ISTANBUL UNIVERSITY PRESS

EXPERIMED

Volume/Cilt **9** Issue/Sayı **3 December/Aralık** 2019

experimed.istanbul.edu.tr

E-ISSN 2667-5846

EXPERIMED

AVES

Publisher
İbrahim KARA

Publication Director
Ali ŞAHİN

Editorial Development
Gizem KAYAN

Finance and Administration
Zeynep YAKIŞIRER ÜREN

Deputy Publication Director
Gökhan ÇİMEN

Publication Coordinators
Betül ÇİMEN
Özlem ÇAKMAK
Okan AYDOĞAN
İrem SOYSAL
Arzu YILDIRIM

Project Coordinators
Sinem KOZ
Doğan ORUÇ

Graphics Department
Ünal ÖZER
Deniz DURAN
Beyzanur KARABULUT

Address: Büyükdere Cad. No: 105/9 34394 Mecidiyeköy, Şişli, İstanbul-Turkey
Phone: +90 212 217 17 00
Fax: +90 212 217 22 92
E-mail: info@avesyayincilik.com

EXPERIMED

EXPERIMED

CONTENTS

EXPERIMED

İÇİNDEKİLER

Experimed 2019; 9(3): 79-85

ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

DOI: 10.26650/experimed.2019.19020

Expression Levels of Inflammasome Complexes in Experimental Autoimmune Myasthenia Gravis Mouse Model (EAMG)

Deneysel Otoimmün Miyastenia Gravis Fare Modelinde İnflamazom Komplekslerinin Ekspresyon Seviyesi

Ceyda Nur Baltacı[1], Vuslat Yılmaz[2], Canan Ulusoy[2], Erdem Tüzün[2], Burçak Vural[1]

[1]Department of Genetics, İstanbul University Aziz Sancar Institute of Experimental Medicine, İstanbul, Turkey
[2]Department of Neuroscience, İstanbul University Aziz Sancar Institute of Experimental Medicine, İstanbul, Turkey

Cite this article as: Baltacı CN, Yılmaz V, Ulusoy C, Tüzün E, Vural B. Expression Levels of Inflammasome Complexes in Experimental Autoimmune Myasthenia Gravis Mouse Model (EAMG). Experimed 2019; 9(3): 79-85.

ABSTRACT

Objective: Despite the clues that myasthenia gravis (MG) disease may be associated with inflammasomes, there are no studies in the literature on MG disease and inflammasome complexes. Hence, to address this question, we investigated the possible participation of inflammasomes in experimental autoimmune myasthenia gravis mouse model (EAMG).

Material and Method: EAMG was induced in mouse using acetylcholine receptor (AChR) protein, and Anti-AChR IgG antibody levels detected by ELISA in the experimental group confirmed our model. Levels of *CASP1*, *IL-1β, NLRP3, P2X7R,* and *AKT1* of the experimental and control (complete Freund's adjuvant -CFA immunized) groups were measured by qRT-PCR.

Results: After immunization, the AChR IgG antibody levels were significantly higher in the AChR-immunized group than in the control group (p=0.042). *IL-1β* levels in the experimental group were significantly higher, compared to the control group (p=0.01). *CASP1, NLRP3,* and *P2X7R* levels were also higher compared to the control group. However, these differences did not attain statistical significance (p>0.05). *AKT1* levels were lower compared to the control group. There was no correlation between serum antibody concentration and gene expression levels.

Conclusion: Our results suggest that there might be inflammasome involvement in the pathology of MG disease. Increase in *IL-1β* levels indicates the importance of the inflammatory response; however, further studies are necessary to confirm this.

Keywords: Experimental autoimmune myasthenia gravis mouse model, inflammasome, myasthenia gravis

ÖZ

Amaç: Myastenia Gravis (MG) hastalığının inflamazomlarla ilişkili olabileceğine dair ipuçlarına rağmen literatürde MG hastalığı ve inflamazomlarla ilgili bir araştırma yer almamaktadır. Bu çalışmada, inflamazom kompleksinde yer alan genler ile hastalıktaki inflamatuvar yanıt arasındaki ilişkinin belirlenmesi hedeflenmiştir.

Gereç ve Yöntem: Deneysel otoimmün myastenia gravis (DOMG) modeli farelerde asetil kolin reseptör-(AChr) proteini kullanılarak oluşturuldu ve deney grubunda ELISA ile saptanan anti-AChR Ig seviyeleri modelimizi doğruladı. Deney ve kontrol (complete Freund's adjuvant-CFA) immünize grubunda *CASP1, IL-1β, NLRP3, P2X7R* ve *AKT1* gen ekspresyonu seviyeleri qRT-PCR ile incelendi.

Bulgular: İmmünizasyon sonrası AChR IgG antikor düzeyleri AChR-immünize grupta kontrollere göre anlamlı derecede yüksek belirlendi (p=0,042). Deney grubunda *IL-1β* seviyelerinin, kontrol grubuna kıyasla anlamlı derecede yüksek bulunmuştur (p=0,01). *CASP1, IL-1β, NLRP3* ve *P2X7R* seviyelerinin de kontrol grubuna göre arttığı fakat istatistiksel anlamlılığa ulaşmadığı tespit edilmiştir (p>0,05). *AKT1* seviyelerinin ise kontrol grubuna kıyasla azaldığı görülmüştür. Serum antikor düzeyleri ve gen ekspresyon seviyeleri arasında ise korelasyon saptanmamıştır.

Sonuç: Bulgularımız MG hastalığının patogenezinde inflamazom komplekslerinin rolü olabileceğini göstermiştir. *IL-1β* ekspresyon düzeyindeki anlamlı artış inflamasyon yanıtının önemine işaret etmektedir, fakat kesin bir kanıya varabilmek için bu konuda daha ileri çalışmalar yapılması gerektiği sonucuna ulaşmışıltır.

Anahtar Kelimeler: Deneysel otoimmün myastenia gravis fare modeli, inflamazom, myastenia gravis

Corresponding Author/Sorumlu Yazar: Burçak Vural **E-mail:** vburcak@istanbul.edu.tr
Received Date/Geliş Tarihi: 01.10.2019 **Revision Date/Revizyon Tarihi:** 09.10.2019 **Accepted Date/Kabul Tarihi:** 25.10.2019

INTRODUCTION

Myasthenia Gravis (MG) is a rare chronic autoimmune disorder caused by an autoimmune attack against the postsynaptic part of the neuromuscular junction (NMJ). The main characteristic feature of MG is muscle weakness, which can lead to death in its severe forms (1, 2). In MG disease, anti-AChR antibodies are targeted mostly at the acetylcholine receptor (AChR), which results in neuromuscular transmission failure. Anti-AChR antibodies are produced by B lymphocytes, but the exact cause of the autoimmune response in MG is still not known (3, 4).

Due to its economic and social burden, MG poses a significant health issue. Thus, treatments that contribute to the prevention and progression of MG are substantial. Current treatment is aimed at reducing symptoms. In this context, immunosuppressive drugs, plasmapheresis, thymectomy and supportive therapies are in use. However, these treatment methods cause adverse effects, such as opportunistic infections, osteoporosis, diabetes mellitus, and 2-3% of MG patients die due to these adverse effects. Therefore, there is a need for new treatment approaches with a more favorable adverse effect profile and a much more specific mechanism of action (5).

Inflammasomes are multimeric protein complexes that regulate the activation of caspase-1 (*CASP1*) and cause an inflammatory response. Inflammasomes act by activating caspase-1, which converts pro-inflammatory cytokine interleukin -1β (*IL-1β*) into its active form (6).

The experimental animal models generated by immunizations exhibit close clinical and histopathological similarities to MG. Hence, they are suitable for enlightening the pathogenesis of this autoimmune disease (7).

Despite the clues that (MG) disease may be associated with inflammasomes, there are no studies in the literature on MG disease and inflammasome complexes. Hence, to address this question, we investigated the possible participation of inflammasomes in EAMG pathogenesis.

MATERIAL AND METHOD

Mouse and Experimental Set-up

Mouse were obtained from Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Laboratory Animal Science, and their diet and care were carried out under the routine control in the barrier system chambers in this department. Ethics committee approval was given for this study by the Istanbul University Animal Experiments Local Ethics Committee (Decision No: 10.07.2017). We generated two groups of mouse; one group immunized with AChR and complete Freund's adjuvant (CFA) (n=8), and the other group immunized using only CFA as a control group (n=7).

Induction of EAMG

The AChR protein we used in this study was purified by affinity chromatography from Torpedo Californica, and supplied by Dr. Premkumar Christadoss from Texas University. The purity of the protein before immunization was controlled using gel electrophoresis. Before the immunization procedure, propofol diluted with PBS (1:5) was administered intraperitoneally at 20 µl/g per mouse as an anesthetic. The basal weights of all animals before each immunization were recorded, and their weights were measured once a week until the mouse were sacrificed on the termination day. To mimic MG disease in 8 week old male C57Bl/6J (B6) mouse, a mixture of 40 µg of AChR protein (in 100 µl phosphate buffer) and CFA containing 100 µg of Mycobacterium butyricum (100 µl) was prepared, and this 200 µl emulsion was injected subcutaneously into one side of the leg and shoulder on the day 0. The same protocol was applied to the control group with CFA-only immunized mouse. The mouse were monitored. All animals were immunized three times in four weeks. A small amount of blood was withdrawn from the tail vein of the mouse after each immunization, and antibodies in the sera were detected using ELISA. Blood was collected from mouse 10 days after the last immunization, and the serum was stored at -80°C until used.

ELISA

Serum samples were collected after the second and third immunizations, and Anti-AChR IgG antibody levels of AChR-immunized mouse and CFA-only immunized mouse were evaluated by ELISA, using a previously described method (3).

RNA Isolation

RNA was isolated from lymph nodes using RNAzol®RT (MRC, Cincinnati, USA). RNA concentrations, and quality and quantity of all samples were measured by Thermo Scientific Nanodrop 2000 at 260/280 and 260/230 wavelengths. RNAs were stored at -20°C prior to use.

cDNA Synthesis and qRT-PCR (Quantitative Real Time PCR)

cDNA synthesis was performed using commercial Jena Bioscience script cDNA synthesis kit (Jena Bioscience, Jena, Germany), according to manufacturer's instructions. 2 µl of extracted RNA reverse-transcribed into cDNA using reverse transcriptase in a final reaction volume of 20 µl, and amplified respectively for 1 hour at 50°C, 10 minutes at 42°C, 10 min at 70°C and 33 min at 4°C using thermal cycler (Bio-Rad, California, USA).

qRT-PCR was performed to determine the expression levels of *CASP1*, *IL-1β*, *NLRP3* (NLR family pyrin domain containing 3), *P2X7R* (P2X purinoceptor 7 receptor), and *AKT1*(RAC-alpha serine/threonine-protein kinase) genes using qPCR GreenMaster with UNG/lowROX kit (Jena Bioscience, Jena, Germany). Amplification was performed using the CFX Connect Real-Time PCR Detection System (Bio-Rad, California, USA). Data was normalized to GADPH. The $2^{(-\Delta\Delta CT)}$ method was used for relative quantification. Gene-specific primers for qRT-PCR were designed using NCBI Primer Blast tool, and synthesized as the sequences listed in Table 1.

Statistical Analysis

Antibody and gene expression levels were compared by student's t-test, and correlation analysis was done by Pearson test. $p<0.05$ was considered as statistical significant.

Table 1. The forward and reverse primer sequences used for qRT-PCR

Gene name	Forward primer	Reverse primer
AKT1	5′TAGGCCCAGTCGCCCG 3′	5′ AGGTGCCATCGTTCTTGAGG 3′
P2X7R	5′CCTAGGTGAGGGTTTGCTGT 3′	5′GGTGTGCACGGAGCTGATAA 3′
CASP1	5′GGACCCTCAAGTTTTGCCCT 3′	5′GCAAGACGTGTACGAGTGGT 3′
IL-1β	5′TGTCTTTCCCGTGGACCTTC 3′	5′TCATATGGGTCCGACAGCAC 3′
NLRP3	5′TCCCAGACACTCATGTTGCC 3′,	5′GTCCAGTTCAGTGAGGCTCC 3′
GAPDH	5′AGCTACTCGCGGCTTTACG 3′	5′AATCCGTTCACACCGACCTT 3′

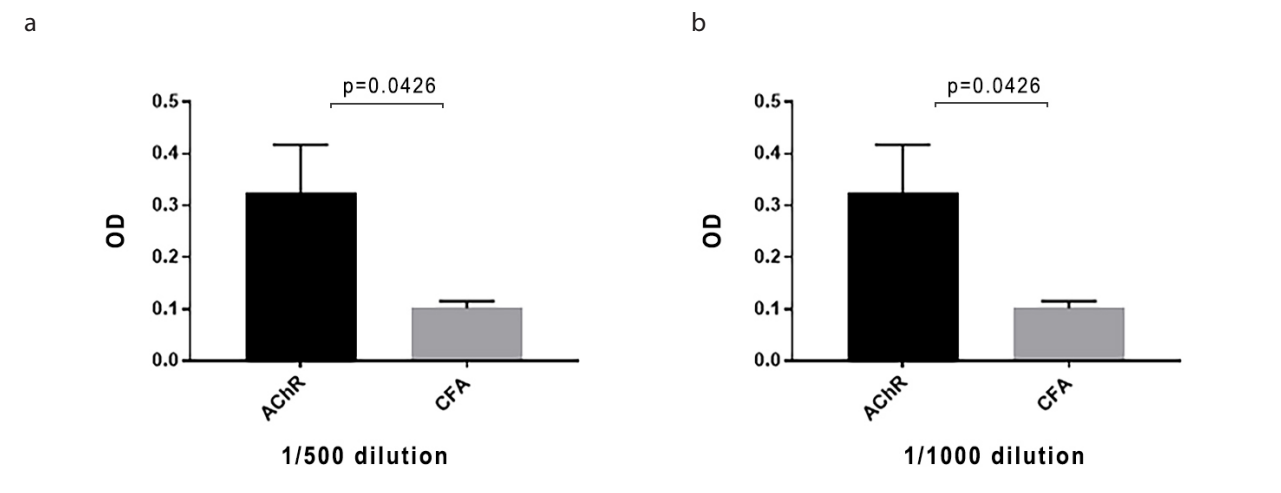


Figure 1. a, b. The AChR IgG antibody levels measured by ELISA

RESULTS

ELISA results of Experimental Autoimmune Myasthenia Gravis Mouse Model

The AChR IgG antibody levels were determined at two different concentrations as 1/500 and 1/1000, respectively. These two concentrations were consistent. After the second and the third immunization, AChR IgG antibody levels were significantly higher in the AChR-immunized group than in the control group for both dilutions (p=0.042) (Figure 1. a, b).

Expression Levels of *CASP1*, *IL-1β*, *NLRP3*, *P2X7R and AKT1* genes

The expression of the *IL-1β* gene showed a statistically significant difference between the experimental and control groups (Figure 2) (p=0.015). Nevertheless, *CASP1*, *NLRP3,* and *P2X7R* genes showed trends towards higher expression levels in the AChR-immunized mouse, whereas *AKT1* gene expression levels were higher in the CFA group (Figure 3-6).

There was no correlation between expression and antibody levels.

DISCUSSION

In this study, expression levels of *CASP1*, *IL-1β*, *NLRP3*, *P2X7R*, and *AKT1* genes in EAMG were investigated for the first time to elucidate the role of inflammasome complexes in the pathogenesis of MG.

The anti-AChR IgG antibody detection in the sera of the experimental group immunized with AChR showed that we successfully induced the EAMG model in mouse. The mean value of serum acetylcholine IgG (AChR) in this experimental group was significantly higher than in the control group.

Many studies suggest the presence of a genetic relationship between autoimmune diseases and variations in genes encoding inflammasome components. However, no such study has been conducted so far in MG disease. *IL-1β* is a pro-inflammatory cytokine produced by activated macrophages, endothelial cells, B cells and fibroblasts. *IL-1β* elicits immune and inflammatory response (8). The caspase-1 inhibitor significantly ameliorates the symptoms of the disease in the EAMG via the *IL-1β* and *IL-17* pathway. This finding suggests that *CASP1* fulfils an important role in the etiology of the disease (9).

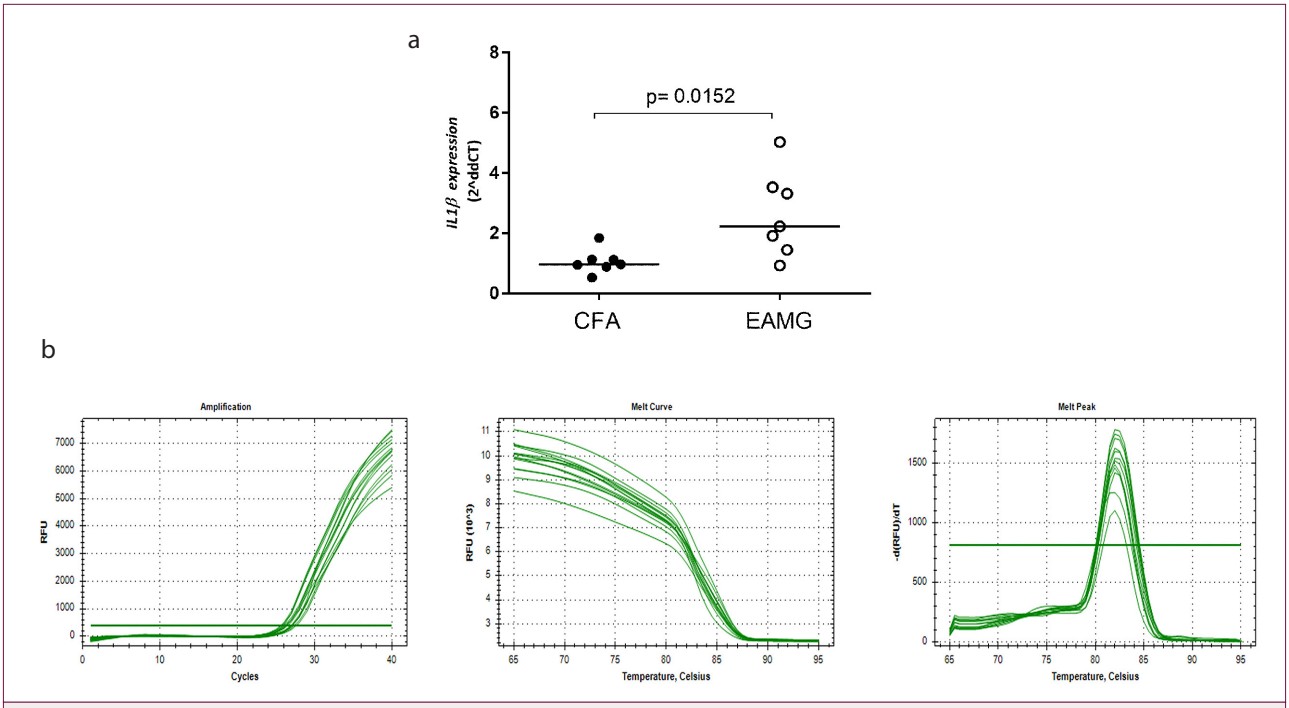


Figure 2. a, b. Comparison of expression levels of EAMG and control group for IL-1β gene (a). Gene profile and melting curves for IL-1β (b)

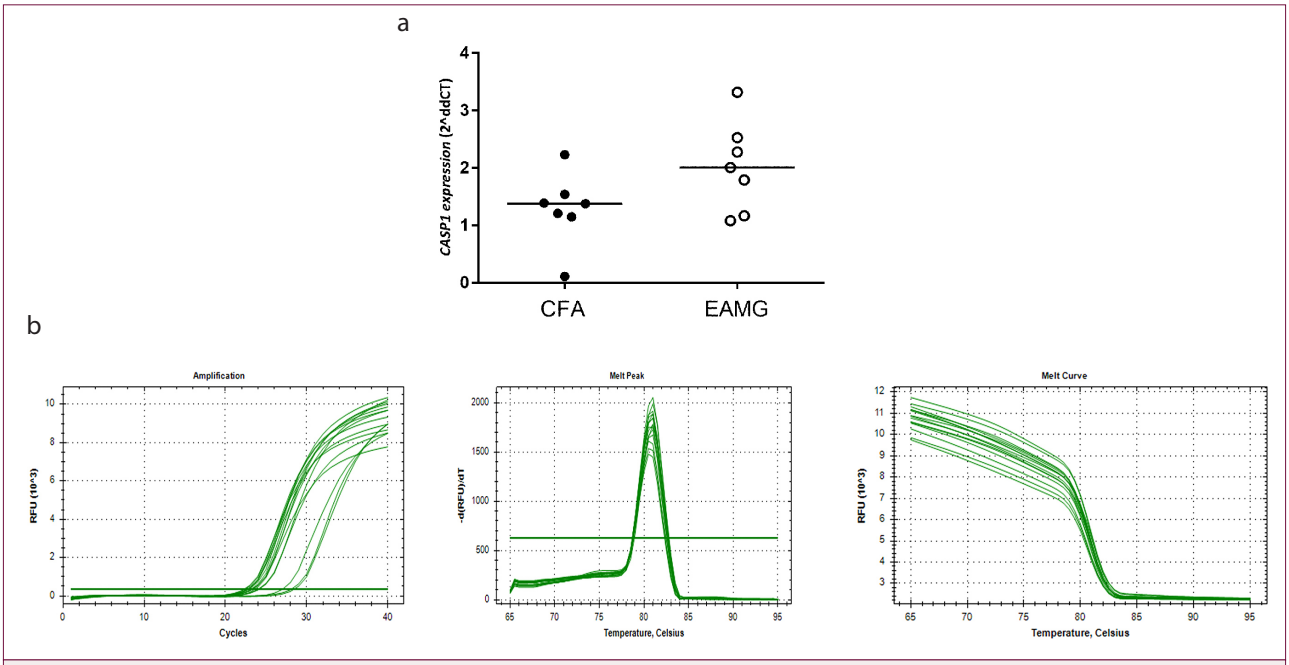


Figure 3. a, b. Comparison of expression levels of EAMG and control group for CASP1 gene (a). Gene profile and melting curves for CASP1 (b)

Considering that the inflammasomes act by activating *CASP1*, which converts *IL-1β* to its active form, an increase in the expression of *IL-1β* and *CASP1* is expected in EAMG. Consistently, the significant increase in the expression level of *IL-1β* (p=0.01) in EAMG compared to the control group indicates the importance of the inflammation response. Furthermore, we detected the increase in the levels of *CASP1*, *NLRP3* and *P2X7R*; whereas decrease in the levels of Akt-1 in the EAMG model compared to the control group. There was no correlation between serum antibody concentration and the expression levels of any gene that were used in this study. Unlike *IL-1β*, the increase in the genes of *CASP1*, *NLRP3,* and *P2X7R* did not attain statistical significance,

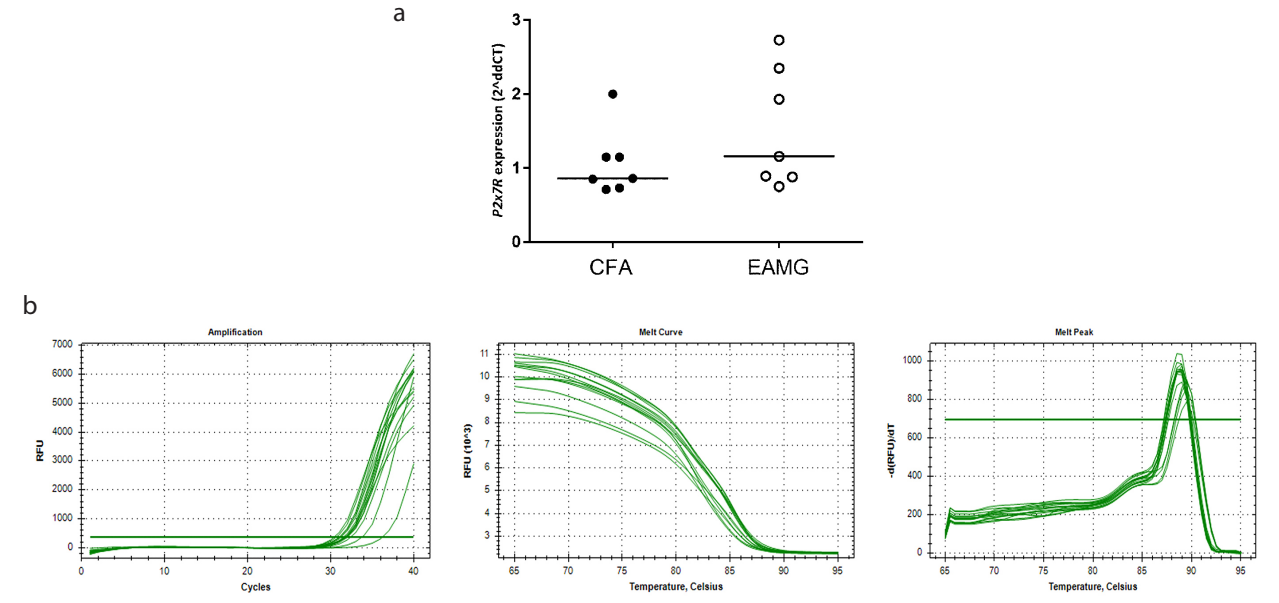


Figure 4. a, b. Comparison of expression levels of EAMG and control group for P2X7R gene (a). Gene profile and melting curves for P2X7R (b)

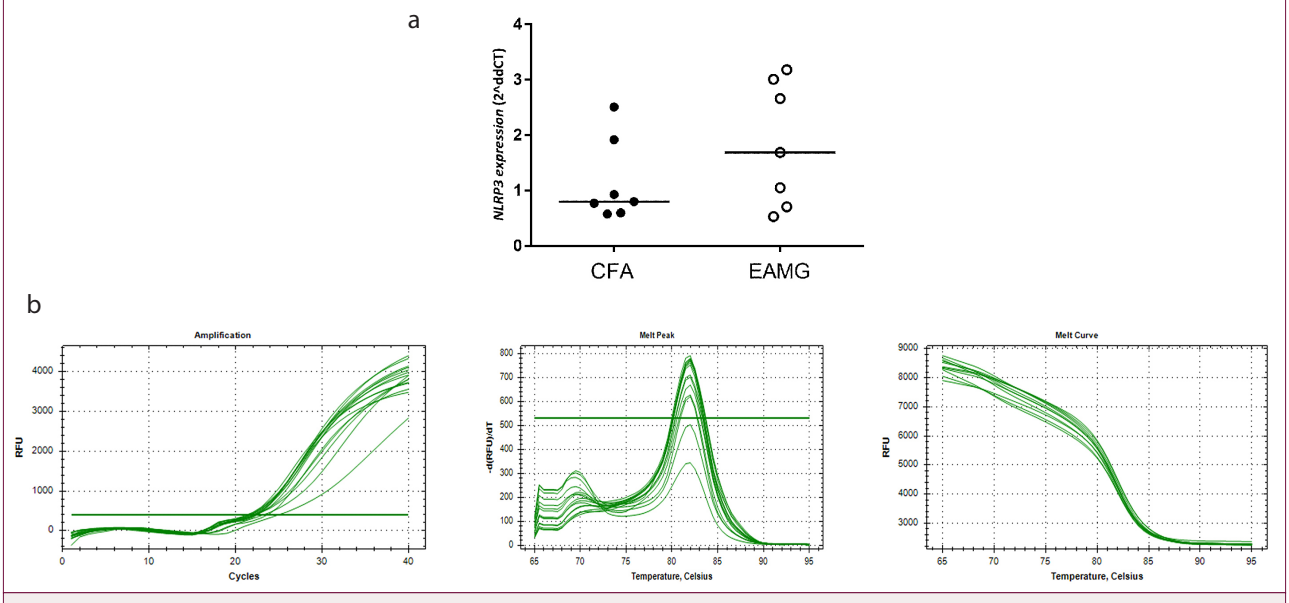


Figure 5. a, b. Comparison of expression levels of EAMG and control group for NLRP3 gene (a) Gene profile and melting curves for NLRP3 (b)

which may have occurred due to the low number of mouse included in our study. *IL-1β* activity is mainly mediated by four cellular signaling pathways. Three of these belong to the MAP kinase (MAPK) pathway. This pathway is mediated by three major enzymes: c-Jun NH2-terminal kinase (JNK) 1/2 (jun kinases), 38-kd protein kinases (p38) and (ERK) 1/2. The fourth signaling pathway that mediates *IL-1β* is the NF-κB pathway (10).

The fact that the expression levels of inflammasome complexes did not increase as much as *IL-1β* levels indicate the involvement of other signaling pathways that activate this cytokine should be taken into account. Moreover, it is known that NF-κB signals regulate the immune response in MG disease, and the PI3K/Akt pathway activates these signals. The PI3K/Akt pathway is important for up-regulation of *P2X7R* expression, which is known to activate caspase-1 (11). A study showed that *P2X7R* expression was increased in blood samples of MG patients which was consistent with our finding (12).

As it is well known, the Akt pathway plays a role in muscle physiology (13). In our study, *AKT1* gene expression levels were decreased in the AChR-immunized group. One possible expla-

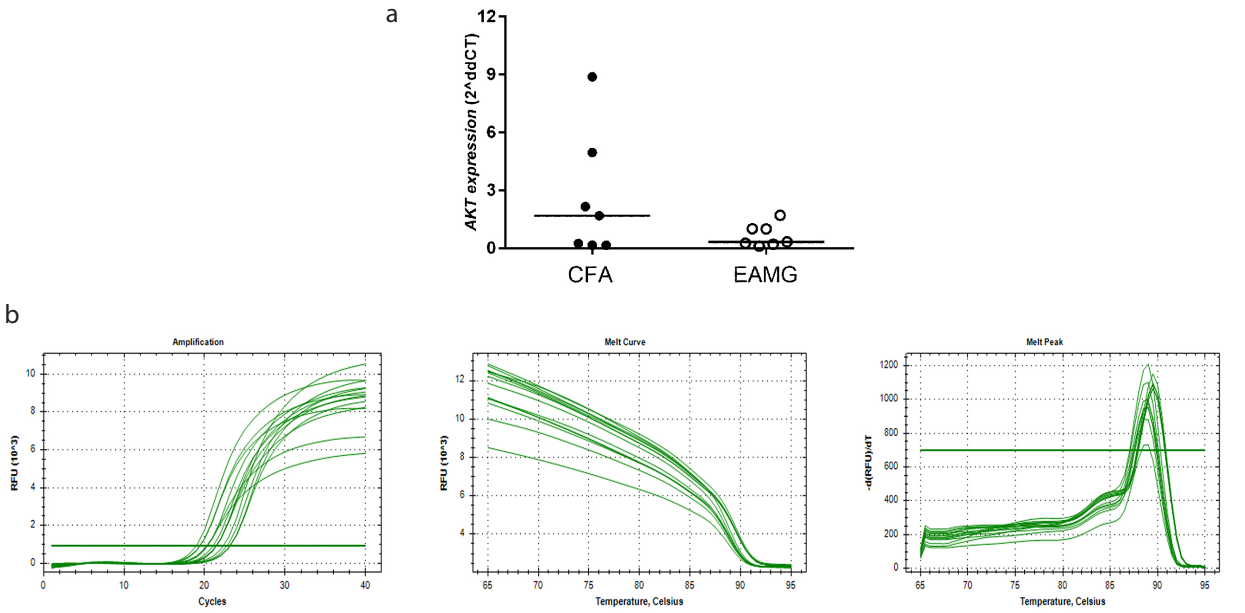


Figure 6. a, b. Comparison of expression levels of EAMG and control group for AKT1 gene (a) Gene profile and melting curves for AKT1 (b)

nation for the decrease in *AKT1* levels may be due to compensation for the inflammation process. This study might highlight the significance of inflammasome complexes in the pathogenesis of MG, and shed light into other studies in this field.

Ethics Committee Approval: Ethics committee approval was received for this study from the Local Ethics Committee of İstanbul University Animal Experiments (10.07.2017).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - E.T., B.V.; Supervision - B.V.; Materials - C.N.B., C.U., V.Y.; Data Collection and/or Processing - C.N.B.; Analysis and/or Interpretation - C.N.B., E.T., B.V., V.Y.; Literature Search - C.N.B.; Writing - C.N.B.; Critical Reviews - E.T., B.V., V.Y.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The present work was supported by a grant from the Scientific Research Projects Coordination Unit of İstanbul University (Project No: TYL-2017-27428).

Etik Komite Onayı: Bu çalışma için etik komite onayı İstanbul Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'ndan (10.07.2017) alınmıştır.

Hasta Onamı: Uygulanabilir değil.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir - E.T., B.V.; Denetleme - B.V.; Gereçler - C.N.B., C.U., V.Y.; Veri Toplanması ve/veya İşlemesi - C.N.B.; Analiz ve/veya Yorum - C.N.B., E.T., B.V., V.Y.; Literatür Taraması - C.N.B.; Yazan - C.N.B.; Eleştirel İnceleme - E.T., B.V., V.Y.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Bu çalışma İstanbul Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından desteklenmiştir (Proje No: TYL-2017-27428).

REFERENCES

1. Tüzün E, Yılmaz V, Parman Y, Oflazer P, Deymeer F, Saruhan-Direskeneli G. Increased complement consumption in MuSK-antibody-positive myasthenia gravis patients. Med Princ Pract 2011; 20: 581-3. [CrossRef]
2. Vincent A. Unravelling the pathogenesis of myasthenia gravis. Nat Rev Immunol 2002; 2: 797-804. [CrossRef]
3. Tüzün E, Scott BG, Goluszko E, Higgs S, Christadoss P. Genetic evidence for involvement of classical complement pathway in induction of experimental autoimmune myasthenia gravis. J Immunol; 171: 3847-54. [CrossRef]
4. Sahashi K, Engel AG, Linstrom JM, Lambert EH, Lennon VA. Ultrastructural localization of immune complexes (IgG and C3) at the end-plate in experimental autoimmune myasthenia gravis. J Neuropathol Exp Neurol 1978; 37: 212-23. [CrossRef]
5. Conti-Fine BM, Milani M, Kaminski HJ. Myasthenia gravis: past, present, and future. J Clin Invest 2006; 116: 2843-54. [CrossRef]
6. Guo H, Callaway JB, Ting JP. Inflammasomes: mechanism of action, role in disease, and therapeutics. Nat Med 2015; 21: 677-87. [CrossRef]
7. Christadoss P, Poussin M, Deng C. Animal models of myasthenia gravis. Clin Immunol 2000; 95: 75-87. [CrossRef]
8. Zhang JM, An J. Cytokines, inflammation and pain. Int Anesthesiol Clin 2007; 45: 27-37. [CrossRef]
9. Wang CC, Li H, Zhang M, Li XL, Yue LT, Zhang P, et al. Caspase-1 inhibitor ameliorates experimental autoimmune myasthenia gravis by innate dendric cell IL-1-IL-17 pathway. J Neuroinflammation 2015; 10.1186/s12974-015-0334-4. [CrossRef]

10. Fan Z, Söder S, Oehler S, Fundel K, Aigner T. Activation of Interleukin-1 signaling cascades in normal and osteoarthritic articular cartilage. Am J Pathol 2007; 171: 938-46. **[CrossRef]**
11. Hoesel B, Schmid JA. The complexity of NF-κB signaling in inflammation and cancer. Mol Cancer 2013; 12: doi: 10.1186/1476-4598-12-86. **[CrossRef]**
12. Zhang Y, Zhang Y, Li H, Jia X, Zhang X, Xia Y, et al. Increased expression of P2X7 receptor in peripheral blood mononuclear cells correlates with clinical severity and serum levels of Th17-related cytokines in patients with myasthenia gravis. Clin Neurol Neurosurg 2017; 157: 88-94. **[CrossRef]**
13. Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. Nat Cell Biol 2001; 3: 1014-9. **[CrossRef]**

Experimed 2019; 9(3): 86-92

ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

DOI: 10.26650/experimed.2019.19021

Correlation Between Salivary Anxiety Markers and Salivary Biochemical Markers in Children with Primary and Mixed Dentition

Süt ve Karışık Dişlenme Dönemindeki Çocuklarda Tükürük Anksiyete Belirteçleri ve Tükürük Biyokimyasal Belirteçler Arasındaki Korelasyon

Sehkar Oktay[1], Sezin Demirel[2], Ünsal Veli Üstündağ[3], Betül Korkmaz[1], Gizem Eskiocak[1], Serap Akyüz[2], Ebru Emekli Alturfan[1]

[1]Department of Basic Science Biochemistry, Marmara University School of Dentistry, İstanbul, Turkey
[2]Department of Pediatric Dentistry, Marmara University School of Dentistry, İstanbul, Turkey
[3]Department of Medical Biochemistry, Medipol University School of Medicine, İstanbul, Turkey

Cite this article as: Oktay S, Demirel S, Üstündağ ÜV, Korkmaz B, Eskiocak G, Akyüz S, et al. Correlation Between Salivary Anxiety Markers and Salivary Biochemical Markers in Children with Primary and Mixed Dentition. Experimed 2019; 9(3): 86-92.

ABSTRACT

Objective: Anxiety due to the dentist and dental treatment is a problem encountered in many children. The aim of the present study is to determine salivary nitric oxide, lactoferrin, α-amylase and cortisol levels of children in primary and mixed dentition, and to evaluate their relation with stress due to dental treatment.

Material and Method: The study consisted of 50 children in primary and mixed dentition. The children were evaluated clinically and according to Frankl Behavior Rating Scale. Salivary flow rate was calculated, and nitric oxide, lactoferrin, α-amylase and cortisol levels were measured in saliva.

Results: 68% percent of the children were found to be negative according to the Frankl Behavior Rating Scale (category 2), and significantly decreased salivary flow rate was evident in these children when compared with children that were categorized as completely negative (category 1). The DMFT+dft index was 7.56±4.29, and positive correlations were found between DMFT+dft indices and salivary nitric oxide, lactoferrin, cortisol and α-amylase levels ($p<0.05$). These parameters were not different between genders. Positive correlations were found between salivary nitric oxide and α amilase, cortisol and α amilase, cortisol and lactoferrin; and also between α-amilase and lactoferrin levels ($p<0.05$).

Conclusion: Salivary lactoferrin, α-amylase and cortisol may be suggested as important parameters of oral health.

Keywords: Anxiety, biochemical markers, saliva

ÖZ

Amaç: Diş hekimi ve diş tedavisine bağlı anksiyete birçok çocukta karşılaşılan bir sorundur. Bu çalışmanın amacı, süt ve karışık dişlenme dönemindeki çocuklarda tükürük nitrik oksit, laktoferrin, α-amilaz ve kortizol seviyelerini tespit etmek ve diş tedavisinden kaynaklanan stresle ilişkilerini değerlendirmektir.

Gereç ve Yöntem: Çalışma süt ve karışık dişlenme dönemindeki 50 çocuktan oluşmaktadır. Çocuklar klinik olarak ve Frankl Davranış Değerlendirme Ölçeğine göre değerlendirildi. Tükürük akış hızı hesaplandı ve tükürükte nitrik oksit, laktoferrin, α-amilaz ve kortizol seviyeleri ölçüldü.

Bulgular: Çocukların %68'i Frankl Davranış Değerlendirme Ölçeğine göre negatif (Kategori 2) olarak bulundu ve bu çocuklarda, tamamen negatif olarak sınıflandırılan çocuklarla karşılaştırıldığında (Kategori 1) anlamlı derecede azalmış tükürük akış hızı belirlendi. DMFT + dft indeksi 7,56±4,29 idi ve DMFT + dft indeksleri ile tükrük nitrik oksit, laktoferrin, kortizol ve α-amilaz seviyeleri arasında pozitif korelasyon bulundu. Bu parametreler cinsiyetler arasında farklı değildi. Tükürük nitrik oksit ile amilaz, kortizol ve amilaz, kortizol ve laktoferrin ve ayrıca a-amilaz ve laktoferrin seviyeleri arasında pozitif korelasyon bulundu.

Sonuç: Tükürük laktoferrin, α-amilaz ve kortizol, ağız sağlığı ve anksiyete için önemli parametreler olarak önerilebilir.

Anahtar Kelimeler: Anksiyete, biyokimyasal belirteçler, tükürük

Corresponding Author/Sorumlu Yazar: Sehkar Oktay **E-mail:** nsehkar@yahoo.com
Received Date/Geliş Tarihi: 27.09.2019 **Revision Date/Revizyon Tarihi:** 23.11.2019 **Accepted Date/Kabul Tarihi:** 08.11.2019

Experimed 2019; 9(3): 86-92

INTRODUCTION

The importance of saliva as a non-invasive diagnostic fluid has increased in recent years. Accordingly, there is constantly increasing evidence supporting the use of saliva as a non-invasive tool for monitoring biomarkers in health and pathological human status (1). Saliva has unique functions to maintain dental health and in protecting against the harmful effects of microorganisms.

The composition of saliva consists of hormones, peptides, electrolytes, mucus, antibacterial compounds and different enzymes, as well as organic and inorganic compounds (2). Cortisol is one of the most important steroid hormones detectable in saliva (2). Lactoferrin and α-amylase, together with immunoglobulins, are the markers of mucosal immunity that are detectable in saliva. Lactoferrin is available in a variety of body fluids, including saliva. It is an iron-binding glycoprotein and protects the organism from infectious diseases by directly passing pathogens such as bacteria through the oral cavity viruses. Lactoferrin is present in the first line of defense in the face of pathogens in the mouth mucosa (3).

Nitric oxide (NO) is a short-lived gas that acts as a strong reactive radical, NO takes part in the defensive mechanisms of the oral cavity. Accordingly, antimicrobial effects of salivary NO metabolites, nitrates and nitrites, on protection against oral diseases, have been shown in recent years (4).

Cortisol, the main glucocorticoid of the organism, is an important component of the reactions called 'stress response', and can be reliably detected in saliva. Salivary cortisol is measured and used as a biomarker of psychological stress. On the other hand, salivary cortisol does not only reflect the hypothalamus-pituitary-adrenal axis (HPAA); different factors regulating HPAA reactivity such as the hippocampus, hypothalamus, pituitary and adrenals, as well as their modulators, receptors, or binding proteins, have all been reported to affect salivary cortisol measurements (5).

α-Amylase is an enzyme found in saliva that digests starch. Salivary α-amylase has also been shown to play a role in the digestive function, as well as the ability to fight bacteria in the mouth (6). In recent years, salivary α-amylase has been shown to be closely related to stress like cortisol, and has been suggested to increase in patients with chronic psychosocial stress, and may be used as a biomarker of chronic stress (7).

Dental anxiety, which is a major problem in pediatric dentistry, is more specific and important than general anxiety, and is a reaction to bad dental experiences. Dental fear and anxiety are problems that affect large populations, especially children (8). Avoidance of treatments and dental care may lead to serious consequences that adversely affect the oral health of the patients. It is important for dentists to identify the fearful patient group and patients who need special attention. Children express their anxiety in different ways, and dental anxiety in children should be assessed as early as possible (8).

Many methods have been developed for evaluating dental fear, in order to obtain the feelings hidden unconsciously. Detecting the anxiety level of patients and treating them accordingly, will have a positive effect on the patient's treatment experience and dental health (9). We hypothesized that biochemical parameters, such as cortisol, α-amylase, lactoferrin and nitric oxide, might be related to anxiety levels in children.

In this study, we aimed to evaluate the levels of salivary NO, lactoferrin, α- amylase and cortisol in children with primary and mixed dentition, and to assess the link between their behavior, evaluated according to the Frankl Behavior Rating Scale.

MATERIAL AND METHOD

Subject Population

The study consisted of 50 children (30 girls and 20 boys), in primary (n=25, aged 5-7 years) and mixed (n=25, aged 9-11 years) dentition, who visited the Department of Pedodontics in Marmara University School of Dentistry. Children who did not have past systemic illness and undergoing any dental treatment, nor were taking drugs at least 6 months, were included the study.

Calculation of DMFT+dft Index

According to the clinical examination conducted at Marmara University School of Dentistry Pedodontic Clinic, for the permanent teeth of children; DMFT index was calculated by number of decayed, missing and filled permanent teeth, and dmft for number of decayed, missing and filled primary teeth. The children that were selected for this study at the Pedodontics clinic had not started treatment yet, had no systematic disease, and had not used antibiotics for the last month.

Frankl Behavior Rating Scale

Behavior assessment of the children was done using the Frankl Behavior Rating Scale (10).

Category 1: Absolutely negative: The child refuses treatment fearfully and shows a marked negative.

Category 2: Negative: The child is reluctant to accept treatment and there is a sign of negative attitude although it is not evident.

Category 3: Positive: The child accepts the treatment but there is a sign of being undecided. They listen to the dentist's message, but there are some suspicions.

Category 4: Absolutely positive: The child is in good agreement with the dentist and is involved in the dental procedures.

Collection of Saliva

Two hours before salivary collections, the children were requested to avoid eating food and drinking beverages. Whole saliva was collected by spitting into a tube. Saliva samples which were collected from children with dental examinations done by a pedodontist, were stored at -20˚C until analyses were made in the Basic Medical Science, Department of Biochemistry.

Salivary Flow Rate Measurement

The salivary flow rate of the samples was calculated as saliva volume (mL) collected per minute, using saliva collection volume and saliva collection time.

