, E-mail: kengeribrahim@gmail.com. ORCID ID: 0000-0002-9848-954X.

1. Introduction

Information on the structure and functions of antibodies as we know them today began to become clear only after the 1950s. When we look at the information obtained from 1950 until today, antibodies are generally known as the most effective components of humoral immunity. There are 5 types of antibodies in humans. These are called immunoglobulin A (alpha), D (delta), E (epsilon), G (gamma), M (mu).(1).

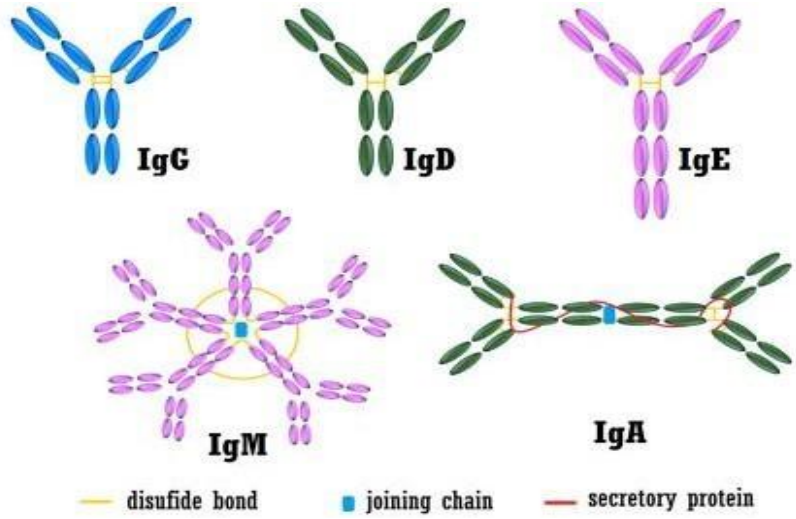

Figure 1: Types of immunoglobulins (2).

Antibodies generated from a single cloneof B cells are called monoclonal antibodies. Monoclonal antibodies (mAbs) only react against one epitope. Lymphoid or myeloid cells are responsible for generating the immunological response. These cells within the lymphoid cells are called "B lymphocytes". Antibodies that occur as a result of stimulation of B cells inthe body and have different activities are called polyclonal antibodies. If the resulting antibody consists of a single cell and its cloning, it is called a monoclonal antibody (3, 4). mAbs are antibodies with high specificity for a single antigen or a single epitope on the antigen (5). mAbs were first produced in 1975 by scientists named Köhler and Milstein using hybridoma technology. To date, a large number of mAbs and their derivatives havebeen used in many clinical applications (6).

2. Uses, Advantages, and Disadvantages of Monoclonal Antibodies

mAbs find a wide variety of uses due to their high specificity. It is used in laboratory tests such as the treatment of diseases, protein purification, suppression of immune response, some allergy tests, identification of special cells, hormone testing, for diagnostic purposes, and basic scientific research. (7).

The diseases in which antibodies are used for therapeutic purposes are increasing day by day. It finds use in cancer, prevention of organ transplant rejection, cardiovascular diseases, inflammatory diseases, and autoimmune diseases. This number is predicted to increase in the future (8, 9).

mAbs are also used effectively and widely to detect various agents threatening human health that can be found in food, water, and soil. There are various mAb-based diagnostic systems for the detection of aflatoxin, which can be found in high

amounts in traditional foods and causes significant harm (10, 11). In addition, monoclonal antibodies are used in the diagnosis of *Bacillus anthracis*, which can be found in water and soil and can cause human death (12, 13).

Oncology and Hematology Treatment

mAbs are preferred because of their high target-specific binding properties. (3). The main purpose of cancer immunotherapy is to reactivate the immune system that has been silenced by the tumor cell in various ways and to make the tumor cells recognizable. The first of the three basic approaches in this regard is monoclonal antibody-mediated cell death targeting tumor-related antigens. Others are immune system control inhibitors and cancer vaccines. (14).

Although there are many effective drugs in cancer treatment, their use is limited due to their strong side effects. Compared to conventional chemotherapeutic drugs, mAbs stand out because they can distinguish antigenic differences between normal and malignant tissues and have minimal effects on healthy tissues. (15).

mAbs show their effects with various mechanisms of action in cancer treatment. These mechanisms show their effects by inhibition of cell signaling and apoptosis, antibody-dependent cellular cytotoxicity (16), apoptosis dependent on modulation of the receptor by signal inhibition (15), as well as tumor-associated antigens blocking the proliferation of tumor cells.

