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Niçin bu çalışmayı yapmaya İhtiyaç duyduğunuzu ve amacınızı sadece en önemli makalelere atıfta bulunarak kısaca belirtiniz.

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İstatistiksel metodlarla desteklenmiş bulgularınızı ayrıntılı olarak belirtiniz. Şekil ve tablolar metin içinde verilen bulguları desteklemeli tekrar etmemelidir; verinin metin, tablo veya şekil şeklindeki sunumların bir tanesinde gösterilmesi yeterlidir. Sadece en önemli bulgularınızı vurgulayınız; bu bölümde bulgularınızı diğer araştırmalarla karşılaştırmayınız. Bu tip karşılaştırmalar tartışma bölümüne saklanmalıdır.

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Bulgularınızın önemini ve farkını vurgulayın ancak sonuç bölümünde sunulan detayları tekrarlamayın. Görüşlerinizi sadece çalışmanızda bulduğunuz gerçeklerle desteklenecek şekilde sınırlayınız, araştırmadığınız ya da gösteremediğiniz varsayımları tartışmaya eklemeyiniz. Bulgularınızı başka araştırmalarla karşılaştırınız. Bu bölümde bulgular bölümünde belirtilmemiş yeni veri sunulmamalıdır.

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Örnekler:

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1. Dilaveris P, Batcvarov V, Giafalos J, et al. Comparison of different methods for manual P wave duration measurement in 12" lead electrocardiograms. Pacing and Clin Electrophysiol 1999;22:1532-8.

Kitap bölümü;

1. Schwartz PJ, Priori SG, Napolitano C. The Long QT Syndrome. In: Zipes DP, Jalife J, eds. Cardiac Electrophysiology. From Cell to Bedside. Philadelphia: WB Saunders Co, 2000:597-615.

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(Bailar JC III, Mosteller F, Ann Intern Med 1988; 108:266-73)

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Introduction

State concisely the purpose and rationale for the study and cite only the most pertinent references as background.

Material and Methods

Describe the plan, the patients, experimental animals, material and controls, the methods and procedures utilized, and the statistical method(s) employed. Address "Institutional Review Board" issues as stated above. State the generic names of the drugs with the name and country of the manufactures.

Results

Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Emphasize only your Important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

Discussion

State the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly Indicated by the facts in your report. Compare your findings with those of others. No new data are to be presented in this section.

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Number references in Arabic numerals alphabetically starting with number "(1)". The numbers should be written in parentheses at the end of sentences. Use the form of the "Uniform Requirements for Manuscript Submitted to Biomedical Journals" (http://www.amaassn.org/public/peer/wame/uniform.htm). List all authors, If authors are more than six, use "et al". Journal titles should conform to the abbreviations used in"Cumulated Index Medicus".

Examples:

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1. Dilaveris P, Batcvarov V, Giafalos J, et ai. Comparison of different methods for manual P wave duration measurement in 12 " lead electrocardiograms. Pacing and Clin Electrophysiol 1999; 22:1532-8.

Book chapter;

1. Schwartz PJ, Priori SG, Napoiitano C. The Long QT Syndrome. In: Zipes DP, Jalife J, eds. Cardiac Electrophysiology. From Cell to Bedside. Philadelphia: WB Saunders Co, 2000:597-615.

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Tables and figures should work under "Windows". Color figures or gray-scale images must be at least 300 dpi. Figures using "Miff", "*.jpg" or "*.pdf" should be saved separate from the text. All tables and figures should be prepared on separate pages. They should be numbered in Arabic numerals. Each table must have a title indicating the purpose or content of each table. Each figure must have an accompanying legend defining abbreviations or symbols found in the figure.

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The Effect of Momordica Charantia Extract on Gene Expression in Wound Healing

Momordica Charantia (Kudret Narı) Ekstraktının Yara İyileşmesindeki Gen Ekspresyonu Üzerine Etkisi

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ABSTRACT

Objective: Momordica charantia is traditionally used in Anatolia for a wide range of diseases from stomach to burns. In our study, usage of momordica for wounds and burns make us to interest possible wound and burn healing effects of it. We investigated possible effects of momordica on the wound and burn models of mice and evaluated expression changes of VEGFA, VEGFB, VEGFC, FGF2 and EGF genes.

Materials and Methods: In our study, experiment and control groups were designed for both wound model and burn model. Healing effects were compared clinically between control groups with both wound model and burn model. After that, tissue samples were obtained from wound model and burn model animals. RNA isolation and cDNA synthesis were done and gene expression changes were evaluated with Real-time PCR (rPCR) method.

Results: Clinical evaluation showed that wound closure and healing effect of momordica were better in wound model ($p<0.05$) but results in burn model they were not different from control group ($p>0.05$). All gene expressions were elevated in wound model ($p<0.05$), but in the burn model, gene expression levels of VEGFB, VEGFC and EGF were not statistically different from control group ($p<0.05$).

Conclusion: Due to our results, we can say momordica has a positive wound healing effect and this effect occurs via

ÖZET

Amaç: Kudret narı- acı kavun (Momordica Charantia) Anadolu'da yüzyıllardır geleneksel olarak mide rahatsızlıklarından yanığa kadar farklı nedenlerle yaygın olarak kullanılmaktadır. Çalışmamızda farede oluşturulacak yara ve yanık modellerinde acı kavunun yara ve yanık iyileşmesine ve alınacak doku örneklerinde VEGFA, VEGFB,VEGFC, FGF2 ve EGF genlerinin ekspresyon düzeylerine etkisini ortaya koymayı amaçladık.

Yöntem: Çalışmamızda hem yara modeli hem de yanık modeli için deney ve kontrol grupları oluşturuldu. Çalışma sonunda öncelikle klinik açıdan yara ve yanık iyileşme düzeyleri karşılaştırıldı. Daha sonra yara ve yanık bölgelerinden doku örnekleri alındı. Alınan doku örneklerinden RNA izolasyonu ve cDNA sentezi yapılarak gen ekspresyon değişimleri Real-time PCR (rPCR) yöntemi ile değerlendirildi.

Bulgular: Çalışma sonucunda klinik değerlendirmelerde yara modeli oluşturulan gruplardan deney grubundaki yara iyileşmesinin kontrol grubuna göre daha iyi olduğu ($p<0.05$), yanık modeli oluşturulan grupta ise kontrol grubuna göre iyileşme açısından bir fark olmadığı bulundu ($p>0.05$). Gen ekspresyon değişimlerine bakıldığında, yara modeli oluşturulan gruplarda deney grubundaki tüm genlerin ekspresyon düzeylerinin kontrol grubuna göre arttığı bulundu ($p<0.05$). Yanık modeli oluşturulan gruplarda ise VEGFB, VEGC ve FGF genlerinin ekspresyonu kontrol grubuna göre istatistiksel

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VEGFA, VEGFB, VEGFC, FGF2 and EGF genes. But momordica has no effect on burns. Our study is valuable for guiding possible future studies about momordica.

Key Words: *Momordica charantia, wound healing, gene expression*

olarak anlamlı ölçüde farklılık göstermedi (p>0.05).

Sonuç: Çalışmamızda kudret narının yara iyileşmesine olumlu etkisinin olduğu ve bu etkisini VEGFA, VEGFB, VEGFC, FGF2 ve EGF genlerinin ekspresyonlarını arttırarak yaptığı, ancak yanık iyileşmesinde böyle bir etkisinin olmadığı bulundu. Çalışmamız kudret narının insan sağlığına yararlı olması maksadıyla yapılacak diğer çalışmalara öncülük yapması bakımından değerlidir.

Anahtar Kelimeler: *Momordica charantia (kudret narı), yara iyileşmesi, gen ekspresyonu*

INTRODUCTION

Momordica charantia, also known as bitter melon, is a tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit, which is extremely bitter. Bitter melon has been used in various Asian and African herbal medicine systems for a long time[1,2]. In Turkey, it has been used as a folk remedy for a variety of ailments, particularly stomach complaints[3]. In traditional medicine of India different parts of the plant are used to relieve diabetes, as a stomachic, laxative, antibilious, emetic, anthelmintic agent, for the treatment of cough, respiratory diseases, skin diseases, wounds, ulcer, gout, and rheumatism[4]. In a manuscript, Yeşilada et al. (1999) were studied the anti-Helicobacter pylori effect of the momordica extracts which are used in folk medicine by using the agar dilution method. They found that momordica extracts were effective on the clinical isolates of H. pylori strains[5]. *Momordica charantia* contains many biological active molecules, such as momordicin I, momordicin II and cucurbitacin B[6]. Despite of these findings, studies on the effect of *Momordica charantia* on wound healing are limited.

Wound healing is a regeneration and recreation process of the skin or another organ after an injury. Wound healing process has four grades; homeostasis, inflammation, proliferation and remodeling[7]. The most active grade is proliferation phase. This phase includes angiogenesis, collagen depositing, granulation tissue forming, epithelialization and wound contraction. During angiogenesis vascular endothelial cells forms new blood vessels. Fibroblasts grow up and contribute fibroplasias and formation of granulation tissue. Re-epithelialization begins in the epidermis and the wound area begins to full with new tissue8. In the second part of our study, we investigated the possible effect of *Momordica charantia* on the genes especially active in the proliferative phase of wound healing process[9].

In our study, thus we created both control and study groups for wound model and burnt model of mice. For the first part of study, the levels of healing of these areas were compared clinically. The tissue samples were collected from the wound and burnt areas for comparing the gene expression differences. The expression profiles of VEGFA, VEGFB, VEGFC, FGF2 and EGF genes from the tissues of the wound areas were analyzed with Real-time Polymerase Chain Reaction (RT-PCR) method.

MATERIAL AND METHODS

This study was designed due to Gülhane Military Medical Academy, ethic council decision (20/10/2013-13-117). The project was granted by Gülhane Military Medical Academy, Health Science Institute (GATA-AR2013/44).

Prepearing the *"Momordica charantia"* Solution;

"Momordica charantia" solution was prepared by using traditional methods (Fruit part of *Momordica charantia* was minced and mixed with olive oil)10. The extract includes momordicin I and II, cucurbitacin B11 and the other terphenoidecompounds (momordicin-28, momordicinin, momordicilin, momordenol and momordol), bioactive glikozides (momordin, Charantin, charantosides, goyaglycosides, momordicosides)

[6,12,13,14,15]. The extract was applied twice a day to the wounded and burnt areas.

Preparing the wounded areas:

Fourteen mice were assigned into two different groups. Seven mice were used for control group (group 1) and seven mice were used for study group (group 3). In each group, sterile cuts were carried out for ensuring clean wounds. The cuts were sutured and the wounded areas were clearly dressed16. In the control group daily dressings were applied only. In study group, "*Momordica charantia*" solution was applied twice a day for one week.

Preparing the burnt areas:

Fourteen mice were assigned into two different groups. Seven mice were used for control group (group 2) and seven mice were used for study group (group 4). In each group, sterile burns (first and second degree) were carried out for ensuring clean wounds. In the control group daily dressings were applied only. In study group "*Momordica charantia*" solution were applied twice a day till for one week.

Assessment of wound healing in wounded and burnt areas:

The wound healing areas of the mice in the control and the study groups were evaluated clinically.

Tissue sample preparation: In the 7th day of study, the wounded and burnt areas of control and study groups were completely collected for RNA isolation. At least one mouse from every group was left alive for final comparison.

RNA isolation from tissue samples: After thawing the tissue samples taken from control group and study group in the liquid nitrogen, RNAs were isolated according to the manufacturer company's method (NucleoSpin RNA-Machenery Nagel).

Revers Transcription Polymerase Chain Reaction (RT-PCR): The obtained RNAs were converted to cDNAs by reverse transcriptase by using RT-PCR method according to the manufacturer's method (RevertAid First Strand cDNA Synthesis Kit - Life Technologies- Fermantas)12. The PCR conditions were 42º 60', 70º5' and the final volume was 20 µl for each run.

Real Time Polymerase Chain Reaction (RT-PCR): The cDNAs were used as master for RT-PCR. In the procedure, the primers of *EGF, VEGF, PDGF* and *FGF* were used. Primer lists were taken from Primer Bank (http://pga.mgh.harvard.edu/primerbank). As house-keeping gene, β-actin was used. Each reaction was performed as 20 µl final concentration (10 µl Mastermix, 5 µl DNA, 0,5 µl primer, 4 µl H2O) (SYBR® Green PCR Master Mix- Roche Life Science). RT-PCR conditions were 55º for 45 cycles. The gene expression alterations were evaluated with relative quantification method with Roche Applied Sciences LightCycler 1.5 software program.

Statistical Analyses: Chi-Square test for wound healing evaluation and Student T-Test for RT-PCR evaluation were used.

RESULTS

Group 1 and 3 were evaluated together as wound healing group. Group 2 and 4 were evaluated as burn healing group.

In our study, the healing effect of *Momordica charantia* was evaluated on wound and burn models clinically. The wounds were evaluated macroscopically in each group. Due to the results, in the wound healing group; the wound healing process was better than the control group (group 1) in the study group (group 3) (Figure 1). But in the burn healing group; the healing process was found similar in the control group (group 2) and the study group (group 4) (Figure 2). In the second part of our study, we evaluated the effects of *Momordica charantia* in a molecular view. In our study the gene expression levels of *VEGFA, VEGFB, VEGFC, FGF2* and *EGF* were studied in each group.