Determination of Nitric Oxide

Nitric oxide (NO) determination is based on reducing nitrate to nitrite by vanadium (III) chloride. In an acidic media, nitrite and sulfonylamide reacted with N-(1-Naphtyl) ethylenediaminedihydrochloride, and complex diazonium compound was formed. The colored complex was measured at 540 nm by a spectrophotometer, and the results were expressed as µmol NO/dL (11).

Determination of Lactoferrin

The salivary lactoferrin level was measured by ELISA commercial kit, using lactoferrin- specific polyclonal antibody (Catalog no: EL 2011-1 AssayMax Human Lactoferrin ELISA KIT 96 Test Assaypro, St. Charles, MO, USA). The process followed the manufacturer's instructions. Briefly, standard and diluted samples were adsorbed in a polystyrene 96 well microplate and incubated for 2h at 25ºC. After five-times repeated washing of wells with wash buffer, a biotinylated lactoferrin antibody was added to each well, and incubated for 1h.

After washing the microplate, 50 µl of streptavidin-peroxidase conjugate was added per well, and incubated for 30 min. Subsequently, the third washing was applied. 50 µl chromogenic substrate was used per well for detection, and incubated for 15 min. After 50 µl of stop solution was added, the plate was read at a wavelength of 450 nm, using on a microplate reader.

Determination of α-Amylase

The α-amylase assay was performed using a commercial kit (Catalog no: 1-1902 Salivary Alpha-amylase kinetic Research, Salimetrics, LLC, USA). The process followed the manufacturer's instructions. Briefly, the plate reader was set to incubate at 37ºC. Controls and samples were adsorbed in 96 well microtiter plates. 320 µl of the preheated (37ºC) α-Amylase Substrate was added to each well simultaneously, using a multichannel pipette.

Then, a timer was started immediately, and mixed (500 rpm) at 37ºC. The plate was transfered to the reader in time, the Optical Density (OD) was read at a wavelength of 405 nm exactly 1 min.

After saving the 1 min. OD readings, the plate was transfered to the reader again, the OD was read at a wavelength of 405 nm exactly 3 min., and saved.

Table 1. The results of the Frankl Behavior Scale

Category	%
1	10% (n=5)
2	68% (n=34)
3	22% (n=11)
4	0

Determination of Cortisol

The Cortisol level in saliva was assessed using the commercial kit by EIA (Enzyme Immun Assay) method (Catalog No:1-3102 Salivary ER Cortisol EIA Kit Diagnostic, Salimetrics, State College, PA). All reagents were brought to room temperature and mixed before use. Standards and samples were adsorbed in 96 well microtiter plates, then 200 µl of diluted enzyme conjugate were added to each well. After, the plate was mixed on a plate rotator for 5 min. at 500 rpm, and incubated at room temperature for 1h. After washing the plate 4 times, 200 µl of TMB Substrate Solution were added to each well and mixed on a plate rotator for 5 min. at 500 rpm, then, the plate was incubated in the dark (covered) at room temperature for 25 min. After 50 µl of stop solution were added, the plate was mixed on a plate rotator for 3 min. at 500 rpm, and read at a wavelength of 450 nm, using on a microplate reader.

Statistical Analysis

For all statistical analysis, GraphPad Prism 5.0 (GraphPad Software, San Diego, USA) was used. All data were expressed as mean ± standard deviations (SD). The Kruskal Wallis test was used for the comparison of groups of data, followed by Dunn's multiple comparison tests. An unpaired, two tailed Student's T Test was used to compare two independent groups. Correlation analysis of clinical and laboratory data was performed by Spearman test. A value of $p<0.05$ was considered significant.

RESULTS

Anxiety Results

The results of the Frankl Behavior Rating Scale, which assessed fear situations of the children participating in the study before and during dental procedures, are shown in Table 1.

According to Frankl Behavior Rating Scale results, category 1 (absolutely negative) is 10%, category 2 (negative) is 68%, category 3 (positive) is 22% and category 4 (absolutely positive) is 0%.

Clinical and Biochemical Results

The salivary flow rate and DMFT+dft index, NO, lactoferrin, α-amylase and cortisol values of the children participating in the study are given in Table 2. The children's salivary flow rate averaged 0.57 ± 0.34 ml/min and the DMFT+dft index averaged 7.56 ± 4.29. The lowest value of these parameters was 0, the highest were 1.5 and 17, respectively. The average, lowest and highest levels of NO (µmol/dL): 193.0±55.09; 122.26 and 286.78 respectively, lactoferrin (ng/mL): 8.93±3.75; 3.73 and 16.26 respectively, α-amylase (U/mL): 57.37±30.33; 22.30 and 123.3 respectively, and cortisol (ug/dL): 0.64±0.22; 0.25 and 0.99 respectively. There was no significant difference between girls and boys in terms of the parameters examined (Table 2).

The comparative results of salivary flow rate, DMFT-dft index and salivary nitric oxide, cortisol, α-amylase and lactoferrin values of children participating in the study, according to the Frankl Behavior Rating Scale, are given in Figure 1. Salivary flow rate decreased significantly in the Frankl 2 group compared to the Frankl 1 group (p=0.004) (Figure 1a).

Table 2. The salivary flow rate and DMFT+dft index, nitric oxide, lactoferrin, α-amylase and cortisol values of the children

	Average (n=50)	Lowest Level (n=50)	Highest Level (n=50)	Girls (n=30)	Boys (n=20)
Salivary flow rate (mL/min)	0.57±0.34	0	1.5	0.53±0.35	0.63±0.34
DMFT+dft	7.56±4.29	0	17	7.87±4.31	7.1±4.33
Nitric oxide (µmol/dL)	193.0±55.09	122.26	286.78	191.0±57.73	190.5±52.63
Lactoferrin (ng/mL)	8.93±3.75	3.73	16.26	8.97±3.54	8.87±4.16
A-Amylase (U/mL)	57.37±30.33	22.30	123.3	58.05±32.62	56.35±27.32
Cortisol (ug/dL)	0.64±0.22	0.25	0.99	0.65±0.23	0.62±0.21

Table 3. The results of correlation between the parameters examined

	Salivary flow rate (mL/min)	DMFT-dft	Nitric Oxide (µmol/dL)	Lactoferrin (ng/mL)	α-Amilase (U/mL)	Cortisol (ug/dL)
Salivary flow rate (mL/min)		-0.106	-0.066	-0.094	-0.163	-0.035
DMFT-dft	-0.106		0.902*	0.890*	0.884*	0.882*
Nitric Oxide (µmol/dL)	-0.066	0.902*		0.105	0.937*	0.204
Lactoferrin (ng/mL)	-0.094	0.890*	0.105		0.820*	0.810*
α-Amilase (U/mL)	-0.163	0.884*	0.937*	0.820*		0.849*
Cortisol (ug/dL)	-0.035	0.882*	0.204	0.810*	0.849*	

p<0.001

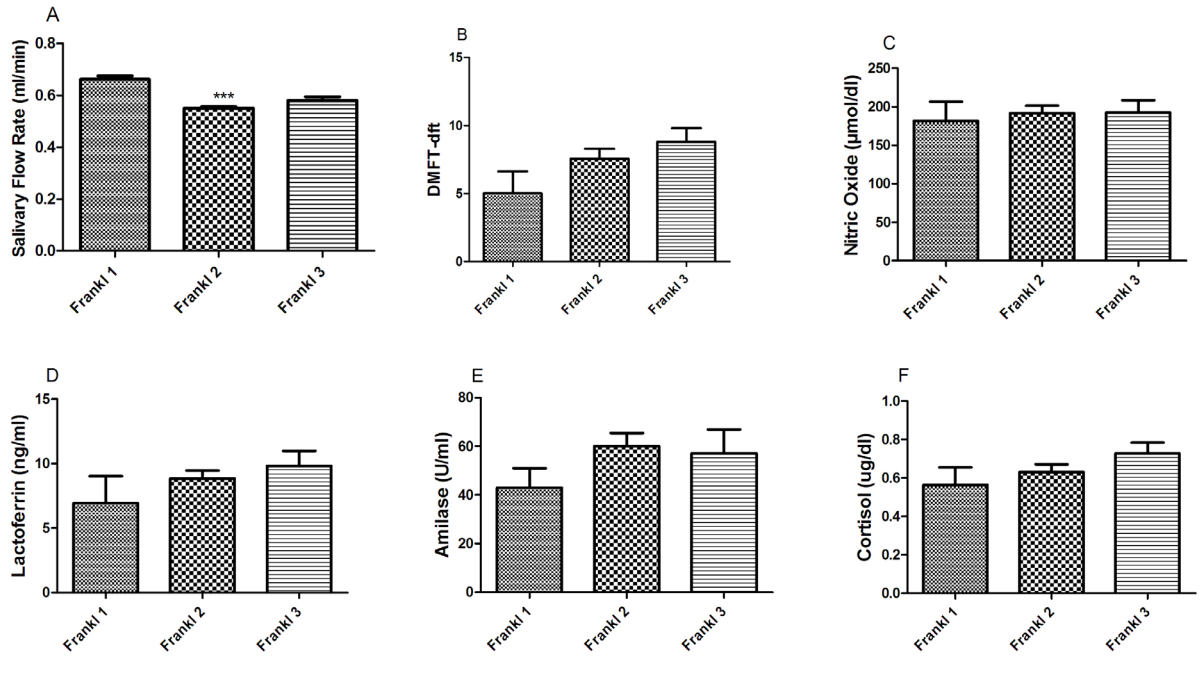


Figure 1. The comparative results of salivary flow rate, DMFT-dft index and salivary nitric oxide, lactoferrin, α-amylase and cortisol values of children according to the Frankl Behavior Rating Scale.***p<0.01 compared with the Frankl 1 group

Except that, there was no significant difference between salivary parameters according to the Frankl 1, 2 and 3 scale. According to the Frankl Behavioral Rating Scale, there are no children in the definite positive group (Frankl 4), therefore, they are not included in the table. No significant difference was found between the groups when the examined parameters were classified for the Frankl Behavioral Rating Scale ($p>0.05$).

The results of correlation between the parameters examined are given in Table 3. There was a significant and positive correlation between DMFT+dft and salivary NO, lactoferrin, α-amylase and cortisol values ($r=0.902$, $r=0.890$, $r=0.884$, $r=0.882$, $p<0.001$). In addition, there was a significant and positive correlation between salivary nitric oxide and α-amylase ($r=0.937$, $p<0.001$), between cortisol and α-amylase ($r = 0.884$, $p<0.001$), between cortisol and lactoferrin ($r=0.810$, $p<0.001$) and between α-amylase and lactoferrin ($r=0.820$, $p<0.001$).

DISCUSSION

The results of the Frankl Behavior Rating Scale showed that the highest ratio of children is in category 2, which means the majority of the children participating in the study (68%) were reluctant to accept treatment and they were showing a sign of negative attitude, although not obvious.

This negative attitude may be related to the high DMFT+dft index and low salivary flow rate in this group, compared with the Frankl 1 group.

The results of our study also showed significant correlations between salivary NO, lactoferrin, cortisol, α-amylase levels and DMFT+dft indexes in children. It was shown that dental caries affect the immune system by causing inflammation, and lead to increase in various salivary biomarkers (12). The correlation between DMFT+dmft indices and salivary proteins in our study is consistent with this information. Salivary flow rate, NO, lactoferrin, α-amylase and cortisol levels were not different between girls and boys. As supported by the results of our study, anxiety due to the dentist and dental treatment is a common problem in children. As completely negative children would be expected to have more decreased salivary flow rate than negative children, the low number of children in category 1 and category 2 may have caused this finding. According to our literature search details, in the present study, the relationship between dental anxiety and salivary NO was investigated for the first time in the literature. There was no correlation between the results of the Frankl Behavioral Rating Scale and the salivary NO levels of the children participating in the study. The low number of patients participating in the study may be the reason for no significant correlation between saliva NO and anxiety. It was reported that NO is one of the agents involved in neurotransmitter dysfunction during anxiety and depression, and if anxiety and depression are an adaptation, NO may be involved during this adaptation (13). Also, it was shown that salivary NO levels of patients with periodontitis were higher than healthy individuals (14).

Because of individual differences in salivary secretion rates and gingival health, salivary antimicrobial factors show individual differences. There was a positive and strong correlation between saliva lactoferrin levels and the DMFT+dft index in our study. Felizardo et al. reported that 58.8% of the children did not have decay in their teeth and 63.3% of them had caries experience, and their lactoferrin concentration correlated positively with both DMFT and restored teeth number (15). Sikorska et al. (16) determined saliva lactoferrin levels in children aged 15 years, and reported that there was a significant relationship between caries surface index and saliva lactoferrin levels. As one of the defence factors in saliva, the direct bacteriostatic effect of lactoferrin, is not only depriving the most important elements necessary for bacteriological growth by binding the bacterium, but it is also achieved by destroying the outer membrane of the bacterium and building up NO in the macrophages (16).

It has been shown that a high α-amylase concentration in saliva affects oral health positively, and is associated with both physical and physiological stress conditions (6). In our study, it was found that salivary α-amylase correlates strongly with the DMFT-dft index and salivary NO. It was reported that α-amylase may play a role in plaque formation (17). Plaque formation and damage caused by bacteria may lead to increase NO in oral tissue. This relationship may be the cause of salivary α-amylase DMFT-dft index and NO correlation.

Collection of saliva is easier and less invasive than blood collection in children. The determination of salivary cortisol can provide great convenience, especially in field studies, large cohort studies and studies with children, due to stress-free collection and working conditions. Considering the correlation between saliva and blood, saliva can be used as an alternative to blood in free cortisol measurements (18).

In our study, cortisol levels in saliva samples collected from children participating in the study were examined to investigate the stress that the dental examination environment created. Sadi et al. (19) did not find any relationship between salivary cortisol levels and the patient's anxiety levels, also there was no correlation between salivary cortisol levels and those determined by the Frankl Behavior Rating Scale in this study. The lack of correlation between the Frankl Behavior Rating Scale and salivary cortisol and α-amylase may depend on the measurement method used to determine stress. The Frankl Behavior Rating Scale method is open to interpretation since it is observational. The grouping may vary according to the observer (20). Since children cannot express themselves as clearly as adults, the grouping of their current situation may vary.

A positive correlation between salivary cortisol levels and DMFT+dft indeces was found in our study. Rai et al. evaluated salivary cortisol levels in children with rampant caries (21).

These children reported acute pain and distress prior to dental treatment, and an increased level of cortisol was recorded in their saliva. Also, after dental treatment, a decreased level of sal-

ivary cortisol, which was attributed to the absence of pain and reduced level of stress, was observed in their study. Additionally, Patil et al. (22) found a correlation between salivary cortisol and stress in dental procedure of healthy children undergoing routine dental procedures. Dental treatment is generally considered stressful and anxiety producing. These emotional states lead biochemical changes, such as elevation of salivary cortisol. Also, this may affect dental fear, and lead to the refusal of dental treatment, which may cause an increase in caries. Measurement of salivary cortisol is an accurate way of measuring adrenocortical function, and may be used as an index for stress (23).

Additionally, in the present study, a significant and positive correlation was found between salivary cortisol and α-amylase values. The role of salivary α-amylase in stress was investigated, and it was demonstrated that stress causes a significant increase in salivary α- amylase levels (24). Dental treatment itself can induce anxiety and fear in children, and these emotions cause significantly increased levels of salivary α-amylase immediately after dental treatment (25). Also salivary α-amylase was suggested as a marker of the autonomic nervous system response to stress in youth and adults (26). Unlike most salivary analytes that are actively transported or passively diffused into saliva from plasma, salivary α-amylase is locally produced in the oral mucosa of the salivary glands. The salivary glands are innervated by sympathetic and parasympathetic nerves, and salivary secretions from the glands arise in response to neurotransmitter activation. This suggests that salivary α-amylase may be a noninvasive marker of psychosocial stress autonomic activity (27).

A significant and positive correlation detected between α-amylase and lactoferrin is another important finding of our study. Lactoferrin is an important constituent of the innate immune system. Stressful conditions can affect the immune response, and stress may lead to physiobiochemical alterations in the constituents of saliva (28). Furthermore, dental caries may be suggested as being a triggering factor for a nonspecific immune response, and may lead to an increase in levels of these salivary proteins (29).

The limitation of the present study is the lack of children in category 4 according to the Frankl Behavior Rating Scale. Finding a study group as 'positive children' was not very applicable. It was very difficult to find enough children to make up this group because a 'completely positive group' means that the children loved the dentist treatment.

CONCLUSION

Our findings show the potential of salivary nitric oxide, lactoferrin, cortisol and α-amylase to reflect oral health. However, more studies are needed to prove the interaction of these parameters with dental anxiety in children.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethic Committee of Marmara University (dated 06.01.2012; Cert No. 10).

Informed Consent: Written informed consent was obtained from the parents of the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept -E.E.A, S.A.; Supervision - E.E.A, S.A.; Materials - S.O;S.D;U.V.U; B.K;G.E ; Data Collection and/or Processing - S.O;S.D;U.V.U.; Analysis and/or Interpretation - S.O;S.D;U.V.U;E.E.A, S.A; Literature Search - S.O.; Writing - S.O; Critical Reviews - E.E.A, S.A.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Etik Komite Onayı: Bu çalışma için etik komite onayı Marmara Üniversitesi Etik Kurulu'ndan (tarih: 06.01.2012; Sert. No. 10) alınmıştır.

Hasta Onamı: Yazılı hasta onamı bu çalışmaya katılan hastaların ebeveynlerinden alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Yazar Katkıları: Fikir - E.E.A, S.A; Denetleme -E.E.A, S.A; Gereçler - S.O;S.D;U.V.U; B.K;G.E; Veri Toplanması ve/veya İşlemesi - S.O;S.D;U.V.U.; Analiz ve/veya Yorum - S.O;S.D;U.V.U;E.E.A, S.A; Literatür Taraması -S.O; Yazan - S.O; Eleştirel İnceleme - E.E.A, S.A.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışmada finansal destek almadıklarını beyan etmişlerdir.

REFERENCES

1. Berlutti F, Pilloni A, Pietropaoli M, Polimeni A, Valenti P. Lactoferrin and oral diseases: current status and perspective in periodontitis. Ann Stomatol 2011; 2: 10-8.
2. Chicharro JL, Lucía A, Pérez M, Vaquero AF, Ureña R. Saliva composition and exercise. Sports Med 1998; 26: 17-27. [CrossRef]
3. Legrand D, Pierce A, Elass E, Carpentier M, Mariller C, Mazurier J. Lactoferrin structure and functions. In: Bioactive components of milk. Springer, New York, NY, 2008. p. 163-94. [CrossRef]
4. Eagappan ARS, Rao VAP, Sujatha S, Senthil D, Sathiyajeeva J, Rajaraman G. Evaluation of salivary nitric oxide level in children with early childhood caries. Dent Res J 2016; 13: 338-41. [CrossRef]
5. Hellhammer DH, Wüst S, Kudielka BM. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology 2009; 34: 163-71. [CrossRef]
6. Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. Psychoneuroendocrinology 2009; 34: 486-96. [CrossRef]
7. Vineetha R, Pai KM, Vengal M, Gopalakrishna K, Narayanakurup D. Usefulness of salivary alpha amylase as a biomarker of chronic stress and stress related oral mucosal changes - a pilot study. J Clin Exp Dent 2014; 6: 132-7. [CrossRef]
8. Seligman LD, Hovey JD, Chacon K, Ollendick TH. Dental anxiety: An understudied problem in youth, Clin Psychol Rev 2017; 55: 25-40. [CrossRef]
9. Peretz B, Efrat J. Dental anxiety amoung young adolescent patients in Israel. Int J Paediatric Dent 2002; 10: 126-32. [CrossRef]

10. Frankl SN. Should the parent remain with the child in the dental operatory? J Dent Child 1962; 29: 150-63.
11. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 2001; 5: 62-71. [CrossRef]
12. Monica M, Valad R, Stoica A. Analysis of salivary level of alpha-amylase as a risk factor for dental caries. Acta Medica Transilvanica March 2018; 23: 93-5.
13. Yildiz F, Erden BF, Ulak G, Utkan T, Gacar N. Antidepressant-like effect of 7-nitroindazole in the forced swimming test in rats. Psychopharmacology 2000; 149: 41-4. [CrossRef]
14. Inasu S, Thomas B, Kumari S, Ramesh A, Rao A. Evaluation of serum and salivary sialic acid and nitric oxide levels in chronic periodontitis patients. Int J Appl Dent Sci 2016; 2: 74-6.
15. Felizardo KR, Gonçalves RB, Schwarcz WD, Poli-Frederico RC, Maciel SM, de Andrade FB. An evaluation of the expression profiles of salivary proteins lactoferrin and lysozyme and their association with caries experience and activity. Rev Odonto Ciênc 2010; 25: 344-9. [CrossRef]
16. Sikorska MHJ, Mielnik-Blaszczak M, Kapec' E. The relationship between the levels of SigA, lactoferrin and proteinase inhibitor in saliva and permanent dentition caries in 15-year olds. Oral Microbiol Immunol 2001; 17: 272-6. [CrossRef]
17. Scannapieco FA, Torres G, Levine MJ. Salivary α-amylase: role in dental plaque and caries formation. Crit Rev Oral Biol Med 1993; 4: 301-7. [CrossRef]
18. Akyuz S, Pince S, Hekin N. Children's stress during a restorative dental treatment: assessment using salivary cortisol measurements. J Clin Pediatr Dent 1996; 20: 219-23.
19. Sadi H, Finkelman M,Rosenberg M. Salivary cortisol, salivary alpha amylase, and the dental anxiety scale. Anesth Prog 2013; 60: 46-53. [CrossRef]
20. Aartman IH, van Everdingen T, Hoogstraten J, Schuurs AH. Appraisal of behavioral measurement techniques for assessing dental anxiety and fear in children: a review. J Psychopathol Behav Assess 1996; 18: 153-71. [CrossRef]
21. Rai K, Hegde A, Shetty S, Shetty S. Estimation of salivary cortisol in children with rampant caries. J Clin Pediatr Dent 2010; 34: 249-52. [CrossRef]
22. Patil SJ, Shah PP, Patil JA, Shigli A, Patil AT, Tamagond SB. Assessment of the changes in the stress-related salivary cortisol levels to the various dental procedures in children. J Indian Soc Pedod Prev Dent 2015; 33: 94-9. [CrossRef]
23. Nater UM, Rohleder N, Gaab J, Berger S, Jud A, Kirschbaum C, etal. Human salivary alpha-amylase reactivity in a psychosocial stress paradig. Int J Psychophysiol 2015; 55: 333-42. [CrossRef]
24. Rohleder N, Wolf JM, Maldonado EF, Kirschbau C. The psychosocial stress-induced increase in salivary alpha-amylase is independent of saliva flow rate. Psychophysiology 2006; 43: 645-52. [CrossRef]
25. Noorani H, Joshi HV, Shivaprakash P. Salivary alpha amylase as a noninvasive biomarker for dental fear and its correlation with behavior of children during dental treatment. Int J Clin Pediatr Dent 2014; 7: 19-23.
26. van Stegeren A, Rohleder N, Everaerd W, Wolf OT. Salivary alpha amylase as marker for adrenergic activity during stress: effect of beta-blockade. Psychoneuroendocrinology 2006; 31: 137-41. [CrossRef]
27. Tanaka Y, Ishitobi Y, Maruyama Y, Kawano A, Ando T, Okamoto S, et al. Salivary alphaamylase and cortisol responsiveness following electrical stimulation stress in major depressive disorder patients. Prog Neuropsychopharmacol Biol Psychiatry 2012; 36: 220-4.[CrossRef]
28. Lonnerdal B, Iyer S. Lactoferrin: molecular structure and biological function. Annu Rev Nutr 1995; 15: 93-110. [CrossRef]
29. Felizardo KR, Goncalves RB, Schwarcz WD, Poli-Frederico RC, Maciel SM, de Andrade FB. An evaluation of the expression profiles of salivary proteins lactoferrin and lysozyme and their association with caries experience and activity. Rev Odonto Cienc 2010; 25: 344-9. [CrossRef]

Experimed 2019; 9(3): 93-8

ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

DOI: 10.26650/experimed.2019.19017

Investigation of The Vitamin D Receptor (VDR) Gene Polymorphisms in Lumbar Disc Herniation in Turkish Patients

Lomber Disk Hernisi Tanısı Konan Türk Hastalarda Vitamin D Reseptör (VDR) Gen Polimorfizmlerinin Araştırılması

Bahar Toptaş Hekimoğlu[1], Hakan Eraltan[2], Hidayet Sarı[2], Mehmet Tolgahan Hakan[3], Dilara Sönmez[1], Cem Horozoğlu[4], Ümit Zeybek[1], Arzu Ergen[1], İlhan Yaylım[1], Turgay İsbir[5]

[1]Department of Molecular Medicine, İstanbul University Institute of Experimental Medicine, İstanbul Turkey
[2]Department of Physical Therapy and Rehabilitation, İstanbul University Cerrahpasa, Cerrahpasa School of Medicine, İstanbul, Turkey
[3]Department of Biology, Science and Art Faculty, Hitit University, Çorum Turkey
[4]Department of Technical and Medical Services, İstanbul Gelişim University, Medical Laboratory Techniques Program, İstanbul, Turkey
[5]Department of Medical Biology, Yeditepe University School of Medicine, İstanbul, Turkey

Cite this article as: Toptaş Hekimoğlu B, Eraltan H, Sarı H, Hakan MT, Sönmez D, Horozoğlu C, et al. Investigation of The Vitamin D Receptor (VDR) Gene Polymorphisms in Lumbar Disc Herniation in Turkish Patients. Experimed 2019; 9(3): 93-8.

ABSTRACT

Objective: Lumbar disc herniation (LDH) is a common degenerative disease. It is still not clear if there is a possible association between the vitamin D pathway and the etiopathogenesis of the disease. In this study, we investigated certain VDR polymorphisms which are known to affect vitamin D levels in patients with lumbar disc herniation.

Material and Method: TaqI (rs731236) and Fok-I (rs2228570) polymorphisms were studied by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 72 LDH patients and 81 healthy controls.

Results: After evaluation of our results, the frequency of LDH patients who have VDR Taq-I Tt genotype was significantly higher than the controls and carriers of Taq-I Tt genotype and t allele who had an increased risk for lumbar disc hernia cases, respectively p=0.002, OR:1.688, 95%CI:1.206-2.360; p=0.006, OR:1.420, 95%CI:1.104-1.825. VDR Fok-I genotypes did not differ significantly between lumbar disc herniation and control cases. (p=0.079). But, Ff genotype and f allele carriers had a higher risk for lumbar disk hernia than those with other genotypes, respectively p=0.025, OR:1.594, 95%CI:1.052-2.414; p=0.037, OR:1.514, 95%CI:1.019-2.250.

Conclusion: Our study contributes to the identification of genetic risk factors for specific subgroups of patients with LDH, and emphasizes the contribution of these biomarkers to the detailed clinical evaluation of patients with genetic biomarkers.

Keywords: Vitamin D receptor, polymorphism, lumbar disc herniation

ÖZ

Amaç: Lomber disk hernisi (LDH) yaygın bir dejeneratif hastalıktır. D vitamini yolu ile hastalığın etyopatogenezi arasında olası bir ilişkinin varlığı çalışmalarda tam olarak gösterilememiştir. Bu çalışmada LDH hastalarında D vitamini düzeyini etkilediği bilinen VDR polimorfizmlerini araştırdık.

Gereç ve Yöntem: TaqI (rs731236) ve Fok-I (rs2228570) polimorfizmleri 72 LDH hasta ve 81 sağlıklı kontrol örneğinde polimeraz zincir reaksiyonu- restriksiyon fragman uzunluk polimorfizmi (PCR-RFLP) yöntemi kullanılarak incelendi.

Bulgular: Elde edilen bulguların değerlendirilmesi sonrası VDR Taq-I Tt genotipine LDH hastalarında görülme sıklığı kontrollerden anlamlı olarak yüksek olduğu ve Taq-I Tt genotip ve t alel taşıyıcılarının LDH vakaları için yüksek risk taşıyıcısı olduğu tespit edildi, sırasıyla; p=0,002, OR: 1,688,% 95CI: 1,206-2,360; p=0,006, OR: 1,420,% 95CI: 1,104-1,825. VDR Fok-I genotipleri LDH ve kontrol vakaları arasında değerledirildiğinde anlamlı farklılık gözlemlenmemiştir (p=0,079). Ancak, Ff genotipi ve f allel taşıyıcıları LDH hastaları için diğer genotiplere göre daha yüksek bir risk taşımaktadır. Sırasıyla; p=0,025, OR: 1,594, %95 CI: 1,052-2,414; p=0,037, OR: 1,514, %95 CI: 1,019-2,250.

Sonuç: Çalışmamız, LDH'li hastaların belirli alt grupları için genetik risk faktörlerinin tanımlanmasına katkıda bulunmaktadır ve genetik biyobelirteçlerin hastaların ayrıntılı klinik değerlendirmesine katkısının önemini vurgulamaktadır.

Anahtar Kelimeler: D vitamini reseptörü, polimorfizm, lomber disk hernisi

Corresponding Author/Sorumlu Yazar: İlhan Yaylım **E-mail:** ilhanyaylim@gmail.com
Received Date/Geliş Tarihi: 09.10.2019 **Revision Date/Revizyon Tarihi:** 26.10.2019 **Accepted Date/Kabul Tarihi:** 12.11.2019

INTRODUCTION

The intervertebral disc, a bond between two adjacent vertebrae, contributes to the flexibility of the spine (1, 2). An intervertebral disc disorder involves several pathological conditions such as, deterioration, herniation, or other defects of intervertebral discs. Degenerative changes of a disc lead to several symptoms. Generally, these disorders are usually characterized by low back pain (LBP). (1). More than 80% of people suffer from LBP during their life time (3). LBP has been defined as one of the most common musculoskeletal disorders in the world, especially in the working population (4, 5) and affects up to 50-80 % of people at least once during their lifetime (4). Studies have shown that lumbar disc degeneration (LDD) is associated with LBP. The etiology of LDD has complex features (4, 5). According to the general opinion, there are several potential risk factors for a lumbar disc herniation, such as, age, weight, gender, occupation and smoking, and probably contribute to the genesis or to the acceleration of spinal degeneration (1, 4). The etiology of disc degeneration is based on environmental factors as well, as recent studies have shown that the physiological, molecular and genetic characteristics of herniated intervertebral disc tissues play a very important role in explaining the pathogenesis of human diseases (6, 7). Recent studies have demonstrated that, the effect of genetic factors was found to be more important in the progression of the lumbar disc hernias (1). Moreover, it is also proposed that intervertebral disc disease is very similar to complex disorders with multiple genetic forms. In the studies conducted so far, some genes were analyzed in the pathogenesis of lumber disc disease. Previous studies implied that, many genes are connected with lumbar disc disease, such as collagen IX (*COL9A2*), matrix metalloprotease-3 (*MMP-3*), vitamin-D receptor (*VDR*), estrogen receptor (*ER*) genes. These genetic factors have been reported to influence the regeneration and degeneration degree of the spine (1, 8).

Vitamin D is one of the critical determinants involved in bone metabolism and development (6, 9). The *VDR* gene, one of the top members of the nuclear receptor family, is encoded by chromosome 12 (8, 10). VDR are expressed in the growth plate of the bone and cartilage cells osteoblastic cells (11). Several studies determined that VDR play an important function in healthy bone structure. Therefore, VDR gene variants were thought to be related with various bone diseases, such as osteoporosis, osteoarthritis and degenerative disc disease (1, 11). The *VDR* gene has been investigated as a genetic factor according to development of spine pathologies since 1998 (12). Recent studies have shown that VDR gene polymorphisms have an effect on the development of various degenerative disc diseases (8). The Fok-I (rs10735810, merged into rs2228570) polymorphism, one of the most important variants in the *VDR*, is located on exon 2, and known as the main responsible agent for creating an alternative transcription initiation region that leads to alterations in the activity of the VDR protein. Because of the replacement of cytosine (C) by thymine (T), the Fok-I polymorphism causes different translation initiation sites to occur. These variations are associated with a different capacity to induce transcription of the VDR gene and VDR related genes (12). The VDR Fok-I polymorphism prompts a change translation promoter site, prompting the formation of a longer than wild VDR isoform. As predicted, this caused it to be less active (7, 13). In the end of the studies that have examined the relationship between LDD and the Fok-1 polymorphism have presented conflicting data. For this reason, studies to be carried out in different populations are important (7, 14, 15). Another significant SNP of the VDR gene, the the Taq-I (rs731236T / C) polymorphism is caused by a *switch* from ATT to ATC, which leads to a synonym change in codon 352 (isoleucine) (12, 16, 17). In a recent study by Toktaş et al., the intensity level of disk degeneration has been shown to be enhanced by Taq-I. This study suggests that the Taq-I SNP variant is associated with the severity and development of intervertebral disc degeneration (IVDD) (18).

The aim of this study was to determine any relationship tween the Taq-I and Fok-I polymorphisms of the VDR gene in lumbar disc hernias.

MATERIAL AND METHOD

Subject selection: In our study, in 72 patients with lumbar disc herniation, we analyzed the Taq-I and Fok-I gene polymorphisms in the VDR gene, and in 81 healthy individuals who applied to the Department of Neurosurgery of İstanbul University Cerrahpaşa School of Medicine. The mean age between the groups of the patients and the control group was 44.75±15.63 and 47.22±10.63 years, respectively. Samples from both groups were taken after the informed consent form was signed, and the study was conducted prospectively. İstanbul Medical Faculty Clinical Research Ethics Committee approved our study. The protocol followed during the study is consistent with the Declaration of Helsinki World Medical Association (Ethical Principles for Medical Research Involving Human Subjects).

Polymorphism Analysis

All blood samples were collected in tubes containing EDTA, and DNA was taken from whole blood using the salting-out method (19). Genotyping by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) was performed using these methods (Table 1).

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 21.0 (IBM Corp.; Armonk, NY, USA) revision software package. Clinical laboratory data, which are expressed as mean ± SD, were compared between the patients and the control group by the unpaired Student's t test. The differences in the distribution of VDR genotypes or alleles between the patient and the controls were tested using chi-square statistics. VDR alleles frequencies were calculated by gene counting methods. $p<0.05$ was considered statistically significant.

Table 1. Taq-I / Fok-I RFLP methods

Gene	Primers	PCR reaction mixture	Restriction Enzym	Genotype	PCR conditions
Taq-I polymorphism	Forward:5' CAGAGCATGGACAGGGAGCAAG 3'; Reverse:5' GCAACTCCTCATGGGCTGAGGTCTCA 3'	3mM $MgCl_2$, 0,2mM dNTP, 0.2mM primers 0.2mM Taq polymerase (in a 50 µl reaction volume for 1x polymerase chain reaction)	TaqI (65 °C)	TT (490, 245 bp) Tt (490, 290, 245, 205 bp) tt (290, 245, 205 bp)	Initial denaturation step of 94°C for 4 min followed by 5 cycles of 94°C for 45 sec, 64°C for 60 sec and 72°C for 2 min; and a further, 25 cycles of 94°C for 30 sec, 64°C for 30 sec and 72°C for 45 sec.
Fok-I polymorphism	Forward :5' GATGCCAGCTGGCCCTGGCACTG 3'; Reverse: 5'ATGGAAACACCTTGCTTCTTCTCCCTC 3'	3mM $MgCl_2$ 0.2mM dNTP 0.25 mM primer 0.25mM Taq polymerase (in a 50 µl reaction volume for 1x polymerase chain reaction)	Fok-I (37°C)	FF (272) Ff (272, 198, 74) Ff (198, 74)	Initial denaturation step of 94°C for 4 min followed by 5 cycles of 94°C for 45 sec, 64°C for 60 sec and 72°C for 2 min; and a further, 25 cycles of 94°C for 30 sec, 64°C for 30 sec and 72°C for 45 sec. For Fok-I; initial denaturation of 4 min at 94°C , followed by 30 cycles of 94°C for 1 min, annealing at 60°C for 1 min and extension at 720C for 1 min. A final elongation step occurs at 72°C for 4 minutes.

RESULTS

The analysis included 72 lumbar disc hernia patients (32 female and 40 male) and 81 healthy controls (47 female and 34 male). The patient and control groups had similar distributions for age and sex differences. The genotype and allele frequencies of the VDR polymorphisms (Fok-I and Taq-I) of lumbar disc herniation patients and controls are demonstrated in Table 2. After evaluation of our results, a significant difference was found in Taq I genotype distribution of *VDR* between patients and the control group (p=0.006) (Table 2). The VDR Taq-I Tt genotype was significantly higher in lumbar disc hernia patients (60.9%) compared with controls (39.2%), and carriers of the Taq-I Tt genotype and the t allele had an increased risk for lumbar disc hernia cases, respectively, as shown in the clinical features of the study groups (p=0.002, OR:1.688, 95%CI:1.206-2.360; p=0.006, OR:1.420, 95%CI:1.104-1.825). VDR Fok-I genotype frequencies did not differ significantly between lumbar disc herniation and control cases. (p=0.079). But, Ff genotype and f allele carriers

Table 2. Allele and genotype frequencies of lumbar disc hernia cases and controls

SNP	Controls (n=81) n (%)	Lumbar disc hernia (n=72) n(%)	p
Fok-I Genotype			
FF	55 (67.9)	37 (51.4)	0.079
Ff	24 (29.6)	34 (47.2)	
ff	2 (2.5)	1 (1.4)	
Taq-I Genotype			
TT	39 (48.1)	19 (26.4)	0.006
Tt	30 (37)	45 (62.5)	
tt	12 (14.8)	8 (11.1)	

Table 3. Lumbar function assessment test results in patient group

	Mean	Std. Deviation
VAS (Visual Analog Scale)	6.4559	1.59695
SLR - Right (Straight Leg Raise)	59.4444	20.13320
SLR - Left (Straight Leg Raise)	60.1786	17.55418
LFA (Lumbar Flexion Angle)	50.5147	19.41571
LFA-ROM (Lumbar Flexion-Range of Motion)	4.6812	1.42066

Table 4. Comparison of homozygous genotypes according to VDR Taq-I Polymorphism with lumbar function tests

	Taq-1	Mean	Std. Deviation	p
VAS	TT Genotype	6.1111	1.32349	0.617
	tt Genotype	6.4286	1.61835	
LFA-ROM	TT Genotype	2.1765	0.72761	0.534
	tt Genotype	2.3750	0.74402	
SLR - Right	TT Genotype	63.9286	20.20839	0.125
	tt Genotype	47.0000	19.87461	
SLR – Left	TT Genotype	67.1875	18.43626	0.025*
	tt Genotype	46.6667	15.05545	
LFA	TT Genotype	51.7647	22.28657	0.824
	tt Genotype	50.0000	17.32051	

*:p<0.05

had a higher risk for lumbar disk hernia than those with other genotypes (p=0.025, OR:1.594, 95%CI:1.052-2.414; p=0.037, OR:1.514, 95%CI:1.019-2.250, respectively). In our study, according to gender, there is not any statistical significance of the genotypes distribution. In addition, according to lumbar flexion degrees in lumbar disc herniation patients compared to the distribution of VDR Taq-I and Fok-I polymorphisms genotypes, the Fok-I polymorphisms genotypes for the ff genotype in individuals (73.3±16.9) were determined to be statistically significantly higher than the Fok-I Ff genotype in individuals (48.4±15.3); (p=0.018). Functional lumbar evaluation tests (Table 3) applied in the diagnosis and evaluation process of our patient group were evaluated for VDR Taq-I and VDR Fok-I polymorphisms. SLR-Left who have TaqI TT genotype was found to be 1.44 times than patients with tt genotype (Table 4; p=0.025). For the Fok-I polymorphism, the VAS value was found to be 1.14 times statistically higher in patients with the f allele than in patients carrying the FF genotype (Table 5; p=0.04). The lumbar functional evaluation data of the patient group are presented in Table 4.

Table 5. Comparison of f allele carriage or FF genotype carriage with lumbar function tests in VDR Fok-I polymorphism

	Fok-I	Mean	Std. Deviation	p
VAS	f allele	6.8485	1.62252	0.048*
	FF genotype	6.0857	1.50238	
LFA-ROM	f allele	5.9412	2.51857	0.386
	FF genotype	3.4571	1.37528	
SLR - Right (Straight Leg Raise)	f allele	63.3333	20.39833	0.113
	FF genotype	54.5833	19.10592	
SLR – Left	f allele	60.9259	15.13002	0.762
	FF genotype	59.4828	19.79109	
LFA	f allele	48.1818	16.94745	0.340
	FF genotype	52.7143	21.50044	

*:p<0.05

DISCUSSION

Some of the polymorphisms in the *VDR* gene encoding vitamin D, which is an important factor in the regulation of cell division and differentiation, were investigated for their functional significance and potential effects on disease sensitivity (20). Several studies have shown that cellular effects of *VDR* may be associated with cell proliferation of disc cells. In addition, expression rates of matrix genes are related to specific cytokines and protein production (21, 22). Despite the recent research on lumbar disc disease, knowing that genetic factors play a critical role as the VDR gene, these genes have not yet been fully described (23). Researchers have rapidly turned to polymorphisms of these genes to determine the expression and effects of the full functions of these genes (23). In our study, although there was no statistically significant difference in terms of genotype distribution according to sex, we found a positive correlation between Taq-I genetic variant of *VDR* gene and lumbar disc herniation. We also determined that the VDR Taq-I Tt genotype might affect the development of lumbar disc hernia. These results are correlated with the work of Toktaş et al. (18). At the same time, this work constitutes evidence for the suggestion of TaqI SNP, which Taq-I SNP of VDR could be associated with both escalated developing IVDD and violent IVDD, in the compilations of Martrosyan et al. (3) in 2016.

Another gene associated with the VDR in our study, Fok-I polymorphism FF genotype, could be a less active variant, therefore, this alternation may lead to a more aggressive disease prognosis (24, 25). In several studies, the association of the Fok-I polymorphism in the VDR with the hernia, disc degeneration in different ethnic groups or lumbar spinal stenosis was analyzed (12). Colombini et al. According to the data obtained from the study, VDR Fok-I polymorphism in Italian population reported that there is no correlation with the risk of lumbar spine disease (12). In addition, several studies have determined similar results according to various types of disc pathologies (26). On the other hand, several previous studies have reported a relationship between the Fok-I polymorphism in VDR and the specific signs of disc degeneration in different populations such as Turkish (14), Brazilian (13) and Finnish (27). But some studies with Italian (12) and Mexican Mestizo patients (28), and a study of Norway case/control found an association with Fok-I genotypes (7). According to the findings of our studies, the individuals who have the genotype Ff and ff are associated with a worse prognosis than the individuals having the genotype FF.

The results of VDR Fok-I polymorphism in Italian athletes showed that f allele carrying was associated with LBP (29). However, it has been shown in the Italian population that the F allele is associated with a two-fold increase in risk for lumbar spine pathologies. In addition, the protective effect of f allele was emphasized (12). In our results, f allele carriage and Ff genotype carriage were associated with lumbar disc hernia. At the same time, the f allele was found to be associated with pain scores (VAS). This may be due to allele frequency differences between populations. In terms of VDR Taq-I polymorphism, SLR-Left was found to be higher in TT genotype than tt genotype in our study. The fact that this data have not been reported in different populations and similar spectrum of diseases is unique in terms of its contribution to the literature.

We observed a positive correlation between the levels of Vitamin D in lumbar disk degeneration patients. But these genetic polymorphisms play two important roles in regional disparities of race and ethnicity. Some polymorphisms tend to be more potent or less effective in some races, and there are two important genetic polymorphism aspects that occur in variations originating from race and ethnic origin. Fok-I SNP, for example, has been associated with more intervertebral disk degeneration risk in Hispanics than in Asian populations, but no association with intervertebral disk degeneration has been found in Caucasians (3, 30).

Our limitation is small size of the sample. A stronger statistical result and our findings are needed to verify the number of patients with more advanced studies.

Ethics Committee Approval: Ethics committee approval was received for this study from the Clinical Research Ethics Committee of İstanbul University School of Medicine (2009/1861).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - T.İ., İ.Y.; Supervision - T.İ., İ.Y.; Materials - H.E., H.S.; Data Collection and/or Processing - B.T.H., M.T.H., C.H.; Analysis and/or Interpretation - C.H., İ.Y., A.E., Ü.Z.; Literature Search - B.T.H., D.S. M.T.H.; Writing - B.T.H., C.H.; Critical Reviews - H.S., İ.Y., B.T.H.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This study was funded by Scientific Research Projects Coordination Unit of İstanbul University (Project number: 4364).

Etik Komite Onayı: Bu çalışma için etik komite onayı İstanbul Üniversitesi Tıp Fakültesi Klinik Araştırmalar (2009/1861) alınmıştır.

Hasta Onamı: Yazılı hasta onamı bu çalışmaya katılan hastalardan alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir - T.İ., İ.Y.; Denetleme - T.İ., İ.Y.; Gereçler - H.E., H.S.; Veri Toplanması ve/veya İşlemesi - B.T.H., M.T.H., C.H.; Analiz ve/veya Yorum - C.H., İ.Y., A.E., Ü.Z.; Literatür Taraması - B.T.H., D.S. M.T.H.; Yazan - B.T.H., C.H.; Eleştirel İnceleme - H.S., İ.Y., B.T.H.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Bu çalışma İstanbul Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından desteklenmiştir (Proje No:4364).