Autoimmune Diseases

mAbs act in the prevention of autoimmune reactions by targeting the basic cells of the immune system such as T and B lymphocytes. The movements and functions of mAb-activated cells are inhibited, the levels of proinflammatory cytokines are reduced, and thus the pathological effects are alleviated by suppressing the excessive immune response. In the first studies conducted in autoimmune diseases, it was determined that in the treatment of rheumatoid arthritis and Crohn's disease, it binds to tumor necrosis factor (TNF) and by neutralizing it, pathologies related to these diseases are prevented. (17). There are mAbs developed against many autoimmune diseases (Table 1). In the coming years, it is expected that new antibodies will be discovered in the treatment of different autoimmune diseases.

Infectious Diseases

The number of bacteria that develop resistance to antibiotics is increasing over time. Especially coping with hospital infections has become important problems. The development of resistance to existing antimicrobials and the inability to develop new antimicrobial drugs have increased the interest in mAbs in this field. mAbs with antimicrobial activity neutralize bacterial toxins and alleviate the pathological activities of bacteria. It is stated that the possibility of developing resistance against antibodies is low because mAbs show their effects by different mechanisms than classical antibiotics (18). Monoclonal antibodies such as Raxibacumab and Obiltoxaximab are used in the treatment of anthrax of bacterial origin (*Bacillus anthracis*). These mAbs prevent thebinding of protective antigens in bacteria to cell receptors, thereby preventing edema and theformation of deadly toxins (19, 20).

Infusion Reactions

Side effects that may occur during the infusion of mAbs may occur locally or systemically (21). Common symptoms include fever, flushing, chills, chest discomfort, abdominal pain, nausea,

vomiting, diarrhea and skin rash. Rarely, anaphylaxis may occur. Side effects due to mAb infusion may occur within the first two hours, but may be delayed up to 14 days after treatment. The period when the risk of side effects is highest is during the first or second application, but the risk may decrease with repeated applications. (22). Side effects such as anaphylactic reactions to the mAb, serum sickness, tumor lysis syndrome (TLS), and cytokine release syndrome (CRS) may occur after infusion of mAb (23).

3. Techniques used in the production of monoclonal antibodies

The use of antibodies for diagnostic and therapeutic purposes dates back to the first years when their existence was proven (24). For this purpose, the collection and purification of polyclonal antibody-containing serum of an animal immunized with a specific antigen and its use for the specified purpose have been tried. However, this situation has led to the development of a monoclonal antibody molecule gaining importance due to the drawbacks in the use of polyclonal antibodies (25). First, Köhler and Milstein, in 1975, "The foundations of hybridoma technology were laid by obtaining continuous cell lines of fused cells that secrete specific antibodies for a particular antigen (26). Molecules that can bind specifically to a certain antigen and have a uniform (homogeneous) immunoglobulin (IgG) structure are called “monoclonal antibodies” (Mabs) (Costa et al., 2010). Since the formation and production of hybridomas, there have been significant developments in the biotechnology industry (27). The main field of use of mAbs is diagnostics and immunotherapy. After the first experiments with mouse Mabs, with the advances in technique and the emergence of new fusion mates, hybrids between rat, hamster, human and other species dec also be obtained. In addition, technologies have been developed that allow the production of important immunological T-cell hybridomas that secrete single reproductive factors (28-30). Hybridoma technology is based on fairly clear and unambiguous foundations, but difficulties may arise during the acquisition of antibodies and clones of the desired characteristics and biological character. The procedures take a lot of time and require intensive laboratory labor (31).