In the wound healing group, the expression levels of VEGFA were found approximately 2,37 in the study group whereas 0,58 in the control group. *VEGFB* gene expression levels were 4,29 in the study group and 0,963 in the control group. In the study group, the gene expression level was found 43,71 for *VEGFC* gene. It was found 1,17 in the control group. The expression levels of *FGF2* was found 0,14 in the study group and 0,03 in the control group. It was found 1,13 for the expression levels of *EGF* gene in the study group as it was found 0,49 in the control group (Table 1).

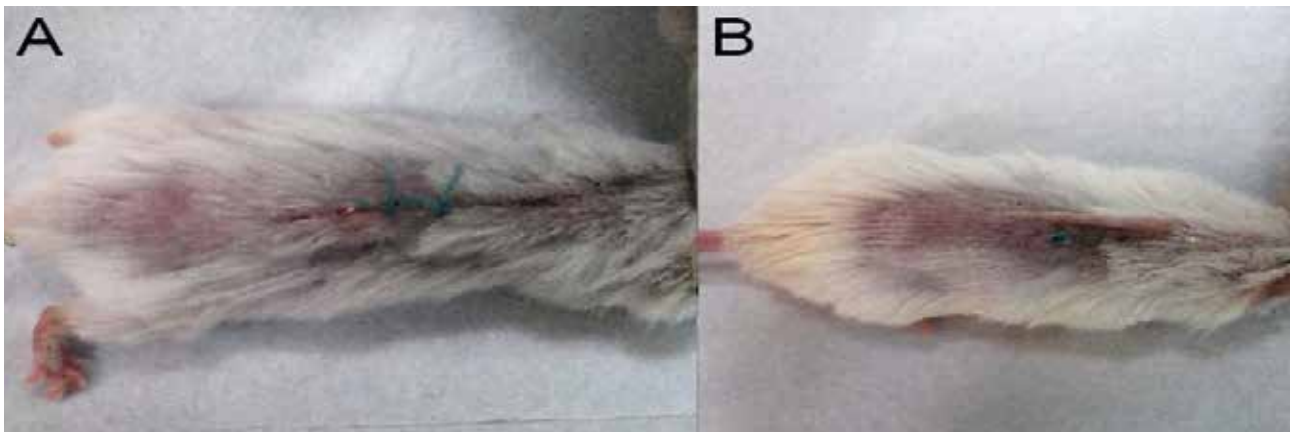


Figure 1: Clinical effect of momordica on control group (group 1, A) and the study group (group 3, B)

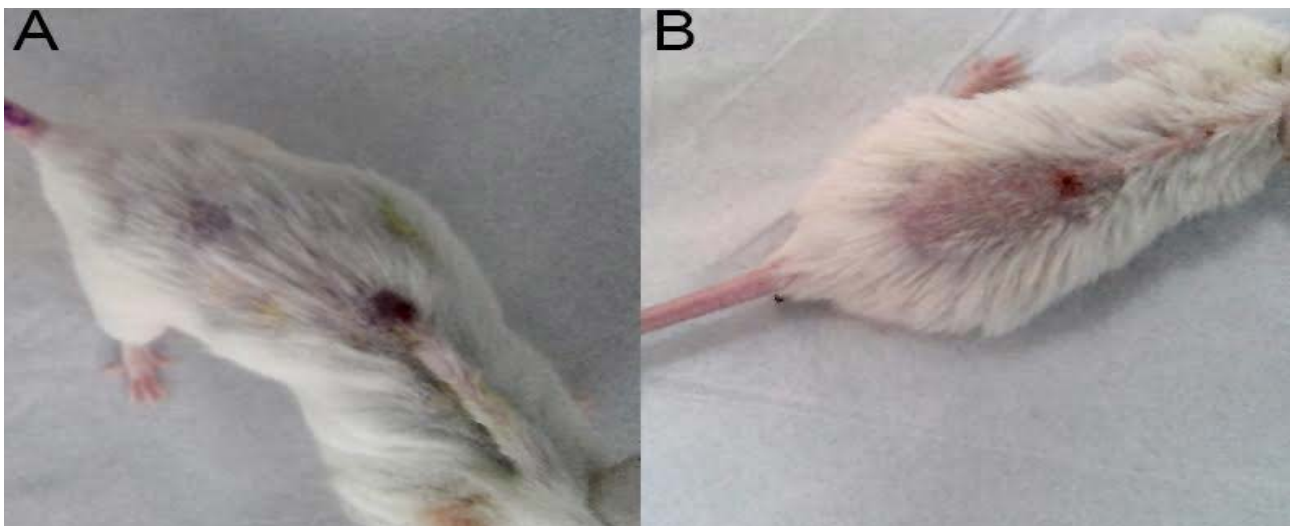


Figure 2: Clinical effect of momordica on control group (group 2, A) and the study group (group 4, B)

Table 1: The gene expression values (± standard deviation) in each group

	Wound healing control group (group 1) group	Burnhealing control group (group 2) (group 3)	Wound healing study group	Burnhealing study (group 4)
VEGFA	0,58±0,11	2,68±0,02	2,37±0,03	2,00±0,11
VEGFB	0,93±0,22	3,85±0,09	4,29±0,01	2,75±0,12
VEGFC	1,17±0,12	4,06±0,11	43,71±0,12	3,10±0,13
FGF2	0,03±0,15	0,12±0,08	0,14±0,11	0,13±0,22
EGF	0,49±0,34	1,12±0,11	1,13±0,22	0,68±0,22

Due to our findings, the expression levels of *VEGFA, VEGFB, VEGFC,FGF2* and *EGF* genes increased significantly in the study group according to the control group ($p<0.05$) (Figure 3).

In the burn healing group, the expression level of *VEGFA* was found approximately 2 in the study group whereas 2,68 in the control group. *VEGFB* gene expression levels were 2,75 (in the study group) and 3,85 (in the control group). For *VEGFC* gene, 3,1 and 4,06 were found in the study and the control groups respectively. The expression levels of FGF2 was found 0,13 in the study group and 0,12 in the control group. For EGF gene, 0,68 and 1,12 were found in gene expression results in the study and the control group (Table 1).

Due to our findings, there was no significant difference between control and study group for the expression levels of *VEGFA, VEGFB, VEGFC,FGF2* and *EGF* genes ($p>0.05$) (Figure 4).

Figure 3: The gene expression differences in wound healing group (red bars) compared to controls (blue bars).

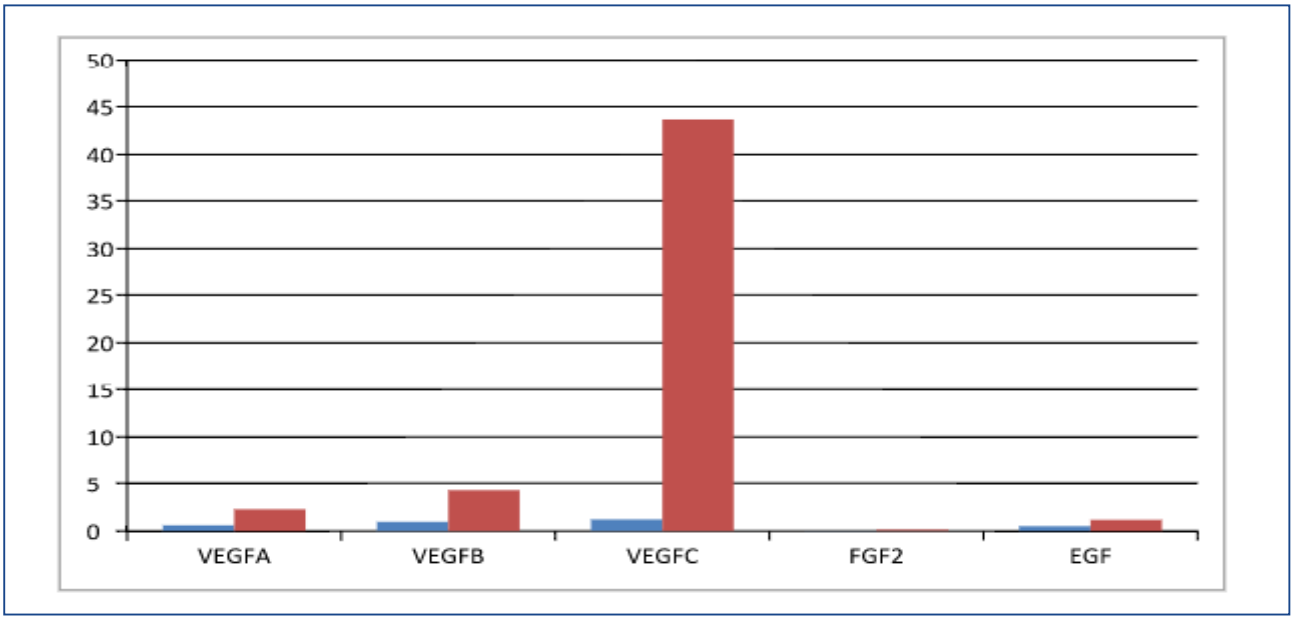
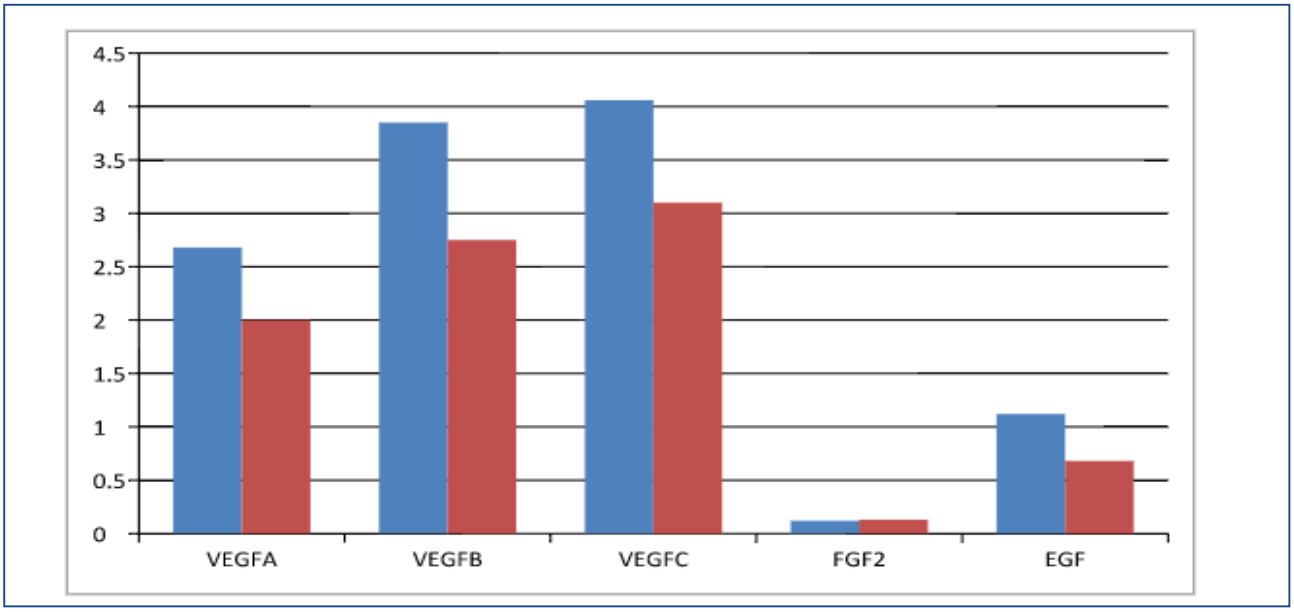


Figure 4: The gene expression differences in burn healing group (red bars) compared to controls (blue bars).



DISCUSSION

Momordica charantia is a plant the family Cucurbitaceae, known as bitter melon. It is used for various diseases from stomach to burn traditionally[11]. In cancer treatment, bitter melon leaf extract is particularly used; the extract has been shown to cause apoptosis in cancer cells and caspase dependent mitochondrial ways[17]. *Momordica charantia* is used for nematode infections as a folkloric medicine in Togo. Beloin at al. reported that momordicins I and II (obtained from momordica leaf) have anthelmintic properties[18]. Bitter melon is used for treating malaria in all South Asian countries, Panama and Colombia[19]. It is also used traditionally against viral diseases such as chickenpox and measles in Togo. Experimental studies have revealed that this plant occurs resistance against human immunodeficiency virus (HIV) and herpes virus[20,21]. One of

the content of *Momordica charantia* named MAP30 (Momordica anti-HIV protein, 30 kDa) is known to be effective against the HIV virus agent. Sun et al. described that it reduces the formation of Kaposi's sarcoma, caused by AIDS[22]. Gadang et al. explained that momordica might increase the expression of "peroxisome proliferator-activated receptor-γ gene" at the fatty tissue and reduces the effects of metabolic syndrome through NF-kB[23]. Sridhar et al. demonstrated that the insulin resistance, insulin sensitivity and glucose tolerance was increased in rats fed with *Momordica charantia*[24]. Tan et al. revealed that *Momordica charantia* has a healing potential in diabetes and obesity[25]. Vardi et al. studied the effect of momordica extract on blood sugar. They found that it was reduced the effects of diabetes on blood glucose in diabetic mice[26]. All these studies suggest that *Momordica charantia* might be used in the treatment of diabetes mellitus[27]. Krawinkel et al. found antioxidant molecules in momordica. It is known that antioxidants are important in maintaining the stability of the cell and prevent cancer development in humans[28]. *Momordica charantia* is widely used in Turkey for the treatment of gastric diseases and wound healing. Yeşilada and his colleagues have found that it may be useful in the treatment of peptic ulcer[5]. So, we aimed to observe the effects of wound healing in *Momordica charantia* application in mice. We also tried to investigate its possible effects on the expression of several selected genes.