REFERENCES

1. Zhang Y, Sun Z, Liu J, Guo X. Advances in susceptibility genetics of intervertebral degenerative disc disease. Int J Biol Sci 2008; 4: 283-90. [CrossRef]
2. Paesold G, Nerlich AG, Boos N. Biological treatment strategies for disc degeneration: potentials and shortcomings. Eur Spine J 2007; 16: 447-68. [CrossRef]
3. Martirosyan NL, Patel AA, Carotenuto A, Kalani MYS, Belykh E, Walker CT, et al. Genetic Alterations in Intervertebral Disc Disease. Front Surg 2016; 3: 59. eCollection 2016. [CrossRef]
4. Colombini A, Cauci S, Lombardi G, Lanteri P, Croiset S, Brayda-Bruno M, et al. Relationship between vitamin D receptor gene (VDR) polymorphisms, vitamin D status, osteoarthritis and intervertebral disc degeneration. J Steroid Biochem Mol Biol 2013; 138: 24-40. [CrossRef]
5. Eskola PJ, Lemmelä S, Kjaer P, Solovieva S, Männikkö M, Tommerup N, et al. Genetic association studies in lumbar disc degeneration: a systematic review. PLoS One 2012; 7: e49995. doi: 10.1371/journal.pone.0049995. [CrossRef]
6. Zawilla NH, Darweesh H, Mansour N, Helal S, Taha FM, Awadallah M, et al. Matrix metalloproteinase-3, vitamin D receptor gene polymorphisms, and occupational risk factors in lumbar disc degeneration. J Occup Rehabil 2014; 24: 370-81. [CrossRef]
7. Taha MM, Sabbah NA, Rezk NA, Mansour H. Vitamin D Receptor Expression in Lumbar Disc Degeneration Patients. Open J Mod Neurosurg 2017; 7: 19-33. [CrossRef]

8. Kalichman L, Hunter DJ. The genetics of intervertebral disc degeneration.Familial predisposition and heritability estimation. Joint Bone Spine 2008; 75: 383-7. [CrossRef]
9. Vuolo L, Di Somma C, Faggiano A, Colao A. Vitamin D and cancer. Front Endocrinol (Lausanne) 2012; 3: doi: 10.3389/fendo.2012.00058. eCollection 2012. [CrossRef]
10. Mittal RD, Manchanda PK, Bhat S, Bid HK. Association of vitamin-D receptor (Fok-I) gene polymorphism with bladder cancer in an Indian population. BJU Int 2007; 99: 933-7. [CrossRef]
11. Amizuka N, Kwan MY, Goltzman D, Ozawa H, White JH. Vitamin D3 differentially regulates parathyroid hormone/parathyroid hormone-related peptide receptor expression in bone and cartilage. J Clin Invest 1999; 103: 373-81. [CrossRef]
12. Colombini A, Brayda-Bruno M, Lombardi G, Croiset SJ, Vrech V, Maione V, et al. FokI polymorphism in the vitamin D receptor gene (VDR) and its association with lumbar spine pathologies in the Italian population: a case-control study. PLoS One 2014; 9: e97027. doi: 10.1371/journal.pone.0097027. eCollection 2014. [CrossRef]
13. Vieira LA, De Marchi PL, dos Santos AA, Christofolini DM, Barbosa CP, Fonseca FLA, et al. Analysis of FokI polymorphism of vitamin D receptor gene in intervertebral disc degeneration. Genet Test Mol Biomarkers 2014; 18: 625-9. [CrossRef]
14. Eser B, Cora T, Eser O, Kalkan E, Haktanır A, Erdogan MO, et al. Association of the polymorphisms of vitamin D receptor and aggrecan genes with degenerative disc disease. Genet Test Mol Biomarkers 2010; 14: 313-7. [CrossRef]
15. Eskola PJ, Kjaer P, Daavittila IM, Solovieva S, Okuloff A, Sorensen JS, et al. Genetic risk factors of disc degeneration among 12-14-year-old Danish children: a population study. Int J Mol Epidemiol Genet 2010; 1: 158-65.
16. Bhanushali AA1, Lajpal N, Kulkarni SS, Chavan SS, Bagadi SS, Das BR. Frequency of fokI and taqI polymorphism of vitamin D receptor gene in Indian population and its association with 25-hydroxyvitamin D levels. Indian J Hum Genet 2009; 15: 108-13. [CrossRef]
17. Xu G, Mei Q, Zhou D, Wu J, Han L. Vitamin D receptor gene and aggrecan gene polymorphisms and the risk of intervertebral disc degeneration - a meta-analysis. PLoS One 2012; 7: e50243. doi: 10.1371/journal.pone.0050243. [CrossRef]
18. Toktaş ZO, Ekşi MŞ, Yılmaz B, Demir MK, Özgen S, Kılıç T, et al. Association of collagen I, IX and vitamin D receptor gene polymorphisms with radiological severity of intervertebral disc degeneration in Southern European Ancestor. Eur Spine J 2015; 24: 2432-41. [CrossRef]
19. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16: 1215. [CrossRef]
20. Toptaş B, Kafadar AM, Cacina C, Turan S, Yurdum LM, Yiğitbaşı N, et al. The vitamin D receptor (VDR) gene polymorphisms in Turkish brain cancer patients. Biomed Res Int 2013; 2013: 295791. doi: 10.1155/2013/295791. [CrossRef]
21. Colombini A, Brayda-Bruno M, Lombardi G, Croiset SJ, Vrech V, Maione V, et al. FokI polymorphism in the vitamin D receptor gene (VDR) and its association with lumbar spine pathologies in the Italian population: a case-control study. PLoS One 2014; 9: e97027. doi: 10.1371/journal.pone.0097027. eCollection 2014. [CrossRef]
22. Colombini A, Lanteri P, Lombardi G, Grasso D, Recordati C, Lovi A, et al. Metabolic effects of vitamin D active metabolites in monolayer and micromass cultures of nucleus pulposus and annulus fibrosus cells isolated from human intervertebral disc. Int J Biochem Cell Biol 2012; 44: 1019-30. [CrossRef]
23. Rajasekaran S, Kanna RM, Senthil N, Raveendran M, Ranjani V, Cheung KMC, et al. Genetic susceptibility of lumbar degenerative disc disease in young Indian adults. Eur Spine J 2015; 24:1969-75. [CrossRef]
24. Yaylım-Eraltan İ, Arzu Ergen H, Arıkan S, Okay E, Öztürk O, Bayrak S, et al. Investigation of the VDR gene polymorphisms association with susceptibility to colorectal cancer. Cell Biochem Funct 2007; 25: 731-7. [CrossRef]
25. Neyestani TR, Djazayery A, Shab-Bidar S, Eshraghian MR, Kalayi A, Shariatzadeh N, et al. Vitamin D Receptor Fok-I polymorphism modulates diabetic host response to vitamin D intake: need for a nutrigenetic approach. Diabetes Care 2013; 36: 550-6. [CrossRef]
26. Omair A, Lie BA, Reikeras O, Brox JI. An Association Study of Interleukin 18 Receptor Genes (IL18R1 and IL18RAP) in Lumbar Disc Degeneration. Open Orthop J 2012; 6: 164-71. [CrossRef]
27. Videman T, Leppävuori J, Kaprio J, Battié MC, Gibbons LE, Peltonen L, et al. Intragenic polymorphisms of the vitamin D receptor gene associated with intervertebral disc degeneration. Spine (Phila Pa 1976) 1998; 23: 2477-85. [CrossRef]
28. Cervin Serrano S, González Villareal D, Aguilar-Medina M, Romero-Navarro JG, Romero Quintana JG, Arámbula Meraz E, et al. Genetic polymorphisms of interleukin-1 alpha and the vitamin d receptor in mexican mestizo patients with intervertebral disc degeneration. Int J Genomics 2014; 2014: 302568. doi: 10.1155/2014/302568. [CrossRef]
29. Cauci S, Migliozzi F, Trombetta CS, Venuto I, Saccheri P, Travan L, et al. Low back pain and FokI (rs2228570) polymorphism of vitamin D receptor in athletes. BMC Sports Sci Med Rehabil 2017 9: 4. doi: 10.1186/s13102-017-0069-x. eCollection 2017. [CrossRef]
30. Zhao J, Yang M, Shao J, Bai Y, Li M. Association Between VDR FokI Polymorphism and Intervertebral Disk Degeneration. Genomics Proteomics Bioinformatics 2015; 13: 371-6. [CrossRef]

Experimed 2019; 9(3): 99-104

ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

DOI: 10.26650/experimed.2019.19024

Preliminary Study: DNA Repair Gene Polymorphisms (RRM1, RRM2, ERCC2) In Left Ventricular Hypertrophy

Sol Ventrikül Hipertrofisinde (RRM1, RRM2, ERCC2) DNA Tamir Gen Polimorfizmleri

Fatma Tuba Akdeniz[1], Seda Güleç Yılmaz[1], Cemil Selim İsbir[2], Yaşar Birkan[2], David Sinan Esensoy[3], Gözde Özcan[1], Atike Tekeli Kunt[4], Turgay İsbir[3]

[1]Department of Molecular Medicine, Yeditepe University Institute of Health Sciences, İstanbul, Turkey
[2]Department of Cardiovascular Surgery, Marmara University Pendik Training and Research Hospital, İstanbul, Turkey
[3]Department of Medical Biology, Yeditepe University School of Medicine, İstanbul, Turkey
[4]Department of Cardiovascular Surgery, Ankara Numune Training and Research Hospital, Ankara, Turkey

Cite this article as: Akdeniz FT, Güleç Yılmaz S, İsbir CS, Birkan Y, Esensoy DS, Özcan G, et al. Preliminary Study: DNA Repair Gene Polymorphisms (RRM1, RRM2, ERCC2) In Left Ventricular Hypertrophy. Experimed 2019; 9(3): 99-104.

ABSTRACT

Objective: Left ventricular hypertrophy (LVH) accounts for one of the most important independent risk factors for cardiac diseases. In the present study, we investigate the relationship of DNA repair gene polymorphisms and LVH.

Material and Method: DNA samples isolated from peripheral blood were genotyped with real-time polymerase chain reaction (RT-PCR) for RRM1 (rs12806698), RRM2 (rs6859180) and ERCC2 (rs13181) genes.

Results: Although there were no significant differences for RRM2 (p=0.365) and ERCC2 (p=0.740) genes. RRM1 (A>C) CC genotype was significantly higher in the LVH than control groups (p=0.018). RRM1gene wild type A allele carriers were significantly higher in the healthy controls than the LVH group (p=0.029).

Conclusion: RRM1 gene CC genotype could be a risk factor, whereas the RRM1 gene AC genotype and the A allele might play a protective role against LVH.

Keywords: LVH, DNA repair genes, polymorphism

ÖZ

Amaç: Sol Ventrikül Hipertrofisi (SVH) kardiak hastalıklar için sık görülen bir risk faktörüdür. Bu çalışmada DNA tamir gen polimorfizimleri ve sol ventrikül hipertrofisi arasındaki ilişkiyi araştırdık.

Gereç ve Yöntem: Periferik kandan izole edilen DNA örnekleri RRM1 (rs12806698), RRM2 (rs6859180) ve ERCC2 (rs13181) genleri için Gerçek Zamanlı Polimeraz Zincir reaksiyon (GZ- PZR) ile genotiplendirildi.

Bulgular: Kontrol ve hasta grupları arasında RRM2 (p=0,365) ve ERCC2 (p=0,740) genleri için önemli fark olmamasına rağmen, RRM1 (A>C) CC genotip frekansı SVH hastalarında kontrol grubuna göre anlamlı şekilde yüksek olarak bulundu (p=0,018). RRM1 geni yabani tip A allel taşıyıcıları sağlıklı kontrolde SVH li hastalara göre anlamlı şekilde yüksek olarak tespit edildi (p=0,029).

Sonuç: RRM1 CC genotipi SVH'ne karşı bir risk faktörü olabilirken RRM1 AC genotipi ve A alleli koruyucu role sahip olabilir.

Anahtar Kelimeler: SVH, DNA tamir genleri, polimorfizm

INTRODUCTION

During mammalian organogenesis heart is the first functionally formed organ. Each year approximately one million newborns are known to be born with heart defects, and one in every three people die because of heart diseases, thus cardiovascular diseases are becoming the leading cause of global mortality (1).

Left ventricular hypertrophy (LVH) is responsible for a compensatory mechanism to keep normal wall tension and to maintain cardiac output. When the left ventricle contracts, oxygenated blood is distributed to the body through the aortic valve. If hypertension exists making it difficult for blood to spread throughout the body, the left ventricle will strain to send the blood. This leads to an abnormal increase in the left ventricular (LV) mass, which is called LVH (2). LVH can occur because of hypertension, aortic valvular stenosis, aortic regurgitation, mitral regurgitation, and hypertrophic cardiomyopathy (3). Patients with LVH may experience car-

Corresponding Author/Sorumlu Yazar: Fatma Tuba Akdeniz **E-mail:** akdeniztf@yahoo.com.tr
Received Date/Geliş Tarihi: 23.10.2019 **Revision Date/Revizyon Tarihi:** 01.11.2019 **Accepted Date/Kabul Tarihi:** 14.11.2019

diac failure, coronary artery disease, peripheral arterial disease and sudden death. Stroke risk is increased six-fold in LVH cases (4).

Ribonucleotide reductase (RR) is an enzyme catalyzing the conversion of Adenosine diphosphate (ADP) into Deoxyadenosine diphosphate (dADP) by removing the hydroxyl group from the second position of the ribose ring. Then, dADP is converted to Deoxyadenosine triphosphate (dATP) with the help of creatine kinase (5). The RR enzyme is required for the synthesis of deoxyribonucleotides, it is the rate-limiting step in DNA synthesis and it has two protein subunits important for its activity (6). Ribonucleotide reductase protein M1 (RRM1), is a catalytic activator, and ribonucleotide reductase protein M2 (RRM2), is a free radical-containing subunit (7, 8). Cardiac muscles prefer dATP as a more effective substrate than ATP for contraction, and with an increase in dATP levels, force generation, cross bridge cycling, and calcium sensitivity in the myocardium are known to be increased (9, 10). Excision Repair Cross-Complementing 2 (ERCC2) gene is a nucleotide excision repair mechanism (11).

There are only few studies examining the association between DNA repair genes and cardiovascular diseases. In this study, we aimed to investigate some DNA repair gene polymorphisms (RRM1, RRM2, ERCC2) and discuss their possible roles in the molecular mechanism of LVH.

MATERIAL AND METHOD

Study participants

All participants were selected from Marmara University Cardiovascular Surgery Department after detailed clinical examinations. All individuals signed an informed consent which was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki". The study consisted of 15 patients with LVH and 24 healthy individuals. Echocardiographic parameters were measured using standard methods. Left ventricular systolic functions were estimated by measuring the Ejection Fraction (LVEF, %).

Genotyping

Blood samples from all participants were collected in EDTA-tubes and DNA isolations were performed using Invitrogen iPrep Purification Instrument and Invitrogen iPrep Pure Linkg DNA Blood Kits (Invitrogen, Life Technologies, Carlsbad, CA, USA). DNA concentrations and optical density ratios were measured using NanoDrop 2000 (Thermoscientific, Waltham, MA, USA). Genotyping was performed using Applied Biosystems Fast Real-Time polymerase chain reaction (RT-PCR) instrument and TaqMan reagents primer-probe sets designed for RRM1 gene (rs12806698), RRM2 gene (rs6859180) and ERCC2 gene (rs13181) polymorphisms (Applied Biosystems, Foster City, CA, USA).

LDL-HDL subfraction analysis: Serum samples were analyzed for LDL and HDL subfractions with the LIPOPRINT SYSTEM (Quantimetrix, CA, USA). This system is performed using high-resolution, polyacrylamide gel electrophoresis to separate lipoprotein particles into various fractions on the basis of size and density. LDL was analyzed with 7 subfractions including Large LDL (1 to 2) and Small LDL (3 to 7), while HDL was separated into 10 subfractions with Large HDL (1 to 3), Intermediate HDL (4 to 7) and Small HDL (8 to 10).

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences version 23 software (IBM Corp.; Armonk, NY, USA). Significant differences between groups were determined using the Student's t-test and demographic information was compared using Chi-square and Fisher's exact tests. Risk estimations were examined with Odds Ratio (OR) at 95% Confidence Interval (CI). $p<0.05$ was denoted as statistically significant.

RESULTS

The demographic characteristics of the study population are given in Table 1. This study was conducted 39 samples; 4 female, 11 male in LVH group and 10 female 14 male in healthy control group. There were no statistically significant difference between healthy group and LVH group as demographic characterization (p=0.359). The mean ages of the patients with LVH and the healthy control groups were 66.87±10.63. and 62.46±5.97 (p=0.105). Systolic Blood Pressures (BP) were significantly higher in patients with LVH than the healthy control group (p=0.017). However there were no significantly difference between the groups, LDL-HDL Subfractions values are given as demographic information and were not associated with polymorphism.

Echocardiographic parameters of LVH patients and healthy control group are given in Table 2. Genotype and allele frequencies between LVH patients and the healthy controls are listed in Table 3. Although there were significant differences for neither RRM2 (A>G) gene (*p*=0.365) nor ERCC2 (G>T) (p=0.740) genes, RRM1 (A>C) gene was significantly different in the LVH and control groups (p=0.018). RRM1 heterozygote AC genotype was significantly higher in the healthy control group (p=0.008), but homozygote mutant CC genotype was higher in the LVH group (p=0.029). Regarding RRM1 (A>C) polymorphism, in the patient group the percentage of wild-type A allele was 30% while mutant C allele was 70%. RRM1 A allele carriers were significantly higher in the healthy control group (*p*=0.029), moreover RRM1 A allele carriers had a ~4.5-fold decreased risk for LVH (OR=0.22, 95%CI=0.056-8.889). Our results indicated that RRM1gene CC genotype carrying might increase the risk of LVH, whereas the RRM1 gene AC genotype may decrease the risk, and the A allele might have a protective role against LVH.

Although there was no statistically significant difference between the echocardiographic parameters and the RRM1 allele group, the echocardiographic parameters in the RRM1 A allele carrier group were lower than the non-A allele carrier group and the RRM1 C allele carrier group was higher than the non-C allele carier group (Table 4).

Table 1. Demographic characteristics and clinical values for the patients with LVH and healthy control groups

	LVH (n=15)	Healthy Control (n=24)	p	95% confidence interval (CI)	
				Lower	Upper
Sex (n) (Female/Male)	4/11	10/14	0.359	0.551	0.794
Age (year)	66.87±10.63	62.46±5.97	0.105	-12.071	0.004
Height (cm)	165.67±8.46	168.73±7.50	0.199	-7.775	1.658
Weight (kg)	83.93±14.95	81.23±12.80	0.508	-5.435	10.851
BMI (kg/m^2)	30.83±6.49	28.67±5.23	0.208	-1.23880	5.55830
BSA(m^2)	1.91±0.17	1.91±0.155	0.997	-0.09612	0.09646
Systolic BP (mmHg)	137.33±19.35	124.75±15.80	0.017*	2.370	22.796
Diastolic BP(mmHg)	83.67±6.11	81.63±10.883	0.497	-3.940	8.024
Total Cholesterol (mg/dL)	186.13±45.41	186.90±42.14	0.953	-26.902	25.368
TC (mg/dL)	157.33±48.79	157.93±103.0	0.983	-56.467	55.284
LDL (mg/dL)	118.27±40.32	114.78±42.15	0.783	-21.819	28.802
HDL (mg/dL)	38.13±6.08	42.20 ± 12.23	0.225	-10.717	2.584
Large LDL (mg/dL)	53.93±19.98	53.66±15.00	0.957	-9.837	10.388
Small LDL (mg/dL)	8.20±8.66	4.11±6.11	0.058	-0.136	8.326
Large HDL (mg/dL)	10.21±4.44	12.44±4.371	0.117	-5.033	0.580
Intermediate HDL (mg/dL)	22.93±6.28	21.68 ± 3.78	0.399	-1.708	4.212
Small HDL (mg/dL)	6.43±3.13	8.15± .767	0.139	-4.019	0.582
VLDL (mg/dL)	30.93±9.65	30.95±20.086	0.998	-10.904	10.871

Demografic and clinical laboratory data were expressed as mean ± standard deviations (SD). LVH: left ventricular hypertrophy; n: number of individuals; BMI: body mass index; BSA: body surface area; BP: blood pressure; TC: triglyceride; LDL: low density lipoprotein; HDL: high density lipoprotein; VLDL: very low density lipoprotein
*statistically significant difference (p<0.05).

Table 2. Echocardiographic parameters of patients with LVH and control groups

		LVH (n=15)	Healthy Control (n=24)	p
Interventricular Septum Wall Thickness	(mm)	1.56±0.27	0.95±0.11	0.000*
LVH end diastolic diameter	(mm)	5.58±0.47	4.09±0.23	0.000*
LVH Mass	(g)	583.48±132.13	218.85±33.00	0.000*
LVH Mass Index	(g/m^2)	310.98±71.67	118.51±23.41	0.000*

Data were expressed as mean ± SD. LVH: left ventricular hypertrophy; n: number of individuals
*statistically significant difference (p<0.05)

DISCUSSION

Despite advances in ventricular dysfunction epidemiology, the mechanism of genotypic and phenotypic variations remains unclear. In this study, the relationship of RRM1 (rs12806698), RRM2 (rs6859180) and ERCC2 (rs13181) polymorphisms on LVH were investigated. The AC genotype of RRM1 gene was higher in the control group, indicating that the AC genotype decreases the risk of LVH; The CC genotype was in a high proportion in LVH patients, so carrying the A allele could lower the risk for LVH as its frequency was higher in the healthy control group than in the LVH patients.

Table 3. Genotype and allele frequencies between patients with LVH and the healthy controls

Polymorphism	LVH n (%)	Healthy Control n (%)	p	Odds ratio (OR)	95% confidence interval (CI) Lower	Upper
RRM1 (rs13181)	n=15	n=24	0.018*			
AA	3 (20.0)	2 (8.3)	0.354	2.750	0.402	18.804
AC	3 (20.0)	16 (66.7)	0.008*	0.125	0.027	0.573
CC	9 (60.0)	6 (25)	0.029*	4.500	1.125	17.993
	Allelic count	Allelic count				
A	9 (30)	20 (41.6)	0.029*	0.222	0.056	0.889
C	21 (70)	28 (58.4)	0.354	0.364	0.053	2.487
RRM2 (rs13181)	n=15	n=16	0.365			
AA	12 (80.0)	11 (68.8)	0.685	1.818	0.350	9.455
AG	3 (20.0)	3 (18.7)	0.930	1.083	0.182	6.439
GG	0 (0)	2 (12.5)	0.484	0.875	0.727	1.053
	Allelic count	Allelic count				
A	18 (85.7)	25 (78.1)	0.484	0.194	0.054	0.691
G	3 (14.3)	7 (21.9)	0.685	0.848	0.188	3.823
ERCC2 (rs13181)	n=15	n=24	0.740			
GG	4 (26.7)	5 (20.8)	0.711	1.382	0.305	6.255
GT	5 (33.3)	11 (45.8)	0.440	0.591	0.155	2.258
TT	6 (40)	8 (33.3)	0.673	1.333	0.350	5.076
	Allelic count	Allelic count				
G	13 (43.3)	21 (43.75)	0.673	0.750	0.197	2.855
T	17 (56.7)	27 (56.25)	0.711	0.724	0.160	3.276

LVH: left ventricular hypertrophy; n: number of individuals
*statistically significant difference ($p<0.05$)

Table 4. The relationship between the echocardiographic parameters of patients with LVH according to RRM1 allele carriers

		RRM1 A allele carriers n=6	RRM1 non-A allele carrier n=9	RRM1 C allele carriers n=12	RRM1 non-C allele carrier n=3
Interventricular Septum Wall Thickness	(mm)	1.53±0.35	1.64±0.12	1.65±0.13	1.36±0.45
LVH end diastolic diameter	(mm)	5.23±0.41	5.46±0.25	5.43±0.25	5.13±0.56
LVH Mass	(g)	577.51±87.35	629.81±85.84	629.15±75.94	526.13±94.43
LVH Mass Index	(g/m^2)	262.64±122.67	335.43±55.49	333.52±48.99	285.01±52.12

Data were expressed as mean ± SD. LVH: left ventricular hypertrophy; n: number of individuals

The clinical and laboratory studies showed that dATP acts as a substrate for de-membranated cardiac muscle contraction and is a more effective substrate than ATP. It increases left ventricular contractions both in normal and infarcted heart regions. dATP can be synthesized through a reductive reaction catalyzed by the RRM1 enzyme. Furthermore it has been asserted that RR inhibition could be a therapeutic target of atheroproliferative disorders (12).

Unrepaired DNA base damage triggers apoptotic pathways (13) and apoptosis accelerates myocyte loss and causes myocardial dysfunctions (14). Apoptosis-induced myocyte damage causes abnormal loading and increased wall stress which results in heart failure. In spite of compensatory mechanisms, insufficient DNA repair could lead to myocyte apoptosis that begins at the onset of hypertrophy (14).

The RRM1 gene, is a molecular target of gemcitabine**,** and has very important roles. Increased RRM1 expression levels were correlated with longer survival rates, while it is a disadvantage to have high levels of RRM1 expression because of a decreased efficacy of chemotherapy. Two single nucleotide polymorphisms were discovered upstream of the first exon of the RRM1 gene. They are an adenine/cytosine change at (-) 37 nucleotide position and a cytosine/thymidine change at (-) 524 nucleotide position. These polymorphisms affect promoter activity highly associated with overall survival (15).

Ribonucleotide reductase activity has a role not only in cell cycle but also in the proper functioning of myocytes. RR overexpression increases dATP, affecting the contractility of cardiomyocytes. Elevated cardiomyocyte dATP levels via RR protein increases muscle contractility, subsequently basal cardiac function increases by actin–myosin binding and cycling. Thus, even in cardiac overload, myocytes could maintain normal myocardial energetics (9). Furthermore, it has been claimed that cardiac-specific RR gene therapy may reverse cardiac dysfunction. In the animal model, heart failure treated with the RR gene was inducing myosin activation (10).

According to analysis of sex differences, genders may account for the risk of cardiovascular diseases. In terms of cardiac adaptation, the left ventricular dimensions are smaller and left ventricular performance is higher in women compared to men. Sex differences can play a role in LVH however, in this study, there was no statistical significance related to gender and LVH (16).

One of the most important factors in the pathogenesis of LVH is systolic BP. It has been shown that there is a direct relationship between the incidence of LVH and the level of the systolic BP. Our study supports this finding due to the fact that the difference in the systolic BP between the groups is statistically significant and the mean is higher in patients with LVH than the healthy control group (17).

CONCLUSION

DNA repair mechanisms have important roles in the molecular mechanisms of LVH. Our results showed that there was a relationship between RRM1 gene polymorphism and LVH risk. To clarify and confirm this association, further studies on larger populations are required. If future studies continue to support the current findings, RRM1 gene could be considered as a therapeutic decision target.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Marmara University School of Medicine.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - T.İ., İ.Y.; Supervision - T.İ., İ.Y; Materials - H.E., H.S.; Data Collection and/or Processing - B.T.H.,M.T.H., C.H.; Analysis and/or Interpretation - C.H., İ.Y., A.E., Ü.Z.; Literature Search - B.T.H., D.S. M.T.H..; Writing - B.T.H., C.H.; Critical Reviews - H.S., İ.Y., B.T.H.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Etik Komite Onayı: Bu çalışma için etik komite onayı Marmara Üniversite Tıp Fakültesi'nden alınmıştır.

Hasta Onamı: Yazılı hasta onamı bu çalışmaya katılan hastalardan alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir - T.İ., İ.Y.; Denetleme - T.İ., İ.Y.; Gereçler - H.E., H.S.; Veri Toplanması ve/veya İşlemesi - B.T.H., M.T.H., C.H.; Analiz ve/veya Yorum - C.H., İ.Y., A.E., Ü.Z.; Literatür Taraması - B.T.H., D.S. M.T.H.; Yazan - B.T.H., C.H.; Eleştirel İnceleme - H.S., İ.Y., B.T.H.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

REFERENCES

1. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al. American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. Circulation 2017; 135: 146-603. [CrossRef]
2. Messerli FH, Schmieder R. Left ventricular hypertrophy. A cardiovascular risk factor in essential hypertension. Drugs 1986; 31: 192-201. [CrossRef]
3. Davidson, BP, Giraud GD. Left ventricular function and the systemic arterial vasculature: remembering what we have learned. J Am Soc Echocardiogr 2012; 25: 891-94. [CrossRef]
4. Pokharel P, Bella JN. Regression of left ventricular hypertrophy: Lessons from clinical trials. OA Evidence-Based Medicine 2013; 1: 13. https://doi.org/10.13172/2053-2636-1-2-1110. [CrossRef]
5. Elledge SJ, Zhou Z, Allen JB. Ribonucleotide reductase: regulation, regulation, regulation. Trends Biochem Sci 1992; 17: 119-23. [CrossRef]
6. Oh IJ, Ban HJ, Kim KS, Song SY, Na KJ, Kim YH, et al. Response to gemcitabine-platinum chemotherapy by single nucleotide polymorphisms of RRM1 and ERCC1 genes in patients with non-small-cell lung cancer. Thorac Cancer 2012; 3: 19-26. [CrossRef]
7. Thomson KS, Odom GL, Murry CE, Mahairas GG, Moussavi-Harami F, Teichman SL, et al. Translation of Cardiac Myosin Activation with

2-deoxy-ATP to Treat Heart Failure via an Experimental Ribonucleotide Reductase-Based Gene Therapy. JACC Basic Transl Sci 2016; 1: 666-79. [CrossRef]

8. Korte FS, Dai J, Buckley K, Feest ER, AdamekN, Geeves MA, et al. Upregulation of cardiomyocyte ribonucleotide reductase increases intracellular 2 deoxy-ATP, contractility, and relaxation. J Mol Cell Cardiol 2011; 51: 894-901. [CrossRef]
9. Nowakowski SG, Kolwicz SC, Korte FS, Luo Z, Robinson-Hamm JN, Page JL, et al. Transgenic overexpression of ribonucleotide reductase improves cardiac performance. Proc Natl Acad Sci U S A 2013; 110: 6187-92. [CrossRef]
10. Kadota S, Carey J, Reinecke H, Leggett J, Teichman S, Laflamme MA, et al. Ribonucleotide reductase-mediated increase in dATP improves cardiac performance via myosin activation in a large animal model of heart failure. Eur J Heart Fail 2015; 17: 772-81. [CrossRef]
11. Cervelli T, Borghini A, Galli A, Andreassi MG. DNA damage and repair in atherosclerosis: current insights and future perspectives. Int J Mol Sci 2012; 13:16929-44. [CrossRef]
12. Gallaugher LD, Henry JC, Kearns PN, Elford HL, Bergdall VK, Cardaunel AJ. Ribonucleotide reductase inhibitors reduce atherosclerosis in a double-injury rabbit model. Comp Med 2009; 59: 567-72.
13. Nowsheen S, Yang ES. The Intersection Between Dna Damage Response And Cell Death Pathways. Exp Oncol 2012; 34: 243-54.
14. Choi YH, Cowan DB, Moran AM, Colan SD, Stamm C, Takeuchi K, et al. Myocyte apoptosis occurs early during the development of pressure-overload hypertrophy in infant myocardium. J Thorac Cardiovasc Surg 2009; 137: 1356-62. [CrossRef]
15. Bepler G, Zheng Z, Gautam A, Sharma S, Cantor A, Sharma A, et al. Ribonucleotide reductase M1 gene promoter activity, polymorphisms, population frequencies, and clinical relevance. Lung Cancer 2005; 47: 183-92. [CrossRef]
16. Garavaglia GE, Messerli FH, Schmieder RE, Nunez BD, Oren S. Sex differences in cardiac adaptation to essential hypertension. Eur Heart J 1989; 10: 1110-4. [CrossRef]
17. Gradman AH, Alfayoumi F. From Left Ventricular Hypertrophy to Congestive Heart Failure: Management of Hypertensive Heart Disease. Prog Cardiovasc Dis 2006; 48: 326-34. [CrossRef]

Experimed 2019; 9(3): 105-12

ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

DOI: 10.26650/experimed.2019.19026

B Cell Immunophenotyping and Expression Analysis of B Cell Specific Molecules of Patients with Benign Multiple Sclerosis

Benign MS Hastalarinin B Hücre İmmünfenotiplemesi ve B Hücresine Özgü Moleküllerin Ekspresyon Analizi

Melis Şen[1], Ece Akbayır[1], Recai Türkoğlu[2], Erdem Tüzün[1], Vuslat Yılmaz[1]

[1]Department of Neuroscience, İstanbul University Aziz Sancar Institute of Experimental Medicine, İstanbul, Turkey
[2]Department of Neurology, Haydarpasa Numune Training and Research Hospital, İstanbul, Turkey

Cite this article as: Şen M, Akbayır E, Türkoğlu R, Tüzün E, Yılmaz V. B Cell Immunophenotyping and Expression Analysis of B Cell Specific Molecules of Patients with Benign Multiple Sclerosis. Experimed 2019; 9(3): 105-12.

ABSTRACT

Objective: Multiple Sclerosis (MS) is a progresive and an immune mediated inflammatory central nervous disease. The focus of this study was to determine the possible relationship between B cell immunophenotypes and related gene expressions in the benign MS (BMS) group with disease and cognitive processes.

Material and Method: Twenty BMS patients, 16 non-BMS and 28 healthy volunteers were included in the study. Gene expression was performed by real-time PCR (RT-PCR). Immunophenotyping of peripheral B cells was also evaluated by flow cytometry. The relationship between cognitive functions and gene expression levels and B cell subtypes was investigated.

Results: It was observed that naïve ($CD19^+$ IgD^+CD27^-) cells were higher in the BMS group compared to the healthy group (HC), and memory B cells showed opposite changes. Un-switched memory B cells($CD19^+IgD^+CD27^+$) were found to be higher in the benign group than in the HC. The expression of BANK and BLNK was found to be lower in both MS groups than in the HC. As a result of neuropsychological examinations and cognitive tests; it was observed that motor processes in BMS were better protected than Non-BMS.

Conclusion: These findings support that B cell functions may have molecular and cellular effects, and may lead to regression in inflammation and clinical progression. Molecules showing significant changes in our study may play a role as prognostic biomarkers in MS.

Keywords: Benign Multiple Sclerosis, B cell, immunophenotyping, gene expression

ÖZ

Amaç: Multipl Skleroz (MS), aksonal dejenerasyona, demiyelinizasyona ve inflamasyona bağlı gelişen, merkezi sinir sistemini etkileyen progresif bir hastalıktır. Bu çalışmada benign MS (BMS) grubunda periferik kan B hücre immünofenotiplerinin ve B hücresi ile ilişkili gen ekspresyonlarının hastalık ve bilişsel süreçlerle ilişkisinin araştırılması hedeflenmiştir.

Gereç ve Yöntem: Yirmi BMS hastası, 16 benign olmayan MS (Non-BMS) hastası ve 28 sağlıklı gönüllü çalışmaya dahil edildi. Daha önce periferik kan hücrelerinde yapılan gen mikroarray çalışması ile gruplar arasında ekspresyonu değişikliği gözlenen genlerin validasyonu gerçek zamanlı PZR ile yapıldı. Periferik B hücrelerinin immünofenotiplemesi akım sitometrisi ile değerlendirildi. Bilişsel fonksiyonlar ile gen ekspresyon seviyeleri ve B hücre alttipleri arasındaki olası ilişki araştırıldı.

Bulgular: Naif ($CD19^+$ IgD^+CD27^-) B hücrelerinin BMS grubunda sağlıklılara göre yüksek olduğu, hafıza B hücrelerinin zıt yönde değişiklik gösterdiği gözlendi. Dönüşmemiş hafıza B hücrelerinin ($CD19^+IgD^+CD27^+$) ise benign grupta sağlıklılara göre yüksek olduğu belirlendi. BANK ve BLNK gen ekspresyonları her iki MS grubunda da sağlıklılardan düşük olarak belirlendi. Nöropsikolojik incelemeler ve kognitif testler sonucunda, BMS'te motor süreçlerin Non-BMS'ye göre korunduğu gözlendi.

Sonuç: Bu bulgular B hücresi işlevlerinin moleküler ve hücresel etkileri olabileceği ve inflamasyon ile klinik progresyonda gerilemeye yol açabileceği yönündeki görüşleri desteklemektedir. Değişiklik gösteren moleküllerin MS hastalığında prognostik biyobelirteç olarak rol oynaması da mümkündür.

Anahtar Kelimeler: Benign Multipl Skleroz, B hücresi, immünfenotipleme, gen ekspresyonu

Corresponding Author/Sorumlu Yazar: Vuslat Yılmaz **E-mail:** vuslaty@hotmail.com
Received Date/Geliş Tarihi: 18.11.2019 **Revision Date/Revizyon Tarihi:** 21.11.2019 **Accepted Date/Kabul Tarihi:** 27.11.2019

INTRODUCTION

Multiple sclerosis (MS) is a multifactorial chronic demyelinating disease that affects the patient's quality of life, and the exact cause of disease is unknown. It is a disease characterized by repetitive demyelination in the central nervous system (CNS), with autoimmunity in the pathophysiology (1). Although the pathogenesis of MS is not fully known, migration of autoreactive lymphocytes through the blood brain barrier into the CNS is thought to initiate the inflammation process (2). Clinical subtypes of MS; attacks (RRMS), primary progressive (PPMS), secondary progressive (SPMS), progressive-recurrent and benign MS (BMS) are classified as (3). BMS is a retrospective diagnosis characterized by low lesion burden on MRI, which is characterized by rare attacks without serious sequelae. Patients with EDSS scores ≤ 3-15 years after the onset of the disease are considered BMS (3, 4). Although a relatively slow progression is observed in the somatic neurological findings of BMS patients, other nervous system functions may be severely impaired (5).

T lymphocytes have played a major role in MS immunopathogenesis until recently. Autoreactive T lymphocytes and antibodies that develop against CNS elements in MS cases play a role in the formation of tissue lesion and inflammation, and T lymphocytes react to myelin by causing demyelination (6, 7). However, recent studies also have shown the importance of B cells in the pathogenesis of MS. There is evidence that B cells have different effects other than antibody production, such as antigen uptake and presentation, stimulation of T lymphocytes, cytokine, chemokine and neurotropic factors. Treatment approaches for the removal of antibodies from the circulation leads to improvement in MS, and is an indication that B cells play an active role in the pathogenesis of the disease (7). The fact that monoclonal antibody-based treatment methods targeting B cells are effective in stopping the progression of the disease suggested that B cells also play a role in the development of disability (8, 9).

Cognitive impairment is frequently seen in MS and affects 70% of patients. Cognitive functions are affected in the late and early stages of the disease (including clinically isolated syndrome), and impairments in the course of the disease may occur. There are many studies showing that cognitive findings deteriorate in BMS cases (5).

The aim of our study was to evaluate the role of peripheral blood B cells in the BMS group, which had not previously been studied in the literature. Immunophenotyping of B cells and their subgroups (plasma, plasmablast, naïve, memory and regulatory), and expression of genes associated with B cells, are aimed to determine the possible relationship with cognitive processes. In light of this information, the goal of this research was to determine the importance of B-cell immunophenotyping in BMS and the importance of B-cell-related genes in expression analysis, and the possible relationship of MS subgroups to cognitive processes.

MATERIAL AND METHOD

Study Groups

A total of 36 multiple sclerosis patients (BMS, n=20 and non-Benign MS (Non-BMS), n=16), who were followed up from the Multiple Sclerosis Outpatient Clinic of Neurology Department of Istanbul Medical Faculty, and age/sex-matched healthy individuals as the control group (n=28) were included in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of İstanbul University, İstanbul School of Medicine, Clinical Research Ethical Committee (Project Number 2018/449). Participants were selected from patients who were over 18 years of age, diagnosed according to the revised McDonald criteria for 2017, and had no attacks in the last three months, and the healthy control group was selected from participants who were over 18 years of age, had no neurological and autoimmune disorders, had no infectious diseases in the last 3 months, and had not used immunosuppressive drugs (Table 1).

Isolation of Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells (PBMC) were isolated using the gradient method. For this purpose, donor blood collected in EDTA containing tubes was diluted with the same volume of phosphate buffered saline (PBS) and added slowly to Ficoll (Lympho-paque). Tubes were centrifuged at 3000 rpm for 20 minutes at 20°C with brake. In the resulting cell gradient, the mononuclear cell layer was collected, centrifuged at 1800 rpm for 10 minutes at 4°C by adding the same volume of PBS and the supernatant was removed. The viability of the cells was determined by trypan blue and freezed in -80°C with $1x10^6$ cells in fetal bovine serum (FBS) with 10% Dimethyl sulfoxide (DMSO).

Immunophenotyping

Frozen cells were dissolved in a water bath that sets at 37°C and centrifuged in medium (RPMI 1640 and 10% FBS) at 1800 rpm at + 4°C for 10 minutes. Then, cells were stained with anti-human monoclonal CD19-APC, CD24-PerCP, IgD-APC/Cy7, CD138-PE, CD27-FITC, and CD38-Alexa fluor 700 (Biolegend) conjugates for 30 min at 4 °C, then, washed with PBS and resuspended in PBS. Immunofluorescence staining was performed (BD FACS Aria II), and data were analyzed using the FlowJo software.

RNA Isolation and Determinating Target Genes

For identification of candidate genes, a microarray assay was performed. RNA expression profiles obtained from PBMCs of 16 participants, 5 RRMS, 6 BMS and 5 healthy control groups, were determined with the *Sureprint G3 Human Gene Expression V3 microarray* (MA) system. In this context, a total of 26083 Entrez genes were evaluated, and microarray analysis determined the target genes that showed changes between the groups.

In order to do validation of these candidate genes, RNA isolation from PBMC was performed according to the instructions of the QIAGEN RNeasy Mini kit (Hilden, Germany). The quality and quantity of the obtained RNA were evaluated spectrophotometrically. For the purity of RNA, the OD value at 260 nm/280 nm between 1.9 and 2.1 were included in the study.

Table 1. Clinical and demographic data

	BMS (n=20)	Non-BMS (n=16)	Healthy Control (n=28)	p
Sex (F/M)	17/3	10/6	18/10	0.221
Age (years)	40.5±9.54	47.1±8.4	39.3±8.7	0.024
Age at disease onset (years)	25.3±8.39	32.4±9.36		0.022
Disease duration (years)	13.5±4.35	14.1±4.12		0.69
EDSS score	2.42±0.52	5.1±0.95		<0.0001
Total Attack Scores	7.75±4.33	7.3±4.25		0.76

Table 2. RT-PCR programme

Program	Temperature (°C)	Time (h:min:sec)	Cycle
Pre-incubation	95	00:08:00	1
Amplification	95	00:00:15	40
	55	00:00:05	
	72	00:00:10	
Melting Curve	95	00:00:05	1
	65	00:01:00	1
	97	Continuous	1
Cooling	4	00:00:20	1

Table 3. Target genes and primers

Gene	Primer Sequence
BLK_Frw	TAGATCACAGGGTCG-GAAGG
BLK_Rev	GGCAGCGGATCTTATAGTGC
TGFB1_Frw	GTACCTGAACCCGTGTTGCT
TGFB1_Rev	CAACTCCGGTGACATCAAAA
ATP1B3_Frw	CAGTCTGTCCTGATGGAGCA
ATP1B3_Rev	TGGCACTCCTTCAGGCTTTA
BANK1_Frw	GTTCAGACCCCGCACATATT
BANK1_Rev	CCTTCCCCTTCCATTTCATT
BLNK_Frw	GAGCAGTGGTCCGATGACTT
BLNK_Rev	TGGGCTTACTGGGAAGTGTC
FCRL2_Frw	CTCTGGGGACTGTTTGGTGT
FCRL2_Rev	GGTTGGGCTTGAATAGGTGA
SWAP70_Frw	CGGTGCTGAAGGTTCCTCAT
SWAP70_Rev	GACACAGAGGGTCCAACACA
CCL19_Frw	CCTGCTGGTTCTCTGGACTT
CCL19_Rev	GTGAACACTACAG-CAGGCAC
GAPDH_Frw	CCATCAATGACCCCTTCATT
GAPDH_Rev	TTGACGGTGCCATGGAATTT

Synthesis of cDNA and real-time PCR Studies

cDNA synthesis was carried out with the Transcriptor First Strand cDNA Synthesis Kit (Basel, Switzerland), according to the manufacturer's instructions. All samples were prepared with 10 ng / del cDNA in the tubes. real-time PCR (RT-PCR) reactions were performed on the LightCycler 480 instrument, and following the instructions of the Fast Start DNA Master SYBR Green I kit (Roche, Basel, Switzerland). Gene-specific reverse and forward primers were used at a concentration of 600-800 nM. For cDNAs, the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene, which is expressed in equal amounts in each tissue and responsible for basic cellular functions (house-keeping gene, reference gene), was used. cDNA samples 100ng and forward / reverse primers (Table 2) 600-800 nM were used and the program in Table 3 was applied. Amplification curves and melting peaks were evaluated after the procedure.