The basis of hybridoma technology is “creation of cells capable of indefinite reproduction and antibody formation *in-vitro*”. However, antibody-producing B-lymphocytes usually die within a few weeks in cell culture (in-vitro) (32). Therefore, antibody-producing B-lymphocytes must be modified to live in cell culture for a long time. For this reason, lymphocyte tumor cells (myeloma) that are capable of proliferating indefinitely are used. The fusion of antibody-producing B-lymphocytes and myeloma cells, which are capable of endless reproduction, is carried out (33). Immortal cell lines formed as a result of the fusion of B-cell and myeloma are hybrid in nature and are called "hybridoma". Hybridoma cell lines have characteristics of both fusion partners (34). The basic process steps have changed little since Köhler and Milstein. The critical steps of the process are the achievement of efficient and efficient cell fusion and then selection of clones of appropriate character. The steps of the method can be listed as follows:

- An effective immunization (in-vivo or in-vitro).
- Acquisition of immune B-lymphocytes.
- Preparation of myeloma cells.
- Hybridization (cell fusion) and obtaining hybridoma.
- Control of immunoglobulin synthesis.
- Cloning of hybridomas.
- Production of hybridomas on a large scale.
- Purification of monoclonal antibodies.

Phage Display Technique

This technique stands out as the most popular of the available monoclonal antibody development methods. It hasmany advantages, such as the fact that it allows the development of recombinant antibodies with different properties and their production using protein chains of human origin. It is based mainly on the principle of directed evolution. Accordingly, as a result of somatic mutation, the antibody has become specific to the antigen to be developed and the best- binding M13 bacterial phage has been selected, and the protein chain that provides binding is produced using recombinant DNA technology methods (35-37). Within the scope of the technique, firstly, the gene framework encoding the VH and VL protein chains present in the human B-cell is amplified by PCR (polymerase chain reaction). The transcribed gene region is integrated in front of the pIII surface protein gene region so that the recombinant antibody (in the form of scFv or VH) can be synthesized to coincide with the pIII surface antigen of the M13 bacterial phage (38-40). Escherichia coli strain is infected with the bacterial phage prepared in this way and the phages are reproduced. Replicated phages are superimposed on antigens attached to the surface of the chip or 96- well plate for the first selection step. At this stage, phages that can bind to the antigen are bound, while those that cannot bind are removed by washing. In the next step, the surface of the well is treated with a secondary antibody with a phage-specific and luminescent conjugate. After the last washing process, the presence of the boundantibodies can be detected spectrophotometrically, so that the conjugate bound to the secondary antibody is irradiated and the measurement is made immediately afterwards. According to the results obtained, the phages in the wells determined to be positive are collected by trypsinization and propagated in E.coli (41, 42).

For the phages collected from the positive wells obtained as a result of the first selection, the selection process isperformed two more times in the same way. The secondary and tertiary selection steps performed in this way are performed to mature the affinity of the antibody to be obtained. Thus, it is ensured that the antibody to be obtained will have a high binding ability (43-45).

After the relevant gene chain of the antibody obtained by the binding site phage display technique is reached, the gene chain in question is cloned into a plasmid vector with the appropriate expression cassette. The resulting plasmid is transferred into bacterial (often E.coli), yeast (often Pichia pastoris) or mammalian (often CHO) cells, depending on the final antibody form designed (holistic, mini- structure, scFv, etc.). Thus, the antibodydeveloped to have high specificity and

affinity is produced for use for diagnosis and/or treatment purposes (46-48).

4. Global market for monoclonal antibodies

The first chimeric mAbs were generated in the late 1990s. Subsequently, there was a significant increase in the sales of mAbs in 2013 in parallel with the increase in the speed of approval of humanized and fully human mAbs. In 2013, annual worldwide sales of monoclonal antibodies amounted to approximately $75 billion, accounting for almost half of the biopharmaceutical product market (49).

The last 20 years have seen rapid growth in therapeutics in the monoclonal antibody class. Today, there are over 300 clinically developed monoclonal antibodies (50). The demand for monoclonal antibodies on a global scale is increasing. Global sales revenue for monoclonal antibodies in 2018 reached $115.2 billion (51). It is thought that this sector will be a 300 billion dollar industry by 2025 (52).

5. Conclusions

Recently, depending on the difference in the results obtained in personal treatment, the concept of personalized treatment has begun to gain more and more importance. With this, the importance of monoclonal antibodies in the treatment has begun to increase. The ability of monoclonal antibodies to bind to a single epitope is one of the main reasons for the increased interest in these molecules. The molecular and cellular biology of cancer will be better understood when preclinical studies are completed in many areas such as cancer formation, progression, metastasis, the mechanisms of cancer to render immune system elements unresponsive, to secrete factors that increase tumor growth, and to develop resistance to chemotherapy. The effect of cancer cells at the receptor level, especially in cancerous tissue, hasled to an increase in studies and clinical uses in this field. Today, monoclonalantibodies are one of the groups with the largest market share among all pharmaceutical products. With the increasing interest and studies on monoclonal antibodies, it is expected that even more monoclonal antibodies will be available in the coming years.

6. Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

9. Acknowledgement

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Table 1: List of Monoclonal antibodies approved for clinical use in the United States (53).

Cancer				
Generic Name Brand Name	Type Antigenic Target	Approval	Likelihood Score	Major Uses
Alemtuzumab Campath	Humanized CD52	2001	C	Chronic lymphocytic leukemia
Atezolizumab Tecentriq	Humanized PD-L1	2016	D	Urothelial carcinoma Non-small cell lung cancer
Avelumab	Human	2017	E*	Merkel cell carcinoma

Bavencio	PD-L1			Urothelial carcinoma
Bevacizumab	Humanized	2004	E	Colorectal cancer
Avastin	VEGF	2006		Non-small cell lung cancer
		2009		Macular degeneration (off lbl) Renal
		2018		cancer
				Ovarian cancer
Blinatumomab	Mouse	2014	E*	Acute lymphoblastic leukemia
Blincyto	CD3, CD19			
Brentuximab	Chimeric	2011	E*	Hodgkin lymphoma
Adcetris	CD30	2018		Peripheral T-cell lymphoma
Cemiplimab	Human	2018	C	Squamous cell carcinoma
Libtayo	PD-1			
Cetuximab	Chimeric	2004	E	Head and neck cancer
Erbitux	EGFR			Colorectal cancer
Daratumumab	Human	2015	E	Multiple myeloma
Darzalex	CD38			
Dinutuximab	Chimeric	2015	E*	Neuroblastoma
Unituxin	GD2			
Durvalumab	Human	2017	C	Urothelial carcinoma
Imfinzi	PD-L1			
Elotuzumab	Humanized	2015	D	Multiple myeloma
Empliciti	SLAMF7			
Gemtuzumab	Humanized	2000	A	Acute myelogenous leukemia
Mylotarg	CD33	2017		
Inotuzumab	Humanized	2017	B	Acute lymphoblastic leukemia
Besponsa	CD22			
Ipilimumab	Human	2011	A	Malignant melanoma
Yervoy	CTLA4			
Mogamulizumab	Humanized	2018	C	Mycosis fungoides
Poteligeo	CCR4			Sézary syndrome
Moxetumomab	Mouse	2018	E*	Hairy cell leukemia
Lumoxiti	CD22			
Necitumumab	Human	2015	E	Non-small cell lung cancer
Portrazza	EGFR			
Nivolumab	Human	2015	E*	Malignant melanoma
Opdivo	PD-1	2018		Metastatic small cell lung cancer
Ofatumumab	Human	2009	E*	Chronic lymphocytic leukemia
Arzerra	CD20			
Olaratumab	Human	2016	E	Soft tissue sarcoma
Lartruvo	PDGF			
Panitumumab	Human	2006	E	Colorectal cancer
Vectibix	EGFR			
Pembrolizumab	Humanized	2014	E*	Malignant melanoma
Keytruda	PD-1	2015		Non-small cell lung cancer
		2018		Advanced cervical cancer
Pertuzumab	Humanized	2012	E	Breast cancer
Perjeta	HER2			
Ramucirumab	Human	2014	E*	Gastric, non-small cell lung cancer
Cyramza	VEGF	2015		Colorectal cancer
Rituximab	Chimeric	1997	A	Chronic lymphocytic leukemia
Rituxan	CD20			Non-Hodgkin lymphoma
				Rheumatoid arthritis
Tositumomab	Mouse	2003	E*	Non-Hodgkin lymphoma
Bexxar	CD20	Withdrawn		
Trastuzumab	Humanized	1998	D	Breast and gastric cancer
Herceptin	HER2			
Autoimmune Diseases				
Adalimumab	Human	2002	B	Inflammatory bowel disease
Humira	TNFα			Rheumatoid, Psoriatic arthritis
				Severe psoriasis