Wound healing is a process of the skin or to the self-renewal and repair after damage to any other organs[12]. There are four stages of healing; homeostasis, inflammation, proliferation, and formation of new (remodeling). It regulates many pathways that play a role in the repair of the damage came in many complex biochemical processes occur after scar formation. Damage formation of platelets in the first few minutes arrives at the damaged tissue, they aggregate as activated. Thus the coagulation cascade activates, stops bleeding (homeostasis). In the inflammation stage of wound, bacteria and cell debris are removed by phagocytosis by leukocytes. At the wound site, cell migration and division are activated by PDGF from platelets. After this stage, the proliferation phase is passed. Proliferation phase includes angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction[29]. In angiogenesis, vascular endothelial cells form new blood vessels. Fibroblasts grow and contribute to the formation of granulation tissue and fibroplasia. In addition, they provide plenty of extracellular collagen and fibronectin release by editing the matrix. So wound re-epithelialization of the area is started and the epidermis starts to fill with new tissue. In wound contraction, myofibroblasts narrow the lip wound. Wound lips combined with apoptosis of unnecessary cells. Collagen fibrils rearranged the in the tense region in remodeling stage. Too many cells are removed by apoptosis. Wound healing process is quite complex and delicate embodiment. In this stage if there is any pause or any error, the healing process leads to poorly healed (Scars left) or badly healed scars[12]. In the proliferative phase of wound healing, the VEGF gene family with associated genes such as epidermal growth factor (epidermal growth factor-EGF), platelet derived growth factor (platelet derived growth factor-PDGF), fibroblast growth factor (fibroblast growth factor- FGF) and transforming growth factor (transforming growth factor-TGF) play an active role[13]. Complete and uniform amount of wound healing depends on releasing of these factors at proper amount and proper time and communication with the other growth factors. In this study the effect of *Momordica charantia* application wound and burn healing in was examined. So, we found that *Momordica charantia* has a healing effect on wounds, but has no healing effect on burns. The healing effects of *Momordica charantia* on wounds support literature findings[5,13]. The absence of the healing effect in *Momordica charantia* treatment on burns is an original finding.

The gene expression levels of *VEGFA, VEGFB, VEGFC, FGF2* and *EGF* genes were found significantly increased in wound healing group, showing the healing effect of *Momordica charantia* on wounds (Table 1). These results support the healing effect of *Momordica charantia* on wounds, observed by clinically. Thus, the molecular gene expression results are valuable for showing the healing promoting effect of momordica via inducing angiogenesis in wound areas.

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Celon Radiofrequency Versus Coblator, Alone or With Adenoidectomy

Adenoidektomi Eşliğinde veya Yalnız Yapılan, Celon Radyofrekans ve Koblator Uygulamalarının Karşılaştırılması

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ABSTRACT

Objective: The efficacy of radiofrequency tonsil ablation of the pediatric population were investigated.

Methods: 79 patients (46 males and 33 females) with grade 3-4 tonsillar hypertrophy±concurrent adenoid hypertrophy were included. Group1: Celon radiofrequency, Group2:Celon Radiofrequency+adenoidectomy, Group3: Coblator , and Group 4: Coblator+adenoidectomy were applied. The intertonsillar distance-ITD) was measure. Pain assessment was made at postoperative (PO) period by Wong Baker Faces Pain Rating Scale (WBFPRS).

Results: ITD of group1 was significantly higher than group2; group4 at PO-1st week and 1-12th months. Similarly, ITD of group3 was significantly higher than group2; group4 at PO-1st day, PO-1st week and 1-12th months. In Celon group, significant enlargement of air passage was detected after 7 days of the operation, whereas in Coblator group it was just at the 1st postoperative day and so on.

Conclusion: In early postoperative period and at 1st year controls, Coblator device caused more improvement air passage compared to Celon group.

Key words: *Olympus Celon, Coblator II, radiofrequency, tonsil ablation, adenoidectomy.*

ÖZET

Amaç: Pediyatrik hasta grubunda, radyofrekans tonsil ablasyonun etkinliği araştırılmıştır.

Yöntemi: 79 hasta (46 erkek, 33 kadın) çalışmaya dahil edildi. Hastaların tamamında grade 3 veya 4 tonsil hipertrofisi ve\veya adenoid hipertrofisi mevcuttu. Grup1: Celon radyofrekans, Grup 2: Celon Radyofrekans+adenoidektomi, Grup 3: Koblator, ve Grup 4: Koblator+adenoidektomi uygulamaları yapıldı. Tonsiller arası mesafe ölçüldü. Ağrı değerlendirilmesi ise Wong Baker Faces Ağrı Değerlendirme Skalası(WBFPRS) ile yapıldı.

Bulgular: Tonsiller arası mesafe, Grup 1'de, Grup 2 ve Grup 4'e oranla postoperatif 1. hafta ve 1. ve 12. aylarda anlamlı olarak fazla idi. Benzer olarak tonsiller arası mesafe, Grup 3'te Grup 2 ve Grup 4'e oranla, postoperative 1. gün, 1. ay ve 1 ve 12. aylarda anlamlı olarak fazlaydı. Celon grubunda, hava pasajı postoperative 7. günde anlamlı olarak açılmıştı. Koblator grubunda ise 1. günde ganlamlı hava yolu açıklalığı izlendi.

Sonuç: Erken postoperatif dönem ve kontrolleme kablator grubunda celon grubuna göre daha iyi hava yolu açıklığı sağlandı.

Anahtar kelimeler: *Olympus Celon, Coblator II, radyofrekans, tonsil ablasyon, adenoidektomi.*

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INTRODUCTION

Tonsillar hypertrophy can lead to symptoms of upper airway obstruction such as apnea, dysphagia and dysphonia in children and adults[1]. Besides traditional tonsillectomy, there are many options in the treatment of obstructive tonsillar hypertrophy like laser, electrocautery, cryotherapy, suction diathermy, monopolar and bipolar diathermy, radiofrequency ablation, and Coblation technology. Whereas there is still no consensus on the optimal technique. All of the techniques were designed to solve the problems and to reduce the major morbidities like postoperative dehydratation, pain and bleeding[2].

Three basic surgical procedures were defined for the treatment of tonsillar hypertrophy: (1) Completely excision of tonsil by dissection from tonsillar capsule (tonsillectomy), (2) near-total removal of tonsils by maintaining tonsillar capsule and an amount of tonsillar lymphoid tissue (subtotal/partial tonsillectomy), and (3) reduction of tonsillar lymphoid elements by maintaining the surface mucosa of tonsil[3].

While eliminating the symptoms of obstructive tonsillar hypertrophy, all of these techniques carry the risk of varying amounts of pain, dysphagia, and bleeding[4-6]. Radiofrequency provides a reduction of the total soft tissue volume of tonsils in a period of 4-6 weeks and has a significant number of advantages compared to the procedures of total and partial tonsillectomy[7,8].

This technique usually well-tolerated with minimal postoperative pain, dysphagia and less risk of bleeding; and can be performed under local anesthesia. Return to the daily life may be possible in 1-2 days. Additionally, satisfactory changes have also been reported in improvement in daytime sleepiness, snoring, sound quality,swallowing and throat irritation[9].

There are different radiofrequency devices, used for this purpose: Somnus (Gyrus), Entec Coblator, Ellman, Celon (Olympus) are some of them. Each device basically works on the same principle but the heat energy produced and frequency range varies[3]. Celon and Coblator are devices which produce low heat and target minimal penetration and damage to surrounding tissue. These devices work near radiofrequency energy ranges. However, Celon runs as energy-controlled, but Coblator is not energy controlled and produces less heat to the surrounding tisssue compared to the other radiofrequency energy technologies.

In this study, we aimed to investigate the efficacy and post-operative results of radiofrequency tonsil ablation of the pediatric population. Two different devices of Celon and Coblator were used; and efficacy on treatment outcomes were evaluated.

MATERIAL AND METHODS

This retrospective study was designed to investigate the difference between Celon and Coblator radiofrequency devices used for tonsil ablation. The data was obtained for 2007-2011 years in Antalya Private Anadolu Hospital and Antalya Private Medisu Hospital. The study was approved by Antalya Training and Research Hospital, Clinical Trials Ethics Committee (No:55/8, Date: 26.02.2015)

Subjects

Retrospective data of 79 patients (46 males and 33 females) admitted ENT Clinics with complaints of snoring and tonsillar hypertrophy were included into the study. There were no recurrent tonsillitis history. Tonsillar size was graded on 0-5 scale[10]; and grade 3-4 tonsils were included: (Grade 0: 0 tonsils can not be seen even when lateralized anterior plica, Grade 1: tonsils can be seen when lateralized anterior plica, Grade 2: tonsils can be seen in the level of the anterior plica, Grade 3: tonsils located between the midline and anterior plica, but closer to anterior plica, Grade 4: tonsils located between the midline and anterior plica, but closer to midline, Grade 5: tonsils classifed as the kissing tonsils[10]. In the present study, Grade 5 tonsils were not included into the study due to the risks of edema and obstruction postoperatively. Adenoid size was determined by endoscopic view and the children with over than 50% obstruction of the choana bilaterally were underwent adenoidectomy.

There were 4 groups. In all groups, grade 3-4 tonsillar hypertrophy; and in groups 2 and 4, adenoid hypertrophy was present:

Group 1: Celon radiofrequency (n=13): There were 7 males (53.8%) and 6 females (46.2%) with a mean age of 10.84±2.30 years.

Group 2: Celonradiofrequency+adenoidectomy (n=28): There were 18 (64.3%) males and 10 (35.7%)

females with a mean age of 7.46±1.66 years.

Group 3: Coblator (n=11): There were 7 (63.6%) males and 4 (36.4%) females with a mean age of 11.00±1.78 years

Group 4: Coblator+adenoidectomy (n=27): There were 14 males (51.9%) and 13 (48.1%) females with a mean age of 7.33±1.77 years

Children with acute respiratory infections for last month; having pulmonary and/or neurological diseases, or craniofacial abnormalities were not included into the study.

This study was conducted in accordance with Helsinki Declaration[11].

METHODS

Anestesia and postoperative medication

All surgical procedures were performed under general anesthesia. Iv induction, with 5-7 mg / kg thiopental, 1.0-2.0 mcg / kg fentanyl, 0.1 mg / kg vecuronium using orotracheal intubation was performed. Anesthesia was maintained by nitrogen protoksid 50%, 50% oxygen and was maintained with 1% Isoflurane. Spiral tube intubation was used in all patients. Post-operative analgesia was given in every 6 hours period of 15 mg / kg paracetamol All patients received postoperatively 1 week 50 mg / kg per day in two doses of ampicillin and sulbactam at first day and methylprednisolone was administered 1 mg / kg in a single dose.

Surgical method

In this study, the Olympus Celon radiofrequency device (Olympus Corporation, Teltow, Germany) and Coblator II device (Arthrocare Corporation, Austin, Texas,USA) were used. Features of the devices were demonstrated on Table 1. In both groups of Celon radiofrequency and CoblatorII, 6 to 8 applications were made to the two palatine tonsils using the soft palate probes. There was approximately 1 cm distance from each application points. Attention was paid to the establishment of the probes at submucosal region to prevent mucosal damage. Similarly, penetration of the probe to the tonsil capsule and muscular layer were denied. Operations carried out by the same operator (F.B., 1st author of the study).

Table 1: Features of the Olympus Celon Radiofrequency Device and Coblator II Device

	Olympus Celon Radiofrequency Device	Coblator II device
Probe	Prosleep plus	Reflex ultra 55 plasma wand
Frequency	300 khz-2 MHz	100-500 kHz
Heat	<100 oC	40-70 oC
Application of the device	Includes acoustic feedback and application is made in accordance with this notice	The tissue-specific power Coblator applied for the period determined by the operator. Applied for 20 -25 seconds.

All patients were followed up in the hospital for the first 24 hours post-operatively. Analgesic requirements were also recorded.

