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Experimental and Applied Medical Science

Volume 4, Issue 1

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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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Clerk of Editorial Office/Sorumlu Yazı İşleri Müdürü

Mediha Begüm KAYAR, Asst. Prof.

Aim

Experimental and Applied Medical Science 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 any manuscript is considered to deserve publishing, it may be subject to editorial revisions 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 obtained from 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 beyan eder.

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

If there is a commercial relation that contributes to the study process or an institution that provides financial support; the authors must declare that either they have no commercial relationship with the commercial product, drug, company used, or what kind of relationship (consultant or any other agreement) they have, if any.

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. Değerlendirme ve yayın sürecinin hiçbir aşamasında yazarlardan ücret talep edilmez. Tüm yazılar https://dergipark.org.tr/tr/pub/eams adresinde bulunan çevrimiçi başvuru sistemi 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 sayılı Kanun 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 öneriler tamamen 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 görüşüne dayanacaktır.

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

Aşağıdakileri içeren ayrı bir başlık sayfası olmalıdır:

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

Tam uzunluktaki kağıdın ana gövdesi şu bölümlere ayrılmalıdır:

1. Özet
2. Giriş

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

In general, there are no a maximum specific word length laid down as a condition for any manuscript. The general principle is that a manuscript should be as long as necessary to communicate the scientific message clearly and effectively at the most, but should be as short as possible to avoid undue repetition or redundancy with a complete presentation of the information.

In the *Materials and Methods* section, the source of all compounds, equipment or software should be identified by the full name of the supplier, city, state/country. The chemical names of any drug should precede the trade name.

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 uzun olabileceği, ancak gereksiz tekrar veya fazlalık olmadan bilgilerin eksiksiz bir sunumunu 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, et al. 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 ve ardından 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.
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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.
We accept electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, movies, animation sequences, high-resolution images, background datasets, sound clips and more.
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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.
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.

Görseller, minimum 300 dpi çözünürlükte, yalnızca TIFF veya EPS dosyaları halinde ve ayrı dosyalar olarak gönderilmelidir.
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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The Chancellor's Message

Dear Students and Academicians,

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

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

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

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

Wish you all the best

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

Chief Editor's Message

Dear Readership,

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

Nowadays, academic studies are accelerating, multiplying and diversifying. The need for channels where scientific studies, opinions and ideas can be freely expressed and easily shared with experts, researchers or postgraduate students who are still in the learning phase is increasing day by day. "Experimental and Applied Medical Science" has adopted it as a principle from the first day to bring together original and up-to-date studies, stimulating scientific views and ideas from every field of medicine that will potentially increase the quality of life with its readers both from home and abroad. With this fifth issue of our journal, we will continue to publish in English 4 (four) times a year, more than thirty manuscripts, in different types, research articles, case reports, reviews, etc. will have already been published and met with our readers. Recently, researchers have begun to understand the importance of having their studies published in international double-blind peer-reviewed journals. Since the first day of its publication, "Experimental and Applied Medical Science" has subjected the manuscripts received to an international double-blind peer 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 improve their educational or academic lives by providing on the spot feedback.

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

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

Best Regards…

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

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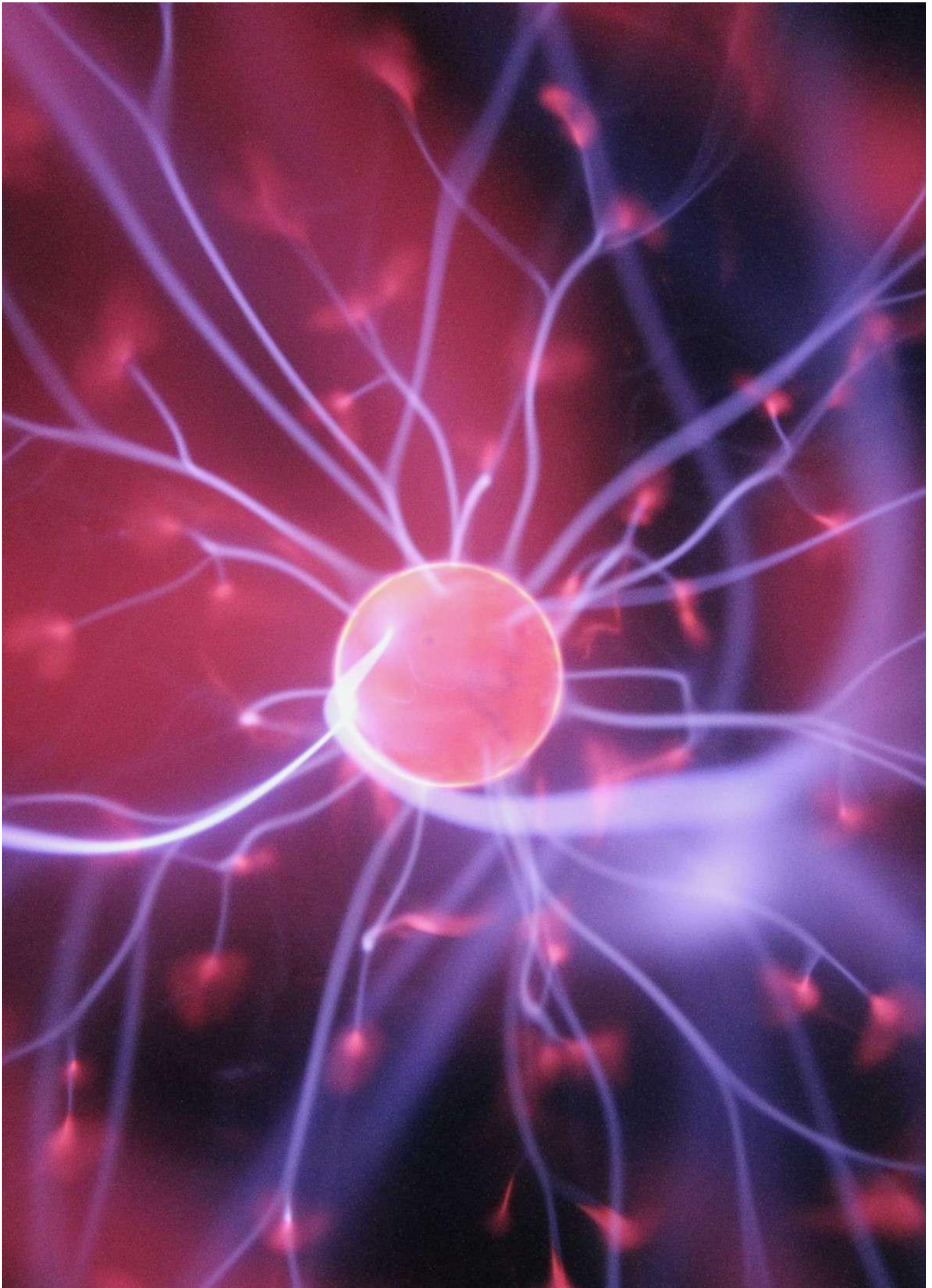

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Pre-Hospital Practices Performed for Children with Burn Injuries

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Abstract

The purpose of this study was to evaluate the first aid practices that the mothers do in their burned children. The study was conducted a descriptive study in burn centers of a university hospital province, between August 2015 and May 2016. The study population consisted of inpatient children aged 1–12 years who were treated in burn centers in hospital during the study period, along with their mothers. The study was carried out in the related hospital with a total 108 children and their mothers who met the research inclusion criteria. In our study, mothers were asked what kind of first-aid they apply when a burn has occurred and found that 48.1% of them spill water over the burn area. When the age of the participant children and the types of burns were examined, it was found that the burn rates of the 1-6 age group were higher in all types of burns than the other age groups.

Key words: *Burn, first aid, child burn, nursing care.*

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Introduction

The tissue damage caused by heat, electricity, chemical substances and radioactive rays is called as burn (1). The majority of burn patients (53.6%) are children (2). Encountering children with burns more commonly in this age group might be because of the children's lack of self-protection, because of being too curious, not being aware of the dangers of heating devices like stoves, poking plugs with metals and getting in their mother's way while they are cooking (3). Burns is the fourth most frequent cause of injury and the top fifteenth among the injuries leading to death in children (4, 5). Deaths linked to burns in children are 10 times more in developing countries than in developed countries (4, 6-8).

The fact that burn has high rates among the causes of injury and death has led to much researches on this subject. Diler et al. have reported that the most common causes of burn in children are hot fluid burn 87.4%, flame burn9.7%, electric burn 2.3% and hot object burn 0.6% (9). Aytaç et al. investigated the causes and rates of burns, and found that 68.8% of them are scalded, 21.5% are flame burns, 3.8% are burns caused by contact with a hot object, and 1.1% are chemical burns (10).

Neglect, abuse, poverty, insecure home environment, interparental conflict, risky behaviors, learning difficulties, depression, arson behavior are among the risk factors for burns in children (11). These risk factors should be assessed and necessary precautions need to be taken. Children need the help of an adult in creating a safe environment for themselves and in protection from accidents (12). The vast majority of burns are due to carelessness and insufficient measures to be taken and about half of them can be prevented by educating parents and children (12, 13). The precautions to be taken and correct first aid practices should be taught to the families. The most important task in this regard falls to professional health professions. Nurses, in particularly, who work in all parts of the community have the opportunity to evaluate accidents, to identify risks and to implement applications for reducing these risk factors with home visits (14).

In the researches conducted, it was found that cold water application is the most commonly used first aid intervention after burns. Additionally, some of the methods used are; to put ice, onion wrap, mud bath, applying toothpaste, yoghurt, tomato paste, shoe polish, inking, unpeeled apple, tomato paste, buttery, egg yolk, iron rust and salt and putting raw potatoes (15-17). It is crucial to know what should be done as well as to

know what shouldn't be done in first aid practices (17). In this respect, foreign substances used for burn treatment are misapplications in terms of infection, prolongation of the healing process and poor cosmetic results (18). For this reason, the first intervention after burn is very important. The purpose of this study is to evaluate the first aid practices that the mothers do in their burned children.

Method

The study was conducted as descriptive study in burn centers of a university hospital province, between August 2015 and May 2016. The study population consisted of inpatient children aged of 1–12 years who were treated in burn centers in related hospital during the study period, along with their mothers. The study was carried out with total 108 children and their mothers who met the research inclusion criteria. Any sample selection was not performed during the study; rather, whole effort was made to reach all inpatient children and mothers during the study period. The inclusion criteria for the study were as follows: 1-12 aged group of children, mothers who agreed to participate in the research and had no audiovisual or psychological problems.

A questionnaire was developed by the researchers. Data were collected through face-to-face interviews with mothers of inpatient children in the burn centers of the respective hospital. Data collection time was approximately 20-25 minutes for each participant. Immediately after data collection, the “First Aid for Burns” pamphlet, which was created by the researcher using the relevant literature to raise awareness about the correct first aid practices for burns, was distributed to mothers (19). Legal permission was obtained from the relevant institutions to conduct the study. The research began after approval from the Ethics Committee was received. Official permissions from the respective hospital was obtained to collect the study data. Informed consent was obtained from mother.

Demographic and other individual characteristics of participants were reported using descriptive statistics. Then we conducted bivariate analyses using percentage distributions, means, and the chi-square test. Statistical significance was considered at $p<0.05$. All analyses were conducted using the Statistical Package for Social Science (PASW) software version 18.

Results

81.5% of the children who participated in the study were between the ages of 1-6, 63% were male. The economic situation of the families was examined and 46.3%

had a moderate income and 63% were living in the countryside. 74.1% of the participant mothers in this study were between the ages of 19-34 and 50% were not literate. (Table 1).

Table 1. Descriptive characteristics of patients and their mothers

Descriptive characteristics	N	%
Age of the child		
1-6	88	81.5
7-12	20	18.5
Gender of the child		
Girl	40	37
Male	68	63
The economic situation of the family		
Good	33	30.6
Middle	50	46.3
Bad	25	23.1
Living Place		
Rural	68	63
City	40	37
Maternal Age		
19-34	80	74.1
35-Above	28	25.9
Mother Education		
Illiterate	54	50
Junior-High School	38	35.2
Literate-primary school	16	14.8

The burn injuries of the children who were participating in the research and the interventions of the mothers to the burns were examined. It was found that 76.9% of the children were scaldburn, and 50% were second degree burn. Mothers were asked about their first aid attempts when a burn was occurred and it was determined that 48.1% of them applied water over the burn and 61.1% of them had learned the application that they use on their own. 50% of the mothers stated that they applied to a health institution within 1 hour and 60.2% of them stated that they went to an emergency services (Table 2).

The descriptive characteristics of the children and the mothers participating in the study were compared with the information about the burn. It was seen that the children of the participant mothers, who are middle school-high school graduates, between 19-34 years old, of this research, mostly had burns in type of scald and the difference between the groups was significant. ($p<0.001$). A large majority of the mothers ages 19-34 reported that they applied cold water to the burn area in the event of burns and that they had benefited from this intervention at a high rate. It was found that the difference between the groups

was significant in terms of the age of the mothers and the first interventions that they applied and benefiting from the application ($p<0.001$). Middle school and high school graduate mothers who participated in the research stated that they learned this practice on their own. It was found that most of the illiterate mothers took their burnt children to the hospital on the same day (Table 3).

Table 2. Informations related to burn

Variables related to burn	N	%
Type of the Burn		
scald	83	76.9
Flame, Stove, Oven	23	21.3
Chemical	2	1.9
Degree of the Burn		
1st Degree Burn	18	16.7
2nd Degree Burn	54	50.0
3rd Degree Burn	36	33.3
First-aid Applications to the Burns		
Doing nothing	9	8.3
Cold water application	52	48.1
Removing clothes	23	21.3
Ointment application	6	5.6
Applying oil	4	3.7
Ice application	8	7.4
Wrapping cloth	6	5.6
Application Learned From		
Self	66	61.1
Environment	28	25.9
Relative	14	13.0
The State of the Application's Benefit		
Some	31	28.7
Yes	63	58.3
No	14	13.0
Time period of Applying to a Health Organization		
In an hour	54	50
In the same day	54	50
First Applied Health Facility		
The health clinic	9	8.3
Children's Hospital	34	31.5
Emergency	65	60.2
Type of the Heating Device		
Stove	88	81.5
Heater	20	18.5

Table 3. Comparison of descriptive characteristics of mother and children with burn-related information

Variables related to burn	Childeren's Age 1-6 years old / 7-12 years old	Gender of the Child Female/ Male	Living Place Rural / City	Mother's Age 19-34 / 35-above	Mother's Education Level Illiterate/Junior-High School /Literacy-Primary School	The State of the Application's Benefit Some/Yes/No
Type of the Burn						
Scald Flame,	69/14	25/58	32/51***	68/15***	31/38/14***	
Stove, Oven	17/6	13/10	8/15	10/13	21/0/2	
Chemical	2/0	2/0	0/2	2/0	2/0/0	
First-aid Applications to the Burns						
Doing nothing				5/4	4/2/3	0/9/0
Coldwater application				42/10***	20/26/6*	10/36/6***
Removing clothes				22/1	9/7/7	3/18/2
Ointment application				4/2	2/4/0	4/0/2
Applying oil				1/3	4/0/0	0/0/4
Ice application				6/2	6/2/0	8/0/0
Wrapping cloth				0/6	6/0/0	6/0/0
From whom the Application is Learnt						
Self					22/28/16***	
Environment					18/10/0	
Relative					14/0/0	

Discussion

Burns are traumas that occur with heat, electricity, chemical substances, and radioactive rays, and has a high mortality rate in younger and older ages (20). One of the most important causes of morbidity and mortality in home accidents is burns (21) and it is one of the top fifteen causes that lead death in children (22). Burn-related deaths in children are 10 times more in developing countries than in developed countries (4, 6).