Alemtuzumab Lemtrada	Humanized CD52	2014	C	Multiple sclerosis
Belimumab	Human	2011	E	Systemic lupus erythematosus
Benlysta	B cell activity factor	2020		Lupus nephritis
Brodalumab Siliq	Human IL-17A	2017	E	Plaque psoriasis
Canakinumab Ilaris	Human IL1β	2009	E*	Autoinflammatory diseases
Certolizumab Cimzia	Humanized TNFα	2008	E*	Inflammatory bowel disease Rheumatoid arthritis
Daclizumab Zinbryta	Humanized CD25	2016	C	Multiple sclerosis
Dupilumab Dupixent	Human IL-4α	2017	E	Atopic dermatitis
Efalizumab Raptiva	Humanized CD11a	2003 Withdrawn	D	Plaque psoriasis
Golimumab Simponi	Human TNFα	2009	E*	Inflammatory bowel disease Rheumatoid, psoriatic arthritis
Guselkumab Tremfya	Human IL-23	2017	E*	Plaque psoriasis
Infliximab Remicade	Chimeric TNFα	1998	A	Inflammatory bowel disease Rheumatoid arthritis Severe psoriasis
Ixekizumab Taltz	Humanized IL-17A	2016	E	Plaque psoriasis Psoriatic arthritis
Ocrelizumab Ocrevus	Humanized CD20	2017	D	Multiple sclerosis
Omalizumab Xolair	Humanized IgE	2003 2014	E	Eosinophilic asthma Chronic idiopathic urticaria
Risankizumab Skyrizi	Humanized IL-23	2019	E	Plaque psoriasis
Rituximab Rituxan	Chimeric CD20	1997	A	Chronic lymphocytic leukemia Non-Hodgkin lymphoma Rheumatoid arthritis
Sarilumab Kevzara	Human IL6R	2017	E*	Rheumatoid arthritis
Secukinumab Cosentyx	Human IL-17A	2015 2016 2018 2020	E*	Plaque psoriasis Psoriatic arthritis, Ankly. Spondylitis Scalp psoriasis Axial spondylarthritis
Siltuximab Sylvant	Chimeric IL6	2014	E	Castleman disease
Tildrakizumab Ilumya	Humanized IL-23	2018	E*	Plaque psoriasis
Tocilizumab Actemra	Humanized IL6R	2010 2011 2017	C	Rheumatoid arthritis Juvenile idiopathic arthritis Giant cell arteritis
Ustekinumab Stelara	Human IL-12, IL-23	2010	E*	Plaque psoriasis Psoriatic arthritis
Vedolizumab Entyvio	Humanized Integrin α4β7	2014	D	Inflammatory bowel disease
Liver Transplantation				
Daclizumab Zenapax	Humanized IL-2	1997 Withdrawn	C	Prevention of transplant rejection
Muromonab-CD3 OKT3	Mouse CD3 T cells	1985 Withdrawn	E	Prevention of transplant rejection
Basiliximab Simulect	Chimeric IL-2Rα	1998	E	Prevention of transplant rejectio

Various mAbs				
Abciximab Reopro	Chimeric GpIIb/IIIa	1993		Inhibition of platelet aggregation
Aducanumab Aduhelm	Human Amyloid β	2021	E	Alzheimer disease
Alirocumab Praluent	Human PCSK9	2015	E	Hypercholesterolemia
Benralizumab Fasenra	Humanized IL5	2017	E	Eosinophilic asthma
Bezlotoxumab Zinplava	Human C. difficile toxin B	2016	E	Prevention of recurrence of C. difficile infection
Burosumab Crysvita	Human FGF 23	2018	E	X-linked Hypophosphatemia
Caplacizumab Cablivi	Humanized vWF	2019	E	Acquired thrombotic thrombocytopenic purpura
Crizanlizumab Adakveo	Humanized P-selectin	2019	E	Sickle cell disease
Denosumab Prolia, Zgeva	Human RANKL	2010	E*	Osteoporosis Bone metastases
Eculizumab Soliris	Humanized C5	2007 2011	D	Paroxysmal nocturnal hemoglobinuria
Emapalumab Gamifant	Human Interferon Gamma	2018	E	Hemophagocytic lymphohistiocytosis
Emicizumab Hemlibra	Humanized Factor IXa & X	2017	E	Hemophilia A
Eptinezumab Vyepti	Humanized CGRP	2019	E	Migraine headache
Erenumab Aimovig	Human CGRP	2018	E	Migraine headache
Evinacumab Evkeeza	Human ANGPTL3	2021	E	Hypercholesterolemia
Evolocumab Repatha	Human PCSK9	2015	E	Hypercholesterolemia
Fremanezumab Ajovy	Humanized CGRP	2018	E	Migraine headache
Galcanezumab Emgality	Humanized CGRP	2018	E	Migraine headache
Ibalizumab Trogarzo	Humanized CD4	2018	E	HIV infection
Lanadelumab Takhzyro	Human Kallikrein	2018	E	Hereditary angioneurotic edema
Mepolizumab Nucala	Humanized IL15	2015	E	Eosinophilic asthma Hypereosinophilic syndrome
Natalizumab Tysabri	Humanized Integrin α4β7	2004	B	Multiple sclerosis Inflammatory bowel disease
Obiltoxaximab Anthim	Chimeric Anthrax toxin	2016	E	Inhalational anthrax
Omalizumab Xolair	Humanized IgE	2003 2014	E	Eosinophilic asthma Chronic idiopathic urticaria
Palivizumab Synagis	Humanized RSV fusion protein	1998	E	Respiratory syncytial virus infection
Ranibizumab	Humanized	2006	E	Macular degeneration