Post-operative follow-up:

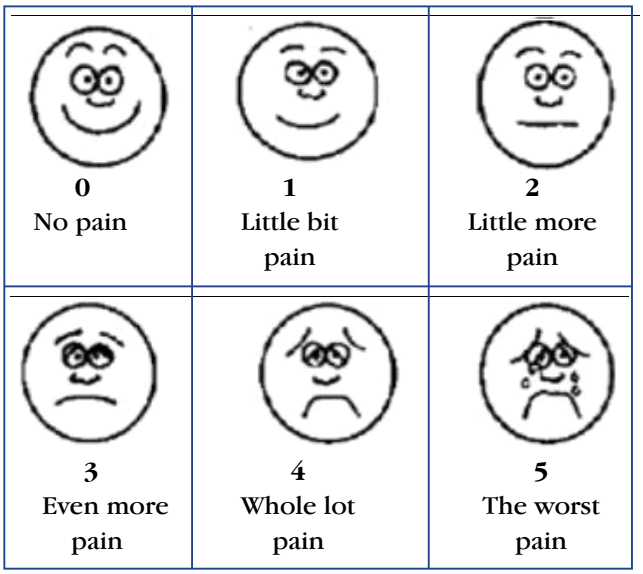
Air passage (Measurements of the Inter Tonsillar Distance-ITD)

The air passage between palatine tonsils (ITD) was measured and recorded at the narrowest point preoperatively and postoperatively (1st day, 1st week; 1st, 6 th and 12th months) in milimeters by the second clinician. In patients with respiratory tract infections, measurements were performed after all the signs and the symptoms have improved.

Wong Baker Faces Pain Rating Scale (WBFPRS)

Pain assessment in patients was made in post-op period at intervals of 6 hours in the 1st day; and between 2nd to 7th days, once a day using Wong Baker Faces Pain Rating Scale (WBFPRS) (Figure 1). Evaluation was performed on 0-5 scale: 0: No pain, 1: Little bit pain, 2: Little more pain, 3: Even more pain, 4: Whole lot pain and 5: The worst pain.

Figure 1: Wong Baker Faces Pain Rating Scale (WBFPRS)



Statistical analysis: All study data were evaluated by "Statistical Packages for the Social Science" (SPSS) 16.0 program. Kruskal Wallis Variance Analysis, Mann Whitney U Test with Bonferroni adjustment, Chi-square test and Linear Regression Analysis (Backward LR) tests were used.

$p<0.05$ value was considered as statistically significant. When Bonferroni adjustment was performed, padjusted <0.0125 was considered as statistically significant

RESULTS

Age, gender and treatment results of groups were shown on Table 2. Between the groups 1-4, in terms of gender, there was no statistically significant difference by Chi-Square test ($p> 0.05$).

Air passage between tonsils (ITD) were demonstrated at pre-operative and post-operative (PO) (1st day, 1st week, 1st month, 6th month and 1st year) period (Table 2, Figure 2). The difference between groups was analyzed by Kruskal Wallis Variance analysis and there was significant difference between group 1-4 at all measurement items ($p<0.05$). To find the value which caused difference, pairwise comparisons were performed by Mann Whitney U Test with Bonferroni adjustment (Table 3)

Figure 2: Intertonsillar distance values of groups

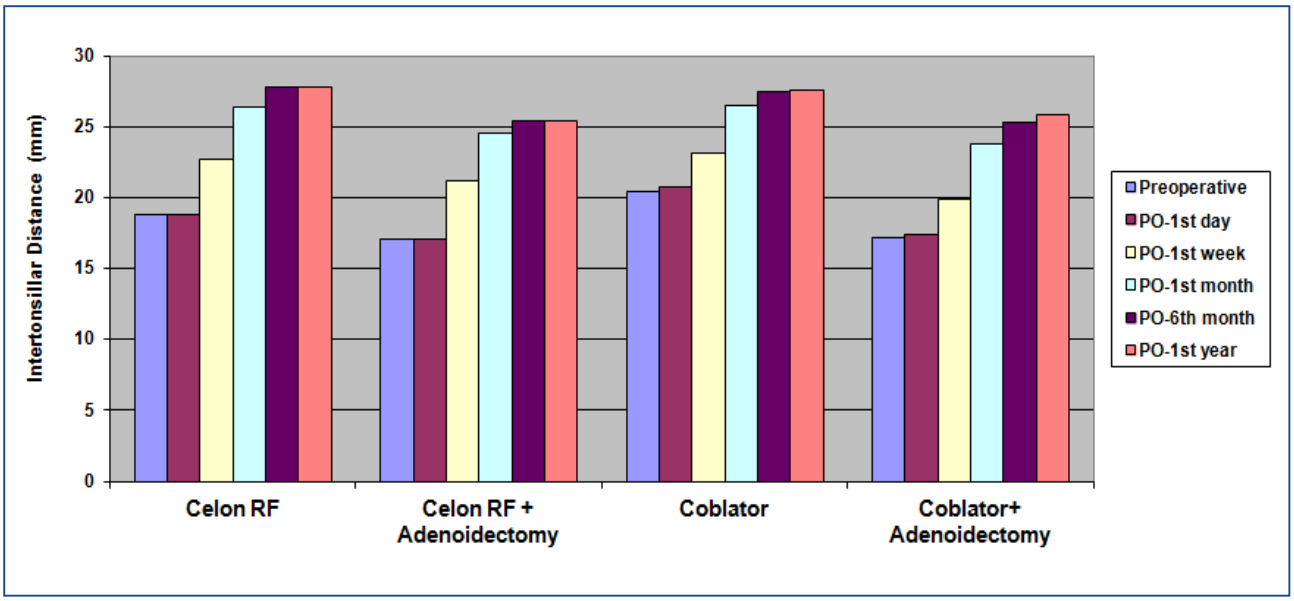


Table 2: Age, gender and treatment results of groups

		Treatment Groups				
		Celon RF (n=13)	**Celon + Adenoidectomy (n=28)**	**Coblator (n=11)**	**Coblator+ Adenoidectomy (n=27)**	
		Mean± Std. Dev.	**Mean± Std. Dev.**	**Mean± Std. Dev.**	**Mean± Std. Dev.**	**P***
Age		10,84±2,30	7,46±1,66	11,00±1,78	7,33±1,77	0.000
		n (%)	n(%)	n(%)	n(%)	p**
Gender	**Male**	7(53.8%)	18(64.3%)	7(63.6)	14(51.9%)	P=0.642
	Female	6(46.2%)	10(35.7%)	4(36.4)	13(48.1%)	$\chi 2$=0.216
		Mean± Std. Dev.	Mean± Std. Dev.	Mean± Std. Dev.	Mean± Std. Dev.	P*
Inter tonsillar distance (ITD)	**Pre-operative**	18,76±2,27	17,10±1,52	20,45±0,93	17,22±1,84	0.000
	PO-1st day	18,84±2,54	17,10±1,49	20,72±1,55	17,37±1,75	0.000
	PO-1st week	22,69±3,06	21,14±1,86	23,09±1,13	19,92±1,70	0.000
	PO-1st month	26,38±2,75	24,50±1,62	26,45±1,29	23,74±1,89	0.000
	PO-6th month	27,76±2,55	25,35±1,49	27,45±1,50	25,33±1,88	0.000
	PO-1st year	27,76±2,55	25,39±1,57	27,54±1,57	25,81±1,59	0.000

Table 2: Age, gender and treatment results of groups

		Treatment Groups				
		Celon RF (n=13)	Celon + Adenoidectomy (n=28)	Coblator (n=11)	Coblator+ Adenoidectomy (n=27)	
		Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	p*
Analgesic stop time		3,0 (1.0-4.0)	3,0 (1.0-5.0)	2,0 (2.0-4.0)	3,0 (1.0-5.0)	0.174
WBFPRS	PO-1st day	4,0 (2.0-6.0)	4,0 (2.0-6.0)	4,0 (2.0-6.0)	4,0 (0.0-6.0)	0.886
	PO-2nd day	2,0 (2.0-4.0)	2,0 (2.0-4.0)	2,0 (2.0-6.0)	4,0 (0.0-6.0)	0.374
	PO-3rd day	2,0 (0.0-4.0)	2,0 (0.0-4.0)	2,0 (0.0-4.0)	2,0 (0.0-4.0)	0.718
	PO-4th day	0,0 (0.0-2.0)	0,0 (0.0-2.0)	0,0 (0.0-4.0)	2,0 (0.0-4.0)	0.352
	PO-5th day	0,0 (0.0-2.0)	0,0 (0.0-2.0)	0,0 (0.0-2.0)	0,0 (0.0-4.0)	0.759
	PO-6th day	0,0 (0.0-2.0)	0,0 (0.0-2.0)	0,0 (0.0-0.0)	0,0 (0.0-2.0)	0.550
	PO-7th day	0,0 (0.0-0.0)	0,0 (0.0-0.0)	0,0 (0.0-0.0)	0,0 (0.0-2.0)	0.829

**p : value shows the results of Kruskal Wallis Variance Analysis*
***p : value shows the results of Chi-Square test*
WBFPRS : Wong Baker Faces Pain Rating Scale

Air passage (ITD) of Celon group was significantly higher than those of Celon+adenoidectomy; and coblator+adenoidectomy groups at PO-1st week and 1-12th months. Similarly, air passage (ITD) of Coblator group was significantly higher than those of Celon+adenoidectomy; and coblator+adenoidectomy groups at PO-1st day, PO-1st week and 1-12th months (padjusted<0.0125) (Table 3).

Table 3: Mann Whitney U Test with Bonferroni adjustment*

		Groups 1-2	Groups 1-3	Groups 1-4	Groups 2-3	Groups 2-4	Groups 3-4
Age	z	-3.873	-0.029	-3.873	-4.177	-0.395	-4.154
	p**	0.000	1.000	0.000	0.000	0.693	0.000
Pre-operative	z	-2.866	-2.109	-2.407	-4.658	-0.146	-4.126
	p**	0.004	0.047	0.016	0.000	0.884	0.000
PO-1st day	z	-2.228	-2.111	-1.960	-4.339	-0.662	-4.027
	p**	0.026	0.041	0.052	0.000	0.508	0.000
PO-1st week	z	-2.688	-0.503	-3.554	-2.965	-2.343	-4.260
	p**	0.007	0.649	0.000	0.003	0.019	0.000
PO-1st month	z	-2.831	-0.537	-3.277	-3.067	-1.420	-3.626
	p**	0.004	0.608	0.001	0.002	0.156	0.000
PO-6th month	z	-3.136	-0.650	-3.007	-3.293	-0.009	-3.037
	p**	0.001	0.531	0.002	0.001	0.993	0.002
PO-1st year	z	-3.074	-0.559	-2.695	-3.212	-1.021	-2.786
	p**	0.002	0.608	0.007	0.001	0.307	0.005

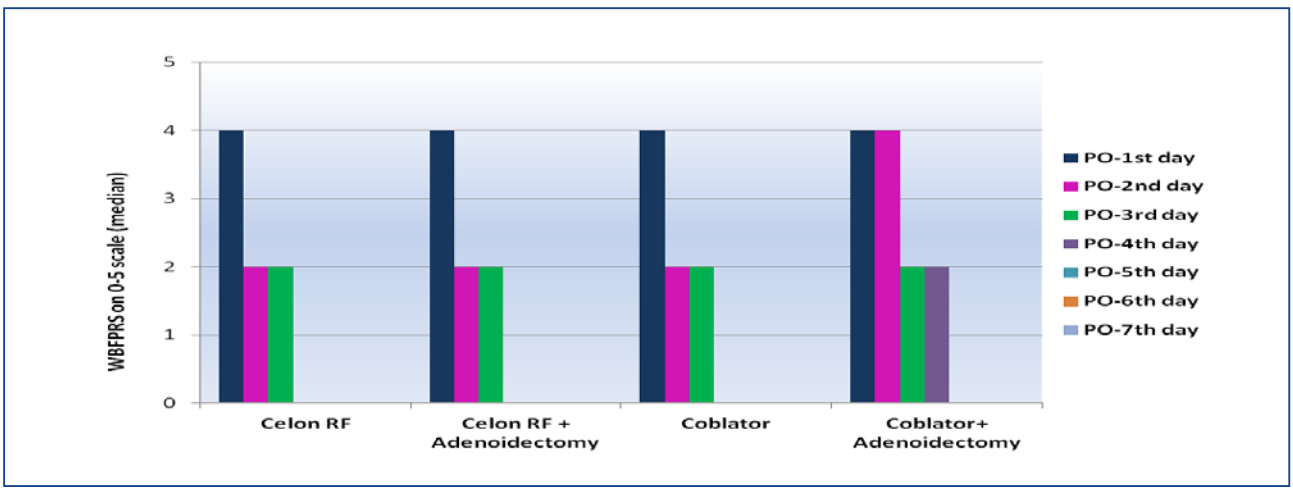
**PO : Post-operative*
***p : adjusted<0.0125 was considered as statistically significant*

In Celon group, significant enlargement of air passage at the narrowest distance between tonsils (ITD) was detected after 7 days of the operation, whereas in Coblator group significant enlargement of air passage at ITD at the narrowest section was detected just at the 1st postoperative day and so on.

There was no significant differences between air passages (ITDs) of Celon and Coblator groups; and Celon+adenoidectomy, and Coblator+adenoidectomy groups (padjusted>0.0125) (Table 3).