Who participated 76.9% of the children in this research had scald type of burnt. Diler et al. found that hot liquid burns were in the first place among the most common burn causes in children with 87.4% (9), it was found that the rate of scald in the 0-18 age group was 76.2%,in the study of Aytaç et al. (10), scalds were in the first place among the causes of burns (%68.8), Kocatürk et al. (23) determined that 65.4% of burns were hot liquid burns caused by the spilling of hot water, tea, pudding, etc. over the body. When the literature is examined it is seen that the rates of scald burns are also high in different regions of the world. According to a study conducted by Lin et al. In Taiwan, it was found that scald burns in children constitute the highest burn type with 76.2% (24). Also in Japan, a study conducted by Fukunishi et al. found that the rate of burns caused by fluid is 80.8% (25).

In our study, mothers were asked what kind of first-aid they apply when a burn has occurred and found that 48.1% of them spill water over the burn area. Battaloğlu et al. found that 44.5% of the mothers applied cold water as first aid in case of burns (26). Also, in the research conducted by Kavurmacı and Küçükoğlu, it was found that in case of burns, one of the primary first aid attempts of mothers is cold water application (18).

When the age of the participant children and the types of burns were examined, it was found that the burn rates of the 1-6 age group were higher in all types of burns than the other age groups. Şayık et al. also found that the rate of burns among 0-5 age group children was higher than the other age groups (27). In the research conducted by Kavurmacı and Küçükoğlu, inthe 0-6 age group children, the rate of burns were found high in all types of burns (18). The high rate of burns of children in this age group, who are in the process of crawling and walking, may be due to insufficient domestic precautions and the lack of adequate watch of the children by the caretaker.

When the sexes of children and types of burns were examined, the burn rate of

girls by flame/ stove/ oven and chemical burns was higher than that of boys. The fact that, according to Turkish culture girls spend more time with their mothers and that their playgrounds are often home, may have caused this finding.

When the type of living place and the type of burn were examined, it was found that the burn incidence rates in the city were higher in all types of burns and the difference between the groups was significant ($p<0.001$). In the research conducted by Kavurmacı and Küçükoğlu, it was found that there are more burn types in rural areas (18). In Turkish society, mothers usually undertake the responsibility of child care. The fact that also mothers work in urban life requires that one of the elderly family members, such as grandmother, to take care of the child. This finding can be attributed to the fact that elderly people are hardly watch andprotect an active child.

According to the study results, the burn rates of children of 19-34 age group mothers were found to be higher in each type of burn and the difference between the groups was significant ($p<0.001$). This can be because of the inadequacy of the young mother's in protection of children, awareness of possible domestic risks and domestic risk reduction behaviors.

When the education levels of the mothers and the types of burns were examined, it was found that the scald burns occurred in middle school and high school graduate mothers' children, and the other burn types occurred in children of illiterate mothers. The difference between the two groups was statistically significant ($p<0.001$). All in all, the education level of themothers participating in the survey seems to be low. Also in the conducted researches, it was found that the level of education of burnt children's mothers was low (18, 26, 27).

The age of the mothers was compared with the first aid application to the burn area, and the mothers in the 19-34 age group were found to apply mostly water to the burnt area. The difference between the groups was significant ($p<0.001$). In the research conducted by Kavurmacı and Küçükoğlu, it was found that the first-aid that young mothers applied in case of burns is cold water (18).

Educational levels of mothers were compared with the first-aid applications that they use in case of burns. It was found that the mothers with high education level preferred to apply cold water as first aid in case of burns and the difference between the groups was significant ($p<0.05$). In the research

conducted by Yalın in 1988 (28) and by Sezen in 1994 (29), it was found that there are many misapplications like applying egg yolk, engine grease, spirit, molasses, tahini etc. In Turkey, the rate of orientation towards traditional practices in case of diseases and accidents is high. Tortumluoğlu et al. found similar results in their study and

found that a great majority of applications (toothpaste, yoghurt, calcium bleach, tomato paste, iron rust, etc.) were harmful applications. It was deduced that these applications are preferred by elderly and undereducated individuals. According to these results, it can be said that as the level of education increases, the level of the usage of traditional practices decreases and the rate of preference of the correct first aid application increases (16). Additionally, in the researches it seems that there is a significant improvement in the first aid attempts being madein case of burns. It can be argued that this situation may also arise from the regional differences in the first aid attempts.

When the applied first aid and the benefit of that firs aid application were examined, it was found that the mothers who applied water were benefited from the application with a high rate. The difference between the groups was statistically significant ($p<0.001$). Considering other applications, cold application to the burn area is known to be both a correct and a useful intervention as a first aid attempt (19). Benefiting from the applied practice can be explained with this ground.

The educational level of the mothers was compared with the information source that they learned the application. It was found that secondary school-high school graduates learned the application on their own and the difference between the groups was significant ($p<0.001$). This finding can be explained with the increased use of mass media and different sources of information such as television and the internet, and also the increase in the level of knowledge about the application of correct first aid attempts in probable accidents.

Conclusion

In this study, the lack of information and practice in preventing domestic accidents, the causesof the inability to create a safe home environment suitable for children and the high rate of exposure of children to burns were found. However, it is seen that the first- aid applications after the burns are correct interventions and there is an improvement in first aid interventions from past to present. In the light of these results, it is considered that especially parents and families should be informed about creating safe home

environment and preventing domestic accidents.

Conflict of Interests

The authors declare that no conflict of interest exists.

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Implicit Motor Imagery Performance in Childhood Recurrent Headaches

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Abstract

This study aims to investigate the implicit motor imagery capacities in children with recurrent headaches. 47 children with recurring headaches and 33 children of a comparable typically developing peers age group were included in the study. The dominant hand, headache localization, intensity, and symptoms accompanying the headache were all determined, in addition to implicit motor imagery abilities and the demographic features of the children participating in the study. These results were compared by measuring the patients' and control groups' right and left lateralization accuracy percentages and response times. According to the study's findings, the group with recurrent headaches had the worse right and left discrimination accuracy percentages and decision-making times, notably in implicit motor imagery performances, than the control group. As a result, whereas children with recurrent headaches have high implicit motor imagery decision-making rates linked to proprioception, they may have impaired accurate decision-making capabilities.

Key Words: Children; Headache; Implicit motor imagery; Left/right judgements

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Introduction

Headache is one of the most common complaints of children. The prevalence of headache complain in children has grown dramatically in recent years (1). Frequency of headaches in children vary with age (3 - 51.5%), and the prevalence rises with age (2,3). The complaint of headache is frequently accompanied by other symptoms such as nausea, vomiting, vision abnormalities, and sore throat (4). While migraine was reported as 4% and non-migrainous headache rate as 24% in elementary school-aged children, this rate was determined as 16% formigraine and 60% for non-migrainous headaches in adolescence (5). It is more frequent in boys at a young age, but it is more common in girls as they become older (6). Children with recurring headaches are less likely to participate in social activities, are more absent from school, and have inferior academic achievement than children who do not have recurrent headaches (7,8).

The chronic pain in children occurs more frequently than expected (9). Approximately 5% to 8% of childrens with chronic pain have a considerable pain-related disability (10). One of the most common causes of chronic pain in children is headaches (11). Patients with chronic pain have been observed to exhibit a variety of peripheral characteristics (cortical disinhibition, the change of motor cortex excitability). These peripheral changes include changes in proprioceptive senses (the sense of where your body is located in space) (12). This peripheral changes are deterioration in proprioception, decrease in tactile acuity, and lateralization decision-making ability (13). Impaired awareness of one's own body form and organs can be caused by a lack of proprioception and motor imagery (lateralization) (14). Motor imagery is also known as the mental simulation of movement without exhibiting any genuine motor activity (15). The capacity to engage with projection and manipulation of the body diagram from a first-person perspective is known as implicit motor imagery (16). In implicit motor imagery, movement imagery is done subconsciously (17). Implicit motor imagery indicates some functional equivalence between observed, imagined, and actual movement (18). When aperson sees a photograph of a hand in an unusual posture, he/she automatically visualizes the hand motions and enters the motor imagery pattern to determine which side the hand belongs to (19).

No study has been found that specifically examines the motor imagery

performances of children with headache problems. For these reasons, the current study was planned to investigate the implicit motor imagery performance of children with recurrent headaches and compare them with their typically developing peers.

Materials and Methods

Participants

This descriptive study included 33 children with typically developing and 47 children with recurring headaches (headache that recurs at least 3 times and aged between 8 and 16). The study was approved by Gaziantep Islam Science and Technology University, the non-interventional clinical research ethics committee (Protocol ID: 2021.47-47). Written informed consent was obtained from all parents of children who participated in the study. All participants provided informed consent, as per the Declaration of Helsinki. Parents and children were inquired about headache complaints and socio-demographic characteristics and the answers were recorded. The study was completed between December 2021 - April 2022. Participants with headache that recurs at least 3 times and volunteering to participate in the study were chosen for the study (Children with headache diagnosed by a pediatric neurologist who is an expert in the field). Patients who had an organic reason for their headache etiology, any other chronic diseases and with complex medical problems other than headache were excluded from the study. The typically developing control group is generated with similar demographic features who don't have any sign or symptom of active infection and chronic disease and also who don't have headache. The children with typically developing were chosen from among the children of the colleagues of the researchers who carried out the study and the children of the close friends of the employees who volunteered to participate in the study.

Assessment of headache severity

Patients were given a visual analog scale (VAS) to score their headaches (0=no pain, 10=unbearable pain). Pain severity was graded as follows: 1-2 was considered mild, 3-4 was considered moderate, 5-6 was considered severe, 7-8 was considered very severe, and 9-10 was considered unbearable (20).

Left/right judgement tasks

Implicit motor imagery capacity was evaluated as a laterality task, that is, choosing which side the hand belonged

to. In this regard, right-left discrimination was tested using the Recognise App Recognise Hand software developed and designed by the NOI group (Neuro Orthopaedic Institute, Adelaide, Australia) (http://www.noigroup.com/ Recognize).

Regarding the identification of the hand's laterality and implicit imagery capacity, two points were evaluated. The first point is decision discrimination accuracy (the percentage of accurate responses), which is the capacity to recognize whether a part of the body belongs to the right

or the left, and the second is the response time of the participants when performing the discrimination task. The participants were asked to judge whether the hand images of the right and left hands from various angles presented on the phone screen belonged to the right or left hand while sitting comfortably. See Fig. 1 for sample images. Each person was shown a total of 20 photos at 5-second intervals. Children were instructed to push the right-side button with their right index finger if they considered the image to belong to the right side and the left side button with their left index finger for the left side. The application computed and logged response times and accuracy percentages. Data from pictures having a reaction time of less than 500 ms were excluded from the study because they were deemed too short to provide the right response (21). Furthermore, if the participant's response time exceeded 5 seconds in eight consecutive images and he or she was unable to answer, the images were deemed failed and were not evaluated.

Statistical Analysis

In descriptive statistics, numerical variables were given mean and standard error mean, whereas categorical variables were given number and percentage values. The Shapiro Wilks test was used to evaluate the normality assumption. The independent sample t-test was performed to see if there was a difference between the two groups. The paired difference test was used to discover which group or groups created the difference when there were differences between the groups. The Spearman correlation coefficient was used to see if there was a difference between numeric variables. Statistical significance level was taken as $p<0.05$ IBM SPSS Statistics (Version 19.0. Armonk, NY: IBM Corp.) was used for statistical analysis.

Results

In the study, there were 28 boys (59.6 %) and 19 girls (40.4 %) in the headache group. The median age of the headache group was 11.4 ± 0.37 years. Forty (85.1 %) of these children were using right-handed dominant and 7 (14.9 %) of them were left-handed dominant (Table 1). Headache duration lasted less than an hour in 22 (46.8 %) of the children, 1-6 hours in 20 (41.6 %) of the children, and 6-24 hours in 5 (10.4 %) of the children. Headache wasaccompanied by nausea-vomiting in 8 (17 %) patients, dizziness in 4 (8.4 %) patients, loss of appetite in 2 (4.2 %) patients, and visual impairment in 2 (4.2 %) patients. There were no

accompanying findings in 31 (65.9 %) patients. The pain intensity was determined as 3-4 (mild) in 24 (51 %) patients, 5-6 (severe) in 4 (8.4 %) patients, and 9-10 (unbearable) in 19 (40.4 %) patients. The pain location was found in the lateral area in 24 (51 %) patients, in the occipital area in 13 (27.6 %) patients, and in the front area and diffuse in 5 (10.6 %) patients. There were 19 boys (57.6 %) and 14 girls (42.4 %) in the control group. The median age of this group was 11.3 ± 0.4 years. In this group, 29 children (87.9 %) were right-handed and 4of them (12.1 %) were left-handed. In terms of laterality task motor imagery right-left discrimination respond times, a significant difference was determined between the groups ($p<0.05$). A statistically significant difference was found between the right-left accuracy percentages between the groups ($p<0.05$). Comparison of laterality task evaluations between groups are shown in Figures 2 and 3. No statistically significant connection was found in the headache group in terms of accompanying findings and right-left lateralization findings ($p>0.05$). The effect of headache duration and severity on lateralization findings was not statistically significant ($p>0.05$). Right-left

response times and accuracy percentages according to the dominant hand are shown in Table 2. The increase in right and left accuracy percentages showed a statistically significant correlation with age in both groups.

Table 1. The physical and sociodemographic characteristics

	Headache Group	Control Group
Male	28 boys (59.6 %)	19 boys (57.6 %)
Years	11.8 ± 0.27 years	11.1 ± 0.6 years
Heights	137 ± 4.1 cm	141 ± 6.5 cm
Female	19 girls (40.4 %)	14 girls (42.4 %)
Years	10.9 ± 0.63 years	11.7 ± 0.27 years
Heights	143 ± 8.7 cm	145 ± 4.21 cm
Right handed	40 (85.1 %)	29 (87.9 %)
Left handed	7 (14.9 %)	4 (12.1 %)

Table 2. Comparison of right-left respond times and accuracy rates according to handedness between groups

		Right Accuracy Percent (%)	Right Respond Time (s)	Left Accuracy Percent (%)	Left Respond Time (s)
Headache Group	Right-Handed (n=40)	46.75 ± 2.3	1.88 ± 0.03	44 ± 1.7	2.02 ± 0.07
	Left-Handed (n=7)	35.7 ± 2.97 $^{\beta}$	1.9 ± 0.07	51.4 ± 4 $^{\beta}$	1.62 ± 0.11 $^{\beta}$
Control Group	Right-Handed (n=29)	67.5 ± 1.96	2.56 ± 0.09	67 ± 1.91	2.63 ± 0.15
	Left-Handed (n=4)	65 ± 2.88	2.5 ± 0.35	75.3 ± 0.5 $^{\alpha}$	2.25 ± 0.3 $^{\alpha}$

n = number of patients, Data are reported as means ± SEM
α: Comparison of left-handed control group and right-handed control group $p < 0.05$
β: Comparison of left-handed headache group and right-handed control group $p < 0.05$.