Cognitive Tests

Based on the literature findings, a test battery has been created for attention, memory, processing speed and executive function areas, which are reported to be the most impaired in MS. The neuropsychological tests to be performed accordingly are Rey auditory and verbal learning, number index, WAIS-R's password subtest, verbal fluency, Wisconsin card matching and Stroop tests. The cognitive tests applied in the study are shown in Table 4.

Statistical Analysis

ANOVA test was used for parametric data, to compare the clinical and demographic characteristics of the patients and healthy control subjects, and paired comparisons between the disease subgroups were performed by the Student t-test or Mann-Whitney U test. Nonparametric data were compared with the chi-square test. Flow cytometry and RT-PCR results were compared with ANOVA and Tukey's post-hoc test in cases with more than two groups, and Student's t-test in cases with more than two groups. Relative quantification of target genes

was performed by 2–ΔΔCT method using the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the reference gene. Pearson correlation test was used for correlation analysis, statistical significance was defined as p value less than 0.05, and a analysis was performed using the SPSS 21.0 program. The GraphPad Prism 5 program was used for the graphs.

RESULTS

Clinical and Demographic Features

The age, onset age, duration of disease, EDSS and number of attacks of the BMS, Non-BMS MS and healthy control subjects were compared. BMS patients' age, disease onset age and EDSS scores were significantly lower than respectively Non-BMS patients (p=0.002, p=0.022 and p<0.0001). Duration of disease and total number of attacks were not different between the groups (p=0.69 and p=0.76). All MS cases included in the study were under an immunomodulatory treatment (inferferon-beta, glatiramer acetate or fingolimod) (Table 1).

Table 4. Cognitive tests

Cognitive Test	Related to Cognitive Process
Selective Reminding Test (Srttl)	Verbal Memory Acquisition
Spatial Recall Test (Sparttl)	Visual Memory Acquisition
Sustained Attention and Speed of Information Processing	Executive Functions
Symbol Digit Modalities Test (SDMT)	Executive Functions
Controlled Oral Word Association Test (COWAT)	Executive Functions
9-Hole Peg Test	Motor Functions
Timed 25-Foot Walk Test	Motor Functions
Beck Test	Depression

Comparison of Peripheral Blood Mononuclear Cell Phenotypes

Distribution of Peripheral Blood B, T and Natural Killer Cells

Peripheral blood mononuclear cell (PBMC) groups of 20 BMS and 16 Non-BMS cases and 28 healthy control donors were evaluated. When the PBMCs of all the subjects were evaluated; the percentage of CD19 expressing B cells was not found to be different between the groups, but $CD3^+$ T cell (p=0.0019) and $CD3^-CD16^+$ $CD56^+$ natural killer (NK) cell (p=0.0349) groups were found to be different. $CD3^+$ T cells were significantly lower in both the BMS group (p<0.01) and the Non-BMS group (p<0.05) compared to healthy subjects. When NK cells were evaluated, it was determinated that these cells were significantly higher in the Non-BMS group than in the healthy ones (p<0.05) (Figure 1).

Distribution of Peripheral Blood B Cell Immunophenotypes Between Groups

In immature ($CD19^+IgD^-CD27^-$) subgroup of immature B cells that did not encounter antigen, there was no difference between the study groups, whereas the percentage of naive ($CD19^+IgD^+CD27^-$) cells was higher in the BMS group compared to healthy subjects (p<0.05). In contrast, memory B cells showed significant changes in the opposite direction. Unswitched memory B cells ($CD19^+IgD^+CD27^+$) were found to be higher in the benign group than in healthy subjects (p <0.01). The switched ($CD19^+IgD^-CD27^+$) (p=0.05) memory B cells were found to be lower in both patient groups compared to healthy subjects (p<0.05). However, there was no difference between the MS subgroups. Plasmablasts ($CD19^+CD38^{++}CD138^-$), which are antibody-producing B cell precursors, had similar percentages in the groups. However, it was observed that antibody-producing B cells, plasma cells ($CD19^+CD38^+CD138^+$)

Table 5. Distribution of cognitive tests in disease groups

	BMS (n=20)	Non-BMS (n=16)	Healty Control (n=28)	ANOVA p	p value (BMS vs. non-BMS)	p value (BMS vs. HC)	p value (non-BMS vs. HC)
Verbal Memory Test	8.1±1.5	7.1±1.61	9.2±1.3	<0.0001		<0.05	<0.001
Visual Memory Test	4.7±1.83	4±1.68	6.2±1.5	<0.0001		<0.01	<0.001
PASAT Test	38.4±13.2	33.3±11.7	48.68±7.67	<0.0001		<0.01	<0.001
SDMT Test	37.5±14.7	27.1±11.8	53.6±19.27	<0.0001		<0.01	<0.001
9-Hole PegTest	20.8±2.63	30±6.19	18.63±2.15	<0.0001	<0.001		<0.001
Timed 25-Foot Walk Test	6.81±1.13	15.13±7.94	6.1±1.48	<0.0001	<0.001		<0.001
COWAT Test	55.9±20.19	45±22.96	75.6±17.48	<0.0001		<0.01	<0.001
Stroop Test	56.95±31.9	68.15±32.82	36.8±14.7	0.0002		<0.05	<0.001
Beck Test	13.55±7.69	13.57±7.67	7±5.72	0.0005		<0.01	<0.01

tended to be lower in the BMS group than in the non-benign group. In the regulatory B cells (Breg, $CD19^{+}CD24^{++}CD38^{++}$) which had the feature of immune suppressor cells, a significant increase was detected in the BMS group compared to healthy subjects ($p<0.05$) (Figure 2).

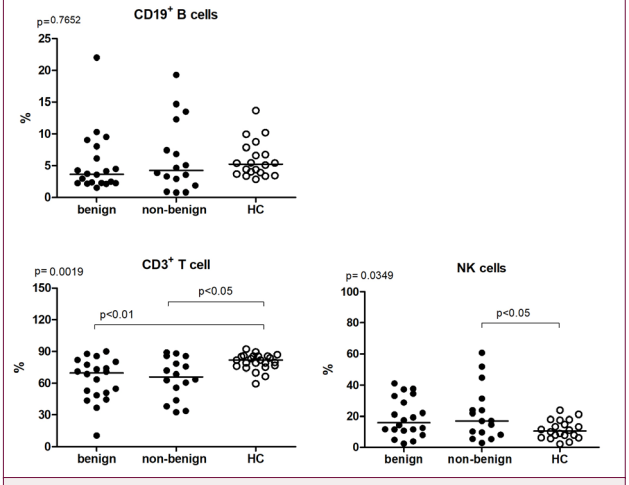


Figure 1. Distribution of peripheral blood B, T and natural killer cells in the study groups

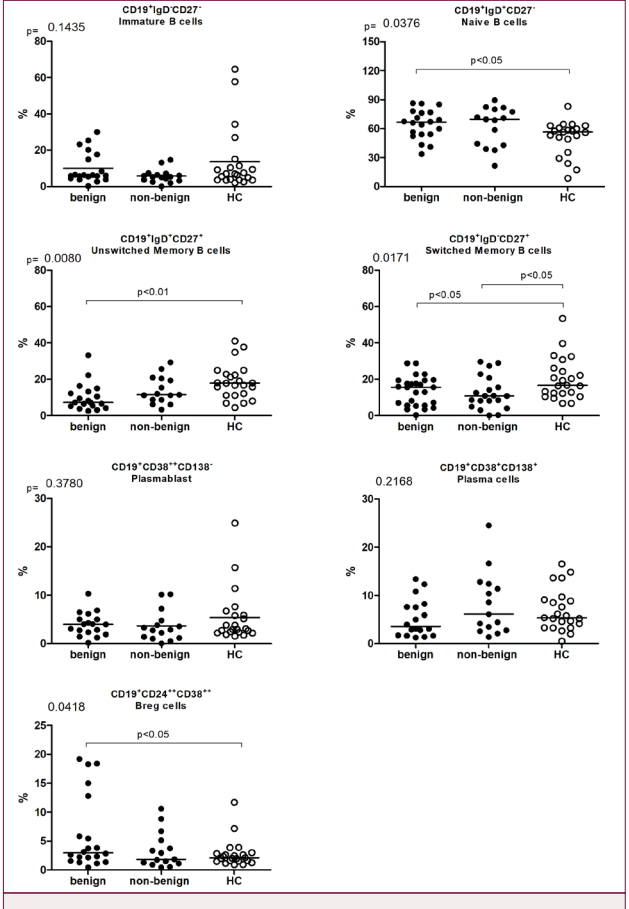


Figure 2. Distribution of peripheral blood B cell immunophenotypes in the study groups

Validation of Genes by RT-PCR

Quantitative PCR primers were designed to validate the expression patterns of target genes determined by microarray data analysis in PBMCs of the study group. First, the sequences of the target genes were reached from http://www.ncbi.nlm.nih.gov/gene, and then, the appropriate primer sequences were designed with the help of http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi (Table 2). Cases with sufficient RNA samples (13 BMS and 12 non-BMS, 10 healthy control) were studied in duplicate for all genes.

Expression analysis of the TGFB1, BANK1, BLNK, FCRL2, CCL19 and BLK genes related to B cell subtypes and ATP1B3, SWAP70 genes related to both T and B cell subtypes were performed. Expression of BLK ($p<0.05$), TGFB ($p<0.05$) and FCRL ($p<0.01$) genes was significantly lower in non-benign MS patients than in healthy subjects. However, expression of KCNS was found significantly higher in the non-BMS group. In the BANK and BLNK genes ($p<0.01$ and $p<0.05$, respectively), significantly lower expression was detected in both MS groups than in healthy subjects (Figure 3).

In particular, its association with B cell functions as a candidate gene supports B cell contribution in the pathogenesis of MS. The genes that have changed as a result of microarray analysis are as follows: BLK (Proto-Oncogene, Src Family Tyrosine Kinase) triggers B cell activation signal after the antigen interacts with the B cell. TGFB is produced by Breg cells and has supressive effects. BANK1 (B Cell Scaffold Protein with Ankyrin Repeats) provides the mobilization of calcium from the stores during B cell receptor interaction. KCNS (Potassium Voltage-Gated Channel Modifier Subfamily S Member 1) are associated with the regulation of the resting membrane potential and the control of the shape and frequency of action potentials. FCRL2 (Fc Receptor Like A 2) mediate the destruction of IgG-coated antigens and of cells induced by antibodies.

There was no correlation between gene expression levels and demographic data, B cell subtype rates and gene expression

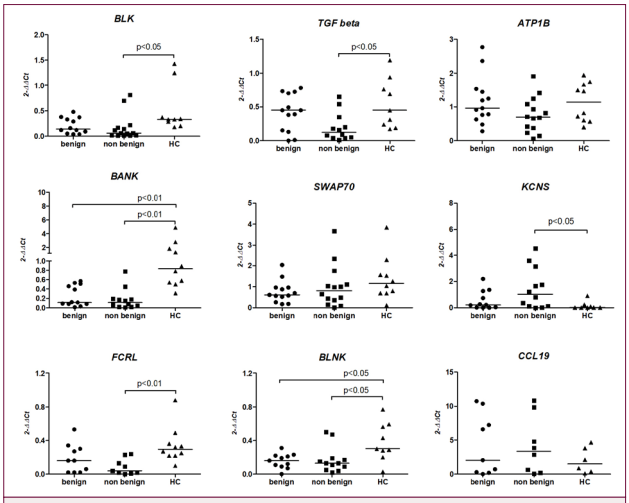


Figure 3. Distribution of gene expression in the study groups

levels, but inverse correlations were determined between BANK1 and executive functions tests (respectively p=0,028 R=-0,439 ve p=0,0086 R=-0,51).

Cognitive Analysis

As a result of neuropsychological examinations and cognitive tests; motor processes in BMS patients were better preserved than in non-BMS. When the cognitive parameters were evaluated, it was found that BMS cases showed the worse performance in all cognitive functions compared to healthy subjects and no significant difference was found in non-BMS BMS comparisons. Beck depression inventory scores were also significantly lower in the benign and non-BMS groups compared to the healthy group (Table 5).

DISCUSSION

Multiple sclerosis is an autoimmune, progressive disease of the CNS with progressive demyelination, inflammation and axonal degeneration (1). In the later stages of the disease, lesions developing due to demyelination in CNS are spread to certain regions of the optic nerves, brain stem, cerebellum and white matter (12). These highly complex etiologic factors of MS (such as autoimmunity, genetic factors, infectious agents, vitamin D, smoking, stress, sleeplessness, drug use) may also vary with age, gender, and ethnicity (13).

The discovery of the key role of B cells in recent years, and the fact that these cells are involved in autoimmune diseases such as MS, also supports the relationship between MS and the interaction with T cells (14). In addition, antibody-producing plasma cells and anti-neuronal antibodies have been reported to play an important role, but B cells with antigen uptake and presentation, stimulation of T lymphocytes and cytokine production have been reported to be effective in the pathogenesis of the disease (6).

In MS, plasmablasts pass to the periphery and migrate to inflamed brain tissue. In this case, plasmablast and plasma cell production in lymphoid tissue is increased or memory cells in brain tissue start to produce pathogenic antibodies (6). Therefore, the most common B cell subtype found in MS plaques is a long-lasting plasma cell (11, 15). The fact that monoclonal antibody-based treatment methods targeting B cells are effective in stopping the progression of the disease suggested that B cells also play a role in the development of disability (8, 9).

In this study, benign MS cases considered to be a good prognostic type of MS because of their low disability despite long disease duration were studied. The data obtained from these cases were compared with those of patients with a higher disability level during the same disease period. The main goal of the study was to determine the importance of B-cell immunophenotyping in BMS patients, and the importance of B-cell-related genes in the expression analysis, and the possible relationship of MS subgroups to cognitive processes.

As a result of immunophenotyping studies, unswitched and switched memory cells of effector B cells, which have an important role in the pathogenesis of autoimmune diseases due to their pro-inflammatory properties, were suppressed in MS groups. Among these, unswitched memory B cells were lower in BMS patients than in non-benign ones. Plasma cells, another effector B cell group, likewise tended to be low in BMS cases. Regulatory B lymphocytes with immunosuppressive properties were significantly higher in the BMS group compared to the other study groups. These findings suggest that one of the factors that may cause MS to remain at a low level of disability over a long period of illness may be the change in inflammatory memory B and anti-inflammatory regulatory B cell ratios.

In a previous microarray study, it was shown that suppression of the RNA-polymerase 1 pathway, which is associated with cell survival in BMS cases, causes the effector lymphocytes to easily develop apoptosis and have a short survival (10). This study showed that mechanisms controlling B cell activity suppress MS progression, thus identifying a mechanism that has not been previously described in the literature. Consistent with the results of our study, another study found that the risk of developing MS was low in patients with clinically isolated syndrome with low memory B lymphocyte ratios in peripheral blood (16). Thus, B cell subtypes have been shown to play a role in preventing the progression of the disease in both early and advanced stages of MS. As an important finding, suppression of T cells and some B cell subtypes was found in benign and non-BMS cases compared to healthy subjects. It is possible that this finding is due to the effect of immunomodulatory therapy, and suppression of memory B cells may be a treatment side effect. In this case, the higher level of suppression of memory B cells observed in BMS may be due to the stronger and more effective response of immunomodulatory therapy to this MS subtype.

There was no significant difference between the groups in RT-PCR analysis due to the low number of cases. However, similar to phenotyping studies, anti-inflammatory TGFβ levels were found to be higher in benign MS cases. In addition, disability levels of patients with high TGFβ levels were found to be low. TGFβ is a well-known cytokine with anti-inflammatory effects in the pathogenesis of MS. In both MS cases and animal models of MS, a relationship was found between TGFβ and disability levels. The association of this cytokine with regulatory B lymphocyte levels is also known (17, 18). However, there are no studies on the effect of TGFβ in BMS. It is possible that the high regulatory B percentage in BMS is one of the factors that determine low memory B cell ratios.

Verbal response test results were found to be high in cases with no expression level change between benign and non-BMS cases, and low levels of expression of BANK1 gene (11) which had an effect on B lymphocyte proliferation. The association of this factor with B cell functions is well known, but there are no studies showing its association with MS. It will be appropriate to test expression levels of all the genes studied with a higher

number of cases and to determine whether these factors may be a predictor of MS prognosis.

Another interesting feature of our study is related to cognitive tests. It was shown that somatic neurological findings (motor, sensory, vision, balance) are preserved in BMS, but cognitive and limbic networks are affected and therefore, the definition of BMS is a deceptive diagnosis. In addition, the lack of correlation between B cell subtype rates and B cell gene expression levels and cognitive test scores suggests that B cells play a role mostly in the progression of physical disability, but different factors are effective in the progression of cognitive findings.

The use of total peripheral blood mononuclear cells instead of isolated B cells in the expression studies is a limitation of the study. In addition, the determination of intracellular cytokine levels in addition to surface markers during immunophenotyping could contribute to a better understanding of the immunological mechanisms.

In conclusion, it was shown that anti-inflammatory B cells were increased, the levels of genes supporting B cell development were decreased, and B cell suppressed genes were increased in a group of MS patients with close clinical and demographic characteristics. These findings support the view that B cell functions may have molecular and cellular effects, and may lead to regression in inflammation and clinical progression. In addition, it is possible that molecules showing significant changes in our study may play a role as prognostic biomarkers in MS. In future studies, validation of the value of BLK, TGFB, BANK, KCNS, FCRL and BLNK gene expressions in isolated peripheral B cells as a biomarker to determine the effect of B cells in the pathogenesis of MS is planned in a wider patient population.

Ethics Committee Approval: Ethics committee approval was received for this study from the Clinical Research Ethics Committee of İstanbul University School of Medicine (2018/449).

Informed Consent: Written informed consent was obtained from the parents' of the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - T.İ., İ.Y.; Supervision - T.İ., İ.Y; Materials - H.E., H.S.; Data Collection and/or Processing - B.T.H.,M.T.H., C.H.; Analysis and/or Interpretation - C.H., İ.Y., A.E., Ü.Z.; Literature Search - B.T.H., D.S. M.T.H..; Writing - B.T.H., C.H.; Critical Reviews - H.S., İ.Y., B.T.H.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This study was funded by Scientific Research Projects Coordination Unit of İstanbul University (Project number 30793).

Etik Komite Onayı: Bu çalışma için etik komite onayı İstanbul Üniversitesi Tıp Fakültesi Klinik araştırmalar Etik Komitesinden alınmıştır (2018/449).

Hasta Onamı: Yazılı hasta onamı bu çalışmaya katılan hastaların ebeveynlerinden alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir - T.İ., İ.Y.; Denetleme - T.İ., İ.Y.; Gereçler - H.E., H.S.; Veri Toplanması ve/veya İşlemesi - B.T.H., M.T.H., C.H.; Analiz ve/veya Yorum - C.H., İ.Y., A.E., Ü.Z.; Literatür Taraması - B.T.H., D.S. M.T.H.; Yazan - B.T.H., C.H.; Eleştirel İnceleme - H.S., İ.Y., B.T.H.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Bu çalışma İstanbul Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından desteklenmiştir (Proje No:30793).

REFERENCES

1. Garg N, Smith TW. An update on immunopathogenesis, diagnosis and treatment of multiple sclerosis. Brain Behav 2015; 5: 1-13. [CrossRef]
2. Samkoff LM, Goodman AD. Multiple Sclerosis and CNS İnflammatory Disorders,Wiley-Blackwell; 2014. [CrossRef]
3. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: Results of an international survey. Neurology 1996; 46: 907-11. [CrossRef]
4. Hawkins SA, McDonell GV. Benign multiple sclerosis? Clinical course, long term follow up, and assessment of prognostic factors. J Neurol Neurosurg Psychiatry 1999; 67: 148-52. [CrossRef]
5. Ozakbas S, Turkoglu R, Tamam Y, Terzi M, Taskapilioglu O, Yucesan C, et al N. Prevalence of and risk factors for cognitive impairment in patients with relapsing-remitting multiple sclerosis: Multi-center, controlled trial. Mult Scler Relat Disord 2018; 22: 70-6. [CrossRef]
6. Tuzun E. Multipl skleroz patogenezinde B hücrelerinin rolü ve B hücre karşıtı monoklonal antikor tedavileri. Nöropsikiyatri Arşivi 2011; 48: 73-8. [CrossRef]
7. Sospedra M, Martin R. Immunology of multiple sclerosis. Annu Rev Immunol 2005; 23: 683-747. [CrossRef]
8. Naegelin Y, Naegelin P, von Felten S, Lorscheider J, Sonder J, Uitdehaag BMJ, et al. Association of Rituximab Treatment With Disability Progression Among Patients With Secondary Progressive Multiple Sclerosis JAMA Neurol 2019; 76: 274-81. [CrossRef]
9. Yamout BI, El-Ayoubi NK, Nicolas J, El Kouzi Y, Khoury SJ, Zeineddine MM.Safety and Efficacy of Rituximab in Multiple Sclerosis: A Retrospective Observational Study. J Immunol Res 2018; 2018: doi: 10.1155/2018/9084759. [CrossRef]
10. Achiron A, Feldman A, Magalashvili D, Dolev M, Gurevich M. Suppressed RNA-polymerase 1 pathway is associated with benign multiple sclerosis. PLoS One 2012; 7: doi: 10.1371/journal.pone.0046871. [CrossRef]
11. Yang J, Ren J, Yang Y, Sun J, Zhou X, Zheng S, et al. BANK1 alters B cell responses and influences the interactions between B cells and induced T regulatory cells in mice with collagen-induced arthritis. Arthritis Res Ther 2018; 20: 9. doi: 10.1186/s13075-017-1503-x. [CrossRef]
12. Kurtzke, J.F., A reassessment of the distribution of multiple sclerosis. Acta Neurologica Scandinavica 1975; 51: 110-36. [CrossRef]
13. Willer CJ, Dyment DA, Sadovnick AD, Ebers GC. Maternal-offspring HLA-DRB1 compatibility in multiple sclerosis. Tissue Antigens 2005; 66: 44-7. [CrossRef]
14. DüzgünN.İmmünSisteminTanıtımı.s:97-122.Availablefrom:URL:http://ichastaliklariromatoloji.medicine.ankara.edu.tr/files/2014/02/%C4%B0mm%C3%BCn-Sistemin-Tan%C4%B1t%C4%B1m%C4%B1.pdf

15. O'Connor BP, Raman VS, Erickson LD, Cook WJ, Weaver LK, Ahonen C, et al. BCMA is essential for the survival of long-lived bone marrow plasma cells. J Exp Med 2004; 199: 91-8. [CrossRef]
16. Aktura ŞD, Yılmaz V, Özkan-Yaşargün D, Ulusoy C, Tüzün E, Türkoğlu R. Peripheral blood memory B cell frequency predicts conversion from clinically isolated syndrome to multiple sclerosis. Mult Scler Relat Disord 2018; 23: 9-14. [CrossRef]
17. Komai T, Inoue M, Okamura T, Morita K, Iwasaki Y, Sumitomo S, et al. Transforming growth factor-β and interleukin-10 synergistically regulate humoral immunity via modulating metabolic signals. Front Immunol 2018; 9: 1364. doi: 10.3389/fimmu.2018.01364. [CrossRef]
18. Molnarfi N, Bjarnadóttir K, Benkhoucha M, Juillard C, Lalive PH. Activation of human B cells negatively regulates TGF-β1 production. J Neuroinflammation 2017; 14: 13. doi: 10.1186/s12974-017-0798-5. [CrossRef]

Experimed 2019; 9(3): 113-9

ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

DOI: 10.26650/experimed.2019.19018

Allergic Rhinitis and Eczema in a Population of School Children from the City of Gjilan in Kosovo

Kosova Gjilan Şehrindeki Okul Çağı Çocuklarını İçeren Popülasyonda Alerjik Rinit ve Egzamanın İncelenmesi

Valbona Gashi[1], Luljeta N. Ahmetaj[1], Bekim Ahmeti[2]

[1]Department of Allergy and Immunology, University of Prishtina School of Medicine, Prishtina, Kosovo
[2]Department of Pharmaceutical Inspectorate, University of Prishtina School of Pharmacy, Prishtina, Kosovo

Cite this article as: Gashi V, Ahmetaj LN, Ahmeti B. Allergic Rhinitis and Eczema in a Population of School Children from the City of Gjilan in Kosovo. Experimed 2019; 9(3): 113-9.

ABSTRACT

Objective: The goal of this study was to investigate the gender difference in manifestation of clinical symptoms related to allergic rhinitis and eczema in a population of school children aged 13-14 years from the city of Gjilan in Kosovo.

Material and method: About 1200 school children aged between 13-14 years, from randomly selected schools, were included in the study, once the passive consent of their parents/guardians had been received. This study covers the data analysis from the questions related to the nose and skin problems (14 out of 53 questions).

Results: Prevalence related to allergic rhinitis; a) sneezing, or a runny or blocked nose ever was 34.20% and in the last 12 months it was 25%; and b) hay fever ever was 14.5%. Prevalence related to eczema,; a) itchy rash at any time in the past 12 months was 7.5%; and b) eczema ever in life was 4.2%.

Conclusion: This study found a higher prevalence of allergic rhinitis symptoms in female children comparing to male, while no significant connection was found between gender and eczema symptoms.

Keywords: School children, allergic rhinitis, eczema, gender, prevalence

ÖZ

Amaç: Çalışmada Kosova Gjilan şehrindeki okullarda 13-14 yaşlarındaki çocuklardan oluşan popülasyonda, alerjik rinit ve egzama klinik semptomlarının cinsiyet farklılığı açısından araştırılması amaçlanmıştır.

Gereç ve Yöntem: Ebeveynlerin/velilerin pasif onayı alınarak, yaşları 13-14 arasında değişen 1200 çocuk farklı okullardan seçilerek çalışmaya dahil edilmiştir. Çalışma burun ve deri problemleri ile ilişkili soruları kapsayan verilerin analizinden oluşmaktadır (53 sorudan 14'ü).

Bulgular: Alerjik rinit ilişkili yaygınlıklar; a) Hapşırma, burun akıntısı veya tıkalı burun gibi durumlar popülasyon genelinde %34,20 olarak saptandı. Bunlardan son 12 aydır bu şikayetlere sahip olanların %25'lik dilimi oluşturduğu görüldü. b) Bahar nezlesi ise %14,5 olarak bulundu. Egzama ilişkili yaygınlıklar; a) Kaşıntılı döküntüler son 12 aylık dönemde incelendiğinde %7,5 olarak görüldü. b) Yaşamı boyunca az bir kere egzama görülenlerin %4,2' lik dilimi oluşturduğu belirlendi.

Sonuç: Alerjik rinitle ilişkili belirtiler kız çocuklarında erkeklere oranla daha yüksek bir yatkınlıkla ilişkili bulunurken, egzama belirtileri ve cinsiyet arasında istatiksel olarak anlamlı bir ilişki bulunamadı.

Anahtar Kelimeler: Okul çocukları, alerjik rinit, egzama, cinsiyet, yaygınlık

INTRODUCTION

Allergic diseases are serious public health problems throughout the world, and they have an economic impact, both in terms of direct medical costs and indirect costs (school absenteeism and work absenteeism) and also with a negative impact on the quality of life of the affected persons (1).

The prevalence of allergic diseases in the last few decades has risen dramatically (2-6). In 2015, the European Academy of Allergy and Clinical Immunology (EAACI) reported that more than 150 million Europeans suffer from allergic diseases and the prediction is that by 2025 half of the entire European Union (EU) population will be affected.

Allergic rhinitis is among the most frequent disorders during childhood. Although it is not considered to be a serious disease, it has a significant impact on the quality of life of the patient. Allergic rhinitis is a chronic disease characterized by inflammation and swelling of the inner parts of the nose after inhalation of allergens. Symptomatology

Corresponding Author/Sorumlu Yazar: Luljeta N. Ahmetaj **E-mail:** luljetaahmetaj@gmail.com
Received Date/Geliş Tarihi: 28.08.2019 **Revision Date/Revizyon Tarihi:** 9.11.2019 **Accepted Date/Kabul Tarihi:** 15.11.2019

of allergic rhinitis is quite specific, with sneezing, runny nose, itching, and nasal congestion. Also, it is often associated with ocular symptoms such asitching, redness or tearful eyes, allergic rhinoconjunctivitis. Allergic rhinitis is a recognized risk factor for the development of asthma, with about 40% of patients with allergic rhinitis reporting for asthma symptoms while 80% of asthmatic patients have symptomatic allergic rhinitis (7-10). In addition, allergic rhinitis is also risk factor for asthma control, and uncontrolled rhinitis exacerbates co-existing asthma (11, 12). According to the World Health Organization, 400 million people in the world suffer from allergic rhinitis and 300 million from asthma (13).

Atopic dermatitis (eczema) affects up to 20% of children and often precedes allergic rhinitis and asthma, a relationship known as "atopic march" (14-16). It is an inflammatory skin disorder, mostly localized on the flexural parts, characterized by itching of these regions that may result in skin damage and secondary infection. In addition, it can result in sleep loss and a serious reduction on the quality of life not only for the affected persons, but also their families (17-19).

Allergies are progressive diseases and neglecting their symptoms may lead to their deterioration (20, 21). Therefore, in the management of allergies it is important to raise the population's awareness of the disease, to identify the risk factors and to take appropriate strategic measures. Genetic predispositions, environmental factors, and social behavior interact in allergic disease manifestation (7-9).

Certain clinical and epidemiological studies have shown gender differences in the prevalence of allergies (22, 23). According to these, the ratio between age and the incidence of allergies is higher in boys before puberty and in girls after puberty. Research related to gender differences in atopic diseases gives the opportunity to investigate the factors responsible for the occurrence and course of these diseases and would improve the ability to effectively manage allergies in clinical practice.

The aim of this study was to investigate the gender difference in manifestation of clinical symptoms related to allergic rhinitis and eczema in a population of school children aged 13-14 years from the city of Gjilan in Kosovo.

MATERIAL AND METHOD

The analytical cross-sectional study was carried out in the city of Gjilan, a municipality located in southeast Kosovo, during the year 2018, as a part of the Project of Global Asthma Network (GAN) Phase I.

Sample of the Study

In the study 1200 school children were included, from both genders, aged between 13-14 years. They were from randomly selected schools in the city of Gjilan, and the passive consent of their parents/guardians was received before the research started.

Study Instrument

In accordance with GAN, standardized self-administrated questionnaires of ISSAC (International Study of Asthma and Allergies in Childhood) Phase III were used, after being translated into the Albanian language and validated, with no additional questions added. The pilot study for evaluation of the translated questionnaire was applied on 50 randomly selected children. All received remarks were incorporated into the final version of the questionnaire. This study covers the data analysis from the questions related to the nose and skin problems (14 out of 53 questions).

In accordance with the study protocol, assessment of the prevalence of rhinitis and eczema was made based on the average prevalence of positive answers on the core questions. Prevalence of allergic rhinitis symptoms was determined through questions "In the past 12 months, have you had a problem with sneezing, or a runny or blocked nose when you did not have a cold or the flu?", "In the past 12 months, has this nose problem been accompanied by itchy-watery eyes?" and "Have you ever had hay fever?". Prevalence of eczema symptoms was determined from questions "Have you ever had a an itchy rash which was coming and going for at least six months?, "Have you had this itchy rash at any time in the past 12 months", "Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?" and "Have you ever had eczema?".

Implementation of the Study

Initially, we measured the weight and height of the children. During the measurements the children were wearing light clothing and were bare footed. Then the questionnaires were given to the children to be filled out, under the supervision of trained representatives from the project team. About 60-90 minutes were given for completing the questionnaire. Children were told to feel free to ask questions related to possible dilemmas.

The Ethics Committee of the Ministry of Health and the Ministry of Education and Science, Kosovo, approved the implementation of the GAN Phase One in Kosovo.

Statistical Analysis

Data was statistically analyzed in Statistical Package for Social Sciences software package, version 22.0 for Windows (IBM Corp.; Armonk, NY, USA). According to The International Study of Asthma and Allergies in Childhood (ISAAC) recommendation, missing or "any other" responses were part of the denominator for the calculation of allergic rhinitis and eczema prevalence figures (ISAAC Phase III Newsletter. Auckland, New Zealand, December 2001). The qualitative series were processed by determining the coefficient of relations, proportions, and rates, and were shown as absolute and relative numbers. Quantitative series were analyzed with measures of central tendency (average, median), as well as with dispersion measures (standard deviation, standard error). Pearson Chi-square test,

Yates corrected, Fischer exact test, and Fisher Freeman Halton exact test were used to determine the association between certain attributive dichotomies. A two-sided analysis with a significance level of $p<0.05$ was used to determine the statistical significance.

RESULTS

The sample of 1200 school children aged 13-14 was shown in the study. About 618 (51.5%) were male and 582 (48.5%) were female with relation between the genders of 1:1.1. The percentage difference between the genders in the sample, for $p>0.05$, was not statistically significant (Difference test: Difference 3% [(-0.99-6.99) CI 95%]; Chi-square=2.159; df=1 p=0.1417). Mean age was 13.4±0.51 with median IQR=13 (13, 14).

Prevalence of rhinitis according to gender

Related to nose problems a total of 7 questions were analyzed. The problem with sneezing or a runny or blocked nose when no cold or flu detected was found:

a) In the category 'ever in life', occurrence was, significantly, 1543 times more frequent in females [OR=1.543 (1.21-1.96) 95% CI]; b) In the category 'last 12 months' occurrence was, significantly, 1467 times more frequent in females [OR=1.467 (1.13-1.91) 95% CI]. A nose problem accompanied by an itchy nose in the last 12 months was 1554 times significantly more frequent in females [OR=1.554 (1.09-2.22) 95% CI]. A nose problem accompanied by itchy-watery eyes in the last 12 months was 1494 times significantly more frequent in females [OR=1.494 (1.04-1.14) 95% CI]. Also, the life experience of hay fever was found 1.525 times significantly more often in female than in male school children [OR=1.525 (1.10-2.11) 95% CI]. Incidents of hay fever confirmed by a doctor occurred in 76 (6.3%) of the school children. No significant association ($p>0.05$) was found between gender and the answers to all other questions related to nose problems (Table 1). Prevalence related to: a) sneezing, or a runny or blocked nose ever was 34.20% and in the last 12 months it was 25%; b) a nose problem accompanied by itchy-watery eyes in the last 12 months was 11.3% and accompanied by itchy nose was 11.7% and c) hay fever ever was 14.5%.

Prevalence of Eczema According to Gender

Analysis of skin problems covered 7 questions. No significant association ($p>0.05$) was found between gender and the answers to all questions related to skin problems presented in Table 2. Prevalence of positive answers related to: a) itchy rash ever which was last for 6 months was 11.8% and at any time in the past 12 month sit was 7.5%; b) itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankle, under the buttocks or around the neck, ears or eyes was 4.3%; c) itchy rash cleared completely at any time during last 12 months was 5.8% and d) eczema ever in life was 4.2%. Eczema confirmed by a doctor occurred in 20 (1.7%) of the school children.

DISCUSSION

This is the first study on this topic conducted in Kosovo, therefore the obtained data can be used as a baseline for assessment of future analysis of prevalence and other epidemiological characteristics of these diseases. Prevalence of allergic rhinitis symptoms in the last 12 months in our study was 25%, whereas prevalence of rhinoconjunctivitis symptoms in the last 12 months was 11.3%. In relation to gender there is a higher prevalence of symptomatology of rhinitis in female children compared with male. Prevalence of eczema symptoms in the last 12 months was 7.5% with no significant association between genders.

Whereas in the past it was thought that the prevalence of allergic diseases was higher in developed countries, from the first global reporting by ISAAC, it is apparent that the prevalence of these diseases was similar or even higher in low-income countries than developed countries, with higher degree of variability at regional and country level (24).

Average global prevalence of current symptoms of rhinoconjunctivitis in children registered during ISAAC Phase III was 14.6%, with the highest rates observed in Africa (18.0%) and Latin America (17.3%) and the lowest in Northern and Eastern Europe (9.2%) (25). Prevalence of current symptoms of eczema was 7.3%, with ranging values from 0.9% in China to 24.6% in Columbia, with the highest values in Africa and Latin America (26). In Europe, the lowest prevalence of symptoms of eczema was found in Northern and Eastern Europe, with intermediate values in Western Europe (24).

In neighboring countries a relatively low prevalence of respiratory allergic disorders has been registered, with average values of prevalence of rhinoconjunctivitis symptoms of 5.5% in Albania and the Republic of Macedonia, 7.6% in Serbia and Montenegro, 8.4% in Bulgaria (25), whereas the prevalence of eczema symptoms was 2.0% in Albania, 2.7% in the Republic of Macedonia, 5.6% in Serbia and Montenegro, 3.0% in Bulgaria (26).There were more girls than boys with symptoms of both conditions, and this held true for most regions.

A female higher prevalence of allergies from puberty and thereafter, including hay fever and eczema is reported by Osman et al. (27), also Fröhlichet al. (28) showed sex-related differences in rhinitis prevalence as well for asthma in a global systematic review with meta-analysis with a prevalence shift from male to female at around puberty. Austin et al. (29) showed gender differences in occurrence of allergic rhinitis symptomatology and eczema with predominate of female children, suggesting that this reverse ratio may be present as early as 12–14 years of age. More frequent occurrences of rhinitis and eczema in girls than in boys also was reported by Arrais et al. (30), in a study of 13-14 years old children.

The main possible mechanisms responsible for the high prevalence of allergies to female gender compared with male gender during and after puberty are suggested to be because female sex hormones increase atopic predispositions, whereas male

hormones have a protective effect (31). In the homeostasis of immunity, the function of sexual hormones is very important (32). Estrogens and progesterone stimulate Th2 response and suppress Th1 response in females, whereas testosterone suppresses Th2 response in males (33). The effect of estrogens on the activation of mastocytes and the development of allergic sensitization has been demonstrated in experiments on rodents, as well as the action of progesterone in the suppression of release of histamine on one side and the strengthening of IgE induction on the other side (32). Testosterone inhibits group 2 innate lymphoid cells (ILC2s), which are potent promoters of Th-2 responses (34).

Table 1. Analysis of questions related to nose problems that occur when there is no cold or flu according to gender

Questions		Gender			p
		male	female	total	
Have you ever had a problem with sneezing or a runny or blocked nose when you DID NOT have cold or flu (n=1200)					
Yes	n	182	228	410	Pearson Chi-square: 12.604; df=1; p=0.0004*
	%	29.45	39.18	34.20	
In the past 12 months, have you had a problem with sneezing or a runny or blocked nose when you DID NOT have a cold or the flu? (n=1200)					
Yes	n	133	167	300	Pearson Chi-square: 8.225; df=1; p=0.0041*
	%	21.52	28.69	25	
In the past 12 months, has this nose problem been accompanied by an itchy nose? (n=1200)					
Yes	n	59	82	141	Pearson Chi-square: 5.964; df=1; p=0.0146*
	%	9.55	14.09	11.75	
In the past 12 months, has this nose problem been accompanied by itchy-watery eyes? (n=1200)					
Yes	n	58	78	136	Pearson Chi-square: 4.813; df=1; p=0.0282*
	%	9.39	13.40	11.33	
In the past 12 months, how much did this nose problem interfere with your daily activities? (n=1200)					
Not at all	n	537	478	1015	Fisher Freeman Halton exact test: p=0.0630
	%	86.89	82.13	84.58	
A little	n	67	80	147	
	%	10.84	13.75	12.25	
A moderate amount	n	13	19	32	
	%	2.10	3.26	2.67	
A lot	n	1	5	6	
	%	0.16	0.86	0.50	
Have you ever had hay fever? (n=1200)					
Yes	n	74	100	174	Pearson Chi-square: 6.4991; df=1; p=0.0104*
	%	11.97	17.18	14.50	
Was your hay fever confirmed by a doctor? (n=1200)					
Yes	n	32	44	76	Pearson Chi-square: 2.867; df=1; p=0.0904
	%	5.18	7.56	6.33	

*significant for p<0.05

Our results are consistent with previous research demonstrating the impact of gender in the prevalence of allergies, except for eczema symptoms where no significant differences have been found between genders. Here we must consider the impact of various environmental factors, apart from gender and also several limitations of the study. Some children can poorly perceive their allergic symptoms, others can exaggerate them, and there may be those who even try to dismiss the disease. Also, a part of this symptomatology may not be of allergic origin and, in addition, itchy skin conditions such as scabies or helminth infestations, should be considered as common problems in developing countries that also can contribute to higher eczema estimates.

CONCLUSION

This study found a higher prevalence of allergic rhinitis symptoms in female children when compared with male children. No significant association was found between gender and

Table 2. Analysis of questions related to skin problems according to gender

Questions		Gender			p
		Male	female	total	
Have you ever had an itchy rash which was coming and going for at least six months? (n=1200)					
Yes	n	67	75	142	Pearson Chi-square: 1.201; df=1; p=0.2729
	%	10.84	12.89	11.85	
Have you had this itchy rash at any time in the past 12 months? (n=1200)					
Yes	n	42	48	90	Pearson Chi-square: 0.910; df=1; p=0.3401
	%	6.80	8.25	7.50	
Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankle, under the buttocks or around the neck, ears or eyes? (n=1200)					
Yes	n	20	32	52	Pearson Chi-square: 3.699; df=1; p=0.0544
	%	3.24	5.50	4.33	
Has this itchy rash cleared completely at any time during the past 12 months? (n=1200)					
Yes	n	32	38	70	Pearson Chi-square: 0.996; df=1; p=0.3182
	%	5.18	6.53	5.83	
In the past 12 months, how often on average, have you been kept awake at night by this itchy rash? (n=1200)					
Never	n	602	564	1166	Pearson Chi-square: 0.293; df=2; p=0.8637
	%	97.41	96.91	97.17	
Less than one night per week	n	11	12	23	
	%	1.78	2.06	1.92	
One or more nights per week	n	5	6	11	
	%	0.81	1.03	0.92	
Have you ever had eczema? (n=1200)					
Yes	n	19	31	50	Pearson Chi-square: 3.8069; df=1; p=0.0514
	%	3.07	5.33	4.17	
Was your eczema confirmed by a doctor? (n=1200)					
Yes	n	8	12	20	Pearson Chi-square: 1.077; df=1; p=0.2993
	%	1.29	2.06	1.67	

*significant for p<0.05

symptoms of eczema. Taking into account that this is the first study of this type in Kosovo, further more extensive research is needed to examine this problem.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of the Ministry of Health and the Ministry of Education and Science, Kosovo.

Informed Consent: Passive informed consent was obtained from the parents' of the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - V.G., L.N.A., B.A.; Supervision - V.G., L.N.A., B.A.; Materials - V.G., L.N.A., B.A.; Data Collection and/or Processing - V.G., L.N.A., B.A.; Analysis and/or Interpretation - V.G., L.N.A., B.A.; Literature Search - V.G., L.N.A., B.A.; Writing - V.G., L.N.A., B.A.; Critical Reviews - V.G., L.N.A., B.A.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Etik Komite Onayı: Bu çalışma için etik komite onayı Kosova Sağlık Bakanlığı ve Eğitim ve Bilim Bakanlığı Etik Komite'lerinden alınmıştır.

Hasta Onamı: Yazılı hasta onamı bu çalışmaya katılan hastaların ebeveynlerinden alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir - V.G., L.N.A., B.A.; Denetleme - V.G., L.N.A., B.A.; Gereçler - V.G., L.N.A., B.A.; Veri Toplanması ve/veya İşlemesi - V.G., L.N.A., B.A.; Analiz ve/veya Yorum - V.G., L.N.A., B.A.; Literatür Taraması - V.G., L.N.A., B.A.; Yazan - V.G., L.N.A., B.A.; Eleştirel İnceleme - V.G., L.N.A., B.A.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışmada finansal destek almadıklarını beyan etmişlerdir.