Analgesic stop times; and Wong Baker Faces Pain Rating Scale (WBFPRS) values at post-operative 1st-7th days of groups 1-4 were shown as median on Table 2, Figure 3. There was no significant difference between groups 1-4 by Kruskal wallis Variance Analysis ($p > 0.05$).

Figure 3: Wong Baker Faces Pain Rating Scales (WBFPRSs) of the groups



By Linear Regression Analysis (Backward LR), the effecting factors for each of the ITDs at PO-1st day to PO-1st year, analgesic stop time and WBFPRSs at PO 1st to 7th days were evaluated in all groups (Covariates: Age, gender, Celon RF (Code 1), Coblator (Code 2) groups; adenoid vegetation condition (Code 1: Present, Code 2: absent), group 1-4:Celon RF (Code 1), Celon RF+adenoidectomy (Code 2), Coblator (Code 3) and Coblator+adenoidectomy: Code 4; ITDs at all measurement times, analgesic stop time, WBFPRS results at all days)

- Coblator versus Celon group: In Coblator group (Group3), the air passage between tonsils (ITD) was larger on the 1st day and 1st year postoperatively compared to Celon group. In this group, enlargement of the air passage occurs earlier than Celon group (Group 1). Whereas in Celon Group (group 1), air passage between tonsils were larger at PO 1st week and PO 6th month significantly ($p<0.05$).

- PO air passage: In children with larger PO air passage (ITD) at 1st year, the air passage were also higher at PO 1st week and PO 6th month. Whereas, ITDs were lower at 1st month ($p<0.05$). It shows that, air passage were enlarged at PO 1st week; and probable reactionary enlargement of tissues after RF applications, tonsil size got bigger at 1st month resulted lower ITDs. Fibrotic effect of RF become obvious again at 6th month and 1st year postoperatively.

- Analgesic stop date was lower in Coblator group (Group 3) compared to Celon group (Group 1) ($p<0.05$).

- WBFPRSs were usually positively related to each other for each days postoperatively. It means, the children had lower pain at the 1st day PO, also had lower pain at the 2nd-7th days PO ($p<0.05$).

- The presence of adenoid vegetation with over than 50% obstruction of the choana bilaterally and underwent adenoidectomy, felt lower pain via lower WBFPRS results at 5th PO day significantly ($p<0.05$). It may be related that obstruction at nasal breathing due to adenoid vegetation caused more discomphort of the children; and after removal of adenoid tissue, the releasing of nasal obstruction disappeared at 5th day.

Table 4: The results of Linear Regression Analysis (Backward LR)*

Dependent variable	Independent confounding factors	p	Beta
PO-1st day ITD	Celon RF (Code 1), Coblator (Code 2) groups	0.026	0.141
	Preoperative ITD	0.000	0.496
	PO-1st week ITD	0.000	0.404
	Analgesic stop date	0.024	-0.128
PO-1st week ITD	Celon RF (Code 1), Coblator (Code 2) groups	0.000	-0.232
	Preoperative ITD	0.013	0.276
	PO-1st day ITD	0.007	0.281
	PO-1st monthITD	0.000	0.547
	PO-6st month ITD	0.010	-0.673
	PO-1st year ITD	0.027	0.515
PO-1st month ITD	PO-1st week ITD	0.000	0.384
	PO-6st month ITD	0.000	0.936
	PO-1st year ITD	0.040	-0.333
	WBFPRS-2nd day	0.023	-0.115
	WBFPRS-4th day	0.022	0.115
PO-6st month ITD	Celon RF (Code 1), Coblator (Code 2) groups	0.026	-0.054
	PO-1st week ITD	0.006	-0.112
	PO-1st month ITD	0.000	0.311
	PO-1st year ITD	0.000	0.785
PO-1st year ITD	Celon RF (Code 1), Coblator (Code 2) groups	0.001	0.089
	PO-1st week ITD	0.004	0.132
	PO-1st month ITD	0.048	-0.145
	PO-6st month ITD	0.000	1.008
Analgesic stop date	Celon RF (Code 1), Coblator (Code 2) groups	0.016	-0.163
	WBFPRS-1st day	0.004	0.263
	WBFPRS-2nd day	0.001	0.343
	WBFPRS-3rd day	0.009	0.240
	WBFPRS-7th day	0.026	0.162
WBFPRS-1st day	WBFPRS-2nd day	0.011	0.307
	Analgesic stop date	0.000	0.452
WBFPRS-2nd day	WBFPRS-1st day	0.048	0.202
	WBFPRS-4th day	0.040	0.208
	WBFPRS-6th day	0.048	0.168
	Analgesic stop date	0.002	0.362

Table 4: The results of Linear Regression Analysis (Backward LR)*

Dependent variable	Independent confounding factors	p	Beta
WBFPRS-3rd day	WBFPRS-2nd day	0.023	0.260
	WBFPRS-5th day	0.002	0.329
	PO-6th month ITD	0.047	0.637
	PO-1st year ITD	0.031	-0.720
	Analgesic stop date	0.013	0.305
WBFPRS-4th day	WBFPRS-2nd day	0.010	0.287
	WBFPRS-5th day	0.000	0.387
	PO-1st week ITD	0.048	-0.266
	PO-1st month ITD	0.007	0.371
WBFPRS-5th day	WBFPRS-3rd day	0.006	0.258
	WBFPRS-6th day	0.004	0.257
	WBFPRS-7th day	0.012	0.199
	Preoperative ITD	0.032	-0.244
	PO-6th month ITD	0.042	-0.581
	PO-1st year ITD	0.024	0.665
WBFPRS-6th day	Adenoid vegetation (Code 1: present, Code 0: absent),	0.037	-0.176
	WBFPRS-2nd day	0.034	0.232
	WBFPRS-5th day	0.000	0.478
WBFPRS-7th day	WBFPRS-5th day	0.002	0.387

**WBFPRS : Wong Baker Faces Pain Rating Scales*

DISCUSSION

Radiofrequency is used in the treatment of snoring soft palate, inferior turbinate, base of the tongue and the palatine tonsils and in different periods of time and in different ranges in order to ensure volume reduction[7, 12-16]. Cottchia et al[17] compared the results of temperature-controlled radiofrequency (TCRF)+adenoidectomy and tonsillectomy+adenoidectomy in patients with polysomnography-proven OSAS. Patients received an average of 12 application for each tonsil, the average energy was 13681 j per treatment. Two groups were compared in terms of weight loss and pain after three months, no significant difference in both groups compared with respiratory disturbance index (RDI) values. TCRF has lower postoperative pain value, weight loss, and analgesic need, time to return to normal diet. Snoring, swallowing, speech disorder symptoms such as daytime sleepiness improved in 1 month in both groups at the same level .

Aim of reducing the volume of tissue with radiofrequency is to get comparable results and less morbibidity related to other minimally invasive office based treatments. Ferguson et al[13] stated that the application of multiple lesions caused more pain after the procedure compared to a solitary lesion, but the increase in the amount of energy supplied caused unplanned down-time away from work. They postulated that multiple lesions were not increasing the complication rates and multiple lesions were safe they suggested that the submucosal application to the soft palate with temperature-controlled radiofrequency energy is the key to reducing complications and post-operative pain.

Nelson[18] provided an average of 75% reduction of tonsil size in 1-year follow-up of radiofrequency. Most of the patients started to be fed with soft food postoperative 6hrs and in 5 days, thay started the normal food. The average time to return to normal daily activities was 3.9 days respectively. 3rd month polysomnography showed 84.2% reduction in the apnea index. Nelson[19] stated that total amount of energy to target tissue and temperature can be determined with RF and

this leads to less complications such as hemorrhage, edema, and infection.

It has been shown that postoperative pain and low morbidity is acceptable even with different radiofrequency devices which have Different properties in different target tissues causing volume reduction and varying amount of pain. complete excision of the tonsils, extended tonsil volume reduction or partial ablation can be performed for hypertrophic tonsils by using radiofrequency. At least one of them, tissue volume reduction, a procedure that can be used in an office environment[7]. Tonsil region is more easily be judged macroscopically after radiofrequency ablation in comparison to the results of the base of the tongue and soft palate.

Temperature controlled RF is a safe and effective procedure which provides significant improvement in symptoms and oropharyngeal air column in adult patients. Reduction of volume started at post-operative 4-6 weeks and continues until weeks of 16-18. Pediatric population, however, the question of whether it is highly effective in children population as compared to the adult population also occurs[8]. In another study with RF tonsil ablation in children have shown that returning to normal activity and amount of analgesics used is less than the adult population[2].

In the present study, only pediatric patients were included into study group; therefore the comparison is not possible with the adult group. We investigated the efficacy and post-operative results of radiofrequency tonsil ablation of the pediatric population. Two different devices of Celon and Coblator were used. In two groups, only tonsil ablation by Celon or Coblator were performed (Groups 1 and 3), whereas in groups 2 and 4, adenoidectomy were also performed besides RF tonsil ablation by Celon or Coblator.

Air passage (ITD) of Celon group was significantly higher than those of Celon+adenoidectomy; and coblator+adenoidectomy groups at PO-1st week and 1-12th months. Similarly, air passage (ITD) of Coblator group was significantly higher than those of Celon+adenoidectomy; and coblator+adenoidectomy groups at PO-1st day, PO-1st week and 1-12th months. In Celon group, significant enlargement of air passage at the narrowest distance between tonsils (ITD) was detected after 7 days of the operation, whereas in Coblator group significant enlargement of air passage at ITD at the narrowest section was detected just at the 1st postoperative day and so on. There was no significant differences of the ITDs between Celon and Coblator groups; and Celon+adenoidectomy, and Coblator+adenoidectomy groups. Analgesic stop times; and Wong Baker Faces Pain Rating Scale (WBFPRS) values at post-operative 1st-7th days of groups were not different.

By Linear Regression Analysis (Backward LR), in Coblator group (Group3), the air passage between tonsils (ITD) was larger on the 1st day and 1st year postoperatively compared to Celon group. In Coblator group, enlargement of the air passage occurs earlier than Celon group (Group 1). Whereas in Celon Group (group 1), air passage between tonsils were larger at PO 1st week and PO 6th month significantly.

In children with larger PO air passage (ITD) at 1st year, the air passage were also higher at PO 1st week and PO 6th month. Whereas, ITDs were lower at 1st month. It shows that, air passage were enlarged at PO 1st week; and probable reactionary enlargement of tissues after RF applications, tonsil size got bigger at 1st month resulted lower ITDs. Fibrotic effect of RF become obvious again at 6th month and 1st year postoperatively. Friedman, in their study, compared Somnus and Entec RF devices and concluded that Entec device is better for tonsil volume reduction and recovery[2].

Nelson[8] showed that a 3-month short-term follow-up study of the tonsil with radiofrequency ablation in adult patients resulted the expansion of the air passages at an average of 1.2 cm, and minimal pain and dysphagia. In this study, all the patients returned to the normal activities in a short period of 1-2 days. In another study, Nelson [9] evaluated results of the 12-month follow-up study and this time the airway expanded as 1.7 cm at 3rd month, 1.8 cm at 6 months and 2.4 cm at 12 months with minimal pain and dysphagia.

Friedman[2] suggested that tonsil ablation with radiofrequency tissue volume reduction of 50% on average is difficult to predict, such as 30 to 70%, and is a disadvantage of having a wide range. In this study, painful periods of pediatric cohort is 1.7 days and of adult group is 1.6 days respectively .

Analgesic stop date was lower in Coblator group (Group 3) compared to Celon group (Group 1).-WBFPRSs were usually positively related to each other for each days postoperatively. It means, the children had lower pain at the 1st day PO, also had lower pain at the 2nd-7th days PO. The presence of adenoid vegetation with over than 50% obstruction of the choana bilaterally and underwent adenoidectomy, felt lower pain via lower WBFPRS results at 5th PO day significantly ($p<0.05$). It may be related that obstruction at nasal breathing due to adenoid vegetation caused more discomphort of the children; and after removal of adenoid tissue, the releasing of nasal obstruction disappeared at 5th PO day.

Babademez et al[10] has determined the average painful peri-

od is 1.3 day. In the same study, the mean airway width is 4.1 cm preoperatively and 2.5 cm postoperatively in 21% of patients and the second session of application is needed . In their study, a shorter painful period were observed in patients treated with coblator than RF and laser therapy. Here, the average time to radiofrequency contradicts other studies that have been determined to be 5.2 days depending on the use of double ablation probe and being explained further edema.

Back et al[20] evaluated the RF ablation with MRI before and after the therapy in 10 patients and showed 70% clinical improvement, 30% of the patients recommended tonsillectomy. 13.6% decrease in the average volume of tonsil (3.7 to 41.5) showed. Day 2 provided an average return to normal daily activities. In the present study, WBFPRS evaluation showed that pain was "none" at 4th day in groups 1-3; and "none" at 5th day in group 4.

In our study, both devices caused improvement of the children's symptoms. In early postoperative period and at 1st year controls, Coblator device caused more improvement air passage compared to Celon group. If tonsil and adenoid hypertrophy were present in the same child, enlargement of air passage was lower than the children with grade 3-4 tonsil hypertrophy alone. Therefore, all childs with tonsil hypertrophy should also be examined in terms of adenoid vegetation and related choanal obstruction.