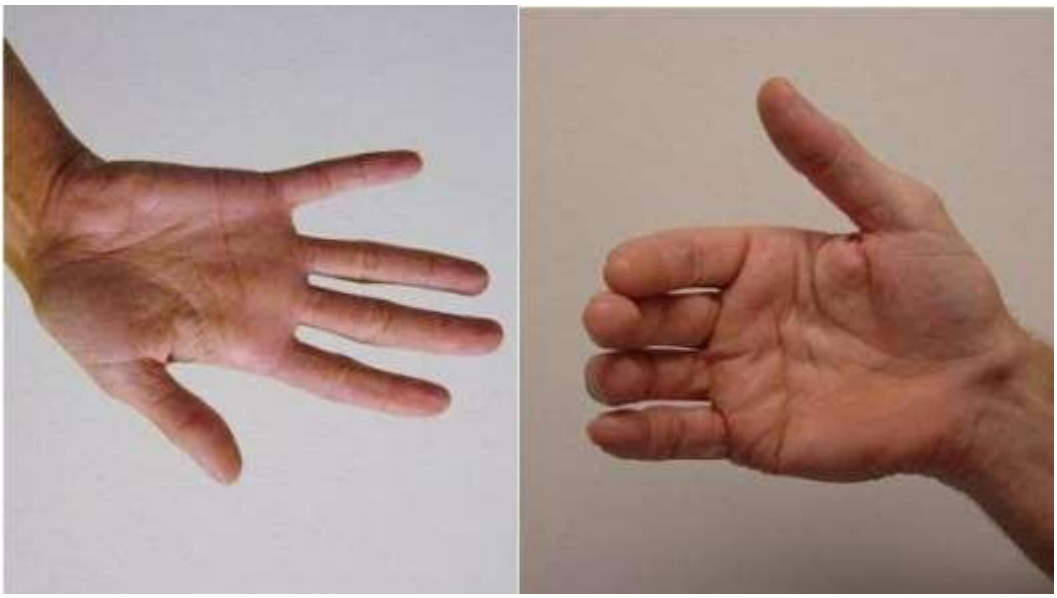

Fig. 1. Sample images used in the left/right judgement tasks

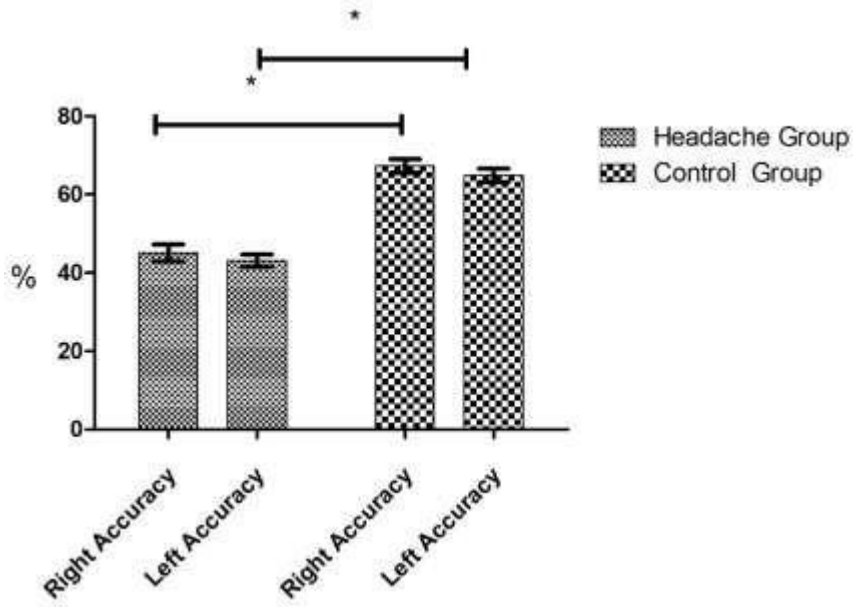

Fig. 2. Comparison of accuracy rates between groups

*P < 0.05

Data are reported as means ± SEM

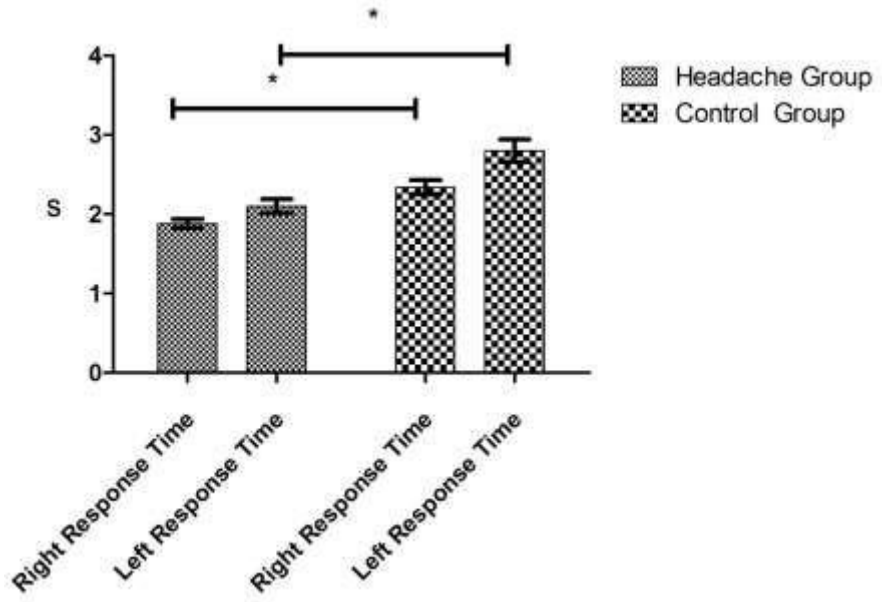

Fig. 3. Comparison of response times between groups

*P < 0.05

Data are reported as means ± SEM

Discussion

We assumed in this study that children with recurrent headaches might have impaired laterality task decision-making times and lower implicit imagery capacity. The data we obtained partially supported our hypothesis. Today, a significant portion of the rate of admission to the emergency department is children with headache complaints, and this rate is increasing. Headache can cause significant disruptions in the daily routine and social life of children (22). Other than organic causes such as migraine, intracranial mass, hypertension, visual impairments, and intracranial infections, tension-type headaches caused by psychosocial stress constitute the majority of causes (23,24). Recurrent headaches affect the way of thinking of children and may cause decreases in academic success (22,25). In this study, when the decision-making times for laterality tasks were examined, the accuracy level of the answers given was found to be significantly lower than in the control group, despite the fact that the decision-making times were relatively fast. When the existing literature was examined, it was discovered that in patients with diseases affecting motor functions such as cerebral palsy, implicit imagery capacities were significantly reduced on the affected side (16). In fact, it has been reported that there is a significant increase in this capacity with motor imagery training in these patients. Furthermore, the imagery capacities of adult patients with chronic pain complaints were examined, and it was discovered that chronic pain have a negative impact on their implicit imagery capacity (26). Also, some studies have reported that localization of pain is ineffective in reducing imagery capacity (26). Since no correlation was found between headache localization and accuracy rate in this study, it was assumed that headache localization did not affect the decision-making process, and this data was found to be compatible with the current literature. In this study, it was determined that the accuracy rate of decision-making process in children with recurrent headache independent of headache localization was lower than in the typically developing group. It is thought that more studies are needed to investigate the related factors affecting motor imagery abilities in children with recurrent headache.

Motor imagery ability follows a

process that develops with age. For this reason, age can be an important factor in tests related to motor imagery. In this study, motor imagery accuracy percentages increase with age and show us a certain correlation, and offer us data compatible with the literature (27). According to the results of the present study the data obtained from children with recurrent headache complaints suggest that not only the age factor, but also other factors affecting the general condition of the child may be effective in determining the motor imagery ability.

Handedness is another effective factor in the evaluation of motor imagery capacity. It has been reported that it gives better results in the evaluation of motor imagery laterality tasks, especially in left-handed children (20). With the data obtained in this study, the results in left- handed patients were determined as significantly better in both groups.

It has been stated that with more severe pain were reported to have more impaired implicit motor capacity than those with less severe pain (28). There was no significant correlation between pain severity and implicit motor capacity according to the data obtained in this study. The likely reason for this is that headaches last for a shorter period in children, although their severity is excruciating.

Conclusion

In conclusion, children with recurrent headaches were found to have a lower implicit motor capability in our study. Age and handedness are two factors that have an affirmative impact on this. We consider that the motor imagery skills of children with recurrent headaches and the factors affecting their skills should be examined in detail by conducting more comprehensive studies.

Conflict of interest

The authors declare no competing interests.

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Antidiabetic and Antioxidant Effects of *Bryonia multiflora* Boiss. & Heldr. in a Rat Model of Streptozotocin-Induced Diabetes

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Abstract

This aim of this study is to investigate the antidiabetic, antioxidant and hypolipidemic potential of Bryonia multiflora BM Boiss. & Heldr. in streptozotocin (STZ) induced diabetes in rats. During 21 days, control group (NC) and diabetes control (DC) were fed only with food and water, while diabetes acarbose group (DAC) was fed with 20 mg / kg of acarbose.
The DB1, DB2, DB3 groups were fed with 100 mg / kg, 200 mg/kg and 400 mg / kg BM plant extract, respectively. The body weight and biochemical parameters and antioxidant parameters were examined for all treated groups and compared against diabetic control group and normal control group. According to the results; significantly higher levels have been observed in DC group serum ALT, AST, BUN, and CRE compared to NC group ($p<0.05$), while declines have been observed in groups treated with BM extract ($p<0.05$). There has been decline in VLDL, cholesterol, and triglycerite levels in the plant extract applied groups ($p<0.05$), while HDL levels increased ($p<0.05$). On the other hand, MDA levels increased while GSH and SOD levels declined as DC group compared to the control group. MDA levels significantly declined ($p<0.05$) while SOD and GSH levels significantly increased in therapeutic groups treated with plant extracts. The results indicate that BM extract have antidiabetic effects by regulating antioxidant activities thereby improving the function of β- cells maintaining normal insulin and glucose levels. Thus the investigation results that BM has significant antidiabetic, antioxidant activity.

Keywords: Antidiabetic, antioxidant, B. multiflora, Diabetes mellitus, Oxidative stress.

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Introduction

Diabetes is a chronic endocrine disorder that is characterized by elevated levels of blood glucose, as well as disturbances in protein, carbohydrate, and lipid metabolism. The condition arises due to a decrease or deficiency in insulin secretion, or increased cellular resistance to insulin. Diabetes can lead to dysfunction or damage in multiple organs and tissues, including the heart, nerves, eyes, kidneys, and blood vessels (1,2). Efforts to complement the treatment of diabetes have recently focused on functional foods and their bioactive compounds. Elevated blood glucose levels in diabetes lead to the production of superoxide anions, which generate hydroxyl radicals, resulting in oxidative damage to cell membranes, as well as other important biomolecules such as carbohydrates, proteins, and DNA (3). The production of reactive oxygen species and oxidative stress associated with hyperglycemia contribute to the pathogenesis and progression of diabetes (4).

The prevalence of diabetes continues to increase globally, leading to a reduction in quality of life, microvascular and macrovascular complications, and even death. In response to this issue, scientists have turned to plants for their healing effects, which have been utilized in traditional medicine for centuries. Numerous studies have investigated the effects of plant extracts on various diseases. This paper aims to examine the effects of *Bryonia multiflora* (BM) extracts on diabetes and diabetes-induced complications through experimental diabetes. BM is a perennial, herbaceous plant with rhizome roots that contain active substances such as ose, steroidal sapanonin, triterpenic sapanonin, essential oil, and cucurbitacin I and cucurbitacin B (5). Literature reviews indicate that cucurbitacins and triterpenic acids are found in B types as triterpenic substances, while sterols are found as steroidal substances. Cucurbitacins possess attributes such as cytotoxic, hepatoprotective, antitumoral, anti- inflammatory, and purgative properties (6). Chemical research has revealed that BM extracts prepared with roots and herbs

using specific reactions contain saponins and fixed oil. Saponins are known to exhibit various biological effects such as hypocholesterolemic, anti-carcinogenic, antioxidant, anti-inflammatory, antimicrobial, antiprotozoal, and antihypertensive effects. *Bryonia multiflora* is a member of the Bryonia L. (Cucurbitaceae) genus and is known to have anti-inflammatory, antimicrobial, cytotoxic, and antioxidant effects (7). The components of this species have been shown to possess anti-tumoral properties (8).

Materials and Methods

Plant Materials and Preparation of Lyophilized Extract

During June 2015, *Bryonia multiflora* (BM) was collected from Hizan town in Bitlis, Turkey, and its authenticity was confirmed by Assoc. Prof. Dr. Fevzi ÖZGÖKÇE from the Department of Botany at Yuzuncu Yil University. The herbarium number of BM was determined to be 165060 and a sample of the plant was stored at the Yuzuncu Yil University Science and Art Faculty Herbarium (VANF). To prepare the extract, dried roots of BM were first weighed as 50 g and then divided into small pieces. The aqueous extract was produced by stirring the 50 g of BM powder in 1000 mL of distilled water (dH2O) for 24 hours using a magnetic stirrer. Afterward, the mixture was centrifuged for 15 minutes at 8,000 rpm, and the filtrate was collected. The solvent was evaporated under reduced pressure at 37°C using a rotary evaporator (Rotavapor R-205; Buchi, Switzerland). The viscous extract was then transferred to a falcon tube and freeze-dried under vacuum and at a temperature of -54°C, resulting in a fine lyophilized powder. The extract was prepared using a modified version of the Dalar and Konzcak methods (9).

Experimental Animals

Female Wistar albino rats between 3-4 months old and weighing between 200-300 g were obtained from the Experimental Animal Research Center at Yuzuncu Yil University.

The rats were divided into six groups, each containing seven

rats, and were housed in controlled environmental conditions with a 12-hour light/dark cycle and a humidity range of 60% to 70% and a temperature of 25°C. The rats were fed a wheat-soybean-based diet and had access to water ad libitum while living in stainless-steel cages. All rats received proper care in accordance with the "Guide for the Care and Use of Laboratory Animals" developed by the National Academy of Science and published by the National Institute of Health. The ethical guidelines and regulations for the protection of animal welfare during experiments were followed according to national and institutional guidelines.

This study was approved by the Ethic Committee of Yuzuncu Yil University under the protocol number 27552122-142.

Induction of experimental Diabetes mellitus

The animals were fasted for 12h prior to the induction of diabetes. Streptozotocin (STZ) freshly prepared in citrate buffer (0,1 M, pH 4,5) was administered intraperitoneally (i.p.) at a single dose of 45mg/kg. After 72 h streptozotocin treated rats with blood glucoselevels higher than 200 mg/dL were considered as diabetic and used in this study.

Chemicals

The substances used in this study, such as Trichloroacetic acid (TCA), thiobarbituric acid (TBA), and reduced glutathione (GSH), were obtained from Sigma Chemical Co. (St. Louis, MO, USA) in technical grade. Other substances used include butylated hydroxytoluene (BHT), ethylenediaminetetraacetic acid (EDTA), and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). β-Nicotinamide adenine dinucleotide phosphate (NADPH), trihydroxymethyl aminomethane (Tris), and 1-chloro-2,4-dinitrobenzene (CDNB) were also used. In addition, kits for antioxidant enzyme analysis were supplied by Randox Laboratories ltd. and α-Glycosidase Activity Colorimetric Assay Kit (Catalog #K690- 100, BioVision, USA) was used for small intestine tissue samples. All substances

used in this study were obtained from reliable sources and were of high quality.

Acute toxicity testing

The current study utilized the techniques introduced by Lorke (10) and Ibeha and EzeaJa (11). The experiment involved with twelve rats, which were divided into three groups randomly. Each group was administered with a different amount of the extract, 250 mg/kg, 500 mg/kg, and 1000 mg/kg, respectively, through oral gastric gavage. The rats were allowed to consume food and water as much as they wanted. No harmful or lethal effects were observed during the 72-hour observation period.

Experimental design

The rats were randomly divided into six groups each containing seven rats.

I. Group: Normal Control (NC) : Rats received citrate buffer (pH 4.5) (1 ml/kg, i.p.).

II. Group: Diabetes Control (DC): Rats received STZ in single dose (45 mg/kg, i.p.).

III. Group: Diabetes + Acarboz (DAC): Rats received STZ in single dose (45 mg/kg, i.p.) and Acarbose (20 mg/kg, per day) was treated to diabetic rat groups by oral gavageduring 21 days experimental period..

IV. Group: Diabetes + BM (DB1): Rats received STZ in single dose (45 mg/kg, i.p.) and BM extract (100 mg/kg, per day) was treated to diabetic rat groups by oral gavage during 21 days experimental period.