REFERENCES

1. Weiss KB, Gergen PJ, Hodgson TA. An economic evaluation of asthma in the United States. N Engl J Med 1992; 327: 571-2. [CrossRef]
2. Lai CK, Beasley R, Crane J, Foliaki S, Shah J, Weiland S, et al. Global variation in the prevalence and severity of asthma symptoms: phase three of the International Study of Asthma and Allergies in Childhood (ISAAC). Thorax 2009; 64: 476-83. [CrossRef]
3. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Lancet 2001; 357: 313-4.
4. Ferkol T, Schraufnagel D. The global burden of respiratory disease. Ann Am Thorac Soc 2014; 11: 404-6. [CrossRef]
5. de Korte-de Boer D, Mommers M, Gielkens-Sijstermans CM, Creemers HM, Mujakovic S, Feron FJ, et al. Stabilizing prevalence trends of eczema, asthma and rhinocon-junctivitis in Dutch schoolchildren (2001-2010). Allergy 2015; 70: 1669-73. [CrossRef]
6. Sybilski AJ, Raciborski F, Lipiec A, Tomaszewska A, Lusawa A, Samel-Kowalik P, et al. Epidemiology of atopic dermatitis in Poland according to the Epidemiology of Allergic Disorders in Poland (ECAP) study. J Dermatol 2015; 42: 140-7. [CrossRef]
7. Bousquet J, Annesi-Maesano I, Carat F, Leger D, Rugina M, Pribil C, et al. Characteristics of intermittent and persistent allergic rhinitis: DREAMS study group. Clin Exp Allergy 2005; 35: 728-32. [CrossRef]
8. Linneberg A, Henrik Nielsen N, Frolund L, Madsen F, Dirksen A, Jorgensen T. The link between allergic rhinitis and allergic asthma: a prospective population-based study. The Copenhagen Allergy Study. Allergy 2002; 57: 1048-52. [CrossRef]
9. Ronmark EP, Ekerljung L, Mincheva R, Sjolander S, Hagstad S, Wennergren G, et al. Different risk factor patterns for adult asthma, rhinitis and eczema: results from West Sweden Asthma Study. Clin Transl Allergy 2016; 6: 28. doi: 10.1186/s13601-016-0112-0. [CrossRef]
10. Downie SR, Andersson M, Rimmer J, Leuppi JD, Xuan W, Akerlund A, et al. Association between nasal and bronchial symptoms in subjects with persistent allergic rhinitis. Allergy 2004; 59: 320-6. [CrossRef]
11. Corren J, Adinoff AD, Buchmeier AD, Irvin CG. Nasal beclomethasone prevents the seasonal increase in bronchial responsiveness in patients with allergic rhinitis and asthma. J Allergy Clin Immunol 1992; 90: 250-6. [CrossRef]
12. Taramarcaz P, Gibson PG. Intranasal corticosteroids for asthma control in people with coexisting asthma and rhinitis. Cochrane Database Syst Rev 2003; CD003570. [CrossRef]
13. Leynaert B, Neukirch C, Kony S, Guénégou A, Bousquet J, Aubier M, et al. Association between asthma and rhinitis according to atopic sensitization in a population-based study. J Allergy Clin Immunol 2004; 113: 86-93. [CrossRef]
14. Hill DA, Spergel JM. The atopic march: Critical evidence and clinical relevance. Ann Allergy Asthma Immunol 2018; 120: 131-7. [CrossRef]
15. Bantz SK, Zhu Z, Zheng T. The atopic march: progression from atopic dermatitis to allergic rhinitis and asthma. J Clin Cell Immunol 2014; 5: 202. doi: 10.4172/2155-9899.1000202 [CrossRef]
16. Pols DH, Wartna JB, van Alphen EI, Moed H, Rasenberg N, Bindels PJ, et al. Interrelationships between atopic disorders in children: A meta-analysis based on ISAAC questionnaires. PLoS One 2015; 10: doi: 10.1371/journal.pone.0131869. [CrossRef]
17. Lawson V, Lewis-Jones MS, Finlay AY, Reid P, Owens RG. The family impact of childhood atopic dermatitis: the Dermatitis Family Impact Questionnaire. Br J Dermatol 1998; 138: 107-13. [CrossRef]
18. Drucker AM, Wang AR, Li WQ, Sevetson E, Block JK, Qureshi AA. The Burden of Atopic Dermatitis: Summary of a Report for the National Eczema Association. J Invest Dermatol 2017; 137: 26-30. [CrossRef]
19. Lindberg M, Isacson D, Bingefors K. Self-reported skin diseases, quality of life and medication use: a nationwide pharmaco-epidemiological survey in Sweden. Acta Derm Venereol 2014; 94: 188-91. [CrossRef]
20. Muraro A, Fokkens WJ, Pietikainen S, Borrelli D, Agache I, Bousquet J, et al. European symposium on precision medicine in allergy and airways diseases: Report of the European Union Parliament Symposium (October 14, 2015). Allergy 2016; 71: 583-7. [CrossRef]
21. Gough H, Grabenhenrich L, Reich A, Eckers N, Nitsche O, Schramm D, et al. Allergic multimorbidity of asthma, rhinitis and eczema over 20 years in the German birth cohort MAS. Pediatr Allergy Immunol 2015; 26: 431-7. [CrossRef]
22. Osman M. Therapeutic implications of sex differences in asthma and atopy. Arch Dis Child 2003; 88: 587-90. [CrossRef]

23. Whitacre CC, Reingold SC, O'Looney PA. A gender gap in autoimmunity. Science 1999; 283: 1277-8. [CrossRef]
24. Mallol J, Crane J, von Mutius E, Odhiambo J, Keil U, Stewart A; ISAAC Phase Three Study Group. The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three: a global synthesis. Allergol Immunopathol (Madr) 2013; 41: 73-85. [CrossRef]
25. Aït-Khaled N, Pearce N, Anderson HR, Ellwood P, Montefort S, Shah J, et al. Global map of the prevalence of symptoms of rhinoconjunctivitis in children: The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three. Allergy Eur J Allergy Clin Immunol 2009; 64: 123-48. [CrossRef]
26. Odhiambo JA, Williams HC, Clayton TO, Robertson CF, Asher MI; ISAAC Phase Three Study Group. Global variations in prevalence of eczema symptoms in children from ISAAC Phase Three. J Allergy Clin Immunol 2009; 124: 1251-8. [CrossRef]
27. Osman M, Tagiyeva N, Wassall HJ, Ninan TK, Devenny AM, McNeill G, et al. Changing trends in sex specific prevalence rates for childhood asthma, eczema, and hay fever. Pediatr Pulmonol 2007; 42: 60-5. [CrossRef]
28. Fröhlich M, Pinart M, Keller T, Reich A, Cabieses B, Hohmann C, et al. Is there a sex-shift in prevalence of allergic rhinitis and comorbid asthma from childhood to adulthood? A meta-analysis. Clin Transl Allergy 2017; 7: doi: 10.1186/s13601-017-0176-5. [CrossRef]
29. Austin JB, Kaur B, Anderson HR, Burr M, Harkins LS, Strachan DP, et al. Hay fever, eczema, and wheeze: A nationwide UK study (ISAAC, international study of asthma and allergies in childhood). Arch Dis Child 1999; 103: 125-38. [CrossRef]
30. Taborda-Barata L, Gama JMR, Rosado-Pinto J, Lulua O, Quifica F, Arrais M. Prevalence of asthma and allergies in 13-14-year-old adolescents from Luanda, Angola. Int J Tuberc Lung Dis. 2017; 21: 705-12. [CrossRef]
31. Bonds RS, Midoro-Horiuti T. Estrogen effects in allergy and asthma. Curr Opin Allergy Clin Immunol 2013; 13: 92-9. [CrossRef]
32. Chen W, Mempel M, Schober W, Behrendt H, Ring J. Gender difference, sex hormones, and immediate type hypersensitivity reactions. Allergy 2008; 63: 1418-27. [CrossRef]
33. Roved J, Westerdahl H, Hasselquist D. Sex differences in immune responses: Hormonal effects, antagonistic selection, and evolutionary consequences. Horm Behav 2017; 88: 95-105. [CrossRef]
34. Cephus JY, Stier MT, Fuseini H, Yung JA, Toki S, Bloodworth MH, et al. Testosterone Attenuates Group 2 Innate Lymphoid Cell-Mediated Airway Inflammation. Cell Rep 2017; 21: 2487-99. [CrossRef]

Experimed 2019; 9(3): 120-9

REVIEW
DERLEME

DOI: 10.26650/experimed.2019.19022

Mesenchymal Stem Cell Signaling Pathway and Interaction Factors

Mezenkimal Kök Hücre Sinyal Yolakları ve Etkileşim Faktörleri

Gülsemin Çicek[1], Selçuk Duman[2], Tahsin Murad Aktan[2]

[1]Kanuni Sultan Süleyman Training and Research Hospital, IVF Center, İstanbul, Turkey
[2]Department of Histology and Embryology, Necmettin Erbakan University, Meram School of Medicine, Konya, Turkey

Cite this article as: Çiçek G, Duman S, Aktan TM. Mesenchymal Stem Cell Signaling Pathway and Interaction Factors. Experimed 2019; 9(3): 120-9.

ABSTRACT

Stem cells are self-renewing and undifferentiated cells with potential to transform into different types of functional cells. These cells can be classified into two types based on their roots; embryonic stem cells that originate from the inner cell mass of preimplanted embryos and can structure the tree germinal layers and, mature stem cells that have the potential to differentiate into at least one type of functional structure. Both types of the stem cells present their own characteristics and capacities by using their own signal pathways in the presence of very different interaction factors. Knowing the characteristics of those stem cells in detail will importantly contribute to therapeutics.

Keywords: Stem cell, signal pathway, interaction factors

ÖZ

Kök hücreler, farklı tip fonksiyonel hücrelere dönüşme potansiyeline sahip, kendi kendini yenileyebilen ve farklılaşmamış hücrelerdir. Bu hücreler, kökenleri temel alınarak iki ana tip olarak sınıflandırılabilir: Preimplante embriyonun iç hücre kitlesinden köken alan ve 3 germ yaprağını da yapılandırabilecek embriyonik kök hücreler ile farklı doku ve organlarda bulunan, en az bir tip fonksiyonel yapıya farklılaşabilme kapasitesine sahip erişkin kök hücreler. Bu her iki kök hücre tipi de çok farklı etkileşim faktörleri varlığında kendi sinyal yollarını kullanarak kendi özelliklerini ve kapasitelerini sunar. Bu kök hücrelerin özelliklerini ayrıntılı olarak bilmek, terapötik maddelere önemli katkı sağlayacaktır.

Anahtar Kelimeler: Kök hücre, sinyal yolağı, etkileşim faktörleri

INTRODUCTION

Cells respond at the receptor level to the signals coming from other cells or to the signals that are produced by themselves. This intercellular communication is called "cell signaling", and it is necessary for the functional regulation and integration of the organism. Signal mechanisms can be through endocrine, paracrine, autocrine, neurotransmitter or neuroendocrine ways (1). These signaling mechanisms are found in both embryonic stem cells and somatic cells (for ex. interleukin secretion of fibroblasts and stromal cells of the bone marrow). Mature stem cells, especially mesenchymal stem cells (MSCs), produce and secrete a large variety of cytokines, chemokines, growth factors, and they stimulate neighboring cells by a variety of mechanisms. While progenitor cells are being stimulated for proliferation and differentiation by the received signals, they also show anti-inflammatory and immune modulator effects by secreting growth factors and mediators (2). It is important to show the secretion of active mediators and their expressions biologically, in order to understand the biology of stem cells. The organization of the secretion of paracrine cytokines occurs by the influence of the microenvironment or by their impact on the microenvironment of the stem cell niches. Additionally, paracrine mediators have a role in pathological cases. The secretion of the paracrine factors as a response to tissue injury, activates the endogenous repair and regeneration mechanisms, and affects the cell survival. Stem cells and progenitor cells protect the tissue for their continuation of life by providing balance between proliferation and differentiation.

Secreted factors of the mature stem cells play an important role in the regenerative process seen after stem cell mobilization, while the proves supporting this hypothesis are increasing. MSCs, that are identified to be safer in the clinical use, are multipotent cells and they are easily obtained and produced. Treatments on the cellular level is mostly done by using autologous bone marrow MSC and fat tissue which reserves a large number of MSC when compared to bone marrow (3). A large part of the

Corresponding Author/Sorumlu Yazar: Gülsemin Çicek **E-mail:** gulseminyuksel@gmail.com
Received Date/Geliş Tarihi: 08.10.2019 **Revision Date/Revizyon Tarihi:** 18.10.2019 **Accepted Date/Kabul Tarihi:** 23.10.2019

stem cells in the adipose tissue are located in the perivascular region (4), and they have a unique capacity for proliferation and transdifferentiation (5). MSCs have the capacity to differentiate into adipocyte, osteocyte, chondrocyte, myocyte and neuronal cell lines. This multipotent differentiation potential is achieved by effect of the large number of secreted cytokines and growth factors (6). Extracellular vesicles (EV) based on the MSCs are structures having therapeutic characteristics for many of the diseases. EVs secreted by the cells are generally called microvesicles, cellular based vesicles, microparticles, secreted vesicles and exosomes. EVs are important mediators of intercellular communication, both in physiological and pathological cases. Vesicle substance varies by cell types and physiological conditions; EVs contain various proteins, lipids and nucleic acids (7). Microvesicles are secreted by the activation of various cells like thrombocytes, endothelial cells, erythrocytes, monocytes, lymphocytes and leucocytes, or by apoptosis. EVs of 50-200 mm in diameter are called exosomes and, exosomes play an important role in intercellular communication (8). In addition to this, alterations in the cytokines and growth factors secreted by the MSCs can affect the (non-migratory) functions of the cell located in the tissue; those effects can even exist after the disappearance of MSCs (9). EVs include the proteins, RNAs and some undefined proteins in their structure, just like a cargo. The content of EV supports the proliferation of the cells, and also provides the inhibition of apoptosis by affecting intracellular signal pathways (8). The first study where this theory became true showed the effect of paracrine factors based on MSC on the myocardial ischemia reperfusion damage (10, 11). It showed that MSC exosomes from autologous origin are effective in enhancing the left ventricular diastolic function in myocardial damage (12). Zhang et al. (13) reported that EVs based on MSCs stimulate the proliferation of epidermal cells in burned skin. An important example is mRNAs of EV secreted from MSCs shown to have therapeutic effect on tissues that have acute renal damage. The mRNA shows its effect here by helping proliferation, transcription regulation and immune modulation (14, 15). Another example is the demonstration of mRNA transfer in the healing of acute lung injury. The mRNA of EVs with KGF (keratinocyte growth factor) is transferred to type II alveolar epithelium from bone marrow stem cells, and they are passed to the protein of type II alveolar epithelium. Increase of KGF protein in type II epithelium turns into an immune modulator effect and, its protective effect in acute lung injury takes place (16).

Various signal pathways are defined as the organizers of self-renewing and stemness in stem cells (Figure 1). Exosomes of the MSCs play an active role in the preservation of the characteristics, like differentiation capacity, self-renewing, stemness and prevention of early differentiation. Those characteristics of the stem cells are essential in development of cell-based therapies. However, it is necessary to optimize the differentiation potential and proliferation rate of MSCs in order to provide the therapeutic efficiency of MSCs.

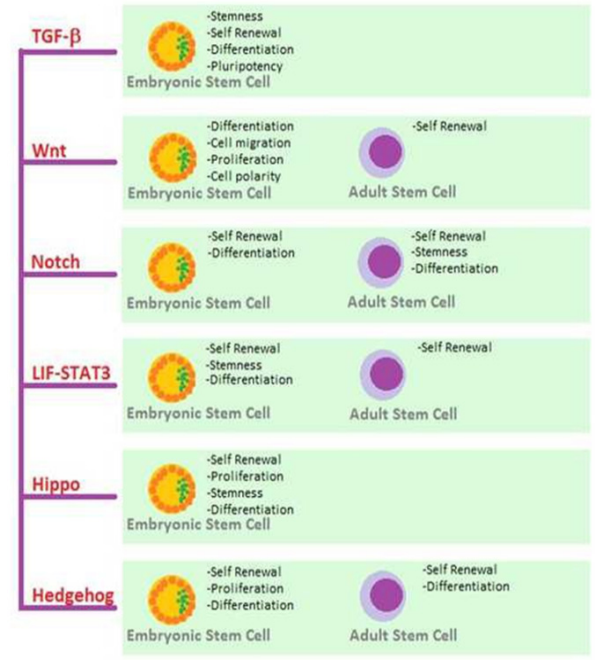


Figure 1. Main signal pathways having role on self-renewal and stemness of stem cell

While signal pathways, epigenetic modulators, cell cycle regulators and transcription factors regulate the balance between self-renewing and differentiation, reorganization for the repair will be shaped. There are many points and signal pathways defined in the human stem cells for stemness and self-renewing.

While the self-renewing concept of stem cells is defined by the continuity of proliferation, differentiation inhibition and apoptosis inhibition, signals related to this include chemical regulators (ex: Prostaglandins, retinoic acid), developmental regulators (ex: BMP- bone morphogenic protein), and signal pathways (ex: Wnt, Notch, Hedgegod signal pathways) (17). The stemness concept is regulated by extrinsic and intrinsic stimuli in stem cells (18). In the meantime, the achievement of the balance between self-renewing and differentiation in stem cells is carried out by the combination of asymmetric and symmetric cell division. Symmetrical division gives rise to two identical cells, while asymmetrical division gives rise to one stem cell and one progenitor cell (19). This discrepancy is also of special importance in embryonic development and cellular treatment periods.

Molecules having a central role in providing the stemness concept, which is predestinating the stem cell, are Oct4, SOX2, NANOG (20). These are main transcription factors regulating the pluripotentiality and self-renewing in the embryonic stem

cell (21). Expression of Nanog is governed by Oct4 , SOX2 (22). Oct4 is expressed highly in embryonic internal cell mass (23). SOX2 stimulates the differentiation in the neuronal ectoderm germ layer (21).

TGF- β Signalling Pathway

The transforming growth factor (TGF)-β superfamily has more than 30 members. It plays a critical role in the regulation of cell growth, development and differentiation (24). It has 3 different subgroups: TGF-β1, TGF-β2 and TGF-β3. TGF-β superβ family includes TGF-β and nodal pathways, which have the characteristics of self-renewal and stemness of stem cell and also, the BMP pathway which induces differentiation (25-27). The signal starts with the lig and based oligomerization of the serin/treonin receptor kinase. Together with the companionship of general signal transduction provider Smad4 to this phosphorylation, the SMAD molecules are translocated to the nucleus, and transcription factors are secreted. Smad4 regulates the transcription of the target genes in the nucleus (28). The main transcription factors of embryonic stem cells in self-renewing and differentiation are Oct4, SOX2 and NANOG.

Activin A is another member of TGF-β family. İt is necessary in pluripotentiality and self-renewing of embryonic stem cells. It induces Oct4, NANOG, Nodal, Wnt3, basic fibroblast growth factor (bFGF) and FGF8 and represses the BMP signals. Recently, TGF-β activin sensitive SMADs which bind to the Nanog promoter region and increase Nanog promoter activity have been demonstrated. So these SMADs play an essential role in maintaining human embryonic stem cell self-renewing (27).

Wingless-Wnt Signalling Pathway

Wnt ligand Low density lipoprotein-related protein (LRP) 5/6 is a secreted glycoprotein which binds to serpertine receptors of the Frizzled family, forming complexes in the cell surface (26). In embryonic stem cells, Wnt signal pathways have an important role in controlling the cell transcription while providing the cell with polarity and enhancing proliferation also in embryonic stem cells differentiation and cell migration is supported by Wnt signals. In hematopoietic stem cell and progenitor cells, there is accumulating knowledge that Wnt signaling mechanisms are necessary for the self-renewal processes (29, 30).

Wnt cascade affects cells as three pathways, whom are:
1. Canonical Wnt pathway (β-catenin pathway)
2. Noncanical Wnt pathway (Planar cell polarity pathway)
3. Wnt calcium pathway

In the active canonical Wnt signal pathway, the dephosphorilization of β-catenin is a key process by keeping β-catenin stable in the nucleus.Through embryonic development, the Wnt/ β-catenin signal pathway regulates the proliferation, and predestinates the embryonic neural stem cells. The canonical Wnt/ β-catenin pathway has an important role in neurogenesis. When the Wnt signal pathway is activated, β-catenin is not phosphorylated, and it accumulates in cytosol. This accumulation enables β-catenin to pass the nuclear site. By β-catenin enterance transcription of target genes (Oct4, SOX2, NANOG) start. When Wnt inactive, β-catenin gets phosphorylated and binds to the destructive complex. Destruction of β-catenin suppresses the transcription of target genes (31, 32). Any defects in the regulation of the Wnt signal pathway can cause many abnormalities and diseases, such as abnormal activation of the Wnt β catenin signal pathway that leads to a disorder called FAP (Familial Adenumatous Polipozis), which shows polyps in the colon and rectum (31).

Notch Signalling Pathway

The notch signalling pathway is important in predestination, and also in the continuity of embryonic and mature stem cells. It is necessary for self-renewal and stemness concepts of hematopoietic stem cells (29, 33). There are four types of notch receptor isoforms (Notch1-4). The canonical notch ligands are Jagged and Delta ligand families (Jag1, Jad2, Dll1,Dll3, Dll4) (34). The notch signal pathway plays a role in the self-renewal of stem cell, and provides the antineurogenic signal during CNS development (35). Notch 1 signals have a critical role in cardiac development and life, in differentiation of cardiogenic stem and progenitor cells and, mutations in Notch1 cause structural abnormalities in the heart, like bicuspid aortic valve (34).

LIF-Stat3 Signalling Pathway

LIF belongs to the IL-6 cytokine family. It binds to the low affinity LIF receptor and the heterodimeric receptor composed of GP130 (36). This formed complex activates JAK (tyrosine kinasenound to Janus). LIF activates 3 pathways in the stem cell:

1- MAPK- differentiation
2- JAK/STAT- self-renewing
3- PI(3)K- surviving (37)

Activation of LIF activates STAT3, and it provides, by affecting the target genes of embryonic stem cells (Oct4, NANOG, c-myc), the reproduction and self-renewal of the cell without differentiation. The Jak/STAT pathway also supports the self-renewal of hematopoietic stem cells. STAT3 contributes to the stemness and self-renewal of pluripotent stem cells by the transcription of target cells (38).

Hippo Signalling Pathway

Hippo signal pathway is important in the balancing of the preserving organ enlargement, cell self-renewing and cell proliferation. Deregulation of this pathway is related to the cancer development (39). It consists of serintreonin kinase MST1/2 (mammalian Ste-2 like kinase) and LATS1/2. The activation of the hippo pathway leads to the inactivation of YAP (Yes-asso-

Table 1. Some environmental effects on MSC fate

Environmental Factors	Some Effects on MSC
Geometry Mechanic Stress	+ Cell actin distribution cell elasticity
	+ partial loss of multipotentiality
High glucose level	+ markers of stemness are increased
	+ proliferation is decreased
	+ neurogenic differentiation in mature stem cell is significantly increased
	+ autophagia and senescence was induced in bone marrow derived MSCs
Ascorbic Acid	+ derivation to osteoblasts, adipocytes, chondrocytes and *in vitro* odontoblasts
	+ acceleration of DNA synthesis
	+ in high concentrations are cytotoxic, may cause the suppression of proliferation and apoptosis
Freezing&Thawing	+ degenerated immune modulator characteristics and their inflammation rate is higher
	+ affects the immune modulator response
	+ heat shock protein (HSP) levels increase
	+ responds to inflammatory conditions decrease
	+ increase their immune modulator characteristics as a reply to inflammation
Hypoxia	+ potent stimulus invoking chondrogenesis
	+ stimulating therapeutically angiogenesis
	+ directly promotes wound healing

ciated protein). This YAP results in the direct phosphorylation ser-127. YAP and TAZ are co-activators of transcription. When LATS1/2 is dephosphorylated, YAP/TAZ is carried to the cell nucleus; it communicates with the transcription factors and induces the inhibition of cell proliferation and apoptosis. Embryonic stem cells YAP/TAZ directly supports stemness (40, 41). TAZ is connected to the Smad2,3,4 proteins in embryonic stem cells, thus it is effective in the self-renewal and proliferation of stem cells. Deletion of YAP leads to myocardial hypoplasia, loss of its function leads to cell death and early neuronal differentiation (41).

Hedgehog Signalling Pathway

This pathway is effective in self-renewal and regeneration of embryonic stem cells and mature stem cells. Hedhedog proteins have 3 different types of isoforms: Sonic (Shh), Desert (Dhh), Indian (Ihh). It is an active pathway both in embryonic and mature life (32). It plays a role in the proliferation and differentiation of embryonic stem cells, and in plerosis and regeneration of mature stem cells. It has a positive effect on osteogenic differentiation, and a negative effect on adipogenic differentiation (32, 42). Regulation of this pathway is responsible from holoprosencephaly and other developmental malformations (42).

Mesenchymal Stem Cell and Interaction Factors

In vivo MSCs are surrounded with extracellular matrix, which is composed of collagen, adhesion proteins, proteoglycans and growth factors called "niche". Besides the importance of molecular structures, physicochemical properties of microenvironment are also important in the differentiation of the stem cells. Some environmental effects are summarized, as seen in Table 1. Stem cells not only answer to the paracrine signals in their local microenvironment, but also undergo changes by being reactivated together with various matrix components, proteases and growth factors secreted from the medium. Matrix proteins play a role in cell adhesion, migration, differentiation and survival. Three dimensional matrix culture systems involving various growth factors *in vitro* are developed by mimicking *in vivo* systems (43). For example, the mesenchymal stem cell goes into osteogenic differentiation in a microenvironment with firmer elasticity and, goes into neuronal differentiation in a looser microenvironment (44). Niches for differentiation have been developed by considering these characteristics. It is supported in the studies that stem cells respond to the mechanical signals presented by the local extracellular matrix. Cells remember the previous mechanical signals, and this memory continues even after the translocation, and predestinates the long term (45). It is reported that with the important communication between

extracellular matrix medium and intracellular signals, YAP and TAZ, by being located in the nucleus and by regulating the mRNA expression, turns the physical information into protein expression (46, 47).

MSCs are mechanosensitive cells. For example: meeting of MSCs with systemic circulation causes them to be exposed to flow stress, and this extracellular matrix mechanical stimulus is perceived by the sensors of the cell membranes; cell behavior is affected rapidly. G-protein coated receptors, intercellular and inter matrix proteins, integrines, and ion channels are mechanoreceptors playing a role in the intracellular kinase activity and the organization of the actinin cytoskeleton. Laminar flow is a type of flow that is unidirectional, stable, with various velocities and different from the flow in humans which is directed by the heart beats. Oscillatory flow and pulsatile flow are in the same direction, and their oscillations are at the same amplitude, but the mean rate of the pulsatile flow is higher than the rate of oscillatory flow. Oscillatory flow stress (0.5 ± 4 dyn/cm^2) affects the regulation of β-catenin and induces the reorganization of f-actin, and predestinates the stem cell. 30 minutes after oscillatory stress, f-actinindepolimerization and β-catenin increase make an adjuvant effect on the factors that inhibits the Wnt. After one hour of oscillatory stress, MSCs continue their fibroblast like appearance but the actin cytoskeleton organization changes. When the effect of oscillatory stress on stemness is searched by measuring the SOX2 , Oct4 and NANOG levels, NANOG and SOX2 expressions were increased, and Oct4 expression was unchanged. Oscillatory stress promotes the adipogenic differentiation of MSCs (48).

Geometry and Stem Cell Interaction

Multipotentiality and self-renewal capacities of MSCs that are often used in clinical practice are important. The spread process of the stem cells *in vitro* culture decreases the multipotent capacity, and this limits the application in clinical practice. Geometric and mechanic control of stem cell spread regulates the differentiation and progression of stem cells (49). Culture size and geometric shape affect the stemness and the cytoskeleton and, the cellular stress formed in this structure affects the multipotentiality of the stem cell (49, 50). Many studies showed that cells respond to the reorganization of the cytoskeleton by biophysical stimulus (51). Cellular stress related to the cytoskeleton is important for the protection of stem cell multipotentiality. Cell elasticity is an important biomechanical parameter that is determined by the existence, number and distribution of specific organelles and by the organization and character of the cytoskeletal elements. Elasticity changes according to the cell functions. For example, fibroblasts have a high elasticity, and chondrocytes have a low elasticity. Elasticity of mature MSCs changes during differentiation (52). If actin structure is highly regular in the cell, elasticity becomes high. Sphere embryonic stem cells present higher Oct4 and NANOG expressions than squamous cells as a result of the loosened membrane-cytoskeleton bounds. While a limited diffusion area has been shown to be helpful to save the undifferentiated form of embryonic stem cells, it has been identified that undifferentiated MSCs have lower contractility than differentiated osteogenic cells. So, this shows that a lower cytoskeletal stress is needed in order to protect the cell multipotentiality. It has also been shown that as the cell's area of diffusion enlarges, the cell's nuclear activity also increases. Parallel stress fibers formed in the slim shaped cells renders the elasticity of MSCs. In different geometric areas (e.g. square, triangle, polygon) MSCs in their center have shown an interrupted actin form which causes low elasticity. Cell diffusion showed similar responses in different geometric areas, if the distribution area is the same. High elasticity of MSCs always exists with low expression of surface molecules, and this shows the partial loss of multipotentiality (49).

Culture and Stem Cell Interaction

The culture medium necessary for feeding and growing of the stem cell is very important in order for them to release their biological effects. Gas percentages (O_2-CO_2-N) and the composition of culture medium (e.g. ph, glucose concentration), cell number in every cm^2, passage time and number, are some important parameters. Fetal Bovine Serum (FBS) has a risk of virus and prion contamination between media, so alternatively, serum-free media are produced with growth factors additions (53). Yet, medium prepared with serum based on autologous source is a good alternative for MSC cultures. The main problem is the identification of serum percentages. Platelet lysate, which is rich in cytokines and growth factors invitro MSC cultures, is a candidate for the usage of FBS (54). Various molecules are stored in platelet granules. Some of these are lysosomal enzymes (elastase, collagenase, catepsine), coagulation factors (factor V, XI, XIII, antithrombin, prothrombin), immunological molecules (IgG, factor D, platelet factor H, C1 inhibitor), adhesion molecules (P selectin, fibrinogen, VWF), chemokines (IL8, RANTES, NAP2), growth and angiogenesis regulators (55). Enriched platelet products like Platelet rich plasma (PRP), Platelet lysate (PL) or platelet gel include various growth factors like platelet derived growth factors (PDGFs), TGF-β, epidermal growth factor (EGF) and bFGF. These growth factors influence MSCs to mitosis. It is shown that cells obtained at the end of cultures stimulate invivo angiogenesis, and play a key role in tissue repair and regeneration (56, 57).

MSCs are traditionally isolated from bone marrow and adipose tissue, and they form fibroblast like cell colonies sticking the plastic in 2-dimensional *in vitro* cultures. While 2-dimensional cultures are artificial and less physiological, 3-dimensional spheroid shaped cultures save more physiological features. Anti-inflammatory, angiogenic, repair and regeneration effects of MSCs are more expanded in spheroid cultures than in 2-dimensional cultures. Oxygen reaches inside the spheroids with only diffusion, which causes the inner cell mass of

the spheroid hypoxic. This condition activates the pathways connected to hypoxia (58). Also, upregulation of the genes showing pluripotentiality enhances multidifferentiation potentiality together with the slowing of the senescence, increases stemness.

Culture and High Glucose Level

Glucose is the vital energy source which is critically important for the biological functions of the cells in culture media. Diabetic hyperglycemic medium is a phenomenon which induces the differentiation in MSCs. Oct4, SOX2 and NANOG gene expressions, which are the markers of stemness, are increased under prolonged high glucose conditions, and their proliferation is decreased. This may be due to reactive oxygen species (ROS) increase, and cultures with low glucose level demonstrated decreased stemness and increased proliferation. Even though diabetic mature stem cells are not the ideal autologous cell therapy because of its degenerated neovascular structure, stemness and differentiation capacities of the mature stem cells in hyperglycemic media are still researched. The inhibition of stemness and proliferation induced by high glucose level is thought to be inhibited by ROS in the environment and, this effect is tried to be decreased by the added antioxidants to the medium. But, this also decreased the positive effects of high glucose on the stemness of stem cell. It is observed that the ratio of neurogenic differentiation in mature stem cell is significantly increased in a high glucose medium, but the potential of adipogenic and osteogenic differentiation does not change. High glucose level, just like hypoxia, medium deprivation and low temperature, is a cellular stress condition which increases the stemness capacity (6). MSCs based on adipose tissue advances adipogenesis more in a high level glucose medium than in a low level glucose medium. In MSCs treated with a high glucose culture, it was shown that chondrogenesis was modulated by ensuring the regulation of TGF-β and protein kinase C (59). While the chondrogenic capacity increases, the adipogenic capacity decreases. In another study (60), MSCs treated with a high level glucose medium (4.5 gr/L) for 28 days and it was shown that autophagia and senescence were induced in bone marrow derived MSCs.

Culture and Ascorbic Acid

Cell metabolism in microenvironment is closely related to the metabolic interactions between different types of cells. When cells are isolated in their actual tissues and cultured, their nutrient needs change, and this need is different for every cell. Ascorbic acid (AA) increases the stimulation effect of *in vitro* cells for intercellular matrix production, and it is understood that it has an important role as a cofactor for the posttranslational modification of AA collagen molecules. It is known that AAs modulates the proliferation of many mesenchymal derived cells including osteoblasts, adipocytes, chondrocytes and *in vitro* odontoblasts. AA works as a growth supporter for the proliferation of the cells and acceleration of DNA synthesis, when it is added to the cultural medium in defined concentrations. But, prominently high concentrations are cytotoxic, and may cause the suppression of proliferation and apoptosis. This relation between cytotoxicity and ascorbic acid is defined by the medium related factors like type of the medium and CO_2 ratio (61). AAs, while increasing the regulation of embryonic stem cell pluripotent markers (Oct4 and SOX2), also induce the proliferation of MSCs based on adipose tissue and, this *in vitro* proliferation expansion is necessary for their use in stem cell therapy (62). Performed studies showed that Vitamin C is a necessary agent for the proliferation of both embryonic and mature stem cells (63).

The Effect of Freezing and Thawing on Stem Cells

With the usage of MSCs in the treatment of degenerative and immunological diseases, further research studies are being conducted for effective treatment methods. After the culture diffusion and isolation of MSCs, they are frozen until brought into use. This process is called cryopreservation. Phenotypical and functional characteristics of the cells are important for the effectiveness and safety of stem cell therapy. After the freezing-thawing process, some characters of MSCs may change. Expected results of *in vitro* and preclinical studies do not always coincide with clinical practices. This may be due to various reasons. Cultured MSCs may not always be compatible with human blood, and when applied intravenously, it may trigger sudden inflammation. As a result of this, on one hand cell survival is conceded, and on the other hand the beneficial paracrine effects increase. MSCs that come directly from a culture flask, frozen and thawed just before usage, show significant differences. Freeze&thawed MSCs have degenerated immune modulator characteristics, and their inflammation rate is higher (64). It is observed that in the first 24 hours of thawed MSCs, heat shock protein (HSP) levels increase, and the response to inflammatory conditions decreases (65). It is shown that the cryopreservation process has a little effect on gene expressions. Unlike traditional medical therapies metabolized by the recipient, cells responding to the microenvironment may undergo dramatic changes in MSC therapy. Freezing&Thawing affects the immune modulator response in MSCs. MSC functions have been completely saved after 24 hours of culture period. After one week of the thawing process, minimal changes in MSC gene expressions have been identified. Cytoskeletal reorganization has occurred and genes including natural immune pathways have undergone changes. Frozen cells have been kept for one week after thawing, and the induction of the increase in cytoskeletal protein expression became possible. The essential question is how this phenotypical changes affecting the functions of MSCs occur. As it is known, MSCs increase their immune modulator characteristics as a reply to inflammation. Increase in the expression of cytokine families, respectively IL11, IL33, IL6 and IL1, is proof of the activation of immunological signal pathways (9).

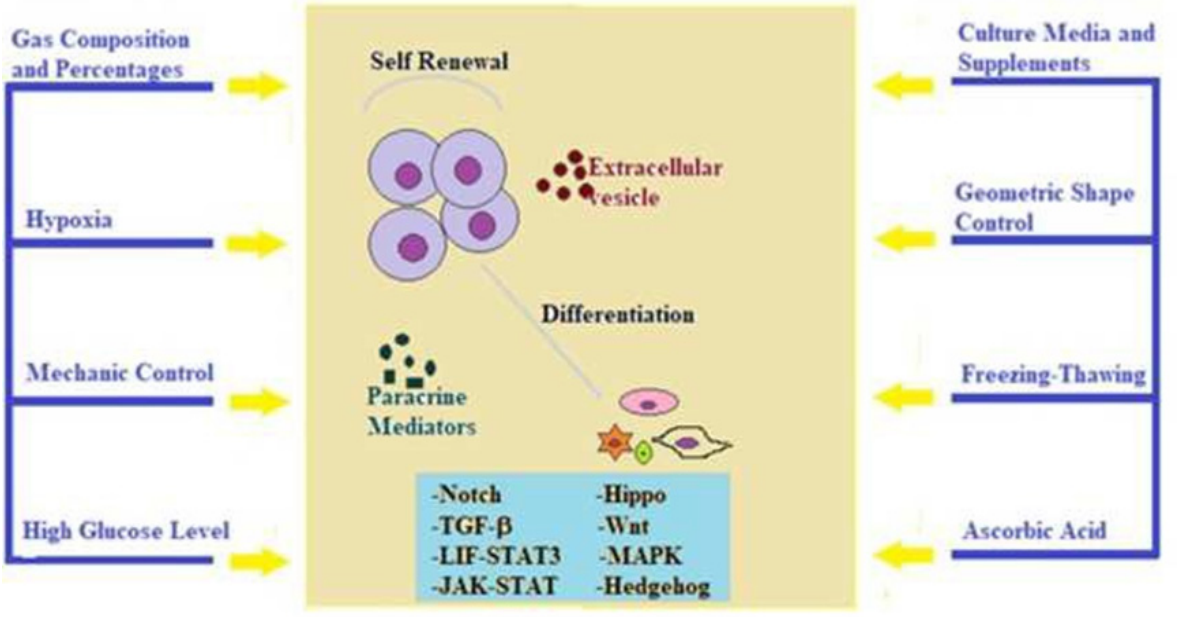


Figure 2. The self renewal and proliferation of stem cells themselves is effected by the signaling pathways of environmental factors

Hypoxia

Oxygen concentration is a very important factor for the maintenance, differentiation and function of stem cells. Oxygen has various effects on embryonic and mature stem cells. Hypoxia plays a role in growth, pluripotentiality, differentiation and growth factor production. Molecular oxygen has characteristics of being a signal molecules and metabolic substrat, both *in vivo* and *in vitro*. The effects of oxygen, self-renewing, differentiation and final function are not completely understood, astheir effects modify according to the cell type and oxygen level. In some mature cell types, a low oxygen level stimulates *in vitro* proliferation and multipotentiality. Low oxygen level is a potent stimulus invoking chondrogenesis in stem cells and in a clinical course, and it is important for functional cartilage production in engineering. Hypoxia also plays an important role in therapeutic angiogenesis by stimulating cytokine production. When stem cells are exposed to hypoxia, it is shown that growth factor, and especially VEGF production, increase. Angiogenesis formed by hypoxia can be both by differentiation of cells directly, or by cytokine production indirectly. In order to understand and optimize the growth functions of the stem cells, it is important to know specific microenvironment conditions *in vivo*. Interpreting *in vitro* experiments containing stem cells shows the need for a minute oxygen level (19). Embryonic stem cell lives under low oxygen levels in the beginning of implementation and during fetal growth. Blocking to reach maternal circulation during embryo implementation results in a hypoxic environment. Uterine surface oxygen concentration is at around 2% level during early the pregnancy period. When the embryo connects with the maternal vessels, the oxygen level rises to approximately 8%. That is why normal physiologic conditions of the embryonic stem cells are more hypoxic compared to *in vitro* conditions. Oxygen is an important signal molecule that stimulates cellular activity. Hypoxia increases the specific gene expressions (Glut-1,EPO,VEGF) comprising glycolysis, erythropoiesis and angiogenesis. VEGF produced by stem cell in hypoxic environment directly affects the surrounding environment (66). Highly perfused organs (e.g. lungs, kidney and liver) have oxygen levels between 4 and 14%. But this level is low in bone marrow (between 0 and 4%). It is especially lower than 3% in adipose tissue. Mature stem cells are located in anatomical regions which have relatively poor levels of oxygen. Both mature and embryonic stem cells express surface markers (Oct4, rex-1 and SOX2) at least for ten passages. Mature stem cell expresses different surface molecules; these are adhesion molecules, receptor molecules, surface enzymes, extracellular matrix proteins and glycoproteins (67). Hypoxia, by some paracrine mechanism which increases the secretion of defined growth factors, advances neoangiogenesis in mature stem cells. The primary mediator of hypoxic adaptation is Hypoxia inducible factor (HIF). It is from transcription factor family and has two subunits: alpha subunit (HIF-1 α and HIF-2 α) and beta subunit (HIF-1 β).HIF-1 β is physically connected to the intracellular region of notch molecule and it is an important component in order to protect undifferentiated stem and progenitor cell populations which are providing an impressive molecular connection between hypoxia and stemness (68). HIF-2 is the transcriptional regulator in embryonic stem cell pluripotentiality and it has a connection with Oct4. HIF-2 α directly connects to Oct4, SOX2, hypoxic response elements (HRE) in the Nanog promoter region in hypoxic conditions (69). Alpha subunit of HIF-1 is stabilized under low oxygen levels and by regulation VEGF expression, it provides functional harmony and characteristics which are necessary for new vascular

growth and maturation. At the same time HIF-1 α, increases dramatically by hypoxia, enhances angiogenic and antiapoptotic growth factors, and this directly promotes wound healing. HIF-1 α rising related increase of Myc, which is a transcriptional factor, affects metabolism with the increase of pleiotropic, proliferative and cell growth factors (70). HIF-1 α directly affects the Wnt/ beta-catenin pathway, and so presents its effect on both embryonic and mature stem cells (68).