CONCLUSION

In our study, both devices caused improvement of the children's symptoms. In Coblator group (Group3), ITD was larger on the 1st day and 1st year postoperatively compared to Celon group. In children with larger PO air passage (ITD) at 1st year, the air passage were also higher at PO 1st week and PO 6th month.

Whereas, ITDs were lower at 1st month. It shows that, air passage were enlarged at PO 1st week; and probable reactionary enlargement of tissues after RF applications, tonsil size got bigger at 1st month resulted lower ITDs. Fibrotic effect of RF become obvious again at 6th month and 1st year postoperatively. If tonsil and adenoid hypertrophy were present in the same child, enlargement of air passage was lower than the children with grade 3-4 tonsil hypertrophy alone. Therefore, all childs with tonsil hypertrophy should also be examined in terms of adenoid vegetation and related choanal obstruction

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Klinik Araştırma / Clinical Research

Koru Proceedings

Serum Protein Elektroforez ve İmmünfiksasyon Elektroforez İstem Endikasyon Uyumu ve Sonuçları Bir Hastane Deneyimi

Serum Protein Electrophoresis & Immunofixation Electropheresis Results And Request Indications' Compliance A Hospital Experience

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ÖZET

Amaç: Serum protein elektroforezi (SPE) testi, klinik laboratuvarda serumda majör protein kompenenti olan albumin ve globulin fraksiyonlarını değerlendirmede kullanılan ucuz, kolay uygulanabilir bir testtir Anormal monoklonal proteinlerin tespiti monoklonal gammapati varlığının ispatı ve plazma hücre diskrazilerinin sıklıkla izlenen karakteristik bir özelliğidir. Biz de laboratuarımıza Eylül 2009-Ekim 2011 yılları arasında başvuran hastalarımızın SPE istemlerini ve endikasyaonlarının değerlendirmeyi amaçladık.

Yöntem: 2009 Eylül-2011 Ekim tarihleri arasında Dr. Abdurrahman Yurtarslan Onkoloji Eğitim ve Araştırma Hastanesi Biyokimya Laboratuvarına SPE istemi ile gönderilen hastaların tanıları, yaşları, cinsiyetleri, gönderen birimler, serum ve idrar immünfiksasyon test sonuçları çalışmaya dahil edildi.

Bulgular: SPE istemi olan 379 kadın 417 Erkek toplamda 796 vaka çalışmaya dahil edildi ve yaş ortalamaları sırasıyla 52,02 ± 15.78 yıl, 51,95 ± 15.79 yıl idi. SPE istemlerinin yapıldığı klinikler incelendiğinde istemlerin % 91.21 Hematoloji Kliniğinden % 2,1 Beyin ve Sinir Hastalıkları kliniğinden ve % 1, ise Nefroloji kliniğinden olduğu görülmüştür. SPE test istemi bulunan hastaların tanıları incelendiğinde ise en sık olarak plazma hücre diskrazisi hastalık grubundan (%40.45) istem yapıldığı görülmüştür. Ayrıca sırasıyla takip eden ilk beş test istem endikasyon nedenleri; lösemiler(% 11.68), kan ve

ABSTRACT

Objective: Serum protein electrophoresis (SPE) is cheap and easily applicable method which measures specific proteins in the serum such as albumin and globulin.

The detection of abnormal monoclonal proteins, which is referred to as monoclonal gammopathy, is a frequent, characteristic feature of plasma cell dyscrasia. In this study we aimed to review SPE results and indications in our laboratory with patients who consulted between September 2009-October 2011.

Methods: Our study has included patients with SPE request, between September 2009- September 2011 in Dr. Abdurrahman Yurtarslan Oncology Education and Research Hospital Biochemistry Laboratory as well as diagnosis, age, gender, department, serum and urine immunofixation electrophoresis test results. Data were analyzed using Excel 2010. Ratios of test request indications and department that demanded SPE test were calculated in percent

Results: 796 patients with SPE request were included in the study. 379 patients were female and 417 were male, while the mean age was 52.02±15.78 years, 51.95±15.79 years respectively. The most frequent diagnoses under indications was plasma cell dyscrasia while the other five indications were leukemia(11.68%), hematopoesis system disorders(10.67%), anemia(9.42%), B lymphoproliferative disorder(4.27%) and lumbago(3.01%) respectively.

Sorumlu Yazar :

Çiğdem SÖNMEZ

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kan yapıcı organların hastalığı (%10.67), anemiler(%9.42), B lenfoproliferatif hastalıklar (%4.27) ve bel ağrısı (%3.01) olarak izlendi. SPE istemlerinin Plazma hücre diskrazileri altında yer alan 322 vaka incelendiğinde 286 MM (% 88.8), 32 Plazmasitom (%9.9), 3 WM (% 0.99), 1 Amiloidoz olduğu görülmektedir.796 SPE istemi olan hastalardan 342 tanesinde SIFE, 223 tanesinde UIFE istemi bulundu. SIFE istemi olan 342 vakanın 167 (% 48)'sinde klonalite izlenirken 175 (% 52) klonalite izlenmemiştir. 223 UIFE'sinde ise 169 (% 75.7) klonalite görülmez iken sadece 54 (% 24.3) hastada klonalite görülmüştür.

Sonuç: Laboratuvarımız SPE testi endikasyonları ve klinikleri incelendiğinde hastanemiz onkoloji hastanesi olması doğrultusunda %100 uygun klinik ve %80 öntanı ve tanılara uygun istem yapıldığı görülmektedir. Takip ettiğimiz vakalar yaş cinsiyet ve monoklonal gammapati tipi olarak literatür ile uyumlu bulunmuştur.

Anahtar kelimeler: *Protein elektroforezi, Klonalite, İmmunfiksasyon elektroforezi, M protein*

The clinics who demanded SPE were Hematology and Bone Marrow Transplantation Center(91.21%), Neurosurgery(2,1%)and Nephrology Department(1%).

When we investigated the plasma cell dyscrasia we observed 286(88.8%)MM, 32(9.9%)plasmacytoma and 3(0.99%) WM and 1amyloidosis. Of 796 patients with SPE request, 342 had SIFE and 223 had UIFE test requests. When the results of the 342 patients with SIFE tests were evaluated, clonality was detected among 167(%48) patients, whereas 175(%52) patients had no monoclonal gammopathy. With the 223 UIFE test, 169(% 75.7) patients had no clonality, however clonality was identified among 54(%24.3) patients.

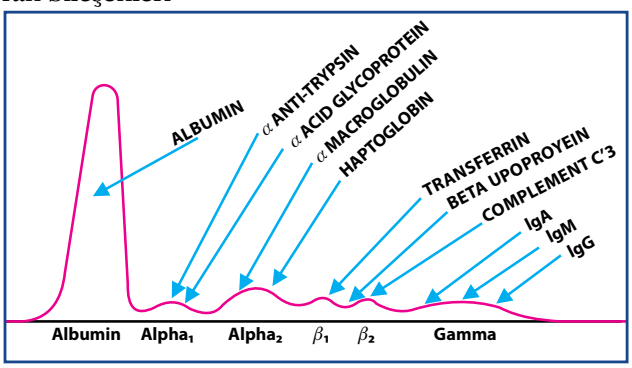
Conclusion: Our laboratory is observed that SPE test request was in accordance with appropriate clinical(100%) while the appropriate indications was 80% accordance with the initial diagnosis. The follow cases age, sex and type of monoclonal gammopathywere accordance with the literature.

Key words: *Protein electrophoresis, clonalite, immunfixation electropheresisi, M protein*

GİRİŞ

Serum protein elektroforezi (SPE) testi, klinik laboratuvarda serumda majör protein kompenenti olan albumin ve globulin fraksiyonlarını değerlendirmede kullanılan ucuz, kolay uygulanabilir bir testtir[1]. SPE 'de protein kompenentleri, uygun medium üzerinde, şekline, büyüklüğüne ve yüküne göre, elektriksel akım ile belli bir zaman aralığında katot ve anot bölgesi arasında yürütülerek fraksiyonlarına ayrılır ve boyanarak dansitometrik taranma sonrasında grafiksel olarak incelenir[2]. SPE ile proteinler, albümin, alfa-1(α), alfa-2, beta (β, β1-β2) ve gama (γ) bölgeleri olarak 5 bölümde değerlendirilir[1,2]. Albumin, majör serum protein bileşenidir ve anoda en yakın bölgede bulunur. Tipik bir protein elektroforez grafiği Şekil 1'de protein komponentleri ve onları oluşturan proteinlerin isimleri ile birlikte izlenmektedir.

Şekil 1. Tipik bir protein elektroforezi grafiği ve albümin, alfa1, alfa 2, beta ve gamma bölgesini oluşturan bileşenleri



SPE, vücuttaki protein komponentlerinin azalması ve/veya artışını değerlendirilerek karaciğer yetmezliği, nefrotik sendrom, demir eksikliği anemisi, gebelik, otoimmün hastalıklar plasma hücre diskrazileri gibi birçok hastalık grubunda da kullanılan bir test olmakla ile birilikte sıklıkla da anormal monoklonal proteinlerin tespiti ve takibi için kullanılır[1,3,4,5]. International Myeloma çalışma grubunun SPE testi istemi için endikasyonları belirlemiştir[4]. Bu endikasyon listesi Tablo 1'de izlenmektedir. Anormal monoklonal proteinlerin tespiti monoklonal gammapati varlığının ispatı ve plazma hücre diskrazilerinin sıklıkla izlenen karakteristik bir özelliğidir[5].

Tablo 1. İnternational Myeloma Çalışma grubunun SPE İstemi için belirlediği endikasyon listesi

Primer olarak monoklonal gamapati testi
Açıklanamayan periferal nöropati
Açıklanamayan anemi/sırt ağrısı/halsizlik/zayıflık
Hipergamaglobulinemi/ hipogamaglobülinemi
>40 yaş hastalarda ağır proteinüri
Açıklanamayan patolojik kırık veya litik lezyonlar
İdrarda Bence Jones proteini varlığı

Plazma hücre diskrazileri içinde multiple myeloma (MM), plazmositom, plazma hücreli lösemi, Waldenstörm Makroglobulinemisi (WM), Amiloidozis (AL) ve premalign hastalık grubu olan Önemi Bilinmeyen Monoklonal Gammopati (MGUS) ve Smoldering Multiple Myeloma (SMM) yer almaktadır[6,7].

Monoklonal gammapatinin tespiti ve tip tayini için serum immünfiksasyon elektroforezi (SIFE) ve idrar immünfiksasyon elektroforezi (UIFE) testleri hastaların, tanı takip ve prognozu açısından istenmesi gereken diğer laboratuvar testlerdir[4,8,9,10]. Bu testler, Monoklonal gammapatiye sahip hastaların tedavi etkinliğinin ve hastalık seyrinin takibi ve değerlendirilmesi açısından oldukça önemlidir. Serum örneklerinin değerlendirmesi yanında idrar testlerinin değerlendirilmesi tanısal hassasiyeti arttırdığı literatürde de izlenmektedir[10,11,12].

Biz de laboratuvarımıza Kasım 2009- Ekim 2011 yılları arasında başvuran hastalarımızın SPE istemlerini ve endikasyonlarının bunun yanında istenmiş olan SIFE ve UIFE testlerinin birlikte istem uygunluğu, yanı sıra monoklonalite görülme oranını ve tiplerini değerlendirmeyi amaçladık.

MATERYAL ve METOD

2009 Eylül -2011 Eylül tarihleri arasında Dr. Abdurrahman Yurtarslan Onkoloji Eğitim ve Araştırma Hastanesi Biyokimya Laboratuvarına SPE istemi ile gönderilen hastaların tanıları, yaşları, cinsiyetleri, gönderen birimler, serum ve idrar immünfiksasyon test sonuçları çalışmaya dahil edildi. SPE ve SIFE çalışmaları için laboratuvarımıza ulaşan jelli tüplerin (BD vacutainer SSTM II Advance tube,plymouth, UK)

3000 rpm'de 15 dakika santrifüj edilmesi ile ayrılan serum örnekleri çalışma gününe kadar 2-8 derece buzdolabında bekletildi.Çalışma günü bekleme süresi 7 günü aşacak olan tüm numuneler -20 derecede saklandı.UIFE çalışması için ise borik asit koruyucusu üzerine toplanan 24 saatlik idrar örneklerinin laboratuvara ulaşmsının ardından idrar örnekleri çalışma gününe kadar 2-8 derece buzdolabında bekletildi.Çalışma günü bekleme süresi 7 günü aşacak olan tüm numuneler -20 derecede bekletildi.İdrar örnekleri UIFE çalışma öncesi konsanstre edildi.

SPE çalışması, SIFE ve UIFE çalışmaları ise otomatize İnterlab G26 (İnterlab Srl,Roma İtalya) elektroforez cihazında İnterlab marka protein elektroforezi jelleri ile, SIFE ve UIFE ise İnterlab marka SCE232 M kiti (İnterlab Srl, Roma İtalya) ve İnterlab marka anti serumlar (İnterlab Srl,Roma İtalya) kullanılarak aynı marka jeller ile çalışıldı.