V. Group: Diabetes + BM (DB2): Rats received STZ in single dose (45 mg/kg, i.p.) and BM extract (200 mg/kg, per day) was treated to diabetic rat groups by oral gavage during 21 days experimental period.

VI. Group: Diabetes + BM (DB3): Rats received STZ in single dose (45 mg/kg, i.p.) and BM extract (400 mg/kg, per day) was treated to diabetic rat groups by oral gavage during 21 days experimental period.

Preparation of tissues supernatant and erythrocyte pellets

At the end of the 21-day experiment, the rats were given anesthesia via intraperitoneal injection of ketamine at a dose of 5 mg per 100 g of body weight. Blood samples were collected through cardiac puncture using a syringe for biochemical analysis. The serum samples were obtained by centrifuging the blood samples at 4 kg for 15 minutes at 4°C, and enzyme levels were measured in the resulting serum samples. The blood samples were immediately placed in two silicon disposable glass tubes with EDTA as an anticoagulant for biochemical analysis. The first tubes were used to measure glycosylated hemoglobin (HbA1c) levels, while the second tubes were centrifuged at 4 kg for 15 minutes at 4°C to obtain erythrocyte pellets. The pellets were then washed three times with physiological saline (0.9% NaCl).

Small intestine, brain, kidney, and liver tissues were dissected and placed in petri dishes. The tissues were washed with physiological saline (0.9% NaCl) and stored at -78°C for analysis. The tissues were homogenized for 5 minutes in 50 mM ice-cold KH_2PO_4 solution (1:5 w/v) using a stainless steel probe homogenizer (20 KHz frequency ultrasonic, Jencons Scientific Co.) and then centrifuged at 7000g for 15 minutes. All procedures were carried out at 4°C. The resulting supernatants and erythrocyte pellets were used to determine the constituents of ADS and MDA contents (12,13). Additionally, α-glycosidase activities in small intestine tissue supernatant samples were investigated.

Biochemical analysis

The concentration of MDA in erythrocytes and tissues was determined using the TBA reactivity method described by Jain et al. (14) while the concentration of GSH in erythrocytes and tissues was measured using the method described by Beutler et al. (15). GST activity was assayed by measuring the conjugation of glutathione with CDNB at 340 nm as described by Mannervik and Guthenberg (16). The

decrease in absorbance of NADPH at 340 nm was used to assay GR activity, according to Carlberg and Mannervik (17). GPx activity was measured by catalyzing the oxidation of glutathione by cumene hydroperoxide using a method based on that of Paglia and Valentine (18). SOD activity was calculated by measuring the inhibition percentage of formazan dye formation at 505 nm (19). CAT activity was determined by measuring the rate of H_2O_2 consumption and the decrease in absorbance at 240 nm using the method described by Aebi (20). The α-glycosidase activity in small intestine tissue samples was measured colorimetrically at 410 nm by hydrolyzing the Substrate Mix to release p-nitrophenol (BioVision kits, USA).

Measurement of biochemical parameters

Several parameters were measured using an automated analyzer (COBAS 8000/ROCHE/Germany/Serial No 1296-08) with Roche kits. These parameters include ALT, AST, LDH, glucose, lipid profile (total triglyceride, total cholesterol, LDL-cholesterol, and HDL-cholesterol), creatinine, blood urea nitrogen, and urea. Insulin and c-peptide levels were measured using an ELISA Enzyme-linked Immunosorbent Assay Kit based on the 450 absorbance.

Measurement of Blood Glucose Levels

Fresh blood samples were collected from the tail vein of the rats. The blood glucose levels were determined with a blood glucose meter (ACCU-CHEK Active, Roche). These measurements were performed on days 0, 7, 14, and 21.

Analysis of data

The data obtained from the experiments were presented as mean values and standard deviations. The statistical analysis of the data was conducted using Minitab 13 for Windows software. The means and standard deviations were calculated for all the parameters using standard methods. One-way analysis of variance (ANOVA) was used as a statistical test to determine any significant differences between the means of the

experimental groups. A significance level of $p < 0.05$ was accepted.

Results

Acute toxicity studies

Animals showed tolerance to testing three (250, 500 and 1000 mg/kg) doses of BM lyophilized extract. Extract in doses as high as 1 g/kg that were found to be non-lethal. Highest dose of extract did not show any noticeable signs of toxicity and mortality after 3 days of administration onceper day. Therefore, the extract is safe for long term administration.

BM extract effect on body weight and blood glucose levels

Table 1. Effect of BM aqueous extract supplements on body weight and glucose level of experimental groups during 21 days

	GROUPS					
	NC	DC	DAC	DB1	DB2	DB3
	Mean 7 SD	Mean 7 SD	Mean 7 SD	Mean 7 SD	Mean 7 SD	Mean 7 SD
Body weight (g)						
Beginning	213,85±5.55	213.71±7.97	205.71±11.81	272.85±22.31	251.01±7.02	249.14±44.63
Finally	219.57±10.21	203.57±5.74*	196.14±8.31	250.57±26.20	245.28±22.04	228.14±11.53*
Three hour period Blood glucose (mg/dL)						
0. Hour (Fasting blood glucose)	84.23±9.28	330.47±159.32	388.81±150.71	158.47±36.25	130.38±38.56	236.91±91.25
1.Hour (After consumption)	85.95±8.39	363.61±142.04	390.09±145.41	158.52±32.59	138.66±39.49	235.91±93.49
3.Hour (After consumption)	90.23±6.61*	388.14±131.79	409.33±128.70	152.09±55.97	130.23±51.92	191.81±62.83
21 day period Blood glucose (mg/dL)						
Day 0	84.42±12.76	261.42±68.73	225.85±21.16	212.01±13.01	291.14±41.58	207.42±4.19
Day 7	82.91±9.85	363.71±146.21	472.14±105.51*	158.85±50.57*	151.81±45.62*	310.57±64.19*
Day 14	88.66±8.41*	377.04±145.34	393.01±134.72*	164.08±28.11*	137.71±30.16*	188.95±39.04
Day 21	88.85±5.33	341.47±146.66	323.09±140.51	146.14.±44.86*	109.76±42.87*	165.09±63,09

Changes in beginning and final body weight in control and experimental groups are shown in Table 1. Significant weight loss was observed in final diabetic control group compared to initial animals. During the study period, the normal control rats gained weight, while STZ- induced diabetic rats exhibited a lower body weight. Table 1 summarizes the levels of glucose in normal and diabetic animals. The NC group blood glucose level increased 3.h compared with 0 hour whereas the levels of blood glucose significantly decreased in diabetic rats treated with BM extract after2

and 3 weeks.

Effect of the BM extract on liver and renal serum biomarkers of experimental groups The serum levels of ALT, AST, URE, CRE and BUN were significantly increased in the diabetic control rats compared to the normal control rats. The serum level of LDH decreased in the diabetic control rats compared to the normal control rats. The serum levels of ALT, were significantly decreased in the treated groups with BM extract compared to the diabetic control group. The treatment groups renal serum biomarkers such as URE, CRE, BUN were decreased compared to the diabetic control group (Table 2).

Table 2. Effect of BM aqueous extract supplements on liver and renal serum biomarkers of experimental groups

GROUPS						
Parameters	NC Mean 7 SD	DC Mean 7 SD	DAC Mean 7 SD	DB1 Mean 7 SD	DB2 Mean 7 SD	DB3 Mean 7 SD
ALT U/L	70.94±18.57	176.55±19.91[a]	139.88±19.91[ab]	143.75±36.53[ab]	98.72±19.83[b]	210.91±49.87[a]
AST U/L	146.81±30.33	239.61±71.33[a]	248.57±17.84[a]	239.05±63.28[a]	289.57±54.08[a]	372.24±66.05[ab]
Üre mg/dL	49.21±4.62	58.52±6.09[a]	87.24±1.85[ab]	48.31±11.51	48.37±16.86	78.61±18.95[ab]
CRE mg/dL	36.10±4.02	40.01±5.71	52.01±5.40[ab]	38.8±3.50	37.8±3.80	61.01±14.70[ab]
BUN mg/Dl	23.14±2.03	27.42±2.82[a]	40.57±1.13[ab]	22.57±5.51	22.42±7.82	36.85±8.74[ab]
LDH U/L	1623.5±274.6	1610.2±247.9	1630.1±211.7	2008.8±462.9	1847.2±470.6	1658.1±449.9

Effect of the B. multiflora extract on lipid profile

Table 3. Effect of BM aqueous extract suppleents on lipid profile of experimental groups

GROUPS						
Parameters	NC Mean 7SD	DC Mean 7SD	DAC Mean 7SD	DB1 Mean 7SD	DB2 Mean 7SD	DB3 Mean 7SD
TG (mg/dL)	134.07±31.06	110.62±33.02	78.71±16.05^{ab}	130.51±32.29	92.97±22.58^{a}	56.71±9.57^{ab}
TC (mg/dL)	68.05±5.81	43.88±9.33^{a}	69.52±2.31^{b}	58.31±6.06^{ab}	57.18±8.51^{ab}	69.81±7.91^{b}
HDL (mg/dL)	51.52±5.06	38.35±5.83^{a}	57.64±6.69^{b}	47.81±8.43^{b}	44.12±8.02	54.28±7.74^{b}
LDL (mg/dL)	5.58±0.99	5.94±1.41	11.42±2.23^{ab}	8.31±1.73^{ab}	6.24±2.05	11.07±2.07^{ab}
VLDL (mg/dL)	28.28±4.61	20.71±4.28	15.82±3.23^{ab}	24.14±5.61	18.57±4.35^{a}	11.71±2.62^{ab}

The serum levels of TG, TC, HDL and VLDL were markedly decreased in the diabetic group compared with the normal group (Table 3). TG levels dropped significantly in plant extract applied rats. VLDL, TC, TG levels notably dropped in all of the groups applied with plant extract, while HDL levels went up close to the healthy control group.

Effect of the B. multiflora extract on HbA1c, serum insulin, c-peptide and α-glycosidase activity in small intestine tissue

Table 4. Effect of BM aqueous extract supplements on HbA1c serum insulin, C-peptide and α-Glucosidase activity in small intestine levels of experimental groups

GROUPS						
Parameters	NC	DC	DAC	DB1	DB2	DB3
	Mean 7SD	Mean 7SD	Mean 7SD	Mean 7SD	Mean 7SD	Mean 7SD
İnsulin (pg/mL)	594.17±44.74	415.28±30.82[a]	516.76±103.42	480.11±32.27[ab]	561.78±45.87[b]	521.59±29.96[ab]
HbA1c (%)	4.45±0.64	7.29±1.42[a]	6.31±1.46	4.01±0.91[b]	4.64±1.36[b]	4.41±0.29[b]
α-Glukozidaz	32.55±5.88	56.35±9.66[a]	52.74±3.21[a]	65.32±8.64[a]	46.89±3.53[a]	67.87±10.33[a]
C-Peptide	577.76±67.77	465.51±33.58[a]	582.16±86.48[b]	473.28±44.16[a]	588.89±109.74	568.52±114,11

As shown in Table 4, at the end of the experiment, the serum insulin, C-peptide levels of diabetic control rats were significantly decreased ($p < 0.05$) compared to those of normal control rats. In contrast, the administration of BM extract to diabetic rats significantly increased all of these parameters compared with the diabetic control rats. However, HbAlc significantly increased in diabetic control group, which were found near to normal in the treatment group.

Effect of the BM extract on lipid peroxidation and antioxidant defense systems

Table 5. Effect of B.multiflora aqueous extract supplements on lipid peroxidation and antioxidant defense systems of experimental groups.

		GROUPS					
	Parameters	NC	DC	DAC	DB1	DB2	DB3
		Mean 7SD	Mean 7SD	Mean 7SD	Mean 7SD	Mean 7SD	Mean 7SD
E	MDA	9.49±2.15	12.5±1.79[a]	10.10±2.47[b]	10.35±1.03[b]	5.39±1.56[ab]	8.41±3.37[b]
	GSH	4.56±0.50	3.77±0.47[a]	4.92±0.69[ab]	4.52±0.57[ab]	4.53±0.24[ab]	3.58±0.86[a]
	GST	9.10±2.40	10.10±1.07	10.01±2.01	9.10±1.80	9.80±1.20	7.50±0.90
	GR	0.17±0.07	0.11±0.05	0.09±0.02[a]	0.10±0.02	0.06±0.03[a]	0.08±0.02[a]
	CAT	371.16±41.70	384.80±53.23	460.65±53.93[ab]	443.90±57.68[ab]	412.32±50.44	321.38±62.46
	GPX	2298.83±527.79	2485.72±307.60	2261.19±256.81	1931.97±282.75[b]	2142.65±272.38	1921.59±272.98[b]
	SOD	2284.78±25.51	2270.41±28.29	2262.58±21.40	2287.06±9.69	2286.37±14.31	2278.71±9.77
Brain (U/g)	MDA	32.19±6.53	72.62±3.13[a]	38.11±8.49[b]	41.54±3.92[b]	44.59±10.57[ab]	53.29±7.67[ab]
	GSH	24.59±5.99	28.39±9.79	25.65±6.64	23.43±2.66	24.17±5.39	26.33±3.09
	GST	12.5±1.79	13.78±0.76	13.21±2.58	14.66±2.65	13.52±2.58	13.21±5.33
	GR	0.98±0.21	0.92±0.12	0.88±0.18	0.64±0.06[ab]	0.45±0.13[ab]	0.87±0.18
	CAT	29.43±1.57	25.84±1.52	35.65±7.15	25.36±1.72	25.84±1.30	23.45±1.41
	GPX	2604.26±75.97	2607.28±38.34	2642.33±46.03	2670.88±74.05	2703.32±45.65[ab]	2495.67±75.29[ab]
	SOD	2175.07±14.92	2196.48±67.47	2166.92±46.73	2174.95±39.91	2114.42±50.61[ab]	2097.06±54.78[ab]
Kidney (U/g)	MDA	169.21±38.81	231.02±21.54[a]	212.42±53.09	186.51±41.59[b]	181.33±33.66[b]	199.56±18.76[b]
	GSH	51.74±2.67	45.71±12.33[a]	43.63±12.48	57.77±6.68[b]	46.83±4.23	47.06±5.43
	GST	8.23±0.85	8.72±1.07	14.06±8.77	10.10±3.19	10.10±2.47	14.24±1.58[b]
	GR	0.80±0.22	0.47±0.10[a]	0.62±0.10[b]	0.67±0.10[b]	0.43±0.08[a]	0.50±0.17[a]
	CAT	318.75±22.59	154.59±30.84[a]	205.80±64.58[ab]	160.33±28.31[a]	145.73±7.81[a]	172.05±32.39[a]
	GPX	1587.17±292.42	1879.62±170.63[a]	1864.48±240.02	1520.98±175.92[b]	1957.49±72.91[a]	1812.13±137.75
	SOD	2166.04±49.06	2189.9±26.28	2235.34±43.27[ab]	2215.07±77.85	2223.78±32.89[ab]	2183.84±32.43
Liver (U/g)	MDA	53.10±9.92	70.12±13.88[a]	44.13±4.00[ab]	46.35±7.00[b]	55.69±5.40[b]	56.71±13.59
	GSH	50.29±4.43	51.64±5.15	56.34±2.31[ab]	57.46±1.23[ab]	53.57±2.79	54.38±2.26
	GST	61.76±10.52	69.75±7.48	74.03±4.82	72.72±8.49	72.24±10.12	58.85±9.86[b]
	GR	0.47±0.11	0.48±0.09	0.37±0.08	0.31±0.08	0.31±0.06[a]	0.20±0.04[ab]
	CAT	446.06±62.27	319.70±62.47[a]	319.70±58.84[a]	325.69±41.64[a]	414.47±64.64[b]	260.84±36.05[a]
	GPX	1394.66±238.55	1233.72±204.32	829.23±130.39[ab]	868.6±158.97[ab]	1586.74±281.17	921.81±164.89[a]
	SOD	2102.89±47.71	1985.05±67.79[a]	1945.73±67.84[a]	2000.87±43.55[a]	2029.74±60.21	2072.76±85.63[b]

According to the results, the levels of MDA content significantly increased ($p < 0.05$) in all tissues of DM

group as compared to normal control (NC). In our study, liver, kidney, and brain and erythrocyte MDA levels increase in diabetic control group compared with control group, while BM extract applied therapeutic groups MDA decreased, while SOD, GSH, and CAT levels increased. The tissues as brain, kidney, erythrocyte and liver levels of GST increased DM groups as compared to NC. In general, fluctuations in the ASS elements level was recorded as close to control values. Summery while STZ induced diabetic rats caused a decrease in antioxidant defense system constituent's level as a result of oxidative stress condition in the rats; BM extract restored the STZ-induced diabetic rats ADS constituents towards to NC group. After treatment, most of these parameters had returned to normal (Table 5).