CONCLUSION

This article reviews the current knowledge of the mesenchymal stem cell signaling pathways and interactions of stem cells with each other in culture. The more cell culture parameters can be optimized; the better the intercellular signaling pathways will work (Figure 2). Although this requires further investigation, it is essential to achieve the desired success in cell culture techniques. The parameters of cell culture to be considered are much more than what we describe in this paper.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - G.Ç., S.D., T.M.A.; Supervision - G.Ç., S.D., T.M.A.; Materials - G.Ç., S.D., T.M.A.; Data Collection and/or Processing - G.Ç., S.D., T.M.A.; Analysis and/or Interpretation - G.Ç., S.D., T.M.A.; Literature Search - G.Ç., S.D., T.M.A.; Writing - G.Ç., S.D., T.M.A.; Critical Reviews - G.Ç., S.D., T.M.A.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir - G.Ç., S.D., T.M.A.; Denetleme - G.Ç., S.D., T.M.A.; Gereçler - G.Ç., S.D., T.M.A.; Veri Toplanması ve/veya İşlemesi - G.Ç., S.D., T.M.A.; Analiz ve/veya Yorum - G.Ç., S.D., T.M.A.; Literatür Taraması - G.Ç., S.D., T.M.A.; Yazan - G.Ç., S.D., T.M.A.; Eleştirel İnceleme - G.Ç., S.D., T.M.A.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

REFERENCES

1. Abraham L, Kierszenbaum MD, Laura L. Tres, Cell Signaling, in Histology and Cell Biology. An Introduction to Pathology. Elsevier 2012. p. 89-109. **[CrossRef]**
2. Gündeşlioğlu AÖ, Altuntaş Z, İnce B, Dadacı M, Aktan M, Duman S. Adipose Tissue Derived Stem Cells and Their Uses in Plastic Surgery. Turk Plast Surg 2013; 21: 1-10.
3. Aktan TM, Duman S, Cihantimur B. Cellular and molecular aspects of adipose tissue. In: Illouz YG, Sterodimas A, editors. Adipose Stem Cells and Regenerative Medicine. Springer; 2011. p. 1-12. **[CrossRef]**
4. Duman S, Aktan TM, Cüce G, Cihantimur B, Tokaç M, Akbulut H. Effects of Lipokit® Centrifugation on Morphology and Resident Cells of Adipose Tissue. Int J Morphol 2013; 31: 64-9. **[CrossRef]**
5. Akbulut H, Cüce G, Aktan TM, Duman S. Expression of mesenchymal stem cell markers of human adipose tissue surrounding the vas deferens. Biomed Res 2012; 23: 0970-938X.
6. Cheng NC, Hsieh TY, Lai HS, Young TH. High glucose-induced reactive oxygen species generation promotes stemness in human adipose-derived stem cells. Cytotherapy 2016; 18: 371-83. **[CrossRef]**
7. Abels ER, Breakefield XO. Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake. Cell Mol Neurobiol 2016; 301-12. **[CrossRef]**
8. Katsuda T, Ochiya T. Molecular signatures of mesenchymal stem cell-derived extracellular vesicle-mediated tissue repair. Stem Cell Res Ther 2015; 6: 212. **[CrossRef]**
9. Hoogduijn MJ, de Witte SF, Luk F, et al. Effects of Freeze-Thawing and Intravenous Infusion on Mesenchymal Stromal Cell Gene Expression. Stem Cells Dev 2016; 25: 586-97. **[CrossRef]**
10. Lai RC, Arslan F, Tan SS, Tan B, Choo A, Lee MM, et al. Derivation and characterization of human fetal MSCs: an alternative cell source for large-scale production of cardioprotective microparticles. J Mol Cell Cardiol 2010; 48: 1215-24. **[CrossRef]**
11. Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, et al. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Res 2010; 4: 214-22. **[CrossRef]**
12. Tokac M, Aktan M, Ak A, Duman S, Tokgozoglu L, Aygul N, et al. Autologous transplantation of arterial cells improves cardiac function in a rabbit model of infarcted myocardium. Stem Cells Dev 2010;19: 927-34. **[CrossRef]**
13. Zhang B, Wang M, Gong A, Zhang X, Wu X, Zhu Y, et al. HucMSC-Exosome Mediated-Wnt4 Signaling Is Required for Cutaneous Wound Healing. Stem Cells 2015; 33: 2158-68. **[CrossRef]**
14. Gatti S, Bruno S, Deregibus MC, Sordi A, Cantaluppi V, Tetta C, et al. Microvesicles derived from human adult mesenchymal stem cells protect against ischaemia-reperfusion-induced acute and chronic kidney injury. Nephrol Dial Transplant 2011; 26: 1474-83. **[CrossRef]**
15. Bruno S, Grange C, Collino F, Deregibus MC, Cantaluppi V, Biancone L, et al. Microvesicles derived from mesenchymal stem cells enhance survival in a lethal model of acute kidney injury. PloS one 2012; 7: e33115. **[CrossRef]**
16. Zhu YG, Feng XM, Abbott J, Fang XH, Hao Q, Monsel A, et al. Human mesenchymal stem cell microvesicles for treatment of Escherichia coli endotoxin-induced acute lung injury in mice. Stem cells 2014; 32: 116-25. **[CrossRef]**
17. Arreba-Tutusaus P, Heidel FH. Signaling Pathways Maintaining Stemness in Adult Hematopoietic Stem Cells. In: Kursad Turksen, editors. Adult Stem Cells 2014; Springer. p. 1-12. **[CrossRef]**
18. Zon LI. Intrinsic and extrinsic control of haematopoietic stem-cell self-renewal. Nature 2008; 453: 306-13. **[CrossRef]**
19. Virant-Klun I, Stimpfel M, Skutella T. Adult Ovary Stem Cells. In: Kursad Turksen, editors. Adult Stem Cells 2014; Springer. p. 239-64. **[CrossRef]**
20. Mathieu J, Ruohola-Baker H. Regulation of stem cell populations by microRNAs. Adv Exp Med Biol 2013; 786: 329-51. **[CrossRef]**
21. Kallas A, Pook M, Trei A, Maimets T. SOX2 is regulated differently from NANOG and OCT4 in human embryonic stem cells during early differentiation initiated with sodium butyrate. Stem Cells Int 2014; doi: 10.1155/2014/298163. **[CrossRef]**
22. Rodda DJ, Chew JL, Lim LH, Loh YH, Wang B, et al. Transcriptional regulation of nanog by OCT4 and SOX2. J Biol Chem 2005; 280: 24731-7. **[CrossRef]**
23. Wu G, Schöler HR. Role of Oct4 in the early embryo development. Cell Regen 2014; 3: 7. **[CrossRef]**

24. Dubon MJ, Yu J, Choi S, Park KS. Transforming growth factor β induces bone marrow mesenchymal stem cell migration via noncanonical signals and N-cadherin. J Cell Physiol 2018; 233: 201-13. [CrossRef]
25. Avery S, Zafarana G, Gokhale PJ, Andrews PW. The role of SMAD4 in human embryonic stem cell self-renewal and stem cell fate. Stem Cells 2010; 28: 863-73. [CrossRef]
26. Cadigan KM, Liu YI. Wnt signaling: complexity at the surface. J Cell Sci 2006; 119: 395-402. [CrossRef]
27. Jiang J, Ng HH. TGFβ and SMADs talk to NANOG in human embryonic stem cells. Cell Stem Cell 2008; 3: 127-8. [CrossRef]
28. Orlowski J. SMAD5 signaling: more than meets the nuclei. Cell Res 2017; 27: 1075-6. [CrossRef]
29. Katoh M, Katoh M. WNT signaling pathway and stem cell signaling network. Clin Cancer Res 2007; 13: 4042-5. [CrossRef]
30. Hülsken J, Behrens J. The Wnt signalling pathway. J Cell Sci 2000; 113: 3545.
31. Nusse R. Wnt signaling in disease and in development. Cell Res 2005; 15: 28-32. [CrossRef]
32. Chen Q, Shou P, Zheng C, Jiang M, Cao G, Yang Q, et al. Fate decision of mesenchymal stem cells: adipocytes or osteoblasts? Cell Death Differ 2016; 23: 1128-39. [CrossRef]
33. Evans AG, Calvi LM. Notch signaling in the malignant bone marrow microenvironment: implications for a niche-based model of oncogenesis. Ann N Y Acad Sci 2015; 1335: 63-77. [CrossRef]
34. Penton AL, Leonard LD, Spinner NB. Notch signaling in human development and disease. Semin Cell Dev Biol 2012; 23: 450-7. [CrossRef]
35. Zhang K, Zhu L, Fan M. Oxygen, a key factor regulating cell behavior during neurogenesis and cerebral diseases. Front Mol Neurosci 2011; 4: 1-11. [CrossRef]
36. Koide H, Yokota T. The LIF/STAT3 Pathway in ES Cell Self-renewal. Embryonic stem cells-The hormonal regulation of pluripotency and embryogen-esis. Rijeka 2011: InTech: p. 61-78. [CrossRef]
37. Nicola NA, Babon JJ. Leukemia inhibitory factor (LIF). Cytokine Growth Factor Rev 2015; 26: 533-44. [CrossRef]
38. Stine RR, Matunis EL. JAK-STAT signaling in stem cells. Adv Exp Med Biol 2013; 786: 247-67. [CrossRef]
39. Park JH, Shin JE, Park HW. The Role of Hippo Pathway in Cancer Stem Cell Biology. Mol Cells 2018; 41: 83.
40. Mo JS, Park HW, Guan KL. The Hippo signaling pathway in stem cell biology and cancer. EMBO Rep 2014; 15: 642-56. [CrossRef]
41. Ramos A, Camargo FD. The Hippo signaling pathway and stem cell biology. Trends Cell Biol 2012; 22: 339-46. [CrossRef]
42. Briscoe J, Thérond PP. The mechanisms of Hedgehog signalling and its roles in development and disease. Nat Rev Mol Cell Biol 2013; 14: 416-29. [CrossRef]
43. Rakian R, Block TJ, Johnson SM, Marinkovic M, Wu J, Dai Q, et al. Native extracellular matrix preserves mesenchymal stem cell "stemness" and differentiation potential under serum-free culture conditions. Stem Cell Res Ther 2015; 6: 1-11. [CrossRef]
44. Brizzi MF, Tarone G, Defilippi P. Extracellular matrix, integrins, and growth factors as tailors of the stem cell niche. Curr Opin Cell Biol 2012; 24: 645-51. [CrossRef]
45. Gilbert PM, Havenstrite KL, Magnusson KE, Sacco A, Leonardi NA, et al. Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. Science 2010; 329: 1078-81. [CrossRef]
46. Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, et al. Role of YAP/TAZ in mechanotransduction. Nature 2011; 474: 179-83. [CrossRef]
47. Yang C, Tibbitt MW, Basta L, Anseth KS. Mechanical memory and dosing influence stem cell fate. Nat Mater 2014; 13: 645-52. [CrossRef]
48. Kuo YC, Chang TH, Hsu WT, Zhou J, Lee HH, Hui-Chun Ho J, et al. Oscillatory shear stress mediates directional reorganization of actin cytoskeleton and alters differentiation propensity of mesenchymal stem cells. Stem Cells 2015; 33: 429-42. [CrossRef]
49. Wang X, Nakamoto T, Dulińska-Molak I, Kawazoea N, Chen G. Regulating the stemness of mesenchymal stem cells by tuning micropattern features. J Mater Chem B 2016; 4: 37-45. [CrossRef]
50. Wan LQ, Kang SM, Eng G, Grayson WL, Lu XL, Huo B, et al. Geometric control of human stem cell morphology and differentiation. Integr Biol (Camb) 2010; 2: 346-53. [CrossRef]
51. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell 2006; 126: 677-89. [CrossRef]
52. Kiss R, Bock H, Pells S, Canetta E, Adya AK, Moore AJ, et al. Elasticity of human embryonic stem cells as determined by atomic force microscopy. J Biomech Eng 2011; 133:101009. [CrossRef]
53. van der Sanden B, Dhobb M, Berger F, Wion D. Optimizing stem cell culture. J Cell Biochem 2010; 111: 801-7. [CrossRef]
54. Siciliano C, Ibrahim M, Scafetta G, Napoletano C, Mangino G, Pierelli L, et al. Optimization of the isolation and expansion method of human mediastinal-adipose tissue derived mesenchymal stem cells with virally inactivated GMP-grade platelet lysate. Cytotechnology 2015; 67: 165-74. [CrossRef]
55. Burnouf T, Strunk D, Koh MB, Schallmoser K. Human platelet lysate: Replacing fetal bovine serum as a gold standard for human cell propagation? Biomaterials 2016; 76: 371-87. [CrossRef]
56. Schallmoser K, Bartmann C, Rohde E, Reinisch A, Kashofer K, Stadelmeyer E, et al. Human platelet lysate can replace fetal bovine serum for clinical-scale expansion of functional mesenchymal stromal cells. Transfusion 2007; 47: 1436-46. [CrossRef]
57. Cobden SB, Oztürk K, Duman S, Esen H, Aktan TM, Avunduk MC, et al. Treatment of Acute Vocal Fold Injury With Platelet-Rich Plasma. J Voice 2015; 30: 731-5. [CrossRef]
58. Cesarz Z, Tamama K. Spheroid Culture of Mesenchymal Stem Cells. Stem Cells Int 2016; doi: 10.1155/2016/9176357. [CrossRef]
59. Tsai TL, Manner PA, Li WJ. Regulation of mesenchymal stem cell chondrogenesis by glucose through protein kinase C/transforming growth factor signaling. Osteoarthritis Cartilage 2013; 21: 368-76. [CrossRef]
60. Chang TC, Hsu MF, Wu KK. High glucose induces bone marrow-derived mesenchymal stem cell senescence by upregulating autophagy. PloS one 2015; 10: e0126537. [CrossRef]
61. Choi KM, Seo YK, Yoon HH, Song KY, Kwon SY, Lee HS, et al. Effect of ascorbic acid on bone marrow-derived mesenchymal stem cell proliferation and differentiation. J Biosci Bioeng 2008; 105: 586-94. [CrossRef]
62. Potdar PD, D'Souza SB. Ascorbic acid induces in vitro proliferation of human subcutaneous adipose tissue derived mesenchymal stem cells with upregulation of embryonic stem cell pluripotency markers Oct4 and SOX 2. Hum Cell 2010; 23: 152-5. [CrossRef]
63. Li CJ, Sun LY, Pang CY. Synergistic protection of N-acetylcysteine and ascorbic acid 2-phosphate on human mesenchymal stem cells against mitoptosis, necroptosis and apoptosis. Sci Rep 2015; 5: 9819. [CrossRef]
64. Moll G, Alm JJ, Davies LC, von Bahr L, Heldring N, Stenbeck-Funke L, et al. Do cryopreserved mesenchymal stromal cells display impaired immunomodulatory and therapeutic properties? Stem cells 2014; 32: 2430-42. [CrossRef]

65. François M, Copland IB, Yuan S, Romieu-Mourez R, Waller EK, Galipeau J. Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties as a result of heat-shock response and impaired interferon-γ licensing. Cytotherapy 2012; 14: 147-52. **[CrossRef]**
66. Abdollahi H, Harris LJ, Zhang P, McIlhenny S, Srinivas V, Tulenko T, et al. The role of hypoxia in stem cell differentiation and therapeutics. J Surg Res 2011; 165: 112-7. **[CrossRef]**
67. Chung HM, Won CH, Sung JH. Responses of adipose-derived stem cells during hypoxia: enhanced skin-regenerative potential. Expert Opin Biol Ther 2009; 9: 1499-508. **[CrossRef]**
68. Larochelle A. Cord blood culture in hypoxia: making the cells feel at home. Cytotherapy 2012; 14: 900-1. **[CrossRef]**
69. Petruzzelli R, Christensen DR, Parry KL, Sanchez-Elsner T, Houghton FD. HIF-2α regulates NANOG expression in human embryonic stem cells following hypoxia and reoxygenation through the interaction with an Oct-Sox cis regulatory element. PloS One 2014; 9: e108309. **[CrossRef]**
70. Hubbi ME, Semenza GL. Regulation of cell proliferation by hypoxia-inducible factors. Am J Physiol Cell Physiol 2015; 309: 775-82. **[CrossRef]**

Experimed 2019; 9(3): 130-7

DERLEME
REVIEW

DOI: 10.26650/experimed.2019.19023

Yara İyileşmesi ve Deneysel Yara Modelleri

Wound Repair and Experimental Wound Models

Gül Baktır

İstanbul Yeni Yüzyıl Üniversitesi Eczacılık Fakültesi, Farmakoloji Anabilim Dalı, İstanbul, Türkiye

Cite this article as: Baktır G. Wound Repair and Experimental Wound Models. Experimed 2019; 9(3): 130-7.

ÖZ

Yara iyileşmesi hücresel, biyokimyasal ve sistemik proseslerde travma ile başlayan bozulmanın yeni doku oluşumu ile normal haline döndürülmesidir. Akut yaralar belirli bir süre içerisinde dokunun normal anatomik ve işlevsel bütünlüğüne geri dönebildiği yaralar olup tedavisi nisbeten problemsizdir. Kronik yaralar ise akut yaraların aksine, genellikle altta yatan bir hastalık nedeniyle yara iyileşme süreçlerinin kesintiye uğraması sonucu anatomik ve işlevsel bütünlüğün sağlanamadığı yaralardır. Kronik yaralar gelişmiş ülkelerde nüfusun önemli bir bölümünü etkileyen, yaşam kalitesini düşüren önemli bir sağlık sorunu olmasının yanı sıra, yara tedavisi sağlık sistemlerine ciddi bir mali yük getirmektedir. Son yıllardaki teknolojik gelişmelerle birlikte, yara tedavisinde kullanılan ilaçlar ve tıbbi cihazlar bakımından önemli ilerlemeler kaydedilmiştir, ancak bu gelişmelere rağmen, yara iyileşme süreçlerindeki karmaşık yapı ve hasta çeşitliliği nedeniyle yara tedavisi alanındaki deneysel araştırmalar, hala önemini korumaktadır. Bu derlemede, yara iyileşmesi ve deneysel yara modellerine odaklanılarak temel kavramlar ve araştırmada kullanılan güncel uygulamalar ele alınmıştır.

Anahtar Kelimeler: Yara, yara iyileşmesi, deneysel yara modelleri

ABSTRACT

Wound healing is the restoration of distorted cellular, biochemical and systemic processes with trauma to normalization with new tissue formation. Acute wounds are wounds in which the tissue can return to normal anatomical and functional integrity over a period of time, and treatment is relatively problem-free. Chronic wounds, on the other hand, are wounds in which anatomic and functional integrity cannot be achieved as a result of disruption of wound healing processes due to an underlying disease. Chronic wounds are a major health problem that affects a significant proportion of the population and reduces the quality of life. Moreover, wound care creates a significant financial burden on health systems in developed countries. With the technological advances in recent years, significant progress has been made in terms of drugs and medical devices used in wound treatment, however, despite these developments, experimental research in the field of wound therapy remains important due to the complex structure in wound healing processes and patient diversity. In this review, basic concepts and current applications used in wound research are discussed focusing on wound healing process and experimental wound models.

Keywords: Wound, wound repair, experimental wound models

GİRİŞ

Yaralar sıyrık, kesik, batma, ezik, yanık gibi fiziksel travmalar veya hastalıklar gibi birçok farklı nedenle cilt veya mukozanın doku bütünlüğünün bozulması sonucu oluşur ve aynı zamanda damarlar, kas ve sinir gibi yapılarla birlikte iç organ ve dokuları da etkileyebilir. Cilt/mukoza bütünlüğünün tamamıyla bozulmadığı "kapalı yaralar" ezilme, burkulma veya çıkık gibi nedenlerle meydana gelirken, kesik, batma, delinme gibi etkenlerle doku bütünlüğünün bozulmuş olduğu yaralara "açık yaralar" denir. Yatalak hastalarda görülen bası yarası gibi durumlarda ise başlangıçta kapalı olan yara, açık yara haline dönüşebilir.

Akut yaralar belirli bir süre içerisinde belli aşamalardan geçtikten sonra dokunun normal anatomik ve işlevsel bütünlüğüne geri dönebildiği yaralardır. Kronik yaralar (bası yaraları, venöz staz ülserleri, diyabetik yaralar, iskemik yaralar vb.) ise akut yaraların aksine belirli dönemlerden geçmeyen, altta yatan bir hastalık nedeniyle yara iyileşme süreçlerinin kesintiye uğraması sonucu anatomik ve işlevsel bütünlüğün sağlanamadığı yaralardır. Böyle durumlarda basit bir fiziksel travma bile bir türlü iyileşemeyen kronik bir yaraya sebep olabilir. Normal yara iyileşme sürecinin desteklenerek doku onarımının hızlandırılması için öncelikle altta yatan hastalığın teşhis ve tedavi edilmesi gerekir.

Kronik yaralar gelişmiş ülkelerde nüfusun önemli bir bölümünü etkileyen, yaşam kalitesini bozan önemli bir sağlık sorunu olmasının yanı sıra tedavisi ciddi bir mali yük getirmektedir (1, 2).

Son yıllarda, biyofilm gelişiminin yara kronikleşmesinin temel nedeni olduğu anlaşılmıştır. Yara biyofilmi, yara yüze-

Sorumlu Yazar/Corresponding Author: Gül Baktır **E-posta:** gul.baktir@yeniyuzyil.edu.tr
Geliş Tarihi/Received Date: 14.10.2019 **Revizyon Tarihi/Received Date:** 21.10.2019 **Kabul Tarihi/Accepted Date:** 25.10.2019

Experimed 2019; 9(3): 130-7

yine tutunan, polisakkarit matrise yerleşmiş polimikrobiyal bir kolonidir. Yara içinde kronik enflamatuvar bir duruma sebep olan enzim ve toksinler üretmeleri, antibiyotiklere dirençli kronik yara enfeksiyonuna sebep olmaları, hastalar için hastanede uzun yatış süreleri, sağlık sistemi için ise yüksek maliyet demektir. Yara biyofilmlerine yönelik yeni tedavilerin geliştirilmesi, kronik yaraların tedavi edilebilme umudunu artırarak birçok hastanın hayatını kurtaracaktır (3). Diğer taraftan son yıllarda yara iyileşmesini hızlandırmak ve hastanın yaşam kalitesini artırmak amacıyla elektrik akımı, lazer ışını, radyo dalgaları ve ultrason uygulamasını da kapsayan yeni teknolojiler üzerinde de yoğun olarak çalışılmaktadır (3, 4).

KLİNİK VE ARAŞTIRMA ETKİLERİ

Yara İyileşmesi

Yara iyileşmesi hücresel, biyokimyasal ve sistemik proseslerde travma ile başlayan bozulmanın yeni doku oluşumu ile normal haline döndürülmesidir. Yaranın bulunduğu bölge ile bu bölgedeki kan akımı, sitokinler ve büyüme faktörleri, genetik ve immünolojik bozukluklar, diyabet, radyoterapi, kemoterapi, uygun olmayan beslenme, steroid ilaç kullanımı gibi faktörler yara iyileşmesini etkiler. Sağlıklı yara iyileşmesi yeterli doku perfüzyonu, oksijenizasyonu ve epitelizasyonu yanında, dokunun iyi beslenmesi ve nemlenmesi ile sağlanır (3). Yara iyileşmesi sırasında gerçekleşen bu süreçlerin başlıca amacı yara gerilim kuvvetinin normal düzeyine getirilmesidir.

Yara iyileşmesinde çeşitli sitokinler ve büyüme faktörleri rol oynar. Sitokinler vücutta hücreler arası iletişimi sağlayan, hücre gelişimini, olgunlaşmasını ve fonksiyonlarını etkileyen protein yapısında moleküller olup büyüme faktörleri sitokinlerin alt grubunu oluşturur. Sitokinler kemotaktik etki ile inflamatuvar hücreler ve fibroblastların yara bölgesine göçü ile hücre proliferasyonunu sağlarlar, anjiogenezi aktive ederler, ekstraselüler matriks yapılanmasını sağlarlar (5).

Yara iyileşmesinde rol oynayan sitokinler:

- PDGF (Platelet derived growth factor) - Trombosit kaynaklı büyüme faktörü
- FGF (Fibroblast growth factor) - Fibroblast büyüme faktörü
- TGF-Beta (Transforming growth factor) - Transforme edici büyüme faktörü
- EGF (Epidermal growth factor) - Epidermal büyüme faktörü
- IGF (Insulin-like growth factor) - İnsülin benzeri büyüme faktörü
- GM-CSF (Granulocyte-macrophage colony-stimulating factor) - Granülosit makrofaj koloni stimüle edici faktör
- IL 1 (Interleukin-1), IL 2 (Interleukin-2) - İnterlökinler
- TNF alfa (Tumor necrosis factor) -Tümör nekroz faktörü alfa

YARA İYİLEŞMESİNİN EVRELERİ

Hemostaz ve İnflamatuvar Faz

Bu evre damarlarda daralma ile kısa süreli hemostazın sağlanmasıyla başlar. Doku hasarı sonrası sitokinler hemen salınarak iyileşme sürecini yönlendirirler. Damar duvarı zedelendiğinde trombositler açılan damar duvarındaki kollajenle temas ederek geçici pıhtı oluşturur ve hemostaz sağlanır ve inflamatuar hücreler yara alanına doğru göçerek apoptotik hücreleri ve bakterileri yara bölgesinden uzaklaştırmaya başlar. Yara alanında inflamasyonun klinik belirtileri olan lokalize ödem, ağrı, kızarıklık ve sıcaklık gözlenir. Bu faz, genellikle 1-4 gün içinde tamamlanır (6).

Proliferasyon Fazı (Kollajen Sentezi Fazı)

Yaralanma sonrası 2. gün başlayan ve 3 hafta kadar devam eden bir süreçtir. Bu safhada temel olarak geçirgen bir bariyer oluşturulur, reepitelizasyon ve kontraksiyon gelişir, kan desteği için mikro dolaşım düzenlenir ve doku güçlendirilir (6). Yara bölgesindeki inflamatuar hücrelerden salınan sitokinler ve büyüme faktörlerine cevap olarak fibroblastlar yeni ekstraselüler matris ve olgunlaşmamış Tip III kollajen sentezlemeye başlar. Kollajen birikimi yaranın gerilmeye karşı direncini hızla artırır. Yara kenarlarındaki bazal tabakadan köken alan epitel hücreleri yaranın üzerinde yeni bir yüzey oluşturur. Yara kontraksiyonu, fibroblastların bir kısmının miyofibroblastlara dönüşümü sonucu, yaranın derinliğine ve konumuna bağlı olarak meydana gelir.

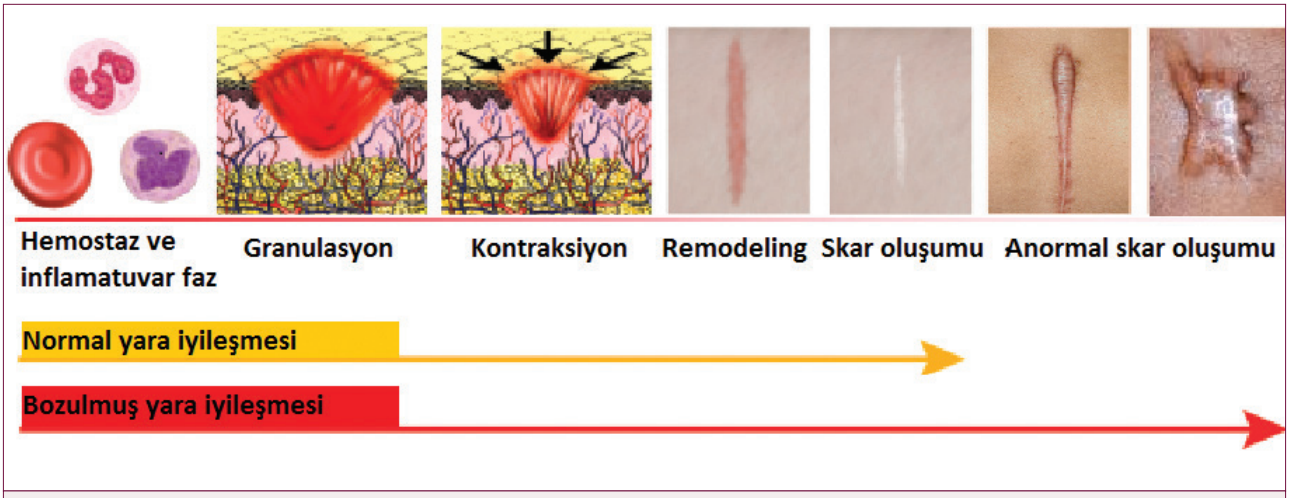
Remodelizasyon (Matürasyon) Fazı

Proliferasyon safhasından sonra 3. haftada başlayan bu evrede yara bölgesindeki fibroblast sayısı azalır, kollajen üretimi dengeye ulaşır ve epitelizasyon tamamlanır. Remodelizasyon, kollajen liflerinin yeniden şekillenmesidir. Bu aşamada yumuşak ve jelatinöz yapıdaki tip III kollajen daha sıkı olan tip I kollajene dönüşür. Kontraksiyonun bir kısmı da bu aşamada gelişir. Yara yaklaşık 6 hafta sonra başlangıçtaki gücünün % 80-95 ini kazanır. Yara alanının renginin soluklaştığı, yara gerilim direncinin arttığı ve skar dokusunun (Şekil 1) oluştuğu bu evre 6-12 ay, hatta 24 aya kadar devam eder (6, 7).

Yara iyileşme süreci ile ilgili veriler *in vitro* deneyler yanında hayvan deneylerine veya özel teknikler gerektiren modellere dayanmaktadır. Hayvanlarda oluşturulan deneysel yara modellerinde insanda meydana gelen yaralara benzer koşullar sağlanabilmekteyse de, fizyopatolojik farklılıklar bulunmaktadır. Hayvan modelleri, incelenecek iyileşme prosesine göre de değişmektedir. Bu derlemede hayvan modelleri iki bölüm altında toplanmıştır. İlk bölümde epitelizasyon, skar oluşumu ve daralma gibi farklı yara iyileşme biçimlerine odaklanan yara modelleri, ikinci bölümde ise bozulmuş doku onarımına odaklanan modeller yer alacaktır.

IN VİTRO MODELLER

İn vitro yara modelleri daha çok hücreler arası ve hücre içi sinyal iletimini değerlendirmek için kullanılmakta, ancak fizyolojik koşulları sağlayamamaktadır. *İn vitro* modeller hücre ve doku kültürleri ile 3D matrisleri kapsar (8). Hücre kültürü ortamında bir veya birkaç hücre tipinin belirli uyaranlara verdiği cevap kaydedilebilir. Hücre kültürleri endotel, fibroblast ve keratinosit hücreler ile bunların kombine olarak kullanıldığı kültürleri kapsar. Büyüme faktörlerine karşı fibroblastlardan salınan kollajen tipi ve miktarı, endotel proliferasyonu, fibroblast kontraksiyonu gibi süreçler incelenebilmektedir (9, 10). Ancak hücrelerin bazı



Şekil 1. Normal ve Bozulmuş Yara İyileşmesi

uyaranlara tek başına verdikleri cevap, diğer hücrelerle birlikte iken verdikleri cevaptan farklı olduğundan yara iyileşmesi araştırmalarında *in vivo* şartlara daha yakın olan organ ve doku kültürü modelleri de kullanılmaktadır (11, 12). 3D Matrisler ise hücre göçünün *in vitro* olarak üç boyutlu yara bölgesinde değerlendirilmesini sağlar (13).

In vitro yara iyileşme modellerinde kısıtlı sayıda uyarana kısıtlı sayıda hücre tipinin verdiği yanıtlar incelenirken, *in vivo* modellerde normal yara iyileşmesinin farklı aşamalarında oynayan hücre tiplerine ek olarak ekstraselüler matriks, sitokinler, büyüme faktörleri, pH, oksijenlenme, sıcaklık, beslenme ve genel sağlık durumu gibi faktörlerin doku onarımına etkisini incelemek mümkündür.

IN VİVO DENEY HAYVANI MODELLERİ

In vivo (deney hayvanı) yara modelleri, yara oluşumunu ve doku onarımını fizyolojik yönden en iyi taklit edebilen kompleks koşulları sağladıkları için halen yara iyileşmesini incelemede kullanılan en fazla tercih edilen modellerdir.

Hayvan modellerinde kemirgenler, domuz, köpek, tavuk, koyun gibi hayvanlar kullanılsa da türler arasında histolojik farklılıklar görülmektedir. Epidermis/dermis kalınlığı insana yakın olduğundan yara iyileşmesinin incelenmesi bakımından insana en yakın deney hayvanı domuzdur. Kemirgen derisi insan deri özelliklerine yakın olmasa da, çalışılması pratik, kolay bakılabilir ve dayanıklı hayvanlar olmaları nedeniyle küçük hayvan modelleri oldukça sık tercih edilmektedir. Fareler üzerinde genetik müdahale yapmak mümkündür ve çok sayıda transjenik fare modeli bulunmaktadır.

Domuzlarda papillalar, subdermis, kıl siklusu, hipodermis kalınlığı insana benzemektedir. Ekrin ter bezleri sadece insanlarda görülürken apokrin ter bezleri insanda perine ve aksillada, domuzlarda ise tüm vücut bölgelerinde yer alır (14-16). Yara iyileşme fare ve sıçan gibi kemirgenlerde kontraksiyonla gelişirken, domuz ve insanlarda granülasyon ve epitelizasyon hakim mekanizmadır. Kemirgenlerin yara modellerinde kullanımına ilişkin en önemli dezavantaj yara iyileşme prosesinin farklı olmasıdır. Bu dezavantajı yenmek için bu modellerde çeşitli modifikasyonlar yapılmıştır (17).

Granuloma Modelleri

Granuloma modelleri deney hayvanının enflamatuvar bir yanıt uyarma yeteneğini ve kollajen serbestleme derecesini incelemek ve ölçmek için kullanılır. Bu amaçla hücrelerin, sıvı ve ekstraselüler matriksin toplanmasını sağlayan polivinil alkol veya selüloz sünger implantlar (18, 19) hayvanın deri altı dokusuna yerleştirilir ve çeşitli zamanlarda toplanan örnekler analiz edilir. Kolajen birikimi, en sık incelenen parametre olup, genellikle kolajen için spesifik bir olan amino asit hidroksiprolin seviyeleri tespit edilerek tahmin edilir. Diğer bir nondinamik yöntemde politetrafluoroetilen endovasküler stent graft (ePTFE; Impra®) ve silikon (silastik) rezervuarlar kullanılmaktadır (20-22). Granülasyon dokusunun toplanmasını sağlayan başka bir non-dinamik yöntem de çelik kafes şeklinde gözenekli silindir "Hunt-Schilling" chamber (23, 24) implantasyonudur. Dinamik yöntemler ise visköz selüloz sünger (21) ve mikrodiyaliz için yarı geçirgen membran probların implantasyonudur.

İnsizyonel Yara Modelleri

Uzun yıllardır bilinen, sık kullanılan bir yara modeli de insizyonel yara iyileşmesi modelidir. Yara gerim gücünün incelenmesi için en sık kullanılan yöntemdir. Modelde cilt insizyonunu takiben yara ya açık bırakılır ya da primer suture edilir. İşlem sonrasında belirlenen zaman aralıklarında insizyon bölgesinden blok çıkartılır ve gerim gücü ölçmek için tasarlanmış bir cihazda yara kenarlarının birbirinden ayrılması için gerekli kuvvet saptanır. Histopatolojik örnekler alınabilir, sistemik veya topikal yara iyileştirici ajanların etkileri kantitatif olarak ölçülebilir (10). Daha çok kemirgenler için kullanılmış olsa da, domuz veya farklı hayvanlarda kullanılabilecek modelleri tanımlanmıştır (25, 26).

Eksizyonel Yara Modelleri

En sık kullanılan ve en basit yara modelidir, açık bir yara oluşturulur ve yaranın zamana bağlı kapanma oranı kaydedilir.

Experimed 2019; 9(3): 130-7

Granülasyon oluşumu, kollajen birikmesi, reepitelizasyon ve konstriksiyon bu modelle araştırılabilir. Yaralar farklı derinlik ve büyüklüklerde, tam kat (eksizyonel) ya da kısmi kalınlıkta oluşturulabilir (27). Aynı deney hayvanında birkaç yara açılarak farklı ajanların etkileri incelenebilir. Yara yüzey alanı dijital fotoğraflar ve bazı yazılımlar aracılığıyla takip edilmektedir. Yaraların histolojik değerlendirmesi ve eksize edilen dokularda moleküler biyolojik analizlerin (mRNA, protein, apoptoz) yapılması da kolaydır. Dikkat edilmesi gereken en önemli konu, bazı türlerin açık yaralarının, granülasyon ve yeniden epitelizasyon oluşumuna kıyasla daha ziyade kontraksiyon ile iyileştirmesidir (örn. başta fare olmak üzere kemirgenler) (8).

Yanık Modelleri

Yara iyileşmesini ve termal yaralanmaya sistemik yanıtı incelemek için kullanılan pek çok yanma modeli tarif edilmiştir. Tam veya kısmi kalınlıkta yanık oluşturmak mümkündür. Yanık yarasının kalınlığı, yanma derinliğini belirleyen faktörlere bağlıdır: temas eden maddenin sıcaklığı, temas süresi, cilt kalınlığı ve kanlanma. Eğer kısmi kalınlıkta yanık isteniyorsa düşük derecele bir sıcaklık kısa süre ve daha kalın ciltlere uygulanmalıdır. Temas yanığı oluşturmak için alev veya kaynar suya yerleştirilmiş metal gibi yöntemler kullanılır. Haşlanma, alev, sıcak cisim gibi modellerin yanı sıra elektrik yanığı, yanık yarası kaynaklı sepsis gibi yanık modelleri tanımlanmıştır (28).

Kemirgenlerde yanık modellerinin önemli dezavantajlarından biri, yanık kaynaklı travmaya verilen cevabın şiddetli olması ve hayvanların araştırma bitmeden kaybedilmesidir. Diğer faktörler, farklı hayvan türlerin vücut yüzey alanlarının orantısal olarak insanlardan farklı olması, hayvanda oluşturulan belirli boyuttaki bir yaranın insandaki büyük yanıklarda meydana gelen sistemik değişikliklerin aynısını meydana getirip getirmediğinin tam olarak kestirilememesidir (8).

Donuk Modelleri

Yanık yara iyileşme modelleri kadar yaygın olmasa da, donma yaralarını inceleme amacıyla da modeller geliştirilmiştir. Bu modellerde temel yöntem aşırı soğutulmuş bir cismi belirli sürelerle hayvan derisine temasta tutmak ve bununla bağlantılı doku hasarını ölçmektir (29).

YARA İYİLEŞMESİNİN BOZULMUŞ OLDUĞU DURUMLAR/HASTALIKLAR İÇİN UYGULANAN MODELLER

İnsanda yara iyileşmesini bozan başlıca faktörler arasında malnütrisyon, diyabet, iskemi, yara enfeksiyonu, steroid ve kemoterapötik kullanımı, radyasyon sayılabilir. Bu faktörleri deney hayvanlarında incelemek için çeşitli modeller kullanılmaktadır. Bunun yanı sıra bası yarası, venöz ülser, hipertrofik skar ve keloid gibi özel yara modelleri de sık görülen bazı klinik durumlar için modellemektedir.

Malnütrisyon

Kötü beslenmenin iyileşmeyi engelleyen bir faktör olduğu, beslenme bozukluklarının yara iyileşmesi yanında genel olarak hastalıkların iyileşmesini de kısıtladığı iyi bilinmektedir. Diyette protein alımının sınırlandığı çeşitli hipoproteinemi modelleri bulunmaktadır.

Yeterli kalori sağlayan fakat protein içeriği açısından sınırlı olan diyetler de dokuların onarılmasını ve yara iyileşmesini kısıtlamaktadır.

C vitamini, A vitamini, tiamin, çinko, bakır, metiyonin, sistein, arjinin, esansiyel yağ asitleri gibi spesifik bir besin maddesinin kısıtlanması da iyileşmenin bozulmasına veya gecikmesine neden olmaktadır (30, 31).

Enfeksiyon

Bakteri kolonizasyonu veya zayıf bir enfeksiyon gelişmesi enflamatuar süreçleri hızlandırarak yara iyileşmesini desteklemektedir. Makrofajlar iyileşme sürecinin temel düzenleyicileri olup bu hücrelerin uyarılması doku onarımını uyararak hızlandırır (32). Buna karşın, yara özellikle virülan bakteriler ile kaplanmışsa bakteri enzimlerinin olumsuz etkileri nedeniyle yara iyileşme süreci bozulur. Enfeksiyon modelleri lokal veya sistemik olarak geliştirilebilir. Lokal enfeksiyon modeli ile çalışmak basittir ve tekrarlanabilir sonuçların elde edilmesini sağlar; mikroorganizmanın bilinen konsantrasyonda yaraya lokal olarak uygulanmasından ibarettir (33).

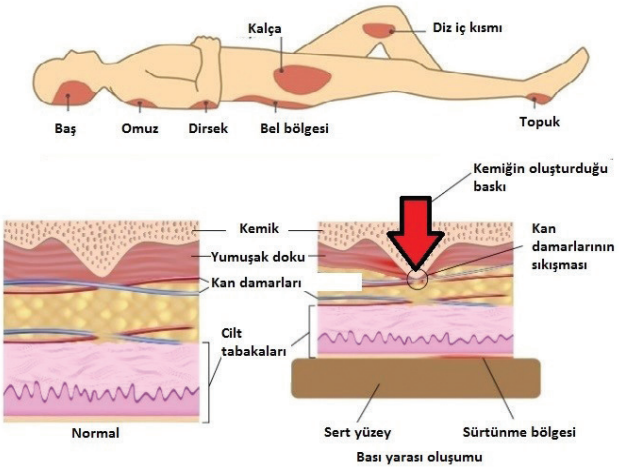
Sistemik enfeksiyon geliştirme yöntemlerinden en iyi bilineni deney hayvanlarına intravenöz veya intraperitoneal *Escherichia coli* infüzyonudur. Diğer bir yöntem, çekal ligasyon ve perforasyon (CLP) yöntemiyle oluşturulan deneysel peritonit modelidir. Kemirgenlerde polimikrobiyal abdominal sepsis oluşturmak için kullanılan oldukça standart diğer bir model asendan kolon stent peritoniti (CASP) modelidir. Bu modelde küçük bir stentin sıçan veya farenin asendan kolonuna cerrahi olarak yerleştirilmesi periton boşluğuna sürekli olarak bağırsak bakterilerinin sızmasına yol açar. Bu süreç peritonit, sistemik bakteriyemi, bağırsak bakterilerinin yol açtığı organ enfeksiyonu, lokal ve sistemik olarak proinflamatuar ve antiinflamatuar sitokinlerin salınımı ile sonuçlanır (34).

İskemi

Zayıf kan akımı, doku ve deride oksijen eksikliğine , dolayısıyla dokularda hasar gelişmesine, mevcut yaraların da zor iyileşmesine neden olur; bacaklarda görülen, iyileşip tekrar oluşan kronik yaralar gibi. İskemik yara iyileşmesi oluşturmak için kullanılmakta olan "flep modeli" ve "damar ligasyonu modeli" ve "tavşan kulağı damar ligasyon modeli" olarak bilinen birkaç model mevcuttur (36, 37).

Diyabet

Diyabetin önemli komplikasyonları olan ateroskleroza yol açan makrovasküler bozukluk, lokal kan dolaşımını sınırlayan mikrovasküler bozukluk, ciltte his kaybına yol açan nöropati, enfeksiyonla savaşabilme yeteneğinin zayıflaması diyabette yara iyileşmesini geciktiren temel faktörlerden olup küçük bir yaranın bile ciddi bir enfeksiyona yol açması ve sonunda ampütasyona sebep olabilir. Ek olarak, hiperglisemi ile ilgili birçok metabolik değişiklik de yara iyileşmesini etkilemektedir (38).



Şekil 2. Bası yarası oluşumu

Diyabet modelleri insüline bağımlı (tip 1) veya insüline dirençli (tip 2) diyabeti temsil etmek üzere geliştirilmiştir. Deney hayvanlarına çeşitli ilaçlar uygulayarak veya genetik değişiklikler yoluyla diyabet oluşturulabilmektedir. Ancak başlıca dezavantajlar, hiperglisemi derecesinin hayvandan hayvana değişmesi ve kullanılan ilaçların T hücre fonksiyonunda değişme ve fagositozda azalma gibi yara iyileşmesinde bozulmaya yol açabilen diğer etkenlere yol açabilmesidir.

Deneysel diyabet oluşturulmasında başta fındık faresi, sıçan gibi kemirgenler yanında tavşan, primatlar gibi pek çok hayvan türü kullanılmaktadır. Genetik olarak diyabetik olan farelerin birçok suşu vardır. En sık kullanılan tür, diyabetik "db / db" faredir (C57BL / KsJdb /db veya C57BL / KsJ $^{Lepr-/Lepr-}$) (39, 40).

Homozigot mutant (db/db) farelerde leptin reseptörü yoktur ve dolayısıyla leptine yanıt alınamadığından bu hayvanlarda tip 2 diyabet gelişir. Bu model büyüme faktörleri ve matris metaloproteinazlar gibi yara iyileşmesinde rol oynayan maddelerin incelenmesi için iyi bir araçtır. "Ob/ob" faresinde ise leptin geni yoktur ancak"db/db" farelerine kıyasla bu farelerde diyabet daha zayıf derecede gelişir (41).

İnsüline dirençli diyabet geliştiren ve doku onarımının bozulmuş olduğu sıçan (rat) türleri ise Zucker "fatty", JCR: LP-cp' dir. İnsüline dirençli diyabet geliştiren diğer fare türleri Agouti, Yeni Zelanda Obez ve Spiny' dir, fakat bu hayvanlarda yara iyileşmesi test edilmemiştir. İnsülin eksikliğine bağlı diyabet geliştiren başka hayvan türlerine ait genetik varyantlar da bulunmaktadır: Obez olmayan diyabetik fareler, BB Wistar sıçanları, Çin hamsterleri, Yucatan minyatür domuzu ve bazı köpek ve primat türleri.

İlaçlarla diyabet oluşturmada kullanılan (diabetojenik) ajanlar arasında ditizon, monosodyum glutamat, altın tiyoglukoz, fruktoz ve glukoz yükleme ve anti-insülin serumu bulunmaktadır. Tip 1 diyabet oluşturan alloksan ve streptozotosin ise yıllardan beri yaygın olarak kullanılan diyabet modelleridir (42). Streptozotosin veya alloksan'ın neonatal sıçanlara uygulanması erişkin hayvanlarda Tip 2 diyabet oluşturur. Diğer taraftan erişkin sıçanlara streptozotosini takiben nikotinamid adenin dinükleotid (NAD) uygulanmasıyla tip 2 diyabet oluşmaktadır.

Cerrahi diyabet modellerinde ise pankerasın rezeksiyonunu takiben Tip 1 diyabet oluşturmak mümkündür.

Bası Yaraları

"Yatak yarası" veya "decubitus ülseri" olarak da bilinen bası yaraları, vücudun fazla basınca maruz kalan kısımlarında oluşur. Sıklıkla yatalak hastalarda, fakat uzun süreli basınca maruz kalan her vücut bölgesinde, örneğin tekerlekli sandalyeye bağlı kişilerde oturma kemikleri üzerinde gelişebilir (Şekil 2).

Literatürde sıçan, tavşan, kobay, köpek ve domuzlarda geliştirilmiş bası yarası modelleri tanımlanmıştır (43-45). Örneğin yaranın altına, kasın üzerine gelecek şekilde bir bariyer yerleştirerek yarayı «germek» (27), cilt altına bir parça paslanmaz çelik ve ardından metal plaka üzerine bir mıknatıs yerleştirerek cilde baskı oluşturmak (46) veya plakayı kas altına yerleştirerek "bası yarası" oluşturma gibi metodlar uygulanmıştır.

Hipertrofik Skar ve Keloid

Hipertrofik skar, yara iyileşmesi sürecindeki bozukluk nedeniyle aşırı hücre üretimi sonucu anormal nedbe dokusu oluşmasıdır. Normalde yara iyileşme süreci tamamlandığında doku onarımı durur. Hipertrofik skarda ise, bağ dokusu oluşma miktarını düzenleyen lokal hormon/enzim mekanizmalarındaki bozukluk sonucu üretimin devam eder, kollajen aşırı miktarda oluşur ve kabarık bir nedbe dokusuna sebep olur. Hipertrofik skarlar başlıca göğüs, omuzlar, kulak memeleri, üst kol ve yanaklarda oluşur, en sık cerrahiden (%40- 70) ve yanık yaralarından (%90) sonra gözlenir, genellikle yara bölgesiyle sınırlıdır.

Keloid ise normal olarak ve tamamen iyileşmiş bir yara izinde, iyileşmeyi takip eden 6 ay 1 sene sonra, kaşıntı ve ağrı ile birlikte bağ dokusu (kollajen) üretiminin yeniden başlamasıyla oluşur. Keloidde oluşan nedbe dokusu yara sınırları içinde kalmayıp çevreye yayılır. Keloidin kanserden farkı, kanser dokusunda hücre sayısı artış gösterirken keloidde bağ dokusu artışı olmasıdır (47).