İmmünfiksasyon Elektroforezinin Yorumlanması:

İlk sütunda yer alan protein elektroforezinde alfa 2, beta ve gama globulin bölgelerinde monoklonal yapıda bir bant olup olmadığı bu bandın karşılığında G,A,M, bölgelerinde sınırları belirgin bir bandın varlığı kontrol edildi. Aynı immünglobulin sınıfında farklı yerlere göç eden birden fazla bant saptanacak olursa bu durumun gerçek biklonal bant olup olmadığı incelendi. Sadece IgG, IgM, IgA bölgelerinde veya kappa ve lambda bölgelerinde göç eden bantların varlığı ağır ve hafif zincir hastalıklar yönünde karar vermeden önce hata kaynağı olabilecek nedenler araştırıldı. Hafif zincir monoklonal gammopatisi tespit edildiği zaman IgD ve IgE anti serumları ile SIFE tekrarlandı.

İstatistik: Veriler istatistiksel olarak Excel 2010 ile değerlendirildi. Test istemi yapılan hastaların tanıları, hangi klinikten istendiği ve oranları yüzde (%) olarak hesaplandı. SPE istemi ile gelen hastların yaşları, cinsiyetleri SIFE ve UIFE istemleri monoklonal gammapati izlenip izlenmediği ve birlikte görülme oranları değerlendirildi.

BULGULAR

Vakalar: SPE istemi olan 796 hasta çalışmaya dahil edildi. Hastaların 379 kadın 417 Erkek iken yaş ortalamaları sırasıyla 52,02 ± 15.78 yıl, 51,95±15.79 yıl idi. Yaşların dağılım aralığı kadınlar için 16-90 yıl, erkekler için 11-84 yıl olarak izlendi. SPE istemi olan hastların tanı endikasyon dağılımları sayı ve yüzde oranları Tablo 2'de görülürken, İstem endikasyonlarının diğer başlığı altında yer alan ön tanılar hipertansiyon, tiroid hastalıkları, Behçet hastalığı, koagülasyon bozuklukları, trombositopeni, dispepsi, lökosit bozuklukları ve genel muayene olarak izlenmiştir. İstem yapan kliniklerin dağılımı Tablo 3'te izlenmektedir. 796 SPE istemi olan hastalardan 342 tanesinde SIFE, 223 tanesinde UIFE istemi bulundu. SIFE istemi olan 342 hastanın sonuçları değerlendirildiğinde 167 (% 48)'sinde klonalite izlenirken 175 (% 52) monoklonal gammopati izlenmemiştir. 223 UIFE'sinde ise 169 (% 75.7) hastada klonalite görülmez iken sadece 54 (% 24.3) hastada klonalite görülmüştür. SIFE ve UIFE sonuçlarının dörtlü tablo ile karşılaştırması Tablo 4'te izlenmektedir. SIFE'de paraprotein izlenen 167 hastanın UIFE'lerine bakıldığında 71 örnekte UIFE'de klonalite gözlenirken 47 hastanın UIFE'sinin negatif olduğu 49 hastada ise UIFE isteminin olmadığı görülmüştür.

SIFE'sinde klonalite görülmeyen 175 hastanın UIFE'lerinde ise 98 hastanın klonalite görülmez iken sadece 7 hastada idrar örneğinde klonalite izlenmiştir. 70 hastanın ise eşzamanlı UIFE istemi yapılmamıştır. SIFE'lerinde görülen paraprotein tiplerinin dağılımları ile ilgili bulgular Tablo 5.'de izlenmektedir. 175 paraproteine sahip hastaların 13 tanesinde biklonal gammopati izlenmiştir. Biklonalite izlenen paraprotein tipleri IgG-kappa, IgA-kappa, ve sadece hafif zincir Kappa ve Lambda' olarak görülmüştür.

Tablo 2. SPE istemi olan hastaların istem endikasyonlarının dağılım tablosu

Tanı (endikasyon) Nedeni	Sayı	Yüzdesi (%)
Lösemiler	93	11.68
Plazma Hücre Diskrazileri	322	40.45
B Lenfoproliferatif Hastalıklar	34	4.27
Malignite	21	2.63
Böbrek Yetmezliği	14	1.75
Anemiler	75	9.42
Lenfadenitler ve Lenadenopaitler	16	2.01
Bel Ağrısı	24	3.01
Myeloproliferatif hastalıklar	8	1.00
Karaciğer yetmezliği	7	0.87
Splenomegali	8	1.00
Kan ve kan yapıcı organların hastalığı	85	10.67
Diğer	89	11.18
Toplam	**796**	**100**

Tablo 3. SPE İstem Yapan Kiniklerin Dağılım tablosu

Klinik Adı	Sayı	Yüzde(%)
Hematoloji Kliniği	726	91.21
Nefroloji Kliniği	14	1.76
Dahiliye Kliniği	5	0.63
Fizik tedavi ve Rehabilitasyon Kliniği	2	0.25
Beyin Cerrahi Kliniği	17	2.14
Medikal Onkoloji Kliniği	15	1.88
Nöroloji Kliniği	2	0.25
Nükleer Tıp Kliniği	1	0.13
Ortopedi ve Travmatoloji Kliniği	5	0.63
Radyoterapi Kliniği	2	0.25
Genel Cerrahi Kliniği	3	0.38
Göğüs Hastalıkları Kliniği	1	0.13
Kulak Burun Boğaz Kliniği	2	0.25
Göğüs Hastalıkları Kliniği	1	0.13
Toplam	**796**	**100,00**

Tablo 4. SPE istemi olan 796 hastaların SIFE ve UIFE istem sayıları ve karşılaştırma tablosu

			UIFE N=223	Eş zamanalı test istemi		
			Pozitif	Negatif	SIFE istemi (+) UIFE istemi (+)	SIFE istemi (+) UIFE İstemi (-)
SİFE	Pozitif	167 (%48)	47	71	118	49
N= 342	Negatif	175 (%52)	7	98	105	70
			54 (%24.3)	**169 (%75.7)**	**223**	**119**

Tablo 5. SİFE'de paraprotein tiplerin dağılım sayıları ve yüzdeleri

Paraprotein tipi	Sayısı	Yüzdesi (%)
IG G-KAPPA	86	% 51.4
IG G-Lambda	29	% 17.4
IG A –KAPPA	13	% 7.8
IG A –LAMBDA	11	% 6.7
IG M-KAPPA	2	% 1.1
KAPPA-LAMBDA	26	% 15.6
Toplam	**167/ (342)**	**% 100**

TARTIŞMA

SPE testi klinik laboratuvarlarda birçok hastalık takibinde sık kullanılan bir test olmakla birlikte en sık abnormal monoklonal proteinlerin tespitinde kullanılmaktadır. Monoklonal gammopati, (paraprotein= M protein) tek bir B lenfosit tarafından üretilen, elektroforetik ve antijenik olarak tespit edilen homojen bir proteindir[13]. Sıklıkla beta ve/veya gama bölgesinde yer alır[5,13]. Monoklonal gammapatinin tipinin tayini için SIFE testi kullanılır[7]. Monoklonal gammapatinin izlendiği plazma hücre diskrazilerinin takibi Myeloma çalışma grubunun yayınladığı rehberde SPE, SIFE, UIFE ve nefelometrik ve biyokimyasal testler ile hastaların tanı, evreleme, prognoz ve tedavi etkinliğini değerlendirmede kullanılmaktadır. Aynı çalışma grubu SPE test istemi ile ilgili endikasyon durumlarını da belirlemiştir[4].

Hastanemiz Biyokimya Laboratuvarına SPE istemi ile ulaşan vakaların istemlerinin yapıldığı klinikler incelendiğinde istemlerin % 91.21 Hematoloji Kliniğinden % 2,1 Beyin ve Sinir Hastalıkları kliniğinden ve % 1, ise Nefroloji kliniğinden oldu-

ğu görülmüştür. İstem yapan bölümler hastanemizin onkoloji alanında faaliyet gösteren bir dal hastanesi olması nedeniyle ağırlıklı olarak Hematoloji ve Kemik İliği Nakil Merkezi olarak izlenmektedir.

Bu doğrultuda bizim çalışmamızda SPE test istemi bulunan hastaların tanıları incelendiğinde ise en sık olarak plazma hücre diskrazisi hastalık grubundan (%40.45) istem yapıldığı görülmüştür. Ayrıca sırasıyla takip eden ilk beş test istem endikasyon nedenleri; lösemiler(% 11.68), kan ve kan yapıcı organların hastalığı (%10.67), anemiler(%9.42), B lenfoproliferatif hastalıklar (%4.27) ve bel ağrısı (%3.01) olarak izlendi. Diğer (%11.18) olarak izlenen grup içinde hipertansiyon, tiroid hastalıkları Behçet hastalığı, koagülasyon bozuklukları, trombositopeni, dispepsi, lökosit bozuklukları ve genel muayene tanıları izlenmiştir. 2003 İnternational Myeloma Working grubunun yayınladığı rehber ile endikasyon nedenlerimiz karşılaştırıldığında SPE endikasyonları içinde yer almayan lösemi durumunda istem yapıldığı görülürken diğer öntanı ve tanıların rehbere uygun olduğu görülmektedir. Ancak istemlerin diğer kısmına toplanmış olan bölümünün de (%11,18) rehbere uygun olarak istenmediği görülmektedir.

SPE istemlerinin Plazma hücre diskrazileri altında yer alan 322 vaka incelendiğinde 286 MM (% 88.8), 32 Plazmasitom (%9.9), 3 WM (% 0.99), 1 Amiloidoz olduğu görülmektedir. Monoklonal gammopati izlenen hastalık grubunda görülen MGUS ve SMM olarak tanılandırılmış hastaların olmamasının nedeni hastanemizin bünyesinde kemik iliği nakil merkezi olması ve daha çok transplantasyon için sevk edilen hasta grubunun fazlalığı olarak değerlendirilebilir. Hastaların yaş dağılımlarına bakıldığında da Monoklonal gammopati izlenen 50 yaş üzeri ile uyumlu olduğu ve cinsiyet konusunda da literatürde benzer olduğu erkeklerde hafif oranda daha yüksek olduğu görülmektedir[14,15].

Bizim çalışmamızda SPE istemi olup MM tanısı olan hastaların yaş ortalamasının 55.02 ±13.03yıl olarak izlenirken Kyle ve arkadaşlarının Mayo Klinikte yaptığı 1027 yeni tanı almış MM vakasında ise yaş ortalamasını 65 yaş civarında olduğu görülmektedir[14]. Öten yandan Renuka ve arkadaşlarının 380 SPE verisi ile yaptığı çalışmada ise SIFE pozitif grupta yaş ortalamasını 67.3± 14.1 SIFE negatif grupta ise 65.1 ±15.5 olarak bulmuştur[5]. Bizim çalışmamızda MM olgularımızın yaş ortalaması diğer iki çalışmaya göre bir dekat daha düşük olduğu görülmektedir.

SPE istemleri bulunan 796 hastalanın SPE bulgularına bakıldığında SİFE istemi bulunan 342 (%42.9), olmayan ise 454(% 57.1) hasta olduğu görülürken tüm hastalarda üç testin eş zamanlı istenmediği görülmüştür. Armin ve arkadaşlarının 3818 SPE istemi olan hastaların incelediklerinde de benzer şekilde SPE istemi olan hastalarda eş zamanlı rutin olarak SIFE ve UIFE istemi olmadığını belirtirken oran vermemiştir[16]. Bizim çalışmamızdaki 342 SIFE istenmiş olan vakaların 167'sinde (% 20.3) monoklonal gammopati izlenmiştir. Benzer şekilde HOA ve arkadaşlarının 10682 vaka taraması sonrasında 2007 vakada (% 18.8) monoklonal gammapati olduğunu göstermiştir[9]. Renuka ve arkadaşlarının hipogammagloblünemili 380 vaka üzerinde yaptığı çalışmada ise 37 vakada (% 9.7) oranında pozitif serum veya idrar IFE saptamıştır[5]. Chang ve arkadaşlarının 327 SPE ve immünsubstraction yöntemi ile İFE istemi bulunan hasalarda yaptığı çalışmada 281 vakada patoloji saptamamış iken 46 hastada klonalte gözlemlediğini belirtmiştir[17].

SIFE sonucunda Monoklonal gammopatiye sahip hastaların immünglobulin ağır ve hafif zincir dağılımları incelendiğinde ise bizim çalışmamızda da literatürdeki diğer çalışmalara[5, 9,11, 17,18] benzer şekilde en sık IgG-Kappa monoklonal gammopatisinin izlendiği görülmektedir.

David ve arkadaşları 2845 hastanın SPE M protein ve SIFE değerlendirdiğinde de 2095 vakada IGG, 456 vakada IGM, 294 vakada IGA monoklonal gammapati tespit ederken hafifi zincir tipini bildirmemiştir. Aynı çalışmadaki nefelometrik değerler ile SIFE karşılaştırılmasında IG A konsantrasyonunun en iyi koralesyona sahip olduğunu göstermiştir[4].