Discussion

Phenolic compounds such as alcaloids, flavonoids, tannins, glycosides, terpenoids, sterols, considered to have antihyperglisemic effect which controls hyperglysemia (21).

Our literature review aimed to reach a limited number of research on effects of BM on various diseases, while no research has been abouton the effect of this plants effect on diabetes.

Chemical research has reveal that BM extracts with water and alcohol prepared with root and herbs using specific reactions hold saponosides and fixed oil. Chemical research has concluded that BM's extracts with water and alcohol prepared with root and herbs using specific reactions hold saponosides and fixed oil. Saponins are 0-glycisides with stereoidal or triterpenic structure forming a complex with cholesterol. They can hemolize erytrons and usually have triterpenic or stereoidal aglycone, stable foaming when its aqueous solutions are shaken. It has been asserted that saponin including plants have various biological effects such as antioksidant, hypocholesterolemic, anti-carcinogenic, antioxidant, anti-inflammatory, antimicrobic, antiprotozoal, and antihypertensive (7,21). Declines have been reported on 0th, 7th,

14th, and 21st days in blood glucose values of extract applied groups starting from the first week. Significant declines have been reported in blood glucose levels of all of the extract applied groups after the second week, while similar values with the healthy group have been reported last week in blood glucose levels of groups treated with BM extract. The reason for this decline might be the active substances in the plant to induce insüline secretion in pancreas, or the plant's built-in phytochemical compounds refunction as islet cells in time to originate a protective effect against hyperglycemia.

Serum ALT, AST, and LDH levels of all of the groups have been measured through on the last day of our study, after sacrificing all the rats. ALT and AST levels of diabetes control group have been higher compared to normal control group. Hepatocyte damage changes the

transport functions and membrane permeability, hence causes enzyme leak (23). Due to the damage, enzyme leak might have caused increase in our diabetic groups. However, ALT level has been significantly lower in DB1 and DB2 groups treated with BM extract, compared to diabetes control group (DC) ($p<0.05$). Based on this, we might consider that active substances in the BM extract regulate liver enzyme activities. Diabetes mellitus is related to abnormal lipid profile (24). Due to the Streptozotosin dose, it damages insulin generating β-cells, causing insulin insufficiency in the organism. In cases of insulin insufficiency, lipid metabolism collapse, and mobilization of fatty acids from fatty tissue increase, hence fatty acid level in the blood goes up as well (25). In our study, HDL and LDL levels have been higher in all of the groups treated with BM extract, compared with diabetes control group, collaterally with the general dose increase. Triglyceride levels dropped significantly in plant extract applied rats. VLDL, cholesterol, triglyceride levels notably dropped in all of the groups applied with plant extract, while HDL levels increased close to the healthy control group. Phytochemical compounds found in the extracts used in our study activate insulin release in order to

provide regeneration of beta cells, and as a result of this, it is possible to say that HDL level increased due to the increase in lipoprotein lipase activity, and hepatic triglyceride lipase activity (26). It is considered that saponins that are found in BM plant structure might regulated lipid metabolism which is effected negatively by diabetes. Many studies have been published asserts that saponins decrease liver lipid, plasma triglyceride concentration and cholesterole, while increasing HDL level (27).

In our study, there have been an increase of insulin levels in all of the groups as a result of the comparison between diabetes control group (DC) and DB1, DB2, DB3 groups with different doses of BM extract applied. On the other hand, increase has been reported in C-peptide levels of all the groups proportional to the increase of extract dose. This might be the result of normalization insulin release, regulating pancreas functions by extracts. HbA1c, known as glycosylated hemoglobin is a compound that its amount where is due to glucose concentration levels, formed by glucose and hemoglobin (28). In case of diabetes, HbA1c level increases notably (29).

In a study, increase occurred in HbA1c levels of diabetes control groups, while there has been decrease in therapeutic groups. This result is stated to occur due to the regulation of insulin release. In our study, glycosylated hemoglobin (HbA1c) level of diabetes control group has been examined higher than control group, as expected. There has been a significant decrease ($p<0.05$) in all of the extract applied groups regarding HbA1c levels compared with diabetes control (DC) group Decreasing of HbA1c levels of all of the plant extract applied groups to

the level of normal control group can be considered as the fact that active substances such as cucurbitacins found in the plants used in this study induce functional cells of pancreas, causing glucose generating in the liver to drop, or effecting the absorbing of glucose in gastro-intestinal channel, and decrease HbA1c levels accordingly. Glucose levels reported

throughout this study confirm these results. Glucose levels reported throughout this study confirm these results.

In a study on the antidiabetic effects of dioscorea batatas, BUN and CRE levels of diabetes groups has been found higher than control group. However, significant decreases have been reported in therapeutic groups as to these parameters, the mentioned extract is suggested to strengthen kidney functioning by inhibiting extra-cell water raise (30).

In our study, serum urea levels of DB1, DB2, DB3 groups with different doses of DC, DAC, and BM extract have been found significantly higher ($p<0.05$) in DC, DAC, and DB3 groups, while urea levels of groups DB1 and DB2 have been found insignificantly less ($p>0.05$) than control group. Urea levels of control group (NC), diabetes acarbose group (DAC), DB1, DB2, DB3 groups compared to diabetes control group (DC) decrease have been found in NC, DB1, and DB2, while only the decrease in control (NC) group has been found significant ($p<0.05$). There has been a significant increase in the groups DAC and DB3 ($p<0.05$). Besides, in our study, levels of creatinin (CRE) and BUN decrease was examined in diabetes control group, as expected. This might be the result of nephropathy, one of the complications of diabetes. Applied different doses of BM extract, DB1 and DB2 groups was shown similar decrease results with the control group. BM extract might have regulatory effects for renal functional disorders by prohibiting diabetic complications with its built in phytochemicals.

Diabetes mellitus is related to abnormal lipid profile (24). Streptozotosin creates insulin insufficiency by damaging insulin generating β cells depending on the dose. Lipid metabolism deterioration of in case of insulin insufficiencies, causing mobilization of fatty acids from fatty tissue, hence increasing the fatty acid level in blood (25).

A study found HDL level in diabetic group significantly low compared to control group (31). In our study, we reported coherent results with previous studies, with the finding of low HDL levels in diabetic control group compared

to control group. The reason for decrease fall is that fatty acid flow from adipose tissue occurs because of the decrease of inhibition of hormone sensitive lipase. Besides in our study, VLDL level of diabetes control group has been found dropping compared to normal control group. As a result of low lipoprotein lipase enzyme activity induced by diabetic insulin insufficiency, the decrease occurred in VLDL carrying endogene triglycerides and chylomicron catabolism carrying exogen triglycerides

taken with food might be considered the reason for VLDL level drop in diabetic rats. Scientific research has stated cholesterol and triglyceride levels increase in experimentally established diebetic rats. (30,31,32). In our study, groups treated with BM extract have reported higher HDL and LDL levels compared with diabetic control group, collaterally with dose increase. Plant extract has found to decrease triglyceride levels significantly in diabetic rats. All of the groups treated with plant extract have shown notable decrease in VLDL, cholesterol, triglyceride levels to the contrary of what was expected in diabetic groups, while HDL levels increased based on dose, yielding similar results with healthy control group. The reason for changes in cholesterol level might be considered as the activity of enzymes joining cholesterol which is effected by the circumstances of diabetes. Saponins which BM plant indicates might be considered as regulating the lipid metabolism effected negatively by diabetes. There are studys on saponins decreasing liver lipid and plasma triglyceride concentration as well as cholesterol, while increasing HDL level (27). Saponins cause cholesterol precipitation by forming compounds with cholesterol within intestine lumen, while reducing the access of including which cholesterol micelles into mucosa cells through effecting their size and/or stability, and ruin the membrane transport functionality because of its effect on mucosa cell membrane's cholesterol. Saponins so reduce the absorbation of cholesterol and increase the excretion of cholesterol and bile acid, together

with neutral sterols such as coprostorol and plant sterols (33,34,35). Decline of hepatic cholesterol level which is closely related to the inhibition of cholesterol absorbation causes liver HMG-CoA reductase activity and low density lipoprotein receptor level increase (33,36,37). Built in plant saponins might reduce blood cholesterol through this mechanism by inhibiting exogen and endogen hypercholesterolemia.

Diabetic hyperglycemia enhances oxidative stress by causing free radical formation, leaving the antioxidant system insufficient (38). A study states that MDA concentrations increase in rats with STZ-formed diabetes, while superoxide dismutase (SOD) and catalase activitydecrease (39). Plasma MDA high levels of diabetic patients was examined , while SOD, GSH, and catalase levels have been examined low (40). Another study asserted that GSH levels were low while MDA levels are high in diabetic subjects, concluding that oxidative stres increases, while antioxidant capacity decreases (41). In our study, liver, kidney and brain and erythrocyte MDA levels increase in diabetic control group compared to control group, while BM extract applied therapeutic groups MDA drops, while SOD, GSH, and CAT levels go up. It is possible to draw the conclusion that the last product of lipid peroxidation, MDA activity's drop in therapeutic groups and SOD, GSH, CAT levels increasing, occurred because

antioxidant phytochemicals in the extract works as a sweeper on free radicals that increase in diabetes, or because they inactivate oxidants, hence inhibiting the diabetes originated oxidative stress. Glutathione S Transferase (GST) enzyme in liver, kidney, lung, erythrocyte, testicles, placenta, carcase, and heart muscle, makes detoxification as a intracell carrier and connector (42,43,44). A study where erythrocyte GST activity of rats with STZ-formed diabetes, show that GST activity in the diabetic group increased compared to control group (45). In our study, GST activity levels of diabetes control group (DC) have been examined high in all of the tissues, compared to the control group. This increase might be considered as a result of resistance

mechanism against the oxidative stress situation. All of the groups treated with BM displayed lower GST activity levels in brain, erythrocyte and in liver, as the dose increases. Extract might be considered to reduce diabetic oxidative stress by directly effecting free radicals or by inhibiting hyperglycemia in rats with STZ formed diabetes. Some studies have shown that plant extract supplementation may have an antioxidative role in diabetic rats (46).

According to the results of our study amount almost all the parameters applied in therapeutic groups treated with plant extracts the levels examined in diabetic individuals as same levels ascould be observed in healthy individuals, indicate that BM has an effect of stimulating or renewing on functional cells of pancreas tissue, also effecting insulin secretion, hence this plant might have healing effect over diabetes related complications, and diabetes as a whole.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest. None of the authors has a commercial interest, financial interest, and/or other relationship with manufacturers of pharmaceuticals, laboratory supplies, and/or medical devices or with commercial providers of medically related services.

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Clinicopathological Results of Percutaneous Transplant Kidney Biopsies: Single Center Nine Years' Experience

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Abstract

Based on clinical criteria alone, the cause of graft dysfunction cannot be accurately predicted in 40-70% of cases. Therefore, renal allograft biopsy is still the gold standard for accurate diagnosis. We performed this study to evaluate the causes of renal graft dysfunction detected in renal allograft biopsies in our center.

The results of 90 patients who underwent renal allograft biopsy between May 2013 and June 2022 were evaluated retrospectively. It was determined that 92 biopsies were performed from 90 patients and all were ''cause'' biopsies. The mean age was 40.03±14.29 years. 82 of the kidney transplants were from living donors. 21 patients had preemptive transplantation. The type of renal replacement therapy before transplantation was hemodialysis in 52 patients, PD in 3 patients, PD and HD in 3 patients. The reason for biopsy was high creatinine in 67 patients, proteinuria in 23 patients, and BK virus viremia in 2 patients. The mean discharge creatinine value was 1.64±1.11mg/dl, and the mean creatinine before biopsy was 3.06±2.07mg/dl. In one

biopsy, although kidney tissue was detected, there was no glomeruli. The mean number of cores taken was 2.94±0.61, and the number of glomeruli was 21.33±11.64. In one biopsy bleeding that required transfusion developed. No other biopsy-related complications were observed. Graft loss was observed in 46 of 90 patients during the follow-up period.

Conclusions: Evaluation of serum creatinine and urinalysis may be useful in predicting histological graft diagnosis, but an allograft biopsy is necessary for definitive diagnosis.

Key words: *Kidney transplantation, allograft biopsy, acute rejection.*

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Introduction

Renal transplantation is the best treatment option for end-stage renal disease. Kidney transplant outcomes have improved due to advances in immunological treatments and surgical techniques (1). However, renal allograft dysfunction may occur due to acute rejection, chronic rejection, calcineurin inhibitor toxicity, infections, and recurrence of the original kidney disease. Each of these causes requires a different therapeutic approach (2). However, based on clinical criteria alone, the cause of graft dysfunction cannot be accurately predicted in 40 to 70% of cases (3- 7). Therefore, renal allograft biopsy is still the gold standard for accurate diagnosis (3,8,9). The causes of graft dysfunction may differ from center to center. We performed this study to evaluate the causes of renal graft dysfunction detected in renal allograft biopsies in our center and to compare our findings with the literature.

Methods

The results of patients who underwent renal allograft biopsy between 13.05.2013 and 22.06.2022, among the kidney transplant patients followed in the Group Florence Nightingale Hospital Kidney Transplantation Center, were evaluated retrospectively. It was determined that 92 biopsies were performed from 90 patients. Renal allograft biopsies were performed in the

presence of unexplained graft dysfunction (≥25% increase in serum creatinine from baseline) and/or proteinuria (>1 gr/day). The procedure was performed by a radiologist under ultrasoundguidance with a 16G automatic biopsy needle.

Demographic data such as transplantation age, gender, dialysis type, post-transplant biopsy time, donor type (living, cadaveric), laboratory data such as posttransplant and before biopsy creatinine, presence of proteinuria were recorded from the files.

The patients were evaluated in terms of hemoglobin values before and after biopsy, biopsy- related complications, number of cores and glomeruli taken by biopsy, biopsy diagnoses, and graft loss.

Statistical Methods

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) software, version 21.0 (IBM Corp, New York, NY, USA). Descriptive statistics of mean ± standard deviation (SD) were used for normally distributed continuous variables, such as age and clinical and laboratory data. For categorical data, such as biopsy diagnoses, numbers (percentages) were used.