Hipertrofik skarları araştırmak amacıyla bir modelde bağışıklık yetersizliği olan atimik ("çıplak") farelere insan hipertrofik skarları nakledilmiş, bir diğerinde iskemik tavşan kulağı modelinde kronik yara oluşturulmuş, ancak skarlar kalıcı olmadığından iki modelde de başarılı olunamamıştır. "Kırmızı Duroc Domuz" larda ise yüzeysel yaralar iz bırakmaz, ancak derin bir yara oluştuğunda iyileşme gecikir ve hipertrofik skar belirtileri gözlenir. En başarılı model ise 3D matrislerde üretilmiş keratinosit kültürlerine insan kaynaklı mezenkimal kök hücre eklenmesini içerir (48).

Steroidlerin, Kemoterapötiklerin ve Radyasyonun Yara İyileşmesine Etkileri

Akut veya kronik steroid ilaç uygulaması hemen hemen her tür iyileşme prosesini bozmak için kullanılabilir (49). Steroidlerin iltihabı baskılayıcı, hücre çoğalmasını engelleyici ve doku rejenerasyonunu bozucu etkileri A vitamini ve bazı büyüme faktörleri

ile reversibldir. Kemoterapötik ilaçlar ise hızlı çoğalan hücreleri öldürerek etki ederler; bu arada yaranın iyileşmesi için elzem olan rejeneratif hücrelerin yaraya göçünü ve proliferasyonunu da önlediklerinden yara iyileşmesini geciktirici ve bozucu tesirleri aşikardır. Radyasyon da kemoterapiye benzer şekilde hücre proliferasyonunu, dolayısı ile doku onarımını engeller (50).

İNSANLARDA DOKU ONARIMI MODELLERİ

İnsan fetüsünde doku onarımının belirgin yara izi bırakmadan gerçekleşmesi, fetal hayvan modellerinin geliştirilmesine yol açmıştır: tavşan (51), fare (52), koyun (53). Bazı hayvan modelleri arasında fetal ciltteki yara iyileşme özelliklerinde farklılıklar olduğu, bu nedenle insandaki duruma ekstrapolasyonun güçleştiği görülmüştür. Örneğin fetal koyun eksizyonel cilt yaraları kontrakte olarak iyileşir, fetal tavşan eksizyonel yaraları iyileşmez, fetüs büyüdükçe genişlemeye devam eder. Etik kısıtlamalar nedeniyle insan fetal cilt yara iyileşmesi çalışmaları yapılamadığından, atimik *(nu/nu)* "çıplak" farelere yerleştirilen insan fetal cilt greftleri kullanılarak bir yara iyileşmesi modeli geliştirilmiştir (54). İnsan fetüs cilt greftlerinin yetişkin "çıplak" farelere subkütan olarak aktarıldıktan sonra oluşturulan yaranın iz bırakmadan iyileşebildiği, skarsız yara iyileşmesinin fetüs serumu ya da intrauterin amniyon sıvısına bağlı olmadığı ve subkütan greftlerin aksine, kütanöz fetal cilt greftlerin skar bırakarak iyileştiği gözlenmiştir. Bu model ile hem subkütan hem de kutan greftlerdeki spesifik büyüme faktörlerinin ve ekstraselüler matriks bileşenlerinin ekspresyonunu sistematik olarak çalışmak mümkündür.

Diğer modellerde insan fetüslerinden elde edilen dokular kullanılmaktadır. "Fetal doku araştırmaları"nda, hücre hatları oluşturmak veya bunları transplantasyon materyali olarak kullanmak amacıyla ölü fetüslerden alınan hücreler kullanılır. Fetal dokuların başlıca kaynağı indüklenmiş veya doğal düşüklerdir.

Deneysel ve klinik kanıtlar, fetüsün yara oluşumuna erişkindekinden farklı şekilde yanıt verdiğini göstermektedir. Akut inflamasyon neredeyse hiç gelişmez, hyaluronik asit, yara matrisinin önde gelen bileşenidir, kollajen ise bol miktarda birikir, doku belirgin yara izi kalmadan onarılır. Yetişkin memelilerde ise sadece oral mukozada minimal veya hiç skar oluşumu olmadan rejeneratif iyileşme gerçekleşir. Skar ve fibroz, pek çok hastalığın ortak sorunu olduğundan, fetal yara iyileşmesini kontrol eden biyolojik mekanizmalar tanımlanarak yetişkin yara iyileşme sürecini modüle etmek için bu bulgulardan yararlanılmaktır.

Son yıllarda fetal ve sağlam erişkin dermisi içeren veya taklit eden ürünler, umut verici klinik sonuçlarla "yara iyileşmesi pazarı" na sunulmaktadır (55).

İNSAN VE HAYVANDA ORAL MUKOZA MODELİ

Fetüs cildine benzer şekilde, insanda oral mukozada yara iyileşmesi ciltekine nazaran daha hızlı ilerler ve mukozal yaralarda nadiren skar oluşur. Bu ilginç bulguyu açıklayabilmek amacıyla Wong JW ve ark (56), yaptıkları deneylerde kırmızı Duroc domuzların oral mukoza ve derilerinde yara iyileşmesi ve skar gelişimini karşılaştırmış, ayrıca domuzlardaki oral mukozal yara iyileşmesini insanlarda oluşturulan benzer yaralarla kıyaslamışlardır. Bulgular, cilt ile karşılaştırıldığında yara oluşumundan 49 gün sonra domuz oral mukozasında hem klinik hem de histolojik düzeyde skar oluşumunda azalmaya işaret etmiştir. Skarlarda tip I prokollajen immünopozitif hücre ve fibronektin içeriği önemli ölçüde artmış, oral mukozal yaralarda uzun süreli tenascin-C birikmesi gözlenmiştir (57).

Domuz oral mukozal yaraları insan oral mukozal yaralarına moleküler kompozisyon, klinik ve histolojik skar skorları bakımından benzediğinden, domuz oral mukozasının skarsız yara iyileşmesini sağlayan biyolojik süreçleri incelemek için uygun bir model olduğu düşünülmektedir.

SONUÇ

Kronik iyileşmeyen yaralar, çok sayıda hastayı etkileyen, gerek hastaya, gerekse sağlık sistemine ciddi finansal yük getiren önemli sağlık sorunlarıdır. Kronik yaralar süregelen iltihaplı bir durum içinde olmalarına rağmen, dinamik özellik taşırlar ve uygun yara ve skar tedavisi, anomalitelerin tanımlanmasını, uygun ilaçların ve büyüme faktörlerinin uygulanmasını ve çevresel koşulların yönetimini gerektirir. Yara tedavisi teknolojileri, ilaç ve tıbbi cihaz pazarının için büyük bir bölümünü oluşturur; yara iyileşmesinde/kapanmasında yer alan ürünlerin pazardaki payları 15 milyar doları aşmış, yara izi önleme ve giderme ürünlerinde ise bu pay 12 milyar dolara ulaşmıştır (58). Son zamanlarda, yara tedavisindeki teknolojik gelişmelerle birlikte önemli ilerlemeler kaydedilmiştir, ancak bu gelişmelere rağmen yara tedavisi alanındaki deneysel araştırmalar, yara iyileşmesindeki karmaşık yapı ve hasta çeşitliliği nedeniyle hala önemini korumaktadır.

Hakem Değerlendirmesi: Dış bağımsız.

Çıkar Çatışması: Yazar çıkar çatışması bildirmemiştir.

Finansal Destek: Yazar bu çalışma için finansal destek almadıklarını beyan etmiştir.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The author has no conflict of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

REFERENCES

1 Mercandetti M, Cohen AJ. Wound healing and repair. Available from: https://emedicine.medscape.com/article/1298129-overview
2. Macdonald J, Asiedu K. WAWLC: World Alliance for Wound and Lymphedema Care. Wounds 2010; 22: 55-9.
3. Rajpaul K. Biofilm in wound care. Br J Community Nurs 2015; Suppl Wound Care: S6, S8, S10-1. **[CrossRef]**
4. Larsen JA, Overstreet J. Pulsed radio frequency energy in the treatment of complex diabetic foot wounds: two cases. J Wound Ostomy Continence Nurs 2008; 35: 523-7. **[CrossRef]**

5. Öztopalan DF, Işık R, Durmuş AS. Yara iyileşmesinde büyüme faktörleri ve sitokinlerin rolü. Dicle Üniv Vet Fak Derg 2017; 10: 83-8.
6. Coşkun Ö, Uzun G, Dal D ve ark. Kronik yarada tedavi yaklaşımları. Gülhane Tıp Derg 2016; 58: 207-28.
7. Shih B, Garside E, McGrouther DA, Bayat A. Molecular dissection of abnormal wound healing processes resulting in keloid disease. Wound Repair Regen 2010; 18: 139-53. [CrossRef]
8. Greenhalgh DG. Models of wound healing. J Burn Care Rehabil 2005; 26: 293-305. [CrossRef]
9. Bettinger DA, Yager DR, Diegelmann RF, Cohen IK. The effect of TGF-beta on keloid fibroblast proliferation and collagen synthesis. Plast Reconstr Surg 1995; 98: 827-33. [CrossRef]
10. Aydın OE, Tan Ö, Çinal H, Kara M, Çakmak MA. Deneysel Yara Modelleri. Turkiye Klinikleri J Plast Surg-Special Topics 2015; 4: 5-11.
11. Strande LF, Foley ST, Doolin EJ, Hewitt CW. In vitro bioartificial skin culture model of tissue rejection and inflammatory/ immune mechanisms. Transplant Proc 1997; 29: 2118-9. [CrossRef]
12. Emanualsson P, Kratz G. Characterization of a new in vitro burn wound model. Burns 1997; 23: 32-6. [CrossRef]
13. Nandi S, Brown AC. Characterizing cell migration within three-dimensional in vitro wound environments. J Vis Exp 2017; 126: doi: 10.3791/56099. [CrossRef]
14. Dorsett-Martin WA. Rat models of skin wound healing: a review. Wound Repair Regen 2004; 12: 591-9. [CrossRef]
15. Sullivan TP, Eaglstein WH, Davis SC, Mertz P. The pig as a model for human wound healing. Wound Repair Regen 2001; 9: 66-76. [CrossRef]
16. Wong VW, Sorkin M, Glotzbach JP, Longaker MT, Gurtner GC. Surgical approaches to create murine models of human wound healing. J Biomed Biotechnol 2011; doi: 10.1155/2011/969618. [CrossRef]
17. Reid RR, Said HK, Mogford JE, Mustoe TA. The future of wound healing: Pursuing surgical models in transgenic and knockout mice. J Am Coll Surg 2004; 199: 578-85. [CrossRef]
18. Diegelmann RF, Lindblad WJ Cohen IK. A subcutaneous implant for wound healing studies in humans. J Surg Res 1986; 40: 229-37. [CrossRef]
19. Kurkinen M, Vaheri A, Roberts PJ, Stenman S. Sequential appearance of fibronectin and collagen in experimental granulation tissue. Lab Med 1980; 43: 47-51. [CrossRef]
20. Sprugel KH, Mcpherson JM, Clowes AW, et al. Effects of growth factors in vivo: I. Cell ingrowth into porous subcutaneous chambers. Am J Pathol 1987; 129: 601-13.
21. Viljanto J. Cellstick: a device for wound healing studies in man. Description of the method. J Surg Res 1976; 20: 115-9. [CrossRef]
22. Diegelmann RF, Kim JC, Lindblad WJ, Smith TC, Harris TM, Cohen IK. Collection of leukocytes, fibroblasts, and collagen within an implantable reservoir tube during tissue repair. J Leukocyte Biol 1987; 42: 667-72. [CrossRef]
23. Schilling JA, Joel W, Shurby HM. Wound healing: a comparative study of the histochemical changes in granulation tissue contained steel wire mesh cylinders and polyvinyl sponges. Surgery 1959; 46: 702-10.
24. Hunt TK, Twomey P, Zedrefeldt B, Dunphy JE. Respiratory gas tensions and pH in healing wounds. Am J Surg 1967; 114: 302-7. [CrossRef]
25. Wong VW, Beasley B, Zepeda J, Dauskardt RH, Yock PG, Longaker MT, et al. A Mechanomodulatory Device to Minimize Incisional Scar Formation. Adv Wound Care (New Rochelle) 2013; 2: 185-94. [CrossRef]
26. Kilpadi DV, Lessing C, Derrick K. Healed porcine incisions previously treated with a surgical incision management system: mechanical, histomorphometric, and gene expression properties. Aesthetic Plast Surg 2014; 38: 767-78. [CrossRef]
27. Galiano RD, Michaels VJ, Dobryansky M, Levine JP, Gurtner GC. Quantitative and reproducible murine model of excisional wound healing. Wound Repair Regen 2004; 12: 485-92. [CrossRef]
28. Greenhalgh DG, Gamelli RL. Immunomodulators and wound healing. J Trauma 1987; 27: 510-4. [CrossRef]
29. Auerbach LJ, Galvez MG, De Clerck BK, Glotzbach J, Wehner MR, Chang EI, et al. A novel mouse model for frostbite injury. Wilderness Environ Med 2013; 24: 94-104. [CrossRef]
30. Levenson SM, Gruber CA, Rettura G, Gruber DK, Demetriou AA, Seifter E. Supplemental vitamin A prevents the acute radiation-induced defect in wound healing. Ann Surg 1984; 200: 494-512. [CrossRef]
31. Alvarez OM, Gilbreath RL. Thiamine influence on collagen during the granulation of skin wounds. J Surg Res 1982; 32: 24-31. [CrossRef]
32. DeHaan BB, Ellis H, Wilks M. The role of infection on wound healing. Surg Gynecol Obstet 1974; 138: 693-700.
33. Levenson SM, Kan-Gruber D, Gruber C, Molnar J, Seifter E. Wound healing accelerated by Staphylococcus aureus. Arch Surg 1983; 118: 310-20. [CrossRef]
34. Traeger T, Koerner P, Kessler W, Cziupka K, Diedrich S, Busemann A, et al. Colon Ascendens Stent Peritonitis (CASP) - a Standardized Model for Polymicrobial Abdominal Sepsis. J Vis Exp 2010; 46: doi: 10.3791/2299. [CrossRef]
35. Buras JA, Holzmann B, Sitkovsky M. Animal Models of sepsis: setting the stage. Nat Rev Drug Discov 2005; 4: 854-65. [CrossRef]
36. Corral CJ, Siddiqui A, Wu L, Farrell CL, Lyons D, Mustoe TA. Vascular endothelial growth factor is more important than basic fibroblastic growth factor during ischemic wound healing. Arch Surg 1999; 134: 200-5. [CrossRef]
37. Serin ve Bayramiçli M. Experimental Rat Flap Models 2018; Available from: https://www.intechopen.com/books/issues-in-flap-surgery/experimental-rat-flap-models [CrossRef]
38. Greenhalgh DG. Wound healing and diabetes mellitus. Clin Plastic Surg 2003; 30: 37-45. [CrossRef]
39. Brown RL, Breeden MP, Greenhalgh DG. PDGF and TGF-alpha act synergistically to improve wound healing in the genetically diabetic mouse. J Surg Res 1994; 56: 562-70. [CrossRef]
40. Tsuboi R, Rifkin DB. Recombinant basic fibroblast growth factor stimulates wound healing in healing-impaired db/db mice. J Exp Med 1990; 172: 245-51. [CrossRef]
41. Goodson WH III, Hunt TK. Wound collagen accumulation in obese hyperglycemic mice. Diabetes 1986; 35: 491-5. [CrossRef]
42. Rerup CC. Drugs producing diabetes through damage of insulin secreting cells. Pharmacol Rev 1970; 22: 485-518.
43. Daniel RK, Wheatley DC, Priest DL. Pressure sores and paraplegia: an experimental model. Ann Plast Surg 1985; 15: 41-9. [CrossRef]
44. Hyodo A, Reger SI, Negami S, Kambic H, Reyes E, Browne EZ. Evaluation of a pressure sore model using monoplegic pigs. Plast Reconstr Surg 1995; 96: 421-8. [CrossRef]
45. Peirce SM, Skalak TC, Rodeheaver GT. Ischemia reperfusion injury in chronic pressure ulcer formation: A skin model in the rat. Wound Rep Reg 2000; 8: 68-76. [CrossRef]
46. Reid RR, Sull AC, Mogford JE, Roy N, Mustoe TA. A novel murine model of cyclical cutaneous ischemia-reperfusion injury. J Surg Res 2004; 116: 172-80. [CrossRef]
47. Shaheen A. Comprehensive review of keloid formation. Clin Res Dermatol 2017; 4: 1-18. [CrossRef]
48. Van den Broek LJ, Limandjaja GC, Niessen FB, Gibbs S. Human hypertrophic and keloid scar models: principles, limitations and

future challenges from a tissue engineering perspective. Exp Dermatol 2014; 23: 382-6. [CrossRef]
49. Laato M, Heino J, Kahari VM, Niinikoski J, Gerdin B. Epidermal growth factor (EGF) prevents methylprednisolone-induced inhibition of wound healing. J Surg Res 1989; 47: 354-9. [CrossRef]
50. Reinisch JF, Puckett CL. Management of radiation wounds. Surg Clin N Am 1984; 64: 795-802. [CrossRef]
51. Somasundaram K, Prathrap K. Intra-uterine healing of skin wounds in rabbit foetuses. J Pathol 1970; 100: 81-6. [CrossRef]
52. Whitby DJ, Ferguson MW. The extracellular matrix of lip wounds in fetal, neonatal and adult mice. Development 1991; 112: 651-68.
53. Longaker MT, Chiu ES, Adzick NS, Stern M, Harrison MR, Stern R. Studies in fetal wound healing. V. A prolonged presence of hyaluronic acid characterizes fetal wound fluid. Ann Surg 1991; 213: 292-6. [CrossRef]
54. Lorenz HP, Longaker MT, Perkocha LA, Jennings RW, Harrison MR, Adzick NS. Scarless wound repair: a human fetal skin model. Development 1992; 114: 253-9.
55. Moore AL, Marshall CD, Barnes LA, Murphy MP, Ransom RC. Longaker MTScarless wound healing: Transitioning from fetal research to regenerative healing. Wiley Interdiscip Rev Dev Biol 2018; 7: doi: 10.1002/wdev.309. [CrossRef]
56. Wong JW, Gallant-Behm C, Wiebe C, Mak K, Hart DA, Larjava H, et al. Wound healing in oral mucosa results in reduced scar formation as compared with skin: Evidence from the red Duroc pig model and humans. Wound Repair Regen 2009; 17: 717-29. [CrossRef]
57. Midwood KS, Chiquet M, Tucker RP, Orend G. Tenascin-C at a glance. J Cell Sci 2016; 129: 4321-7. [CrossRef]
58. Han G, Ceilley R. Chronic wound healing: a review of current management and treatments. Adv Ther 2017; 34: 599-610. [CrossRef]

Experimed 2019; 9(3): 138-42

REVIEW
DERLEME

DOI: 10.26650/experimed.2019.19025

The Effects of Rifampicin on Neuronal Survival

Rifampisinin Nöronal Sağkalım Üzerine Etkileri

İlknur Yurtsever[1], Ebru Emekli Alturfan[2]

[1]Regenerative and Restorative Medicine Research Center, İstanbul Medipol University, İstanbul, Turkey
[2]Department of Biochemistry, Marmara University School of Dentistry, İstanbul, Turkey

Cite this article as: Yurtsever İ, Emekli Alturfan E. The Effects of Rifampicin on Neuronal Survival. Experimed 2019; 9(3): 138-42.

ABSTRACT

Neurodegenerative diseases are characterized by the formation of insoluble aggregates of misfolded proteins in the central nervous system. The β-amyloid protein in Alzheimer's disease and α-synuclein formation in Parkinson's disease (PD) may be given as examples. In addition to α-synuclein accumulation in Parkinson's disease, mechanisms such as oxidative stress, dysfunction of mitochondria, inflammation response, and apoptosis are known to be involved in the disease process. Since the mechanisms underlying these diseases are partially known, the drugs developed are intended to slow the disease process rather than cure them. Rifampicin is an antibiotic commonly used in humans and known to easily penetrate into the brain after oral intake. Studies have shown that rifampicin suppresses mitochondrial oxidative stress, eliminates α-synuclein fibrils and inhibits inflammation in *in vitro* and *in vivo* disease models. In this study, we reviewed recent studies on the neuronal protection of rifampicin and the effects of rifampicin on the pathophysiological mechanisms of PD.

Keywords: Parkinson's disease, rifampicin, α-Synuclein, SUMOylation, inflammation, autophagy

ÖZ

Nörodejeneratif hastalıklar, merkezi sinir siteminde yanlış katlanmış proteinlerin çözünmeyen agregatlarının oluşumu ile karakterizedir. Bunlara örnek olarak; Alzheimer hastalığında β-amyloid protein ve Parkinson hastalığında α-sinüklein oluşumu verilebilir. Parkinson hastalığında α-sinüklein agregasyonuna ek olarak, oksidatif stress, mitokondri fonksiyon bozukluğu, inflamatuvar cevap, apoptoz gibi mekanizmaların hastalık sürecine katıldığı bilinmektedir. Bu hastalıkların altında yatan mekanizmalar tam olarak bilinmediği için, hastalığa ilişkin geliştirilen ilaçlar, hastalığı iyileştirmekten çok, hastalığın seyrini yavaşlatma eğilimindedir. Rifampisin insanlar tarafından sıkça kullanılan bir antibiyotiktir ve ağız yoluyla alındıktan sonra beyne kolaylıkla penetre olmaktadır. Rifampisinin *in vivo* ve *in vitro* hastalık modellerinde mitokondriyal oksidatif stresi baskıladığı, α-sinüklein fibrillerini ayrıştırdığı, inflamasyonu inhibe ettiğini gösteren çok sayıda çalışma mevcuttur. Biz bu çalışmada, rifampisinin nöronal korunumu üzerine raporlanan çalışmaları ve Parkinson hastalığı'nın patofizyolojik mekanizmaları üzerine rifampisinin etkilerini derledik.

Anahtar Kelimeler: Parkinson hastalığı, rifampisin, α-Sinüklein, SUMOlasyon, inflamasyon, otofaji

INTRODUCTION

Although rifampicin is a widely used antibiotic in the treatment of tuberculosis and leprosy, studies have increasingly shown that rifampicin has therapeutic benefits for acute brain injury and chronic neurodegenerative diseases (1-5). Among the neurodegenerative diseases, Parkinson's disease (PD) and Alzheimer's disease are the two major diseases where the therapeutic benefits of rifampicin have been shown. PD is the second most common neurodegeneration disease in the world and is caused by the loss of dopaminergic neurons in the substantia nigra compacta region of the brain. Many pathological mechanisms proposed for PD include mitochondrial dysfunction, increased oxidative stress, protein misfolding-aggregation, apoptosis, inflammatory response and glutaminergic excitotoxicity, and nitrosative stress (6). There are studies showing that rifampicin significantly increases the viability of neurons in in vitro models of PD (7). However, rifampicin inhibited apoptosis in neurons by activating glucose-regulated protein 78 (GRP78), an endoplasmic reticulum stress marker, and inhibiting the expression of α-synuclein multimers (8, 9). There are also studies showing that rifampicin has the ability to suppress inflammation by inhibiting the nuclear transfer of Nf-kB and the release of IL-1β, TNF-α and other inflammatory factors in microglia (10). Accordingly, rifampicin has been shown to protect neurons by different mechanisms including its effects on oxidative stress, autophagy, and mitophagy, α-Synuclein aggregation and SUMOylation (Figure 1). Here, we have reviewed recent studies on the effects of rifampicin on the mechanisms involved in the pathophysiology of PD.

Corresponding Author/Sorumlu Yazar: İlknur Yurtsever **E-mail:** iyurtsever@medipol.edu.tr
Received Date/Geliş Tarihi: 24.10.2019 **Revision Date/Revizyon Tarihi:** 30.10.2019 **Accepted Date/Kabul Tarihi:** 11.11.2019

Experimed 2019; 9(3): 138-42

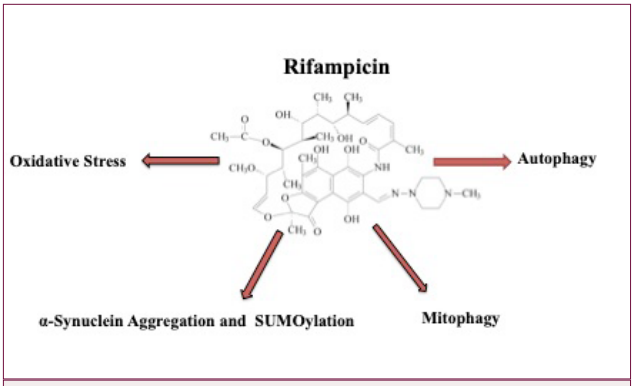


Figure 1. Rifampicin has been shown to protect neurons by different mechanisms including its effects on oxidative stress, autophagy, and mitophagy, α-Synuclein aggregation and SUMOylation

Rifampicin and Oxidative Stress

Researchers have shown that mitochondrial dysregulation plays an important pathological role in dopamine loss in various PD models (11, 12). It is known that PD-causing chemicals, such as rotenone and MPTP, inhibit mitochondrial complex I in dopaminergic neurons, reduce ATP production, and cause increased reactive oxygen species (ROS), as well as oxidative stress (13-16). Mitochondrial dysfunction can result from both increased damage and a reduced ability to repair or clear damaged mitochondria (17).

One of the common ways described in experimental models of neurodegenerative diseases is oxidative/nitrosative stress (OS/NS). This event triggers a series of harmful actions involving the primary formation of reactive oxygen and nitrogen species (ROS/RNS), affecting the structure and function of different biological molecules, leading to specific toxic processes that endanger cell redox status (18, 19). Mitochondria have been shown to be the main source of ROS and responsible for oxidative stress-induced cell death in neurodegeneration (20, 21).

Being the first-line antituberculosis, rifampicin is recommended by the World Health Organization (WHO). However, hepatotoxicity which is the main limiting factor for eliminating the clinical use of rifampicin is accepted to be the major side effect in the treatment of tuberculosis. Although rifampin alone has low hepatotoxicity it may show additive/synergistic hepatotoxicity when it is used with isoniazid during the treatment of tuberculosis (22). Accordingly, rifampicin has been shown to cause liver damage through the induction of cholestasis, with a serious increase in serum bilirubin levels. As oxidative stress is sometimes related to cholestasis and suggested to have a role in the endocytosis of envelope proteins; rifampicin has also been related to oxidative stress in the liver (23). Moreover, Xu et al. showed that rifampicin decreased multidrug resistance-associated protein 2 levels through the induction of oxidative stress in HepG2 cells (23).

In contrast, rifampicin has been shown to act differently in the neuronal system. It has been shown that rifampicin-treated animals had decreased oxidative stress in the nigrostriatal dopaminergic neuronal pathways and provided neuronal protection (24). As rifampicin has been reported to reduce ROS release and secondary brain injury in *Streptococcus pneumoniae meningitis,* it can be a potential treatment for the disease (25). In *in vitro* studies, rifampicin pretreatment protected PC12 cells against rotenone-induced cell death and inhibited the formation of α-synuclein multimers as well. Qualitative and quantitative analyses showed that rifampicin significantly prevented rotenone-induced apoptosis by relieving mitochondrial oxidative stress (26). As the oxidative process changes mitochondrial respiration and leads to changes in the permeability of the transition pores in the mitochondria of the brain; prevention of oxidative stress by rifampicin may have important functions for the development of new treatment strategies.

Rifampicin and Autophagy

Mitochondrial dysfunction is related very closely with the pathogenesis of PD. It may be the result of impaired mitochondrial biogenesis, high ROS formation, impaired mitophagy and electron transport chain dysfunction as well as alterations in the dynamics of mitochondrial functions and calcium homeostasis. Neuroinflammation is a mitochondrial and autophagic dysfunction, associated with the pathophysiology of PD. In order to remove damaged proteins and the mitochondria, a very well regulated lysosomal-mediated autophagy pathway is needed. This autophagy pathway integrates various signals including nutrient availability, cellular stress and oxidized proteins and lipids (27).

Rifampicin inhibits the formation of α-synuclein multimers and neuronal apoptosis by the activation of GRP78 via the PERK-eIF2a-ATF4 pathway (8, 9). Furthermore, rifampicin administration is useful for lipopolysaccharide (LPS) -stimulated microglia-damaged neurons by suppressing the nuclear factor kappa B activation, phosphorylation of MAPKs and the toll-like receptor-4 (TLR-4) pathway (10, 28).

Studies have shown that inhibition of autophagy, in particular, mitophagy, leads to a reduced degradation of damaged mitochondria and an increased production of ROS (29). Previous studies have shown that pre-administration of rifampicin inhibits neuroinflammation by the suppression of 26S protease regulatory subunit 7 (MSS1). Thus, the production of inducible nitric oxide synthase, TNF-α and IL-1β are reduced (30). Chloroquine treatment, an autophagy inhibitor, inhibits the effect of rifampicin pretreatment on rotenone-stimulated IL-1β and IL-6 secretion. This indicates that rifampicin inhibits inflammation by modulating autophagy (31). In recent studies, various effects of rotenone have been shown on autophagy depending on the different dosages used and the cellular systems analyzed. In one study, rotenone reduced all autophagy but increased mitophagy in neurons (32), while in another study it was shown that rotenone increased autophagy and stimulated ROS induced autophagic cell death (33).

Removal of damaged mitochondria is necessary to protect cells from ROS and pro-apoptotic molecules released by dysfunctional mitochondria. In a study, it was found that pre-treatment of rifampicin partially reduced mitochondrial membrane potential (MMP) induced by rotenone and partially reversed ROS production. Moreover, the protective effect of rifampicin on mitochondrial function is suppressed after the addition of the autophagy inhibitor. These results convince us that rifampicin leads to a reduction in ROS production through a tendency towards mitophagy (31).

Rifampicin, α-Synuclein Aggregation, and SUMOylation

α-Synuclein is the main protein component of Lewy Body in PD brains (34, 35) and is a protein of 140 amino acids. α-Synuclein aggregation is a critical step in the pathogenesis of PD. There is a strong association between α-synuclein upregulation and increased cytotoxicity and neurodegeneration (36). The aggregation of α-synuclein has been suggested to be one of the mechanisms linking mitochondrial dysfunction, another important pathway leading to PD pathogenesis (37).

Pathological forms of α-synuclein, which are spread in the parenchymal tissue of the brain, can contribute to the disease process by stimulating inflammatory-type reactions through microglial cells (38). In one study, by using pure microglial cell culture, the inflammatory potential of three different forms of α-synuclein was tested and TNF-α and IL-6 release as inflammation markers were examined (39). As a result, a fibril form of α-synuclein was found to be the most inflammatory form of protein. This result shows us that the inflammatory potential of α-synuclein is dependent on the aggregation state of the protein.

In another study, α-synuclein fibrils were reported to activate the THP-1 monocyte cell line and activate the release of IL-1β by TLR-2 and NLRP3 activation (40). In another study, α-synuclein fibrils in microglial BV-2 cell culture were shown to be more effective in increasing the production and release of proinflammatory cytokines than in monomeric and oligomeric species (41). In previous studies, it has been shown that rifampicin can increase neuronal survival by inhibiting the inflammatory process induced by LPS-activated microglial cells (10, 28). Recent studies have shown that posttranslational modification by the small ubiquitin-like modifier called SUMOylation regulates mitochondrial dynamics. This mechanism is accepted to be one of the underlying mechanisms of PD (42, 43). Some of the proteins encoded by the genes involved in genetic changes in PD are regulated by SUMO.

Covalent binding of SUMO protein to the lysine residue of the target protein is an important control process in eukaryotic cells and regulates the function of hundreds of proteins in many different pathways. SUMOylation causes different results depending on the pathway in which it is located, but the basic principle is to change the interactions between substrate proteins for the molecule and between molecules (protein or DNA). Thus, it regulates the activities, localization, and stability of the substrates (44, 45). SUMO also affects cytoplasmic and membrane proteins, including ion channels and receptors (46-48), so that SUMOylation not only acts in the nucleus but also in different cellular processes including cell signaling, plasma membrane depolarization and signal transduction (45, 49).

SUMOylation of proteins has been shown to play an important role in synaptic transmission, plasticity and neuron conservation (50). Decreasing solubility and pathological accumulation of specific disease-related proteins such as α-synuclein is a common feature among neurodegenerative diseases such as PD. Cytoplasmic filamentous inclusions, whose main component is α-synuclein (51), are abundant in the neurons of PD patients that exhibit other important pathological features. In PD brains and Lewy Body disease (LB) with dementia, SUMO-1 is located in the outer part of LB, which is colocalized by α-synuclein (52). α-synuclein is a SUMO target and SUMOylation occurs on 2 lysine residues K96 and K102 on the protein, this was confirmed by transgenic mice expressing His6-tagged SUMO-2 (53). Reduced α-synuclein SUMOylation by mutation of SUMO-modified lysines, has been found to increase α-synuclein aggregation and toxicity in heterogeneous cells and in dopaminergic neurons of substantial nigra in PD rat models (53).

In a different study, the relationship between rifampicin and SUMOlation was evaluated. It was reported that rotenone-stimulated PC12 cells were prevented from increased apoptosis by increasing the SUMOylation of α-synuclein by pretreatment of rifampicin. In this study, pretreatment of rifampicin caused an early increase in SUMOylation, thereby increasing the solubility of α-synuclein, aggregates-prone neurodegeneration-related proteins. Subsequent treatment of rotenone-stimulated cells with rifampicin resulted in less formation of damaged and misfolded α-synucleins. However, the late generation of SUMOylation in cells has been found to cause a more difficulties in the reversal of toxicity from α-synuclein accumulation (54).

Rifampicin (Rif) and its Oxidated Product (RifQ)

Although many studies have shown the anti-inflammatory effects of rifampicin, there have been only a few studies performed using the oxidative product of rifampicin. It was found that when rifampicin was dissolved in aqueous solution, as a result of the spontaneous oxidation reaction, different oxidized species are produced - such as the rifampicin quinone (RifQ). This molecule differs from rifampicin in that the naphthyl core structure is converted into a naphthoquinone (Figure 1). This confers to the molecule's distinctive biochemical properties.

Rifampicin is defined as a potential immunosuppressive agent in rats, but these effects were obtained only with stocked solutions of the antibiotic, not with freshly prepared solutions. Therefore, the anti-inflammatory effects of rifampicin are attributed to the oxidant product RifQ of rifampicin (55). In addition, other studies have indicated that the oxidation product of rifampicin inhibits α-synuclein fibrillation and strengthens the disaggregation of formed fibrils (56).

Rifampicin and its oxidized derivative, RifQ, have been shown to inhibit the activation of primary microglial cells induced by α-synuclein fibrils, which are inflammatory factors in PD (39, 57). RifQ has been shown to have the potential to inhibit neurotoxic effects induced by microglial cells activated by α-synuclein fibrils.

CONCLUSION

Numerous *in vivo* and *in vitro* studies suggest that rifampicin may have therapeutic effects in PD treatment. These results suggest that rifampicin may slow down the process by reducing oxidative stress, inhibiting inflammation, inhibiting the formation of α-synuclein aggregates, and separating the resulting aggregates and providing neuronal protection. Thus, rifampicin may be a novel method of therapy in the treatment of PD and may be used in the treatment of neurodegenerative diseases which have similar mechanisms.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - İ.Y., E.E.A.; Supervision - İ.Y., E.E.A.; Materials - İ.Y., E.E.A.; Data Collection and/or Processing - İ.Y., E.E.A.; Analysis and/or Interpretation - İ.Y., E.E.A.; Literature Search - İ.Y., E.E.A.; Writing - İ.Y., E.E.A.; Critical Reviews - İ.Y., E.E.A.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir - İ.Y., E.E.A.; Denetleme - İ.Y., E.E.A.; Gereçler - İ.Y., E.E.A.; Veri Toplanması ve/veya İşlemesi - İ.Y., E.E.A.; Analiz ve/veya Yorum - İ.Y., E.E.A.; Literatür Taraması - İ.Y., E.E.A.; Yazan - İ.Y., E.E.A.; Eleştirel İnceleme - İ.Y., E.E.A.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışmada finansal destek almadıklarını beyan etmişlerdir.

REFERENCES

1. Ambrosi G, Cerri S, Blandini F. A further update on the role of excitotoxicity in the pathogenesis of Parkinson's disease. J Neural Transm 2014; 121: 849-59. [CrossRef]
2. Giráldez-Pérez R, Antolín-Vallespín M, Muñoz M, Sánchez-Capelo A. Models of α-synuclein aggregation in Parkinson's disease. Acta Neuropathol Commun 2014; 2: 176. doi: 10.1186/s40478-014-0176-9. [CrossRef]
3. Blesa J, Trigo-Damas I, Quiroga-Varela A and Jackson-Lewis V R. Oxidative stress and Parkinson's disease. Front Neuroanat 2015; 9: https://doi.org/10.3389/fnana.2015.00091 [CrossRef]
4. Franco-Iborra S, Vila M, Perier C. The Parkinson disease mitochondrial hypothesis: where are we at? Neuroscientist 2016; 22: 266-77. [CrossRef]
5. Vivekanantham S, Shah S, Dewji R, Dewji A, Khatri C, Ologunde R. Neuroinflammation in Parkinson's disease: role in neurodegeneration and tissue repair. Int J Neurosci 2015; 125: 717-25. [CrossRef]
6. Guerra de Souza AC, Prediger RD, Cimarosti H. SUMO-regulated mitochondrial function in Parkinson's disease. J Neurochem 2016; 137: 673-86. [CrossRef]
7. Bi W, Zhu L, Jing X, Zeng Z, Liang Y, Xu A, et al. Rifampicin improves neuronal apoptosis in LPS-stimulated co-cultured BV2 cells through inhibition of the TLR-4 pathway. Mol Med Rep 2014; 10: 1793-9. [CrossRef]
8. Jing X, Shi Q, Bi W, Zeng Z, Liang Y, Wu X, et al. Rifampicin protects PC12 cells from rotenone-induced cytotoxicity by activating GRP78 via PERK-eIF2alpha-ATF4 pathway. PLoS One 2014; 9: doi: 10.1371/journal.pone.0092110.[CrossRef]
9. Xu J, Wei C, Xu C, Bennett M C, Zhang G, Li F, et al. Rifampicin protects PC12 cells against MPP+-induced apoptosis and inhibits the expression of an alpha-synuclein multimer. Brain Res 2007; 1139: 220-5. [CrossRef]
10. Bi W, Zhu L, Wang C, Liang Y, Liu J, Shi Q, et al. Rifampicin inhibits microglial inflammation and improves neuron survival against inflammation. Brain Res 2011; 1395: 12-20. [CrossRef]
11. Greenamyre JT, Betarbet R, Sherer TB. The rotenone model of Parkinson's disease: genes, environment, and mitochondria. Parkinsonism Relat Disord 2003; 9 (Suppl 2): S59-S64. [CrossRef]
12. Testa CM, Sherer TB, Greenamyre JT. Rotenone induces oxidative stress and dopaminergic neuron damage in organotypic substantia nigra cultures. Brain Res Mol Brain Res 2005; 134: 220-5. [CrossRef]
13. Watanabe Y, Himeda T, Araki T. Mechanisms of MPTP toxicity and their implications for therapy of Parkinson's disease. Med Sci Monit 2005; 11: 17-23.
14. Sherer TB, Betarbet R, Stout AK, Lund S, Baptista M, Panov AV, Cookson MR, Greenamyre JT. An in vitro model of Parkinson's disease: linking mitochondrial impairment to altered alpha-synuclein metabolism and oxidative damage. J Neurosci 2002; 22: 7006-15. [CrossRef]
15. Starkov AA. The role of mitochondria in reactive oxygen species metabolism and signaling. Ann N Y Acad Sci 2008; 1147: 37-52. [CrossRef]
16. Tretter L, Sipos I, Adam-Vizi V. Initiation of neuronal damage by complex I deficiency and oxidative stress in Parkinson's disease. Neurochem Res 2004; 29: 569-77. [CrossRef]
17. Zhu J, Chu CT. Mitochondrial dysfunction in Parkinson's disease. J Alzheimers Dis 2010; 20 (Suppl 2): S325-S34. [CrossRef]
18. Tobo'n-Velasco JC, Carmona-Aparicio L, Ali SF, Santamarı'a A. Biomarkers of cell damage induced by oxidative stress in Parkinson's disease and related models. Cent Nerv Syst Agents Med Chem 2010; 10: 278-86. [CrossRef]
19. Fariss MW, Chan CB, Patel M, Van Houten B, Orrenius S. Role of mitochondria in toxic oxidative stress. Mol Interv 2005; 5: 94-111. [CrossRef]
20. Beal MF. Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? Ann Neurol 1992; 31: 119-30. [CrossRef]
21. Adams JD Jr, Chang ML, Klaidman L. Parkinson's disease-redox mechanisms. Curr Med Chem 2001; 8: 809-14. [CrossRef]
22. Steele MA, Burk RF, DesPrez RM. Toxic hepatitis with isoniazid and rifampin. A meta-analysis. Chest 1991; 99: 465-71. [CrossRef]
23. Xu B, Tang X, Chen J, Wu H, Chen W, Chen L. Rifampicin induces clathrin-dependent endocytosis and ubiquitin-proteasome degradation of MRP2 via oxidative stress-activated PKC-ERK/JNK/p38 and PI3K signaling pathways in HepG2 cells. Acta Pharmacol Sin 2019; doi: 10.1038/s41401-019-0266-0. [CrossRef]

24. Oida Y, Kitaichi K, Nakayama H, Ito Y, Fujimoto Y, Shimazawa M, Nagai H, Hara H. Rifampicin attenuates the MPTP-induced neurotoxicity in mouse brain. Brain Res 2006; 1082: 196- 204. [CrossRef]
25. Bo¨ttcher T, Gerber J, Wellmer A, Smirnov AV, Fakhrjanali F, Mix E, et al. Rifampin reduces production of reactive oxygen species of cerebrospinal fluid phagocytes and hippocampal neuronal apoptosis in experimental Streptococcus pneumoniae meningitis. J Infect Dis 2000; 181: 2095-8. [CrossRef]
26. Chen S, Sun Y, Zeng Z, Tao E. Rifampicin inhibits apoptosis in rotenone-induced differentiated PC12 cells by ameliorating mitochondrial oxidative stress. Neural Regen Res 2010; 5: 251-6.
27. Zhang J, Culp ML, Craver JG, Darley-Usmar V. Mitochondrial function and autophagy: integrating proteotoxic, redox, and metabolic stress in Parkinson's disease. J Neurochem. 2018; 144: 691-709. [CrossRef]
28. Bi W, Zhu L, Jing X, Zeng Z, Liang Y, Xu A, et al. Rifampicin improves neuronal apoptosis in LPS-stimulated cocultured BV2 cells through inhibition of the TLR-4 pathway. Mol Med Rep 2014; 10: 1793-9. [CrossRef]
29. Yang S, Xia C, Li S, Du L, Zhang L, Zhou R. Defective mitophagy driven by dysregulation of rheb and KIF5B contributes to mitochondrial reactive oxygen species (ROS)-induced nod-like receptor 3 (NLRP3) dependent proinflammatory response and aggravates lipotoxicity. Redox Biol 2014; 3: 63-71. [CrossRef]
30. Bi W, Jing X, Zhu L, Liang Y, Liu J, Yang L, et al. Inhibition of 26S protease regulatory subunit 7 (MSS1) suppresses neuroinflammation. PLoS One 2012; 7: doi: 10.1371/journal.pone.0036142. [CrossRef]
31. Liang Y, Zhou T, Chen Y, Lin D, Jing X, Peng S, et al. Rifampicin inhibits rotenone-induced microglial inflammation via enhancement of autophagy. Neurotoxicology 2017; 63: 137-45. [CrossRef]
32. Giordano S, Dodson M, Ravi S, Redmann M, Ouyang X, Darley Usmar VM, et al. Bioenergetic adaptation in response to autophagy regulators during rotenone exposure. J Neurochem 2014; 131: 625-33. [CrossRef]
33. Chen Y, McMillan-Ward E, Kong J, Israels SJ, Gibson SB. Mitochondrial electron-transport-chain inhibitors of complexes I and II induce autophagic cell death mediated by reactive oxygen species. J Cell Sci 2007; 120: 4155-66. [CrossRef]
34. Forno LS. Neuropathology of Parkinson's disease. J Neuropathol Exp Neurol 1996; 55: 259-72. [CrossRef]
35. Martin FL, Williamson SJ, Paleologou KE, Allsop D, El-Agnaf OM. Alpha-synuclein and the pathogenesis of Parkinson's disease. Protein Pept Lett 2004; 11: 229-37. [CrossRef]
36. Bennett MC. The role of alpha-synuclein in neurodegenerative diseases. Pharmacol Ther 2005; 105: 311-31. [CrossRef]
37. Lee SJ. alpha-Synuclein aggregation: a link between mitochondrial defects and Parkinson's disease? Antioxid Redox Signal 2003; 3: 337-48. [CrossRef]
38. Couch Y, Alvarez-Erviti L, Sibson NR, Wood MJA, Anthony DC. The acute inflammatory response to intranigral-synuclein differs significantly from intranigral lipopolysaccharide and is exacerbated by peripheral inflammation. J Neuroinflamm 2011; 8: doi: 10.1186/1742-2094-8-166. [CrossRef]
39. Acuña L, Hamadat S, Corbalán NS, González-Lizárraga F, Dos-Santos-Pereira M, Rocca J, et al. Rifampicin and Its Derivative Rifampicin Quinone Reduce Microglial Inflammatory Responses and Neurodegeneration Induced In Vitro by α-Synuclein Fibrillary Aggregates. Cells 2019; 8: doi: 10.3390/cells8080776. [CrossRef]
40. Gustot A, Gallea, JI, Sarroukh R, Celej MS, Ruysschaert J-M, Raussens V. Amyloid fibrils are the molecular trigger of inflammation in Parkinson's disease. Biochem J 2015; 471: 323-33. [CrossRef]
41. Hoffmann A, Ettle B, Bruno A, Kulinich A, Hoffmann AC, von Wittgenstein J, et al. Alpha-synuclein activates BV2 microglia dependent on its aggregation state. Biochem Biophys Res Commun 2016; 479: 881-6. [CrossRef]
42. Gareau JR, Lima CD. The SUMO pathway: emerging mechanisms that shape specificity, conjugation, and recognition. Nat Rev Mol Cell Biol 2010; 11: 861-71. [CrossRef]
43. Eckermann K. SUMO and Parkinson's Disease. NeuroMolecular Med 2013; 15: 737-59. [CrossRef]
44. Dohmen RJ. SUMO protein modification. Biochim. Biophys. Acta 2004; 1695, 113-131. [CrossRef]
45. Hay RT. SUMO: a history of modification. Mol Cell 2005; 18: 1-12. [CrossRef]
46. Silveirinha V, Stephens GJ, Cimarosti H. Molecular targets underlying SUMO-mediated neuroprotection in brain ischemia. J Neurochem 2013; 127: 580-91. [CrossRef]
47. Wilkinson KA, Nakamura Y, Henley JM. Targets and consequences of protein SUMOylation in neurons. Brain Res Rev 2010; 64: 195-212. [CrossRef]
48. Luo J, Ashikaga E, Rubin PP, Heimann MJ, Hildick KL, Bishop P, et al. Receptor trafficking and the regulation of synaptic plasticity by SUMO. Neuromolecular Med 2013; 15: 692-706. [CrossRef]
49. Anckar J, Sistonen L. SUMO: getting it on. Biochem Soc Trans 2007; 35: 1409-13. [CrossRef]
50. Henley JM, Craig TJ and Wilkinson KA. Neuronal SUMOylation: mechanisms, physiology, and roles in neuronal dysfunction. Physiol Rev 2014; 94: 1249-85. [CrossRef]
51. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature 1997; 388: 839-40. [CrossRef]
52. Kim YM, Jang WH, Quezado MM, Oh Y, Chung KC, Junn E, et al. Proteasome inhibition induces α-synuclein SUMOylation and aggregate formation. J Neurol Sci 2011; 307: 157-61. [CrossRef]
53. Krumova P, Meulmeester E, Garrido M, Tirard M, Hsiao HH, Bossis G, et al. Sumoylation inhibits a-synuclein aggregation and toxicity. J Cell Biol 2011; 194: 49-60. [CrossRef]
54. Lin D, Jing X, Chen Y, Liang Y, Lei M, Peng S, et al. Rifampicin pre-treatment inhibits the toxicity of rotenone-induced PC12 cells by enhancing sumoylation modification of α-synuclein. Biochem Biophys Res Commun 2017; 485: 23-9. [CrossRef]
55. Konrad P, Stenberg P. Rifampicin quinone is an immunosuppressant, but not rifampicin itself. Clin Immunol Immunopathol 1988; 46: 162-6. [CrossRef]
56. Li J, Zhu M, Rajamani S, Uversky VN, Fink AL. Rifampicin inhibits alpha-synuclein fibrillation and disaggregates fibrils. Chem Biol 2004; 11: 1513-21. [CrossRef]
57. Bi W, Zhu L, Jing X, Liang Y, Tao E. Rifampicin and Parkinson's disease. Neurol Sci 2013; 34: 137-41. [CrossRef]

REVIEWER LIST 2019

A. Ata Alturfan

Afşin Kariper

Ali Osman Gürol

Arzu Ergen

Atike Tekeli

Aslı Uğurlu

Ayten Kandilci

Cevdet Özdemir

Elif Özkök

Elif Sinem İplik

Elif Şahin

Emel Ergül

Emrah Yücesan

Evrim Bayrak Kömürcü

Ezel Uslu

Feyza Tuncer Kılınç

Gülşen Altınkanat Gelmez

İsmail Öğülür

Jülide Duymaz

Kamber Demir

Korkut Ulucan

Mehmet Demirci

Mehveş Poda

Merve Bilgin

Özlem Küçükhüseyin

Selçuk Daşdemir

Selçuk Sözer

Sema Bilgiç Gazioğlu

Sema Ekmekçi

Sibel Penpe Yentur

Sinem Özdemir

Zeynep Birsu Çinçin

EXPERIMED

AIMS AND SCOPE

Experimed is an international, scientific, open access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is the official online-only publication of İstanbul University Aziz Sancar Institute of Experimental Medicine and it is published triannually on April, August, and December. The publication languages of the journal are Turkish and English.