Lucentis	VEGF-A			
Ravulizumab Ultomiris	Humanized Complement C5	2018	E*	Paroxysmal noctural hemoglobinuria
Raxibacumab	Human Anthrax toxin	2012	E	Inhalational anthrax
Reslizumab Cinqair	Humanized IL5	2016	E	Eosinophilic asthma
Romosozumab Evenity	Humanized Sclerostin	2019	E	Osteoporosis
Teprotumumab Tepezza	Human IGF1R	2019	E	Graves ophthalmopathy

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Letter to Editor

Interpretation of the Area Under the Receiver Operating Characteristic Curve

Serdar ÖZDEMIR[1*], Abdullah ALGIN[1]

[1]*Health Sciences University, Ümraniye Education and Research Hospital, Department of Emergency Medicine, İstanbul, Turkey.*

Dear editor,

In medicine, it is very important to be able to intervene in diseases with early and correct diagnosis. In recent years, there has been an increasing interest in medical decision-making methods and the applications of these methods find a wide place in the medical literature. Most of the studies on diagnostic tests are devoted to investigating the reliability of these methods and comparing the methods. Diagnostic test is a general name given to evaluation methods based on laboratory techniques, clinical observations or specific instrument measurements used to identify a disease. It is aimed to distinguish between sick and healthy individuals by using various diagnostic methods and the results of laboratory tests (1).

In order to analyze the results of a diagnostic test comprehensively and reliably, the actual level of effectiveness of the diagnostic test must first be checked. There are many statistical decision-making methods used for this purpose today. ROC (Receiver Operating Characteristic) curve is the most widely used method for these purposes. In order to examine and interpret the results of the diagnostic test, the actual diagnostic results of the cases on which the test was applied should be known. For the actual diagnosis, the gold standard method should be used, which is accepted to give accurate results in determining the same disease. gold standard; The clinical process functions as surgical confirmation, autopsy and, in some cases, the consultation of a specialist (2).

The area under the ROC curve determines the accuracy of the test in distinguishing between patients and non-patients. The size of the area under the ROC curve indicates the statistical significance of the discrimination ability of the diagnostic test studied (2). The expected value of the area under the ROC curve is 0.50 when the diagnostic test being studied has no discrimination ability. This value represents random. That is, the area under the curve of the non-diagnostic test is 0.50. If it is a perfect test, with zero false positives and zero false negatives, the value of the field would be 1.00. If the value under the curve is 0.90-1.00, it is excellent, 0.80-0.90 is good, 0.70-0.80 is medium, 0.60-.070 is weak, 0.50-0.60 is unsuccessful (3). Researchers often make mistakes in interpreting these values (4). Researchers do not have to know advanced statistics. Statistical counseling can be obtained, especially in medical studies, but researchers should master basic statistical information such as the interpretation of the area under the ROC curve.

***Corresponding Author:** Serdar Özdemir, E-mail: dr.serdar55@hotmail.com. ORCID ID: 0000-0002-6186-6110.

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