Results

It was determined that 92 biopsies were performed from 90 patients between May 2013 and June 2022 in group Florence Nightingale Hospital Kidney Transplantation Center. All were "cause" biopsy. The mean age of the patients was 37.32±14.34 at the time of kidney transplantation. The mean age was 40.03±14.29 years at the time of biopsy. The males were predominant among recipients (76.7 vs. 23.3%). Eighty-two (91.1%) of the kidney transplants were from living donors and 8 (8.89%) from cadaveric donors. Twenty-one (23.3%) patients had preemptive transplantation. The type of renal replacement therapy before kidney

transplantation was hemodialysis (HD) in 52 (57.8%) patients, peritoneal dialysis (PD) in 3 (3.33%) patients, PD and HD in 3 (3.33%) patients. The type of renal replacement therapy in 11 (12.2%) patients was unknown. The mean biopsy time after kidney transplantation was 2.84±3.02 years. The reason for biopsy was high creatinine in 67 (72.8%) patients, proteinuria in 23 (25%) patients, and BK virus viremia in 2 (2.5%) patients. The mean creatinine value at the time of discharge from the hospital after transplantation was 1.64±1.11mg/dl, and the mean creatinine before biopsy was 3.06±2.07mg/dl. Although there was kidney tissue in one of the allograft biopsies, there was no glomeruli. The mean number of cores taken was 2.94±0.61, and the number of glomeruli was 21.33±11.64. Histopathologically, focal segmental glomerulosclerosis (FSGS) (21 patients), BK virus nephropathy (BKVN) (13 patients), T-cell mediated rejection (11 patients) and antibody-mediated rejection (ABMR) (10 patients) were in the top four ranks. The biopsy results of the patients are given in table 1. In one of the 92 biopsies performed, bleeding requiring transfusion developed. No other biopsy-related complications were observed. Graft loss was observed in 46 of 90 patients during the follow- up period. The distribution of pathological diagnoses of patients with graft loss is shown in Table 1.

Table 1. Pathological diagnoses of the patients and the number of graft loss.

Diagnosis	**Number (n)**	**Graft loss (n)**
ABMR	5	4
CABMR	5	3
T-cell-mediated rejection	3	2
Bordeline changes	6	
ABMR+ T-cell-mediated rejection	3	2
BKVN+ T-cell-mediated rejection	2	2
Toxicity results of Calcineurin Inhibitor	1	1
BKVN	10	8
FSGS	20	4
Diagnosis	**Number (n)**	**Graft loss (n)**
BKVN+FSGS	1	1
IgAN	4	3
IgAN+T-cell- mediated rejection	3	3
IgAN+CABMR	1	1
Amyloidosis	3	1
C3 glomerulopathy	2	2
Membranoproliferative glomerulonephritis	2	2
Membranous Glomerulonephritis+ABMR	1	1
Thrombotic Microangiopathy	1	1
ATN	2	-
Nonspecific changes	8	-
Tubulointerstitial nephritis	3	3
IF/TA	5	2
ABMR+ATN	1	-

ABMR: Antibody-mediated rejection, CABMR: Chronic active antibody mediated rejection, BKVN: BK virus nephropathy, FSGS: Focal segmental glomerulosclerosis, IgAN: IgA nephritis, ATN: Acute tubular necrosis. IF/TA: interstitial fibrosis/ tubular atrophy.

Discussion

Allograft biopsy is still used as a reliable method to evaluate transplant kidney function. Localization of the transplanted kidney in the inguinal fossa facilitates both the procedure and hemostasis (9). Nevertheless, hemorrhage is stated as the most important complication in the literature, and the complication rates related to transplant kidney biopsy are reported to be between 0.06% and 13% (10-15). In our study, we detected bleeding requiring transfusion in only 1 (1.1%) patient and no other biopsy-related complications were observed. This difference in complication rates can be explained by many factors such as the operator's experience, the use of the imaging guide, and the size of the biopsy needle. We used a 16 g biopsy needle in our study group and the procedure was performed by an experienced radiologist. In our study, the rate of transplantation from cadaver was 8.89% (8 patients). This rate was similar to our country's 2020 data (10%) (16).

The primary indication for allograft biopsy is to differentiate between acute rejection and other causes of renal dysfunction. This indication is mainly established when the creatinine level, which indicates renal dysfunction, rises above the basal level. In our study, allograft biopsy was performed in 67 (72.8%) of the patients due to increased creatinine. Proteinuria, one of the indications for allograft biopsy, is an important marker and has been studied in many studies (17-20). Massive proteinuria after transplantation is common in the first three months and resolves spontaneously during follow-up (17). Persistent proteinuria occurs in 30% of transplants and is positively correlated with the presence of glomerular lesions. In the present study, proteinuria was used as an indicator for allograft biopsy in 25% of the cases and was present in 57.6% of the renal biopsies.

The sensitivity of the kidney biopsy

depends on the biopsy size number of cores and amount of cortex sampled. The reported sensitivity of two core biopsies is close to 99% (21,22). Although the adequacy of the sample depends on the underlying pathology, at least 7 non-globally sclerotic glomeruli and 2 arterial sections must be present for accurate assessment. In our studygroup, less than 7 glomeruli were detected in 7.6% biopsies, and no glomeruli were detected inonly one biopsy sample. The mean number of cores taken was 2.94±0.61, and the mean numberof glomeruli was 21.33±11.64.

It has been stated that, in the early period of kidney transplantation the biopsy results are easier to interpret because the lesions are more specific. In the late period many lesions can be seen together and chronic damage makes the interpretation of existing lesions difficult. (23). In our population, the mean follow-up time between kidney transplantation and allograft biopsy was found 2.84±3.02 years. The shortest biopsy time was 5.7 days and the longest 11.9 years. When the histopathological results of our patients were examined, single and combined diagnoses were detected. A combined result was found in twelve (13.04%) biopsies. Focal segmental glomerulosclerosis was observed in 21 patients, BKVN in 13 patients, T cell-mediated rejection in 11 patients, and ABMR in 10 patients. Graft loss developed in 46 (51.1%) of 92 biopsies performed on 90 patients. The diagnoses of patients with graft loss are shown in Table 1. In onestudy, they attributed about half of the causes of graft failure to ABMR and mixed rejection. In the same study, three other non-rejection causes were specified as glomerulonephritis, BKVN, and intercurrent disease. (24). In our study group, we found that graft loss was most common in the patient group accompanied by BKVN, T-cell- mediated rejection and ABMR. Other studies have also associated BKV nephropathy with early renal dysfunction, graft loss, and

renal histology changes (25–28), and it has been observed that BKV nephropathy may cause graft dysfunction in >90% of affected individuals and graft loss in more than 50% (29). In our study, graft loss developed in 11 (84.6%) of 13 patients whose biopsy was accompanied by BKV nephropathy. This rate seems consistent with the literature. On the other hand, ABMR was detected in the biopsy of 10 patients, and graft loss developed in 7 patients. Graft loss developed in 2 of 3 patients with only T-cell-mediated rejection in histology, while graft loss developed in 9 out of 11 patients with combined T-cell-mediated rejection. Our study has limitations such as being retrospective and consisting small number of patients.

Conclusion

The leading indication for allograft biopsy in our center is increased creatinine. Focal segmental glomerulosclerosis and BK virus nephropathy constitute an important part of biopsy diagnoses. These results do not require antirejection therapy. Performing allograft biopsy in patients with graft dysfunction is important to prevent unnecessary anti-rejection treatments.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Hydrotherapy for Fall Prevention in Elderly Individuals

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Abstract

Falls and fall-related injuries of elderly individuals are an important health problem that threatens their lives. Particularly, regressions related to aging in postural control and challenging dual tasks cause falls. It has been reported that physical training and exercises related to balance are effective in reducing falls in elderly individuals. However, there are inherent difficulties for the elderly in making these exercises effective. Especially as the number of chronic diseases increases, this difficulty increases even more. For this reason, it is important to create alternative methods for performing exercises that increase balance in a more effective and safe environment. As one of these alternatives, hydrotherapy can play a fall-preventing role for elderly individuals with the properties of water that support the balance. With the reduction of the fear of falling in water, the motivation of elderly individuals to exercise may increase. They may also feel more confident when performing balance, motor, and cognitive tasks. With hydrotherapy; It is also possible to provide biomechanical and psychological well-being by making use of the buoyancy, resistance and temperature properties of water. Hydrotherapy and balance training protocols tailored to the individual needs of the elderly population are seen as an effective approach in preventing falls.

Key Words: *Hydrotherpy, Elderly Individuals, Fall Prevention.*

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Introduction

The rapid increase in the elderly population all over the world and in our country has made it important to plan and implement health measures related to the elderly population (1). It is expected that the world's elderly population will cover approximately 17% of the total population by 2050 (2). In our country, according to the data of TUIK for 2021, the elderly population exceeded 8 million and reached 9.7% of the total population. It is estimated that this increase will increase to 11.0% in 2025 and to 16.3% in 2040, increasing in our country as well as in the rest of the world (3).

Aging; it is a period in which changes that continue from birth to death are experienced (4). It refers to a physical, psychological and social multidimensional process that includes changes in physical shape and physiological function of tissues and organs, changes in perception, learning, problem solving, personality traits, as well as changes in function and behavior in social life (4). Biological changes seen with aging increase the susceptibility to fall directly or with different factors (5, 6).

The inclusion of changes in cognitive and sensory areas that cause balance disorders in the natural aging process causes relapses (7). In addition, the probability of recurrent falls doubles after the first fall, which would qualify such an elderly individual as a high-risk patient (8). It is known that falls occur due to many factors. Impairment of postural control is an important factor that can lead to falls and fall-related injuries. Postural control is the ability to maintain balance and spatial orientation in standing upright with the inhibition of gravitational forces. Postural control is necessary to maintain daily activities such as walking and balance (9). Finding effective ways to prevent falls in the elderly population can reduce disability from falls and fall-related injuries. It can also increase the life expectancy of these individuals.

Cause of Falls in the Elderly Individuals

Definition of Falls

Falling is defined as the inactivity of the individual below the level he/she is at for different reasons (10). The World Health Organization defines a fall as “an individual lying on the ground or at a level lower than his/her own level due to carelessness or accident” (11). Falls, which increase with age, are an important condition that causes high mortality and morbidity in the elderly (12, 13). Problems such as decrease in visual perception level, difficulty in fixed posture, immobilization, orthostatic hypotension, weakness in the lower

extremities, and dizziness that occur with aging increase the risk of falling (13).

Falling in elderly individuals; It occurs due to intrinsic (self-induced) and extrinsic (environmental) reasons (12,13). Intrinsically caused falls; It generally occurs due to age- related physiological changes, cognitive disorders, gait, strength and balance disorders, sensory disorders, acute and chronic diseases, preferences and behaviors of the individual (12,13). Extrinsic-caused falls are; It occurs due to reasons such as the use of multiple drugs, the wrong choice of shoes, the use of walking aids, and the wrong design of the furniture in the house. Extrinsic falls mostly occur when the elderly walk or change position. Minimizing the factors that cause falls is important in terms of preventing falls. Elderly individuals often think that falls are inevitable with aging, and they underestimate their personal risks or they are not aware of it (10,13).

Postural Control Changes

For the human body, the trunk and upper extremities constitute more than half of its mass (14). This is a challenging task for most individuals, especially elderly individuals, for the control of balance. About 33% of older adults fall, which causes moderate to severe injuries (8, 15). Therefore, falls have become an important public health problem (16). Postural control is a complex skill maintained by multiple sensorimotor systems (14, 17). Factors responsible for postural control include higher central processing, control of dynamics, spatial orientation, biomechanical factors, sensory and movement strategies (17). This complex mechanism that provides postural control tends to decrease with aging, and this decrease causes an increase in the prevalence of falls in the elderly population (16, 17).

The natural course of aging adversely affects balance performance in dynamic and static postural control, and causes accidents related to falls and changes neuromuscular and sensorimotor systems (18). These changes cause deterioration of the postural control of the elderly individual. Disturbance in the postural control system or exposure to a perturbation results in impaired standing upright balance and subsequent fall (1).

Regression in the sensorimotor system in the elderly; it may be a result of muscle weakness, loss of sensation, and cognitive dysfunction (18). These deficiencies are directly related to immobilization and negatively affect stair climbing, walking and activities of daily living (19). Proprioception is an important aspect of postural control and balance, especially in the elderly population. Since proprioception decreases in the elderly, it makes it more prone to loss of balance

and falls (20). One of the main problems in the elderly is the difficulty in controlling the timing of muscle contraction during multiple joint movements. This is related to age-related decreases in proprioceptive feedback and paves the way for physical inactivity (20).

Balance and Dual Task Changes:

Balance deficits are the most common risk factor contributing to falls in the elderly (8). Elderly people are 5.4 times more likely to fall while performing poor balance and cognitive functions (8, 17). They also experience falls when performing two activities at the same time. dual mission; It involves performing one task (postural control task) while completing a second task (cognitive or motor task) at the same time (8). Some individuals in the geriatric population have limited cognitive function abilities due to neurological deficiencies. Therefore, the existing cognitive function network may be insufficient to control posture (17). The high number of falls due to dual-task activities has led to an increase in research on this subject (7). It has been reported that especially verbal tasks cause more loss of postural control and falls (21).

Balance Training and Hydrotherapy in Fall Prevention Balance Training

Balance control and education in the elderly are necessary to maintain basic daily living activities (17, 22). Static and dynamic balance training can improve postural control and reduce the risk of falls for the geriatric population (23). Balance training on fall prevention aims to improve postural control by opposing the alignment of the individual's body center of gravity with respect to the support surface (24). The dose-response relationship is important in a balance training. Multiple balance training protocols can be 91-120 minutes of balance training per week, with a training period of 6-12 weeks, frequency of three sessions per week, 31-45 minutes in a single session, 36-40 training sessions in total. This training is an exemplary protocol recommended for improving balance (18).

Hydrotherapy

While most land-based exercises are effective, they have their own inherent complications and physical challenges that hinder successful training for older individuals. This increases the need for alternative forms of balance training for the elderly in a safe and efficient way.

For older individuals, there are risk factors leading to falls such as age, previous fall history, lower extremity weakness, environmental conditions, proprioceptive deficits, and fear of

falling. Safe physical activity is recommended to reduce these risk factors (25). Fall prevention exercise programs have been created and implemented for elderly individuals. However, these land-based exercises can be difficult for the elderly due to joint pain and muscle weakness (26). Hydrotherapy is the use of various exercises while the body is in water (25). Hydrotherapy is frequently used in therapeutic areas for injury rehabilitation, improving muscle strength, maintaining balance and cardiovascular compliance (25).

Water's properties such as buoyancy, resistance, and temperature, when combined with physical exercise, can help alleviate many of the physiological problems of natural aging and promote physical activity (27). The aquatic environment is considered safe and efficient for the rehabilitation of the elderly. Water also provides a supportive, low-risk exercise environment that can reduce the likelihood of acute injury and fear of falling, while improving participation and compliance with the rehabilitation program (27, 28). Less feeling of weight with the buoyancy of the water provides less painful and softer movements caused by the temperature of the water. Thus, it can help reduce many fall risk factors (19). Weightless physical activity through hydrotherapy has been shown to improve motor tasks and cognitive processes while in a safe environment for the elderly (29). In addition, exercises performed in water prevent falls and reduce the fear of falling, while creating a safe environment for balance training (30).

In-water exercise program for elderly individuals provides a motivation-enhancing environment. Motivation contributes to the development of psychosocial aspects by increasing the adaptation of the individual. In addition, a more positive attitude was observed in the participants after the exercise program (31).

There is limited literature on the efficacy of hydrotherapy with regard to dual-task. However, it has been reported that more improvement is achieved in balance tests, postural control and weight-bearing exercises when performing single-task activities in water (32).

Conclusion

As individuals age, postural stability decreases due to many factors that complicate rehabilitation and exercise. For healthcare professionals, for clinical practice purposes, hydrotherapy is a safe, effective form of rehabilitation and exercise for the elderly. Hydrotherapy programs are appropriate exercises to reduce the number of falls and instill

confidence in patients. It can provide a safe environment for the elderly to improve their balance and coordination. Therefore, hydrotherapy is a possible method that can be recommended to increase balance and prevent falls in the elderly.