Experimed aims to contribute to the literature by publishing manuscripts at the highest scientific level on all fields of basic and clinical medical sciences. The journal publishes original articles, case reports, reviews, and letters to the editor that are prepared in accordance with ethical guidelines.

The scope of the journal includes but not limited to experimental studies in all fields of medical sciences.

The target audience of the journal includes specialists and professionals working and interested in all disciplines of basic and clinical medical sciences.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Processing and publication are free of charge with the journal. No fees are requested from the authors at any point throughout the evaluation and publication process. All manuscripts must be submitted via the online submission system, which is available at http://experimed.istanbul.edu.tr/en/_.The journal guidelines, technical information, and the required forms are available on the journal's web page.

All expenses of the journal are covered by the İstanbul University.

Statements or opinions expressed in the manuscripts published in the journal reflect the views of the author(s) and not the opinions of the İstanbul University Aziz Sancar Institute of Experimental Medicine, editors, editorial board, and/or publisher; the editors, editorial board, and publisher disclaim any responsibility or liability for such materials.

Editor in Chief: Prof. Bedia Çakmakoğlu
Address: İstanbul University, Aziz Sancar Institute of Experimental Medicine, Vakıf Gureba Avenue, 34093, Çapa, Fatih, İstanbul, Turkey
Phone: 0212-4142000-33305
Fax: 0212-5324171
E-mail: bedia@istanbul.edu.tr

Publisher: AVES
Address: Büyükdere Avenue, 105/9 34394 Mecidiyeköy, Şişli, İstanbul, Turkey
Phone: +90 212 217 17 00
Fax: +90 212 217 22 92
E-mail: info@avesyayincilik.com
Web page: avesyayincilik.com

EXPERIMED

AMAÇ VE KAPSAM

Experimed; İstanbul Üniversitesi Aziz Sancar Deneysel Araştırma Enstitüsü'nün çift-kör hakemli, elektronik, açık erişimli bilimsel yayın organıdır. Dergi Nisan, Ağustos ve Aralık aylarında olmak üzere, yılda 3 sayı olarak yayınlanır. Derginin yayın dili Türkçe ve İngilizce'dir.

Experimed, temel ve klinik tıp bilimlerinin tüm alanlarında orijinal araştırma, olgu sunumu, derleme ve editöre mektup türlerinde makaleler yayınladığı yüksek bilimsel standartlara sahip makalelerle literatüre katkı sunmaktadır.

Derginin hedef kitlesi, temel ve klinik tıbbi bilimler ile ilgilenen ve araştırma yapan tüm uzmanlar ve araştırmacılardır.

Derginin editöryel ve yayın süreçleri International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE) ve National Information Standards Organization (NISO) organizasyonlarının kılavuzlarına uygun olarak biçimlendirilir. Experimed, Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice) ilkelerini benimsemiştir.

Makale değerlendirme ve yayın işlemleri için yazarlardan ücret talep edilmemektedir. Tüm makaleler http://experimed.istanbul.edu.tr/tr/_ sayfasındaki online makale değerlendirme sistemi kullanılarak dergiye gönderilmelidir. Derginin yazım kurallarına, gerekli formlara ve dergiyle ilgili diğer bilgilere web sayfasından erişilebilir.

Derginin tüm masrafları İstanbul Üniversitesi tarafından karşılanmaktadır.

Dergide yayınlanan makalelerde ifade edilen bilgi, fikir ve görüşler İstanbul Üniversitesi Aziz Sancar Deneysel Tıp Araştırma Enstitüsü, Baş Editör, Editörler, Yayın Kurulu ve Yayıncı'nın değil, yazar(lar)ın bilgi ve görüşlerini yansıtır. Baş Editör, Editörler, Yayın Kurulu ve Yayıncı bu gibi yazarlara ait bilgi ve görüşler için hiçbir sorumluluk ya da yükümlülük kabul etmemektedir.

Experimed açık erişimli bilimsel bir dergi olup Budapeşte Açık Erişim Girişimi (BOAI) deklarasyonuna dayalı yayın modelini benimsemiştir. Derginin arşivine ücretsiz ve açık erişimli olarak http://experimed.istanbul.edu.tr/tr/_bağlantısından ulaşılabilir. Experimed'in içeriği Creative Commons Alıntı-GayriTicari 4.0 lisansı ile lisanlanmaktadır.

Baş Editör: Prof. Dr. Bedia Çakmakoğlu
Address: İstanbul Üniversitesi, Aziz Sancar Deneysel Tıp Araştırma Enstitüsü, Vakıf Gureba Caddesi, 34093, Çapa, Fatih, İstanbul, Türkiye
Phone: 0212-4142000-33305
Fax: 0212-5324171
E-mail: bedia@istanbul.edu.tr

Publisher: AVES
Address: Büyükdere Avenue, 105/9 34394 Mecidiyeköy, Şişli, İstanbul, Turkey
Phone: +90 212 217 17 00
Fax: +90 212 217 22 92
E-mail: info@avesyayincilik.com
Web page: avesyayincilik.com

EXPERIMED

INSTRUCTIONS TO AUTHORS

Context
Experimed is an international, scientific, open access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is the official online-only publication of İstanbul University Aziz Sancar Institute of Experimental Medicine and it is published triannually on April, August, and December. The publication languages of the journal are Turkish and English.

Experimed aims to contribute to the literature by publishing manuscripts at the highest scientific level on all fields of basic and clinical medical sciences. The journal publishes original articles, case reports, reviews, and letters to the editor that are prepared in accordance with ethical guidelines.

Editorial Policy
The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Council of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME), the Council of Science Editors (CSE), the Committee on Publication Ethics (COPE), the European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal conforms to the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Originality, high scientific quality, and citation potential are the most important criteria for a manuscript to be accepted for publication. Manuscripts submitted for evaluation should not have been previously presented or already published in an electronic or printed medium. The journal should be informed of manuscripts that have been submitted to another journal for evaluation and rejected for publication. The submission of previous reviewer reports will expedite the evaluation process. Manuscripts that have been presented in a meeting should be submitted with detailed information on the organization, including the name, date, and location of the organization.

Peer-Review Policy
Manuscripts submitted to Experimed will go through a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in their fields in order to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation processes of manuscripts submitted by editors or by the editorial board members of the journal. The Editor in Chief is the final authority in the decision-making process for all submissions.

Ethical Principles
An approval of research protocols by the Ethics Committee in accordance with international agreements (World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects," amended in October 2013, www.wma.net) is required for experimental, clinical, and drug studies and for some case reports. If required, ethics committee reports or an equivalent official document will be requested from the authors. For manuscripts concerning experimental research on humans, a statement should be included that shows that written informed consent of patients and volunteers was obtained following a detailed explanation of the procedures that they may undergo. For studies carried out on animals, the measures taken to prevent pain and suffering of the animals should be stated clearly. Information on patient consent, the name of the ethics committee, and the ethics committee approval number should also be stated in the Materials and Methods section of the manuscript. It is the authors' responsibility to carefully protect the patients' anonymity. For photographs that may reveal the identity of the patients, signed releases of the patient or of their legal representative should be enclosed.

Plagiarism
Experimed is extremely sensitive about plagiarism. All submissions are screened by a similarity detection software (iThenticate by CrossCheck) at any point during the peer-review or production process.Even if you are the author of the phrases or sentences, the text should not have unacceptable similarity with the previously published data.

When you are discussing others' (or your own) previous work, please make sure that you cite the material correctly in every instance.

In the event of alleged or suspected research misconduct, e.g., plagiarism, citation manipulation, and data falsification/fabrication, the Editorial Board will follow and act in accordance with COPE guidelines.

Authorship
Each individual listed as an author should fulfill the authorship criteria recommended by the International Committee of Medical Journal Editors

(ICMJE - www.icmje.org). The ICMJE recommends that authorship be based on the following 4 criteria:

1 Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
2 Drafting the work or revising it critically for important intellectual content; AND
3 Final approval of the version to be published; AND
4 Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

In addition to being accountable for the parts of the work he/she has done, an author should be able to identify which co-authors are responsible for specific other parts of the work. In addition, authors should have confidence in the integrity of the contributions of their co-authors.

All those designated as authors should meet all four criterias for authorship, and all who meet the four criteria should be identified as authors. Those who do not meet all four criterias should be acknowledged in the title page of the manuscript.

Experimed requires corresponding authors to submit a signed and scanned version of the authorship contribution form (available for download through http://experimed.istanbul.edu.tr/en/_) during the initial submission process in order to act appropriately on authorship rights and to prevent ghost or honorary authorship. If the editorial board suspects a case of "gift authorship," the submission will be rejected without further review. As part of the submission of the manuscript, the corresponding author should also send a short statement declaring that he/she accepts to undertake all the responsibility for authorship during the submission and review stages of the manuscript.

Conflict of Interest
Experimed requires and encourages the authors and the individuals involved in the evaluation process of submitted manuscripts to disclose any existing or potential conflicts of interests, including financial, consultant, and institutional, that might lead to potential bias or a conflict of interest. Any financial grants or other support received for a submitted study from individuals or institutions should be disclosed to the Editorial Board. To disclose a potential conflict of interest, the ICMJE Potential Conflict of Interest Disclosure Form should be filled in and submitted by all contributing authors. Cases of a potential conflict of interest of the editors, authors, or reviewers are resolved by the journal's Editorial Board within the scope of COPE and ICMJE guidelines.

The Editorial Board of the journal handles all appeal and complaint cases within the scope of COPE guidelines. In such cases, authors should get in direct contact with the editorial office regarding their appeals and complaints. When needed, an ombudsperson may be assigned to resolve cases that cannot be resolved internally. The Editor in Chief is the final authority in the decision-making process for all appeals and complaints.

Copyright and Licensing
Experimed requires each submission to be accompanied by a Copyright License Agreement (available for download at http://experimed.istanbul.edu.tr/en/_). When using previously published content, including figures, tables, or any other material in both print and electronic formats, authors must obtain permission from the copyright holder. Legal, financial and criminal liabilities in this regard belong to the author(s). By signing the Copyright License Agreement, authors agree that the article, if accepted for publication by the Experimed, will be licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (CC-BY-NC).

Disclaimer
Statements or opinions expressed in the manuscripts published in Experimed reflect the views of the author(s) and not the opinions of the editors, the editorial board, or the publisher; the editors, the editorial board, and the publisher disclaim any responsibility or liability for such materials. The final responsibility in regard to the published content rests with the authors.

MANUSCRIPT PREPARATION

The manuscripts should be prepared in accordance with ICMJE-Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (updated in December 2015 - http://www.icmje.org/icmje-recommendations.pdf). Authors are required to prepare manuscripts in accordance with the CONSORT guidelines for randomized research studies, STROBE guidelines for observational original research studies, STARD guidelines for studies on diagnostic accuracy, PRISMA guidelines for systematic reviews and meta-analysis, ARRIVE guidelines for experimental animal studies, and TREND guidelines for non-randomized public behavior.

Manuscripts can only be submitted through the journal's online manuscript submission and evaluation system, available at http://experimed.istanbul.edu.tr/en/_. Manuscripts submitted via any other medium will not be evaluated.

Manuscripts submitted to the journal will first go through a technical evaluation process where the editorial office staff will ensure that the manuscript has been prepared and submitted in accordance with the journal's guidelines. Submissions that do not conform to the journal's guidelines will be returned to the submitting author with technical correction requests.

Authors are required to submit the following:
- Copyright Agreement Form,
- ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors)

during the initial submission. These forms are available for download at http://experimed.istanbul.edu.tr/en/_.

Preparation of the Manuscript
Title page: A separate title page should be submitted with all submissions and this page should include:
- The full title of the manuscript as well as a short title (running head) of no more than 50 characters,
- Name(s), affiliations, ORCID IDs and highest academic degree(s) of the author(s),
- Grant information and detailed information on the other sources of support,
- Name, address, telephone (including the mobile phone number) and fax numbers, and email address of the corresponding author,
- Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfill the authorship criteria.

Abstract: A Turkish and an English abstract should be submitted with all submissions except for Letters to the Editor. Submitting a Turkish abstract is not compulsory for international authors. The abstract of Original Articles should be structured with subheadings (Objective, Material and Method, Results, and Conclusion). Please check Table 1 below for word count specifications.

Keywords: Each submission must be accompanied by a minimum of three to a maximum of six keywords for subject indexing at the

end of the abstract. The keywords should be listed in full without abbreviations. The keywords should be selected from the National Library of Medicine, Medical Subject Headings database (https://www.nlm.nih.gov/mesh/MBrowser.html).

Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. The main text of original articles should be structured with Introduction, Material and Method, Results, and Discussion subheadings. Please check Table 1 for the limitations for Original Articles.

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489-93). Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

Units should be prepared in accordance with the International System of Units (SI).

Editorial Comments: Editorial comments aim to provide a brief critical commentary by reviewers with expertise or with high reputation in the topic of the research article published in the journal. Authors are selected and invited by the journal to provide such comments. Abstract, Keywords, and Tables, Figures, Images, and other media are not included.

Review Articles: Reviews prepared by authors who have extensive knowledge on a particular field and whose scientific background has been translated into a high volume of publications with a high citation potential are welcomed. These authors may even be invited by the journal. Reviews should describe, discuss, and evaluate the current level of knowledge of a topic in clinical practice and should guide future studies. The main text should contain Introduction, Clinical and Research Consequences, and Conclusion sections. Please check Table 1 for the limitations for Review Articles.

Case Reports: There is limited space for case reports in the journal and reports on rare cases or conditions that constitute challenges in diagnosis and treatment, those offering new therapies or revealing knowledge not included in the literature, and interesting and educative case reports are accepted for publication. The text should include Introduction, Case Presentation, Discussion, and Conclusion subheadings. Please check Table 1 for the limitations for Case Reports.

Letters to the Editor: This type of manuscript discusses important parts, overlooked aspects, or lacking parts of a previously published article. Articles on subjects within the scope of the journal that might attract the readers' attention, particularly educative cases, may also be submitted in the form of a "Letter to the Editor." Readers can also present their comments on the published manuscripts in the form of a "Letter to the Editor." Abstract, Keywords, and Tables, Figures, Images, and other media should not be included. The text should be unstructured. The manuscript that is being commented on must be properly cited within this manuscript.

Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Figures and Figure Legends

Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

Table 1. Limitations for each manuscript type

Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	3500	250 (Structured)	30	6	7 or total of 15 images
Review Article	5000	250	50	6	10 or total of 20 images
Case Report	1000	200	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	No tables	No media

EXPERIMED

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References

While citing publications, preference should be given to the latest, most up-to-date publications. Authors are responsible for the accuracy of references. References should be prepared according to Vancouver reference style. If an ahead-of-print publication is cited, the DOI number should be provided. Journal titles should be abbreviated in accordance with the journal abbreviations in Index Medicus/ MEDLINE/PubMed. When there are six or fewer authors, all authors should be listed. If there are seven or more authors, the first six authors should be listed followed by "et al." In the main text of the manuscript, references should be cited using Arabic numbers in parentheses. The reference styles for different types of publications are presented in the following examples.

Journal Article: Rankovic A, Rancic N, Jovanovic M, Ivanović M, Gajović O, Lazić Z, et al. Impact of imaging diagnostics on the budget – Are we spending too much? Vojnosanit Pregl 2013; 70: 709-11.

Book Section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR, editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a Single Author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study Kidney Int: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktiviteleri ve Beden Kitle İndeksleri Kan Lipidleri Arasındaki İlişkiler. H.Ü. Sağlık Bilimleri Enstitüsü, Doktora Tezi. 2007.

Manuscripts Accepted for Publication, Not Published Yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts Published in Electronic Format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodIEID/cid.htm.

REVISIONS

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be canceled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 30-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.

Editor in Chief: Prof. Bedia Çakmakoğlu
Address: İstanbul University, Aziz Sancar Institute of Experimental Medicine, Vakıf Gureba Avenue, 34093, Çapa, Fatih, İstanbul, Turkey
Phone: 0212-4142000-33305
Fax: 0212-5324171
E-mail: bedia@istanbul.edu.tr

Publisher: AVES
Address: Büyükdere Cad. 105/9 34394 Mecidiyeköy, Şişli, İstanbul, Turkey
Phone: +90 212 217 17 00
Fax: +90 212 217 22 92
E-mail: info@avesyayincilik.com
avesyayincilik.com

EXPERIMED

YAZARLARA BİLGİ

İçerik
Experimed; İstanbul Üniversitesi Aziz Sancar Deneysel Tıp Araştırma Enstitüsü'nün çift-kör hakemli, elektronik, açık erişimli bilimsel yayın organıdır. Dergi Nisan, Ağustos ve Aralık aylarında olmak üzere, yılda 3 sayı olarak yayınlanır. Yayın dili Türkçe ve İngilizce'dir.

Experimed, temel ve klinik tıp bilimlerinin tüm alanlarında orijinal araştırma, olgu sunumu, derleme ve editöre mektup türlerinde makaleler yayınlamaktadır.

Yayın Politikası
Derginin editöryel ve yayın süreçleri International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), ve National Information Standards Organization (NISO) organizasyonlarının kılavuzlarına uygun olarak biçimlendirilmiştir. Experimed'in editöryel ve yayın süreçleri, Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice) ilkelerine uygun olarak yürütülmektedir.

Özgünlük, yüksek bilimsel kalite ve atıf potansiyeli bir makalenin yayına kabulü için en önemli kriterlerdir. Gönderilen yazıların daha önce başka bir elektronik ya da basılı dergide, kitapta veya farklı bir mecrada sunulmamış ya da yayınlanmamış olması gerekir. Daha önce başka bir dergiye gönderilen ancak yayına kabul edilmeyen yazılar hakkında dergi önceden bilgilendirilmelidir. Bu yazıların eski hakem raporlarının Yayın Kuruluna gönderilmesi değerlendirme süresinin hızlanmasını sağlayacaktır. Toplantılarda sunulan çalışmalar için, sunum yapılan organizasyonun tam adı, tarihi, şehri ve ülkesi belirtilmelidir.

Değerlendirme Süreci
Experimed'e gönderilen tüm makaleler çift-kör hakem değerlendirme sürecinden geçmektedir. Tarafsız değerlendirme sürecini sağlamak için her makale alanlarında uzman en az iki dış-bağımsız hakem tarafından değerlendirilir. Dergi Yayın Kurulu üyeleri tarafından gönderilecek makalelerin değerlendirme süreçleri, davet edilecek dış bağımsız editörler tarafından yönetilecektir. Bütün makalelerin karar verme süreçlerinde nihai karar yetkisi Baş Editör'dedir.

Etik İlkeler
Klinik ve deneysel çalışmalar, ilaç araştırmaları ve bazı olgu sunumları için World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013, www.wma.net) çerçevesinde hazırlanmış Etik Komisyon raporu gerekmektedir. Gerekli görülmesi halinde Etik Komisyon raporu veya eşdeğeri olan resmi bir yazı yazarlardan talep edilebilir. İnsanlar üzerinde yapılmış deneysel çalışmaların sonuçlarını bildiren yazılarda, çalışmanın yapıldığı kişilere uygulanan prosedürlerin niteliği tümüyle açıklandıktan sonra, onaylarının alındığına ilişkin bir açıklamaya metin içinde yer verilmelidir. Hayvanlar üzerinde yapılan çalışmalarda ise ağrı, acı ve rahatsızlık verilmemesi için yapılmış olanlar açık olarak makalede belirtilmelidir. Hasta onamları, Etik Kurul raporun alındığı kurumun adı, onay belgesinin numarası ve tarihi ana metin dosyasında yer alan Yöntemler başlığı altında yazılmalıdır. Hastaların kimliklerinin gizliliğini korumak yazarların sorumluluğundadır. Hastaların kimliğini açığa çıkarabilecek fotoğraflar için hastadan ya da yasal temsilcilerinden alınan imzalı izinlerin de gönderilmesi gereklidir.

Dergiye gönderilen makaleler, hakem değerlendirme sürecinde ya da yayına hazırlık aşamasında herhangi bir noktada bir benzerlik tespit yazılımı (CrossCheck, iThenticate) tarafından taranmaktadır. Cümleler ve ifadeler yazar olarak size ait olsa dahi, metnin daha önce yayınlanan verilerle kabul edilemez bir benzerliği olmalıdır.

Başkalarının önceki çalışmalarını (veya kendi çalışmalarınızı) tartışırken, lütfen materyali her durumda doğru bir şekilde alıntıladığınızdan emin olunuz.

Yayın Kurulu, dergimize gönderilen çalışmalar hakkındaki intihal, atıf manipülasyonu ve veri sahteciliği iddia ve şüpheleri karşısında COPE kurallarına uygun olarak hareket edecektir.

Yazarlık
Yazar olarak listelenen herkesin ICMJE (www.icmje.org) tarafından önerilen yazarlık kriterlerini karşılaması gerekmektedir. ICMJE, yazarların aşağıdaki 4 kriteri karşılamasını önermektedir:

1. Çalışmanın konseptine/tasarımına; ya da çalışma için verilerin toplanmasına, analiz edilmesine ve yorumlanmasına önemli katkı sağlamış olmak; VE
2. Yazı taslağını hazırlamış ya da önemli fikirsel içeriğin eleştirel incelemelerini yapmış olmak; VE
3. Yazının yayından önceki son halini gözden geçirmiş ve onaylamış olmak; VE
4. Çalışmanın herhangi bir bölümünün geçerliliği ve doğruluğuna ilişkin soruların uygun şekilde soruşturulduğunun ve çözümlendiğinin garantisini vermek amacıyla çalışmanın her yönünden sorumlu olmayı kabul etmek.

Bir yazar, çalışmada katkı sağladığı kısımların sorumluluğunu almasına ek olarak, diğer yazarların çalışmanın hangi kısımlarından sorumlu olduğunu da teşhis edebilmelidir. Ayrıca, yazarlar birbirlerinin katkılarının bütünlüğüne güven duymalılardır.

Yazar olarak belirtilen her kişi yazarlığın dört kriterini karşılamalıdır ve bu dört kriteri karşılayan her kişi yazar olarak tanımlanmalıdır. Dört kriterin hepsini karşılamayan kişilere makalenin başlık sayfasında teşekkür edilmelidir.

Yazarlık haklarına uygun hareket etmek ve hayalet ya da lütuf yazarlığın önlenmesini sağlamak amacıyla sorumlu yazarlar makale yükleme sürecinde http://experimed.istanbul.edu.tr/tr/_ adresinden erişebilinen Yazar Katkı Formu'nu imzalamalı ve taranmış versiyonunu yazıyla birlikte göndermelidir. Yayın Kurulu'nun gönderilen bir makalede "lütuf yazarlık" olduğundan şüphelenmesi durumunda söz konusu makale değerlendirme yapılmaksızın reddedilecektir. Makale gönderimi kapsamında; sorumlu yazar makale gönderim ve

değerlendirme süreçleri boyunca yazarlık ile ilgili tüm sorumluluğu kabul ettiğini bildiren kısa bir ön yazı göndermelidir.

Çıkar Çatışması
Experimed; gönderilen makalelerin değerlendirme sürecine dahil olan yazarların ve bireylerin, potansiyel çıkar çatışmasına ya da önyargıya yol açabilecek finansal, kurumsal ve diğer ilişkiler dahil mevcut ya da potansiyel çıkar çatışmalarını beyan etmelerini talep ve teşvik eder.

Bir çalışma için bir birey ya da kurumdan alınan her türlü finansal destek ya da diğer destekler Yayın Kurulu'na beyan edilmeli ve potansiyel çıkar çatışmalarını beyan etmek amacıyla ICMJE Potansiyel Çıkar Çatışmaları Formu katkı sağlayan tüm yazarlar tarafından ayrı ayrı doldurulmalıdır. Editörler, yazarlar ve hakemler ile ilgili potansiyel çıkar çatışması vakaları derginin Yayın Kurulu tarafından COPE ve ICMJE rehberleri kapsamında çözülmektedir.

Derginin Yayın Kurulu, itiraz ve şikayet vakalarını, COPE rehberleri kapsamında işleme almaktadır. Yazarlar, itiraz ve şikayetleri için doğrudan Editöryel Ofis ile temasa geçebilirler. İhtiyaç duyulduğunda Yayın Kurulu'nun kendi içinde çözemediği konular için tarafsız bir temsilci atanmaktadır. İtiraz ve şikayetler için karar verme süreçlerinde nihai kararı Baş Editör verecektir.

Telif ve Lisans
Experimed, ilk gönderim sırasında http://experimed.istanbul.edu.tr/tr/_adresinden indirilebilen Telif Hakkı Lisans Sözleşmesinin imzalanarak makale ile birlikte derginin çevrimiçi değerlendirme sistemine yüklenmesini zorunlu tutar. Yazarlar, Telif Hakkı Lisans Sözleşmesini imzalayarak, makalenin Experimed tarafından yayınlanmak üzere kabul edilmesi durumunda Creative Commons Atıf-GayriTicari 4.0 Uluslararası Lisansı (CC BY-NC) kapsamında lisanslanacağını kabul ederler.

Yazarlar, basılı ya da elektronik formatta yer alan resimler, tablolar ya da diğer her türlü içerik dahil daha önce yayınlanmış içeriği kullanırken telif hakkı sahibinden izin almalılardır. Bu konudaki yasal, mali ve cezai sorumluluk yazarlara aittir.

Sorumluluk Reddi
Dergide yayınlanan makalelerde ifade edilen görüşler ve fikirler Experimed, Baş Editör, Editörler, Yayın Kurulu ve Yayıncı'nın değil, yazar(lar)ın bakış açılarını yansıtır. Baş Editör, Editörler, Yayın Kurulu ve Yayıncı bu gibi durumlar için hiçbir sorumluluk ya da yükümlülük kabul etmemektedir. Yayınlanan içerik ile ilgili tüm sorumluluk yazarlara aittir.

MAKALE HAZIRLAMA

Makaleler, ICMJE-Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals (updated in December 2015 - http://www.icmje. org/icmje-recommendations.pdf) ile uyumlu olarak hazırlanmalıdır. Randomize çalışmalar CONSORT, gözlemsel çalışmalar STROBE, tanısal değerli çalışmalar STARD, sistematik derleme ve meta-analizler PRISMA, hayvan deneyli çalışmalar ARRIVE ve randomize olmayan davranış ve halk sağlığıyla ilgili çalışmalar TREND kılavuzlarına uyumlu olmalıdır.

Makaleler sadece http://experimed.istanbul.edu.tr/tr/_ adresinde yer alan derginin online makale yükleme ve değerlendirme sistemi üzerinden gönderilebilir. Diğer mecralardan gönderilen makaleler değerlendirilmeye alınmayacaktır.

Gönderilen makalelerin dergi yazım kurallarına uygunluğu ilk olarak Editöryel Ofis tarafından kontrol edilecek, dergi yazım kurallarına uygun hazırlanmamış makaleler teknik düzeltme talepleri ile birlikte yazarlarına geri gönderilecektir.

Yazarların; Yayın Hakkı Sözleşmesi Formu, Yazar Katkı Formu ve ICMJE Potansiyel Çıkar Çatışmaları Formu'nu (bu form, tüm yazarlar tarafından doldurulmalıdır) ilk gönderim sırasında online makale sistemine yüklemeleri gerekmektedir. Bu formlara http://experimed.istanbul.edu.tr/tr/_ adresinden erişilebilmektedir.

Başlık sayfası: Gönderilen tüm makalelerle birlikte ayrı bir başlık sayfası da gönderilmelidir. Bu sayfa;

- Makalenin başlığını ve 50 karakteri geçmeyen kısa başlığını,
- Yazarların isimlerini, kurumlarını, ORCID numaralarını ve eğitim derecelerini,
- Finansal destek bilgisi ve diğer destek kaynakları hakkında detaylı bilgiyi,
- Sorumlu yazarın ismi, adresi, telefonu (cep telefonu dahil ve e-posta adresini,
- Makale hazırlama sürecine katkıda bulunan ama yazarlık kriterlerini karşılamayan bireylerle ilgili bilgileri içermelidir.

Özet: Editöre Mektup türündeki yazılar dışında kalan tüm makalelerin Türkçe ve İngilizce özetleri olmalıdır. rijinal Araştırma makalelerinin özetleri "Amaç", "Gereç ve Yöntem", "Bulgular" ve "Sonuç" alt başlıklarını içerecek biçimde hazırlanmalıdır.

Anahtar Sözcükler: Tüm makaleler en az 3 en fazla 6 anahtar kelimeyle birlikte gönderilmeli, anahtar sözcükler özetin hemen altına yazılmalıdır. Kısaltmalar anahtar sözcük olarak kullanılmamalıdır. Anahtar sözcükler National Library of Medicine (NLM) tarafından hazırlanan Medical Subject Headings (MeSH) veritabanından seçilmelidir.

Makale Türleri
Orijinal Araştırma: Ana metin "Giriş", "Gereç ve Yöntem", "Bulgular" ve "Tartışma" alt başlıklarını içermelidir. Özgün Araştırmalarla ilgili kısıtlamalar için lütfen Tablo 1'i inceleyiniz.

Sonucu desteklemek için istatiksel analiz genellikle gereklidir. İstatistiksel analiz, tıbbi dergilerdeki istatistik verilerini bildirme kurallarına göre yapılmalıdır (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489-93). İstatiksel analiz ile ilgili bilgi, Yöntemler bölümü içinde ayrı bir alt başlık olarak yazılmalı ve kullanılan yazılım kesinlikle tanımlanmalıdır.

Birimler, uluslararası birim sistemi olan International System of Units (SI)'a uygun olarak hazırlanmadır.

Editöryel Yorum: Dergide yayınlanan bir araştırmanın, o konunun uzmanı olan veya üst düzeyde değerlendirme yapan bir hakemi ta-

rafından kısaca yorumlanması amacını taşımaktadır. Yazarları, dergi tarafından seçilip davet edilir. Özet, anahtar sözcük, tablo, şekil, resim ve diğer görseller kullanılmaz.

Derleme: Yazının konusunda birikimi olan ve bu birikimleri uluslararası literatüre yayın ve atıf sayısı olarak yansımış uzmanlar tarafından hazırlanmış yazılar değerlendirmeye alınır. Yazarları dergi tarafından da davet edilebilir. Bir bilgi ya da konunun klinikte kullanılması için vardığı son düzeyi anlatan, tartışan, değerlendiren ve gelecekte yapılacak olan çalışmalara yön veren bir formatta hazırlanmalıdır. Ana metin "Giriş", "Klinik ve Araştırma Etkileri" ve "Sonuç" bölümlerini içermelidir. Derleme türündeki yazılarla ilgili kısıtlamalar için lütfen Tablo 1'i inceleyiniz.

Olgu Sunumu: Olgu sunumları için sınırlı sayıda yer ayrılmakta ve sadece ender görülen, tanı ve tedavisi güç olan hastalıklarla ilgili, yeni bir yöntem öneren, kitaplarda yer verilmeyen bilgileri yansıtan, ilgi çekici ve öğretici özelliği olan olgular yayına kabul edilmektedir. Ana metin; "Giriş", "Olgu Sunumu", "Tartışma" ve "Sonuç" alt başlıklarını içermelidir. Olgu Sunumlarıyla ilgili kısıtlamalar için lütfen Tablo 1'i inceleyiniz.

Editöre Mektup: Dergide daha önce yayınlanan bir yazının önemini, gözden kaçan bir ayrıntısını ya da eksik kısımlarını tartışabilir. Ayrıca derginin kapsamına giren alanlarda okurların ilgisini çekebilecek konular ve özellikle eğitici olgular hakkında da Editöre Mektup formatında yazılar yayınlanabilir. Okuyucular da yayınlanan yazılar hakkında yorum içeren Editöre Mektup formatında yazılarını sunabilirler. Özet, anahtar sözcük, tablo, şekil, resim ve diğer görseller kullanılmaz. Ana metin alt başlıksız olmalıdır. Hakkında mektup yazılan yayına ait cilt, yıl, sayı, sayfa numaraları, yazı başlığı ve yazarların adları açık bir şekilde belirtilmeli, kaynak listesinde yazılmalı ve metin içinde atıfta bulunulmalıdır.

Tablolar
Tablolar ana dosyaya eklenmeli, kaynak listesi sonrasında sunulmalı, ana metin içerisindeki geçiş sıralarına uygun olarak numaralandırılmadır. Tabloların üzerinde tanımlayıcı bir başlık yer almalı ve tablo içerisinde geçen kısaltmaların açılımları tablo altına tanımlanmalıdır. Tablolar Microsoft Office Word dosyası içinde "Tablo Ekle" komutu kullanılarak hazırlanmalı ve kolay okunabilir şekilde düzenlenmelidir. Tablolarda sunulan veriler ana metinde sunulan verilerin tekrarı olmamalı; ana metindeki verileri destekleyici nitelikte olmalılardır.

Resim ve Resim Altyazıları
Resimler, grafikler ve fotoğraflar (TIFF ya da JPEG formatında) ayrı dosyalar halinde sisteme yüklenmelidir. Görseller bir Word dosyası dokümanı ya da ana doküman içerisinde sunulmamalıdır. Alt birimlere ayrılan görseller olduğunda, alt birimler tek bir görsel içerisinde verilmemelidir. Her bir alt birim sisteme ayrı bir dosya olarak yüklenmelidir. Resimler alt birimleri belli etme amacıyla etiketlenmemelidir (a, b, c vb.). Resimlerde altyazıları desteklemek için kalın ve ince oklar, ok başları, yıldızlar, asteriksler ve benzer işaretler kullanılabilir. Makalenin geri kalanında olduğu gibi resimler de kör olmalıdır. Bu sebeple, resimlerde yer alan kişi ve kurum bilgileri de körleştirilmelidir. Görsellerin minimum çözünürlüğü 300DPI olmalıdır. Değerlendirme sürecindeki aksaklıkları önlemek için gönderilen bütün görsellerin çözünürlüğü net ve boyutu büyük (minimum boyutlar 100x100 mm) olmalıdır. Resim altyazıları ana metnin sonunda yer almalıdır.

Makale içerisinde geçen tüm kısaltmalar, ana metin ve özette ayrı ayrı olmak üzere ilk kez kullanıldıkları yerde tanımlanarak kısaltma tanımın ardından parantez içerisinde verilmelidir.

Ana metin içerisinde cihaz, yazılım, ilaç vb. ürünlerden bahsedildiğinde ürünün ismi, üreticisi, üretildiği şehir ve ülke bilgisini içeren ürün bilgisi parantez içinde verilmelidir; "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)".

Tüm kaynaklar, tablolar ve resimlere ana metin içinde uygun olan yerlerde sırayla numara verilerek atıf yapılmalıdır.

Özgün araştırmaların kısıtlamaları, engelleri ve yetersizliklerinden Sonuç paragrafı öncesi "Tartışma" bölümünde bahsedilmelidir.

Kaynaklar
Atıf yapılırken en son ve en güncel yayınlar tercih edilmelidir. Kaynakların doğruluğundan yazarlar sorumludur. Kaynaklar Vancouver referans stiline uygun olarak hazırlanmalıdır. Atıf yapılan erken çevrimiçi makalelerin DOI numaraları mutlaka sağlanmalıdır. Dergi isimleri Index Medicus/Medline/PubMed'de yer alan dergi kısaltmaları ile uyumlu olarak kısaltılmalıdır. Altı ya da daha az yazar olduğunda tüm yazar isimleri listelenmelidir. Eğer 7 ya da daha fazla yazar varsa ilk 6 yazar yazıldıktan sonra "et al" konulmalıdır. Ana metinde kaynaklara atıf yapılırken parantez içinde Arabik numaralar kullanılmalıdır. Farklı yayın türleri için kaynak stilleri aşağıdaki örneklerde sunulmuştur:

Dergi makalesi: Blasco V, Colavolpe JC, Antonini F, Zieleskiewicz L, Nafati C, Albanèse J, et al. Long-term outcome in kidney recipients from donors treated with hydroxyethylstarch 130/0.4 and hydroxyethylstarch 200/0.6. Br J Anaesth 2015; 115: 797-8.

Tablo 1. Makale türleri için kısıtlamalar

Makale türü	Sözcük limiti	Özet sözcük limiti	Kaynak limiti	Tablo limiti	Resim limiti
Özgün Araştırma	3500	250 (Alt başlıklı)	30	6	7 ya da toplamda 15 resim
Derleme	5000	250	50	6	10 ya da toplamda 20 resim
Olgu Sunumu	1000	200	15	Tablo yok	10 ya da toplamda 20 resim
Editöre Mektup	500	Uygulanamaz	5	Tablo yok	Resim yok

EXPERIMED

Kitap bölümü: Sherry S. Detection of thrombi. In: Strauss HE, Pitt B, James AE, editors. Cardiovascular Medicine. St Louis: Mosby; 1974.p.273-85.

Tek yazarlı kitap: Cohn PF. Silent myocardial ischemia and infarction. 3rd ed. New York: Marcel Dekker; 1993.

Yazar olarak editör(ler): Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

Toplantıda sunulan yazı: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992.p.1561-5.

Bilimsel veya teknik rapor: Smith P. Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX) Dept. of Health and Human Services (US). Office of Evaluation and Inspections: 1994 Oct. Report No: HHSI-GOE 169200860.

Tez: Kaplan SI. Post-hospital home health care: the elderly access and utilization (dissertation). St. Louis (MO): Washington Univ. 1995.

Yayına kabul edilmiş ancak henüz basılmamış yazılar: Leshner AI. Molecular mechanisms of cocaine addiction. N Engl J Med In press 1997.

Erken Çevrimiçi Yayın: Aksu HU, Ertürk M, Gül M, Uslu N. Successful treatment of a patient with pulmonary embolism and biatrial thrombus. Anadolu Kardiyol Derg 2012 Dec 26. doi: 10.5152/akd.2013.062. [Epub ahead of print]

Elektronik formatta yayınlanan yazı: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodIEID/cid.htm.

REVİZYONLAR

Yazarlar makalelerinin revizyon dosyalarını gönderirken, ana metin üzerinde yaptıkları değişiklikleri işaretlemeli, ek olarak, hakemler tarafından öne sürülen önerilerle ilgili notlarını "Hakemlere Cevap" dosyasında göndermelidir. Hakemlere Cevap dosyasında her hakemin yorumunun ardından yazarın cevabı gelmeli ve değişikliklerin yapıldığı satır numaraları da ayrıca belirtilmelidir. Revize makaleler karar mektubunu takip eden 30 gün içerisinde dergiye gönderilmelidir. Makalenin revize versiyonu belirtilen süre içerisinde yüklenmezse, revizyon seçeneği iptal olabilir. Yazarların revizyon için ek süreye ihtiyaç duymaları durumunda uzatma taleplerini ilk 30 gün sona ermeden dergiye iletmeleri gerekmektedir.

Yayına kabul edilen makaleler dil bilgisi, noktalama ve biçim açısından kontrol edilir. Yayın süreci tamamlanan makaleler, yayın planına dahil edildikleri sayıyla birlikte yayınlanmadan önce erken çevrimiçi formatında dergi web sitesinde yayına alınır. Kabul edilen makalelerin baskıya hazır PDF dosyaları sorumlu yazarlara iletilir ve yayın onaylarının 2 gün içerisinde dergiye iletilmesi istenir.

Baş Editör: Prof. Dr. Bedia Çakmakoğlu
Address: İstanbul Üniversitesi, Aziz Sancar Deneysel Tıp Araştırma Enstitüsü, Vakıf Gureba Caddesi, 34093, Çapa, Fatih, İstanbul, Türkiye
Phone: 0212-4142000-33305
Fax: 0212-5324171
E-mail: bedia@istanbul.edu.tr

Publisher: AVES
Address: Büyükdere Cad. 105/9 34394 Mecidiyeköy, Şişli, İstanbul, Turkey
Phone: +90 212 217 17 00
Fax: +90 212 217 22 92
E-mail: info@avesyayincilik.com
avesyayincilik.com