Especially in our country, it can be applied in limited environments due to the lack of clinics and facilities for hydrotherapy. Considering the benefits of in-water exercises, especially for the elderly population, it is important that the use of hydrotherapy becomes widespread. Hydrotherapy offers a successful alternative balance training method in preventing falls and fall-related injuries in elderly individuals.

Conflict of Interest

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

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An Overview of the Various Appropriate Types of Cell Lines for the Production of Monoclonal Antibodies

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Abstract

As the first monoclonal antibodies [mAbs] were produced in 1975, the challenge to improve antibody engineering started. Since then, therapeutic antibodies have become the predominant class of new drugs developed recently, and an essential part of progress has affected cell lines. From the first pioneer hybridoma cells to the current vanguard Chinese hamster ovary [CHO] cells dramatic improvement has been seen. An essential part of this process is choosing the suitable cell line to seed the targeted antibody gene. This review encompa

sses all the current evidence to compare various proper cell lines for monoclonal antibody production such as mammalian cells, plant cells, bacterial cells, and yeast cells. Valid long-term data, regarding glycosylation, efficiency, and safety, support the current popularity of CHO cells. At the same time, other types of cell lines also show some promise for emerging needs for more therapeuticantibodies on the market.

Keywords: *Therapeutic antibodies, Chinese hamster ovary [CHO] cells, Cell line.*

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Introduction

Since 1975, when César Milstein and Georges Köhler introduced antibody-secreting by hybridoma cells, monoclonal antibody technology has been progressively applied to a wide variety of biological and medical issues of both theoretical and practical concerns which nowadays they have become the dominant type of biopharmaceutical (1,2). Since their introduction, mAbs have been applied to treat autoimmune disorders, allergic diseases, transplantation rejection, anti-idiotype vaccines, and cancer (3,4). Scientists have developed complete mAbs with the same immunogenic properties as human immunoglobulin [Ig] from endogenous animals (2). They have combined phage display technology with transgenic mice that express the human variable domain. The first therapeutic monoclonal antibody, muromonab- CD3 [Orthoclone OKT3] made by Johnson and Johnson, received the US Food and Drug Administration [FDA] approval in 1986. This mAbs was created from a murine monoclonal antibody [mAb] against CD3 expressed on T cells, which was utilized to treat acute transplantrejection by acting as an immunosuppressant (5,6). Because of the enormous rise in the previous several years, the FDA has authorized 79 therapeutic mAbs for numerous clinical applications, including infectious illnesses, cancer, immunological disorders, and arthritis.[5] This growth showed a 51% rise in the number of mAbs or conjugates between 2014 and 2019, and there is still significant growth potential in this area (5-7).

In recent years, mAbs have become one of the most highly demanded classes of biological products with high efficiency in targeted therapies with raised specificity, potency, and low toxicity quality (2). They account for nearly half of the market and are among the top five therapeutic proteins consumed in recent years. By 2024, the mAb market is predicted to reach $138.6 billion (8).

Despite their impressive performance, mAbs still hold numerous disadvantages, including instability, high-dose prescription, and poor compliance (2).

Antibody Structure and Function

An antibody is a large immunoglobulin glycoprotein, which recognizes foreign antigens, neutralizes them, and triggers additional immune responses (9). Antibodies have a fundamental structure that includes a constant Fc [crystallizable fragment] domain and an antigen-binding domain comprised of the Fv [variable fragment] and Fab region [antibody binding fragment] (9).[Figure 1] (10).

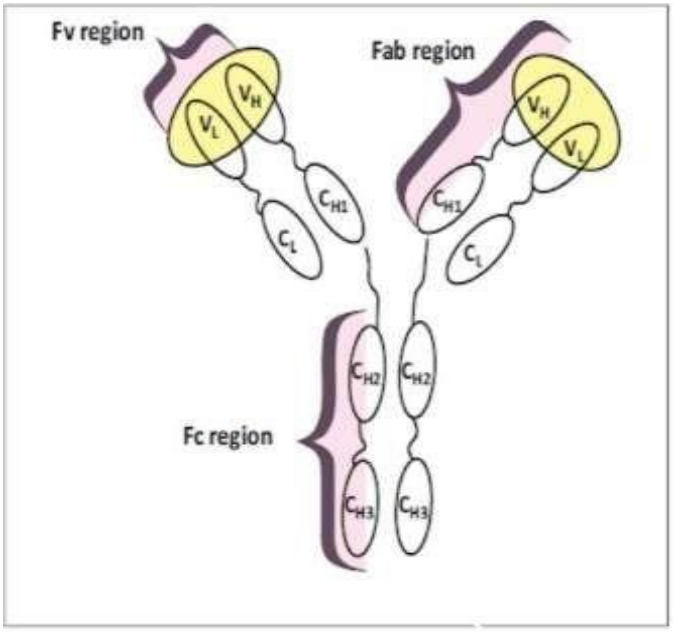


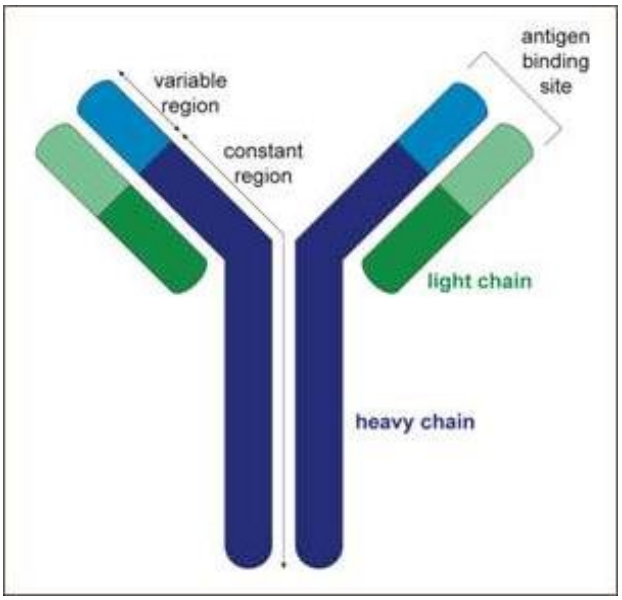
Figure 1: Diagram of a complete antibody [yellow highlights indicate where the antigen binds].[10]

Fc regions are part of antibodies which are called heavy chains [Figure 2] (12). Based on their heav

y chains, antibodies are classified into five types: IgA, IgE, IgD, IgM, and IgG. Fc regions of antibodies are identified by Fc receptors [FcRs]. These receptors are positioned in several classes of immune cells, such as neutrophils, natural killer [NK] cells, eosinophils, dendritic cells, and monocytes (11).

As an example of function antibodies, IgG could be utilized to treat complement-dependent cytotoxicity [CDC], along with antibody-dependent cellular cytotoxicity [ADCC]. There are many subgroups within the IgG class based on the capacity of the Fc region to enable ADCC and CDC: IgG3 and IgG1 can cause ADCC and CDC, but IgG2 and IgG4 cannot. A monoclonal antibody is a large-scale product form of an antibody isotype that specifically targets an antigen epitope (11).

Figure 2: A total antibody diagram demonstrating heavy and light chains



(12).

The term monoclonal antibody [mAb] refers to antibodies that recognize only one epitope. In the human immune system, cloned homogeneous hybrid cells [B cells] make these antibodies (13). Native full-length mAbs are glycosylated during production. While not directly interfering with antigen function, the glycosylated Fc domain stabilizes antibodies and is required for ADCC. Additionally, glycosylation affects the pace at which recombinant mAbs are cleared from the body, and incompatible glycoforms might produce severe immunogenic consequences in patients. A full-length antibody does not need to have a glycosylated Fc domain in order to identify an antigen. The Fv and Fab regions [Figure 1] contain antigen-binding capabilities. Antibody fragments penetrate deeper into tissues and have a shorter retention duration in non-target tissues than mAbs. While the absence of the product's stabilizing Fc domain makes it less stable, the lack of glycosylation on both the Fv and Fab regions makes it easier to construct and culture microbial hosts such as bacteria and yeasts (9,10).

Researchers have discovered the method through which antibodies destroy tumor cells by providing efficient, consistent, and long-lasting cancer-suppressive effects in experimental and clinical tests. In reality, the appearance of spontaneous or increased tumor cells in the body triggers antitumor responses. A high number of antitumor antibodies are generated as part of this antitumor response, which directly destroys tumor cells while also damaging vascular and stromal cells. Scientists have investigated methods to create mAbs that selectively target a certain antigen present on the surface of cancer cells to increase mAb activity encouraging such anticancer processes. Every mAb targets a distinct malignant cells antigen, and their actions vary based on the antigenic targets of the various kinds of cancer cells. There are three categories of mAbs in general. They function in a variety of methods, including stimulating theimmune system to fight cancer cells, blocking signals that tell cancer cells to divide, and delivering chemotherapeutic medications or radiation to those cells (14).

When changes in the manufacture of mAbs occur, biopharmaceutical firms are becoming increasingly interested in creative strategies to supply new protein therapy options. Since antibody treatments require big dosages over a long period of time, the manufacture of large quantities of antibody medications with cost and time efficiency is required to fulfill clinical standards and progress

toward commercialization. Pharmaceutical firms strive to minimize time-to-market, keep costs low, and enable production flexibility while preserving desired quality qualities (15). One of these approaches is to find and use an appropriate cell line for the synthesis of mAbs. The sections that follow will look at current cell lines, vectors, and the best culture types for producing stable mAbs.

Expression systems

To produce mAbs, a suitable cell line must be selected, depending on the type of cell (4). Initially, cell lines used to be selected by their growth patterns and morphologies; yet, as culture conditions and time passed, these characteristics may change. There is an even greater need to identify established cell lines by additional criteria, given the growing number of cell lines (16). Chosen cell lines are expected to have some of the properties that are based on stoichiometric and kinetic parameters; such as high growth rates, the ability to produce high levels of productover a long period while maintaining cell concentration, along with additional characteristics such as cell line stability, virus vulnerability, and be able to process protein after translation and also, the obtained products do not endanger human health. When selecting the suitable cell for the possible cell line, it is important to optimize the medium composition and bioreactor operating conditions for improving specific productivity per cell.[15] In addition to the aforementioned features, there are no constraints on employed cell lines, and the most suitedcell line product should be chosen (4,16).

Mammalian cells have become more vital in the current biotechnology industry because of their capacity to conduct [human-like] post-translational alterations, as indicated by their substantial market share and the importance of proteins obtained from this technique.[16] In addition to prokaryotic [insect and plant cells] and eukaryotic [insect and plant cells] systems, mammalian cell lines are the leading system used to produce pharmaceutical proteins, including mAbs (4).

Developing production culture processes and reforming cell lines allowed biopharmaceutical companies to achieve recombinant antibody titers up to 3–8 g/L for fed-batch mammalian cultures over 10 kilos (17).

Mammalian cell lines

Many expression techniques were used to create antibodies and antibody-based compounds. African green monkey kidney cells [COS], a mammalian cell

line, are an acceptable alternative for producing modest amounts of mAbs for biochemical and biophysical study (4). They are, however, not the best cells for large-scale industrial operations since their capacity to produce diminishes with time. Cell lines from mammals Because of their ability to perform complex posttranslational modifications, which result in the production of proteins similar to those found naturally in humans, Chinese hamster ovary [CHO] cells and murine myeloma cells are the dominant hosts for the commercial production of therapeutic glycoproteins and non-human glycan structures (18-20).

Chinese hamster ovary [CHO] cells

Several industrial-scale recombinant proteins are synthesized utilizing Chinese hamster ovary [CHO] cells (17). The fact that this sort of cell line has been well-defined and effectively employed for the synthesis of a variety of therapeutic biopharmaceuticals implies that this trend will continue (21). According to a survey conducted in 2013 in the US or EU, most mAbs are produced in mammalian cells. The reliance on deliveries is increasing as more progress is being made in this area (22). Theodore Puck isolated the first CHO cells from Chinese hamster ovary tissue in 1957. The CHO-ori cells developed from Chinese hamster ovary cells isolated from these animals were found to be immortal. In 1968 following clonings of the CHO-ori cell line made CHO-K1 cell lines possible. Similar to CHO-ori cell lines this new cell line had limited advantages for large-scale processes and also they both were grown in adherent cell culture conditions. Thompson developed CHO-S, a subline that could grow in suspension culture In 1971. Using this breakthrough of making CHO cells suspension-compatible, researchers wereable to grow this cell line in bioreactors at a much larger scale (23). Since then their population as the dominant cell line has increased due to their easy culturability, and rapid proliferation and they show tolerance to chemically-defined media. It is also a positive characteristic of CHO cells that the downstream purification process is facilitated by the lack of replication of HIV, influenza, and polioviruses. Mentioned features combined with the fact that they typically don't

produce variable outcomes enhance the reliability of mAbs. Furthermore, CHO cells are easily genetically modified, making them an ideal choice for producing mAbs (22).

The regulatory approval procedure for CHO cells is less difficult due to their capacity to create safe, biocompatible, and bioactive mAbs, and they are governed by their product quality attributes [PQAs]. Product

quality features include charge variations, glycosylation, oxidation variants, measure variants, and structural variances. The main criteria defining mAb quality include potency, pharmacokinetics/pharmacodynamics [PKPD], immunogenicity, and product safety (21-24).

In clonal selection, MTX [methotrexate] injection is utilized to select cells that express sufficient amounts of DHFR [dihydrofolate reductase] because recombinant CHO cell lines produce DHFR enzymes that are resistant to MTX effects. Due to the fact that CHO cell lines can produce large amounts of recombinant protein [like mAbs] when methotrexate [MTX] (15), is used as an inhibitor, they have been amplified together with the dihydrofolate reductase [DHFR] gene as well as the intended recombinant gene (25). It is understood that there is a possibility of gaining resistance to MTX by amplifying the DHFR gene. A significant difference exists between the amplification units of gene-specific expression vectors and those of the DHFR gene, owing to the larger gene-specific expression vectors [100–3,000 kilobases [kb]] that co-amplify genes of interest that are adjacent to the host genome. High gene copy numbers may not be enough to boost productivity in highly amplified subclones due to transcriptional and post-transcriptional limitations, even though the level of gene amplification usually correlates with the level of expression. As a result of high levels of amplification and expression, foreign gene products can exert a variety of levels of toxicity and metabolic burden on cells. Since cells regulate gene expression in response to stress, this may lead to instabilities in recombinant protein production. Recombinant proteins can modulate any stage of gene expression, including transcription, which is considered to be the primary determinant of protein expression (26).

Several studies have been conducted on genetically engineering the production of host cells to improve or modify product quality or host cell strength. In recent years, glycosylation of antibodies gained much attention, which involves adding glycan structures to products, thereby affecting their clearance rate and bioactivity (15). Monitoring and controlling qualities such as mAbs N-linked glycosylation of the Fc domains (27) are vital because of their effects on PK/PD [pharmacokinetics and pharmacodynamics], efficacy, and immunogenicity. In addition to immunogenicity, glycan species differ in relative abundance, affecting their circulation half- time, making glycan

species potential CQAs [Critical Quality Attributes]. As a result, glycosylation affects galactosylation levels, which in turn can reduce complement-dependent cytotoxicity [CDC]. On the other hand, Despite maintaining FcRn binding and *in vivo* half-life, non-glycosylated mAbs have minimal effector activity. However, some non-human glycosylation substrates, such as N-glycolylneuraminic acid and *α*-galactosyl epitopes, may induce immune responses. The N-linked glycosylation in the Fab domain of Cetuximab (28) has caused anaphylaxis in some individuals since Some patients have IgE antibodies to *α*- galactosyl-containing N-linked glycoproteins on the Fab (27). Furthermore, core-fucosylation levels are also reduced, which leads to increased antibody- dependent cytotoxicity [ADCC]. And finally, The immunogenicity potential of mAbs was improved by inserting non-human epitopes such as galactose-alpha-1, 3-galactose residues and N-glycolylneuraminic acid residues in the glycosylation process (24).

Glycoform profiles of mAbs are under influence of several factors, including the type of cell line used, the media composition, feeding strategy, and process parameters during cell culture. Glycosylation machinery regulatory mechanisms, which depend on the cell line and protein sequence and structure, are a factor counting as inconsistency on the universal scale. Despite this, some qualitative trends have been observed consistently across many studies (24,27,29). According to these study results, the proportion of mature glycoforms declines during the culture. The culture advances by increasing galactosylation and underprocessed glycoforms, such as high-mannose species. Sugar nucleotide levels, glycosylation enzyme levels, and cofactors like metal ions can all impact the timing of glycosylation network activity (30). During the culture, significant changes in gene expression profiles are also observed in the cell cycle. Throughout the culture, the specific productivity [QP] of the mAb generally shows changes (31). Despite the absence of rigorous investigation to support the assumption that transfection into a similar host cell might lead to significant clone-to-clone variance, it is widely accepted that glycosylation is dictated by the host cell utilized in cell-line formation. When manufacturing scales up, it is also vital to maintain constant N-linked glycosylation throughout batches and large-scale bioreactors. As previously stated, several variables, such as cell culture and/or processing settings, might impact glycosylation. As a result, companies have begun incorporating N-linked glycosylation profiling into their cell-line development processes, forcing them

to choose the final production of cell lines based on another factor known as optimal N-linked glycosylation profile, in addition to the frequently mentioned production yield, intrinsic efficiency, and optimized cell growth conditions (27).

Murine cells

Another frequently used cell line for large-scale mAbs is Murine cells [e.g., NS0, Sp2/0-Ag14]. NS0 and Sp2/0-Ag14 cells are repeatedly cloned and chosen. From BALB/c mouse plasmacytoma cells, immortalized B cells that do not release immunoglobulin G are generated [IgG]. To establish these two parental cell lines, the original cell line was cloned many times and, in the case of Sp2/0-Ag14, fused with spleen cells from another BALB/c animal (32,33). These cells are cholesterol auxotrophs, meaning they require cholesterol in their culture medium for growth (4,18,32). In 1962, Potter and Boyce realized that injecting mineral oil into the intraperitoneal cavity of BALB/c mice caused plasma-cell neoplasms (34,35). In later studies, Potter and Boyce discovered that one of the tumor-induced inbred BALB/c female mice was able to secrete IgG1. Horibata and Harris created a cell line from these tumor cells in 1970. Galfre and Milstein created NS0/1, a non-secreting cell line, after removing IgG1 synthesis from the cell line in 1981. Since then, NS0 has been used to generate mAbs for a range of disorders (22).

To lessen the risk of transmissible spongiform encephalopathies [TSE], producers have employed sheep wool-derived cholesterol instead of bovine traces on a large scale. Apart from animal-derived cholesterols, synthetic cholesterol solutions such as SynthecolTM [Sigma-Aldrich, St. Louis, MO, USA] and lipid concentrate [Life Technologies, Grand Island, NY, USA] are available on the market. A recent study found that electrohydrodynamic spraying cholesterol nanoparticles into NS0 cells lowered toxicity compared to synthetic cholesterol solutions (32).

The NS0 cells are characterized by the absence of glutamine synthetase [GS] enzyme activity (32,33). This feature can be employed as a marker for recombinant antibody expression. Nonetheless, Non-GS NS0 cell lines have also been reported to produce antibodies up to 3 g/l (35). One of the advantages of Sp2/0-Ag14 in contrast to NS0 cells is they experience several spontaneous mutations which make them needless to satisfy glutamine requirements (33).

A disadvantage of Mouse-derived cell lines, including NS0, is the production of N-glycosyl neuraminic acid [NGNA], a

sialic acid that is not found in human antibodies and might have immunogenic properties. This immunogenicity concern might partially explain why NS0 cells are not used as widely for producing therapeutic antibodies as CHO cells (15). α-gal and Neu5Gc are the other immunogenic products of murine cells which they produce at a noticeable amount which is another disadvantage of these cells in contrast to hamster cells (35).

Antibodies are frequently produced using the host cell lines NS0 and Sp2/0-Ag14. Cetuximab [ERBITUX®] and palivizumab [SYNAGIS®] are some of the well-known mAbs produced by utilizing these cell line systems (35).

Due to the more extensive characterization of CHO cells from an 'omics perspective, it might be worthwhile to continue studying and characterizing NS0 cells (22).

Human cell lines

Despite the widespread use of CHO cells in the past few years and the availability of a wide range of mAbs derived from them, In most cases, human cells can produce proteins that are similar to those of humans. This may be a positive characteristic in comparison to other animal cell lines (36,37). Several products made from these human cell lines have been tested in large- scale clinical trials. Furthermore, these cell lines are useful as expression systems in biomedical research since they are high-yielding and contain the same [post-translational modifications] PTMs as normal human cells (37).

In 1951 the first human cell line was created by isolating cervical cancer cells. In the 1960s, human diploid cells made vaccine production possible. Their acceptance, however, was held back because of concerns about latent oncogenic agents in these cell lines without even representative phenotypic characteristics (37). In 1973, human embryonic kidney cells [HEK] were transformed with adenovirus type 5 [Ad5] sheared pieces of DNA, which led to the immortalization of the cells in the form of the HEK293 line. As a result of the adenovirus's integration, the Ad5 genome is believed to have been merged with the 19th chromosome of these cells. Many development characterizations have been done on this type of cell line, which led to the development of numerous subtypes of the cell line, as mentioned previously (38). Because of their rapid growth, high protein output, and investment in system optimization, human cell lines are now commonly employed in the production of viral vaccines. They also serve as a substrate option for the synthesis of

recombinant proteins and mAbs instead of CHOcells (37).

Various types of human cells are used as cell lines. For instance, the HEK293 cell line which originated from epithelial cells, the HT-1080, produced from an epithelial-like phenotypic fibrosarcoma, and the PER.C6 cell line which is originating from immortalized with adenovirus E1 gene transfection human embryonic retinal cells (37,38). Nonetheless, being widely used, the HEK293 cell line was more in the attention of researchers, leading to many derivative cell lines which can produce a wide range of recombinant proteins and mAbs (38). These cell lines can produce with high productivity, and their products are approved as recombinant biotherapeutic products. more are being considered and tested (37,39).

The production of biotherapeutic proteins, such as mAbs, relies almost exclusively on HT-1080 and HEK293 cell lines (37). As an advantage of these cells, both HEK293 and HT-1080 are capable of passing their products through human PTM (40).

HEK cells were initially modified to produce in small-scale quantities such as research amount protein productions. But as their potential to get transfected was realized, they became widely popular in large-scale production, and HEK293 became the predominant cell line today (41). Aside from PTM, which these cells accomplish considerably more promising than the CHO cell line and the murine myeloma NSO cell line, these cells can grow in serum-free suspension cultures and have transfecting capacities (38,42,43). This cell line's performance has been altered bya combination of these traits and genetic alterations across the biological process, including cell growth, apoptosis, metabolism, glycosylation, release, and protein folding, as well as bioprocess, medium, and vector optimization (38).

Inefficient glucose metabolism is a major disadvantage of HEK293, pushing the cell to operate predominantly on glutamine for energy generation. Due to the use of glutamine as an energy source, ammonia and lactate are formed as byproducts. These by-products are toxic to premature cells, which causes their death. Furthermore, high ammonia levels disrupt the glycosylation of proteins. To improve this issue, researchers on HEK293 tend to enhance and reformulate central carbon metabolism. When the glycolysis and tricarboxylic acid cycle were restored by overexpressing the pyruvate carboxylase gene [PC] and removing the pyruvate dehydrogenase gene [PDK], as well as its activator, the hypoxia-inducible factor 1 gene [HIF1], the cell density increased, as

well as lactate and ammonia production decreased. Additionally, these modifications improved recombinant protein expressions and glycosylation, mixed amino acid usage, and enhanced recombinant viral titers by 30 times (38).

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Immunization and Vaccines

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Abstract

Immunity is the ability of the human body to resist almost all types of organisms and toxins that can damage tissues and organs. The most important is acquired immunity, which develops after exposure to bacteria, viruses, or toxins. Natural immunity consists of protective barriers that prevent the passage of microorganisms such as skin and stomach acid, phagocytosing cells, and the complement system. Vaccines are biological products that protect against a specific disease by stimulating the immune system. This review aims to address the concepts of immunization and vaccination. One of the most basic components of children and adults' right to a healthy life is the protection of them against diseases by vaccination. Immunization services are among the most important public health interventions for children in terms of preventing vaccine-preventable diseases and deaths.

Keywords: *Immunization, Active immunity, Natural immunity, Vaccination.*

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Introduction

Immunity is the ability of the human body to resist almost all types of organisms and toxins that can damage tissues and organs. The most important is acquired immunity, which develops after exposure to bacteria, viruses, or toxins. Natural immunity consists of protective barriers that prevent the passage of microorganisms such as skin and stomach acid, phagocytosing cells, and the complement system (1). The body has two types of immunization against pathogens: active immunity and passive immunity. Active immunity is achieved by vaccination or recovery from a disease. This type of immunity is usually long-lasting. Passive immunity, however, is achieved by transferring antibodies (immunoglobulins) from other people or animals. This type of immunity is short-lived and lasts from a few weeks to several months, depending on the amount of immunoglobulin administered. Passing antibodies from the mother to the baby through the placenta and giving blood and blood products (such as whole blood, plasma, erythrocyte and platelet suspensions, and immunoglobulin preparations) also provides passive immunity (2). This review aims to address the concepts of immunization and vaccination.

Material and Method

The data in this review were searched through the Web of Science, PubMed, Cochrane Library, Google Scholar, EMBASE (OVID), WHO Global, Council of Higher Education National Thesis Center, and Dergipark databases. The keywords 'Immunization', and 'Vaccine' were used during the search.

Concept of Vaccine

Vaccines are biological products that protect against a specific disease by stimulating the immune system. Vaccines typically contain an agent similar to the disease-causing microorganism. It is made from attenuated or killed forms of the pathogen itself, its toxins, orone of its surface proteins, and this substance stimulates the body's immune system to recognize, destroy, and memorize the agent. Thus, the immune system can more easily recognize and destroy any of these microorganisms that it will encounter later (3). Vaccines can induce humoral immunity through the production of antigen-specific antibodies as well as cellular immunity (4). Vaccines are divided into two main groups:

a) Live attenuated vaccines: This is an attenuated form of a wild virus or bacteria. The agent reproduces in the body, but since it is attenuated, it produces immunity without causing disease. They usually produce immunity in a single dose. Live vaccines are sensitive to heat and light. This group of vaccines should never be administered to immunocompromised individuals and

pregnant women. Tuberculosis, measles, rubella, mumps, chicken pox, and oral polio vaccines are examples of live vaccines.

b) Inactivated vaccines: These vaccines are produced from the whole, parts, or toxins of the agent. The desired level of immunity is achieved by administering more than one dose. Since antibody levels decrease over time, a booster dose is required. Diphtheria, tetanus, hepatitis B, and pneumococcal vaccines are examples of inactivated vaccines (5).

History of Immunization

The word "vaccine" is derived from "Vaccinia virus", a type of Poxvirus that was used to protect against smallpox (6). Benjamin Jesty observed that dairy women who contracted cowpox were protected from smallpox, and during an outbreak in 1774, he took material from a lesion on a cow's udder and vaccinated his wife and two sons (7). In 1796, based on this observation, Edward Jenner initiated the first systematic immunization. Jenner took a substance from the pustules of a person who had been exposed to smallpox and administered this substance under the skin on the arms or legs of a non-immune person, this method was called the vaccination method and protected against smallpox in a non-immune person (8).

This practice was applied in different parts of the world for centuries (9). In 1771, the wife of the British Ambassador wrote a letter to her friend in England, describing her observations in detail on the application of the variolation method in Istanbul to protect against smallpox. This document is the oldest known document on vaccination (10).

The people who applied this method did not know that the disease was caused by agents such as bacteria or viruses, and applied this method based entirely on observations. Following Jenner, Louis Pasteur (1822-1895) and Robert Koch (1843-1910) proved that there were some pathogenic microorganisms causing diseases (8). In 1885, Louis Pasteur proved that infectious diseases could be prevented by administering the rabies vaccine for the first time (9). In the second half of the nineteenth century, microorganisms causing diseases started to be identified. **History of vaccination and expanded program on immunization in Turkey**

The first vaccination in Turkey started in 1930 with the smallpox vaccination. Afterwards, the following vaccines were added to the current vaccination schedule: 1937: Diphtheria, Pertussis, 1952: BCG, 1963: Live polio, 1968: DPT, 1970: Measles, 1989: Polio Eradication Program, 1995: Polio National Vaccination Days, 1996: Measles Vaccine Acceleration Campaign, 1997: Polio Mop-up, 1998: Hepatitis-B Vaccination and the last polio case, 2003: Measles School Vaccination Days, 2004: Transition to Td

vaccine in all cases where tetanus vaccine is required for adults, 2005: Measles Vaccination Days, 2006: Addition of the Rubella, Mumps, and Hib vaccines to the program, introduction of Hepatitis B adolescent vaccination, 2007-2008:

Completion of Hepatitis B and Rubella vaccination of primary school age groups, 2008: Introduction of the five-component (DaBT-P/Hib) vaccine, November 2008: Introduction of the seven-component Conjugated pneumococcal vaccine into the program, February 2009: Elimination of maternal and neonatal tetanus, 2010: Introduction of DaBT-IPA vaccine in 1st grade primary education instead of Td and live polio vaccine, 2011 April: Introduction of 13- component conjugated pneumococcal vaccine, 2012 November: Hepatitis A vaccine and Varicella vaccine in February 2013 (11). The Expanded Program on Immunization Circular (EPI) states that each family physician and family healthcare professional should form a team to regularly follow the registered individuals on immunization within the relevant legislation. Family Physicians and Family Healthcare Professionals are responsible to the Community Health Center (CHC) for carrying out vaccination activities following the targets set in the EPI(12).

Immunization services in family medicine, relevant legislation and legal situation Turkey Article 7 of the Family Medicine Implementation Regulation states that family physicians should coordinate vaccination procedures by regularly monitoring the population under their care, taking necessary precautions regarding the storage of vaccines and cold chain, and working together with Community Health Centers to achieve national vaccination targets (13). In the Expanded Program on Immunization Circular, it was also stated that each family physician and family healthcare professional should form a team to regularly follow their registered individuals on immunization within the framework of the relevant legislation. Family Physicians and Family Healthcare Professionals are responsible to the CHC for carrying out vaccination activities following the targets set in the EPI (14). In Turkey, parents who did not want their children to receive any vaccine within the scope of the expanded vaccination schedule were reported to the Provincial Directorates of the Ministry of Family and Social Policies according to the Child Protection Law No. 5395, and health measures were taken for these children (15). However, with the individual application to the Constitutional Court for the decision taken on compulsory vaccination and the decision dated 11.11.2015, the Constitutional Court ruled that compulsory vaccination violated the right to protect and improve the corporeal and spiritual existence of the individual guaranteed in Article 17 of the

Constitution (16). Following this decision, in the regulation dated 19.01.2016 published by the Department of Vaccine-Preventable Diseases of the Public Health Institution of Turkey, it was deemed sufficient to fill out a form stating that vaccination is not allowed in case of refusal of compulsory vaccination (17). With this decision, vaccine refusal continues to increase in Turkey.

Conclusion

As a result; One of the most basic components of children and adults' right to a healthy life is the protection of them against diseases by vaccination. Immunization services are among the most important public health interventions for children in terms of preventing vaccine- preventable diseases and deaths. The current national vaccination program implemented in our country is a program that is successfully implemented in many ways and in which vaccines are used against many infectious agents.

Conflict of Interest

There is no conflict of interest between the authors.

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