Bizim çalışmamızda da 342 SIFE çalışılmış iken UIFE örneği sayısı 223'dir. Ayrıca özellikle UIFE testi, metodolojik olarak, idrar konsantrasyon oranı, poliklonal antikorların kullanımı ve boyanma tekniklerine göre etkilenme olasılığı yüksek bir testtir[19]. 24 saatlik idrar numunesinin toplanma şekli, bu tür durumların olasılığı göz önüne alındığında SPE SIFE UIFE testlerinin yanında serbest hafif zincirlerin tayini istemlerinin yapılması önerilmektedir[11,13,16,19,20].

Armin ve arkadaşları 3818 serum örneğinde SPE ve serbest kappa ve lambda hafif zincir düzeyleri oranı ile monoklonal gammapatili bantları takip edebildiklerini SIFE testine ihtiyaç duymadıklarını söyleşmişlerdir[16]. Katzmann ve arkadaşlarının 1877 Plazma hücre prolefaratif hastalığında 5li test paneli SPE, SIFE, UIFE, USPE ve FLC tayini ile taradığı hastalarda 26 vakanın tüm testlerde negatif olduğunu bulurken idrar testini panel dışında bıraktığında 23 vakanın daha tespit edilemediğini FLC testinin çalışılmadığı durumda ise 30 hasta tespit edilemediğini göstermiştir. SIFE ve UIFE testi kullanılmadığında ise 58 vakada tespit edilememesine neden olduğunu göstermişlerdir. SPE SIFE ve FLC paneli yerine SPE ve FLC panelini kullanmak MGUS'da %8) POEMS' %23 Plazmositomda %4 Amilodozda % 1 SMM 'de %0.5 oranında sensitivite azalığını gösterirken MM LCDD ve makroglobunemide azalmadığını göstermiştir[11]. Mayo kliniğin yaptığı çalışmada SIFE SPE ve UIFE istemelerinin yanı sıra serbest düzeylerin birlikte çalışılması monoklonal gammopati tespiti için % 100 duyarlılık sağladığı gösterilmiştir.

Sonuç olarak bizim laboratuvarımıza gelen SPE testi endikas-

yonları ve klinikleri incelendiğinde hastanemiz onkoloji hastanesi olması doğrultusunda %100 uygun klinik ve %80 öntanılar ve tanılar ile istem yapıldığı görülmektedir. Takip ettiğimiz vakalar yaş cinsiyet ve monoklonal gammapati tipi olarak literatür ile uyumlu bulunmuştur. Çalışmalarımızda hassasiyeti arttırmak için SPE SIFE UIFE yanında ek olarak USPE ve FLC oranlarının laboratuvarımız çalışma paneline eklenmesi sunduğumuz hizmet kalitesini arttıracak klinisyenlerin hastaların tanı ve takibinde daha etkin rol almalarını sağlayacaktır.

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Koru Proceedings

Outcomes of Delivery : A 2 Year Experience

Doğum Sonuçlarımız: 2 Yıllık Deneyim

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ABSTRACT

Objective: The aim of this study is to contribute to literature by sharing our hospital birth results.

Methods: This study is done by detailed analysis of the 3163 consecutive births in Ankara Private Koru Hospital and Sincan Private Koru Hospital. All births divided in to 4 groups as term, late preterm, very preterm, and extremely preterm. Each of this 4 group's, delivery methods, maternal age and parity, the assisted reproduction techniques if done, baby gender, ponderal index, birth weight and length, and head circumference, 1st and 5th minute APGAR score were examined and analyzed.

Results: Total 3163 births examined and 2955 of them were term and 208 of them were preterm. 2805 pregnancy were spontaneous and 358 of them achieved by assisted reproductive techniques. 2234 was realized by cesarean section (70.7%) and 923 births (29.3%) occurred vaginally. Our cesarean rate was similar with other private hospitals in Turkey (67.9%). As expected cesarean rate is slightly higher in preterm birth. Cesarean rate of severe preterm birth was low. 51.9% of the infants were male and 48.1% of them were female.

Conclusion: Despite increasing birth rates, cesarean rates increase dramatically. As a result of patient education and support of health ministries to have birth in health institutions will also decrease the cesarean rate.

Keywords: *Birth outcomes, cesarean rate, preterm birth*

ÖZET

Amaç: Bu çalışmanın amacı, hastanemizin doğum sonuçlarını paylaşarak literatürüne katkıda bulunmaktır.

Yöntem: Bu çalışmada Ankara Özel Koru Hastanesi ve Sincan Özel Koru Hastanesinde ardışık 3163 doğumun detaylı analiz yapılmıştır. Tüm doğumlar term, geç preterm, çok erken preterm ve son derece preterm olarak 4 gruba ayrıldı. Bu 4 grup, doğum şekli, anne yaşı ve paritesi, varsa yardımcı üreme teknikleri, bebeklerin cinsiyeti, ponderal indeks, doğum ağırlığı ve boy, baş çevresi, 1. ve 5. dakika APGAR skorları incelenmiş ve analiz edilmiştir.

Bulgular: Toplam 3163 doğum incelendi ve bunlardan 2955 term ve 208 preterm doğum olduğu görüldü. Gebeliklerin 2805 spontan ve 358 ise yardımcı üreme teknikleri ile oluşmuştur. 2234 doğum sezeryan (%70,7) ile 923 doğum ise vajinal yolla (%29,3) gerçekleşti. Sezeryan oranlarımız Türkiye'de ki diğer özel hastaneler ile (%67,9) benzerlik göstermektedir. Beklenen sezeryan oranları preterm doğumlarda biraz daha yüksekti. Sezeryan oranı şiddetli preterm doğumlarda düşük oranda görüldü. Bebeklerin % 51,9'u erkek, % 48,1 'i ise kız olarak doğdu.

Sonuç: Hastanede doğum hızının artması ile sezeryan doğum oranları da dramatik olarak artmaktadır. Sonuç olarak hastaların eğitimi ve Sağlık bakanlığının desteği ile sezeryan oranlarında azalma olacaktır.

Anahtar kelimeler: *Doğum sonuçları, sezeryan oranı, preterm doğum*

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INTRODUCTION

World Health Organization (WHO) defines birth as the birth of the fetus with a weight above 500 grams or with crown-heel length of ≥ 25 mm. WHO also defines the pregnancy that ended after the 20th week of pregnancy as birth[1]. Caesarean section(c-section) delivery is defined as the delivery of the fetus, placenta, and membranes through an incision in the abdominal and uterine wall[2].

There are three different opinions about where the word "Caesarean" derived from. First one is the theory that Julius Caesar was delivered by this way, even though there is no historical evidence that support this. The second one suggests that Caesarean derived from verb "caedere" that was used in Middle Ages which means "to cut" in Latin. The third one suggests that it is derived from the term "lex regia" later changed to "lex caesarea" which was a procedure in Roman laws that was performed on pregnant women, who died in the last weeks of their pregnancy, to save their child[2]. In our country; the number of live born infants was 1.286.828 in 2012, while in 2013, it dropped by 0,3% and became 1.283.062[3]. It is estimated that every year in the world; 500.000 women die from pregnancy or childbirth related causes, 7 million women develop long term complications, and adverse outcomes are observed in 50 million women[4]. These kind of adverse events mostly occur in developing countries.

C-section rate was 4.5% in 1965 in United States of America; while it increased gradually and between 1996 and 2006 it increased by 50% and comprised approximately 32% of the total birth rate[5,7]. According to Turkish Public Health Association's data, c-section rates to all birth rate which were done in hospital were 42,7% in 2009, 45,5% in 2010, 46,6% in 2011, 48% in 2012 and 50,4% in 2013. C-section rates in private hospitals were 61,8% in 2009, 63,7% in 2010, 66,2% in 2011 and 2012, and 67,9% in 2013[8]. C-section rates in our country increase day by day and the reasons of this increase include the rise in women's average marriage age, and thus getting pregnant in older age, their desire to have fewer children than before, infertility problems, increasing the diagnosis of high risk pregnancy and precious baby[9].

MATERIAL - METHODS

In our study; deliveries performed in Sincan Koru Private Hospital and Ankara Koru Private Hospital that resulted in singleton births between 26-42 weeks of pregnancy, 2955 term and 208 preterm cases were evaluated retrospectively. Maternal age, parity, delivery methods, gestational age, newborn's sex, 1st and 5th minute APGAR scores, head circumference and heights were examined.

Newborns are categorized based on gestational age in accordance to the terminology as follows; "term" between 37-42 weeks, "late preterm" between 32-37 weeks, "very preterm" between 28-32 weeks and "extremely preterm" less than 28 weeks[10]. Ponderal index (PI) scores [(Mass(gr) X 100) / (Height3)] were calculated based on birth weight and height. Infants with severe congenital anomalies and chromosome anomalies, multiple births, and intrauterine exitus were excluded from the study.

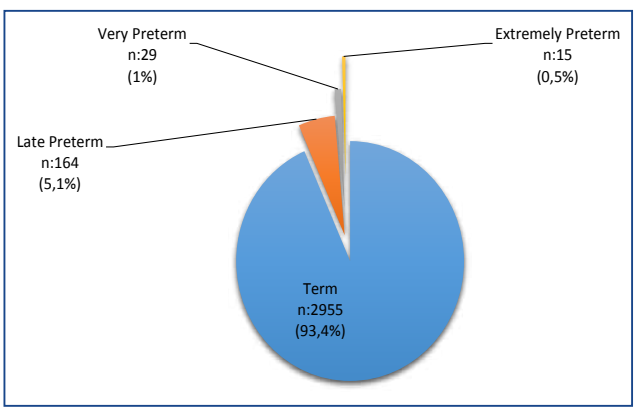
Collected data were analyzed by SPSS 22.0 package program on windows 8. Numbers, percents, medians, and standard deviation values were used as the descriptive statistics.

RESULTS

Out of 3163 birts 2955 (93,4%) were term and 208 (6,6%) were preterm. 2805 of these pregnancies were achieved spontaneously whereas 358 were by assited repruductive tecniques. The patients were diveded into 4 groups according to the time of delivery. The distrubition of these groups were demonstrated in Graphic 1. 208 singleton preterm births were also divided into late, very and extremly preterm categories as shown in Graphic 2.

Maternal age, parity and type of asissted reproductive techniques that yield pregnancy with regard to time of birth were demostrated in Table 1 and 2. C-section rate was 70,7% (2234/3163). 2063 (69,9%) term, 136 (82,9%) late preterm, 27 (93,2%) very preterm, 8 (53,4%) extremly preterm birth were conducted by C-section. The C-section rate were significiantly lower in term group as compared tolate and very preterm groups. However C-section rate was significant lower in extremly group in comparison with term, late preterm and very preterm gropus. Table 3.

Graphic 1. 3163 singleton pregnancies into term, late preterm, very preterm, extremely preterm categories.



Graphic 2. 208 singleton preterm birth cases into late preterm, very preterm, extremely preterm

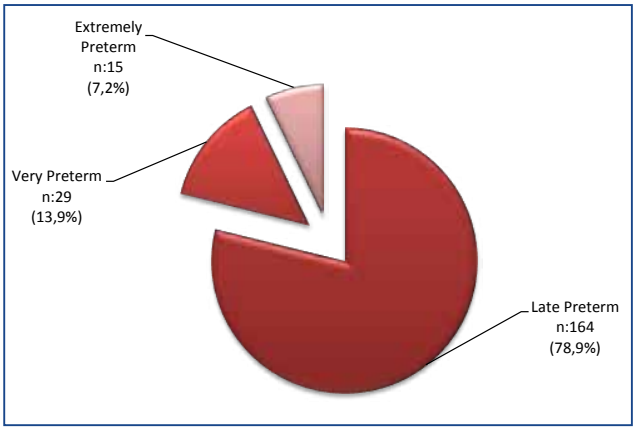


Table 1. Maternal age and parity divisions based on groups.

	Term Birth Group (mdn ± SS)	Late Preterm Birth Group (mdn ± SS)	Very Preterm Birth Group (mdn ± SS)	Extremely Preterm Birth Group (mdn ± SS)
Age	30,1±4,23	29,4±4,9	29,6±6,17	30,5±4,03
Parity	0,43±0,63	0,22±0,56	0,48±0,63	0,66±0,72

Table 2. Pregnancies with assisted reproductive technics.

	Term Birth Group n:2955 (%100)[a]	Late Preterm Birth Group n:164 (%100)[a]	Very Preterm Birth Group n:29 (%100)[a]	Extremely Preterm Birth Group n:15 (%100)[a]
*IVF Pregnancy	179 (6,05)	5 (3)	1 (3,4)	2 (13,3)
**IUI Pregnancy	154 (5,21)	3 (1,8)	1 (3,4)	0
***Oral Ov. Ind.Tre.	12 (0,4)	1 (0,6)	0	0

[a] Column Percentage
** IVF: in vitro fertilization,*
*** IUI: intrauterine insemination,*
****Oral Ov. Ind.Tre.: ovulation induction treatment with clomiphene citrate*

Table 3. Delivery methods based on groups.

	Term Birth Group n:2955 (%100)[a]	Late Preterm Birth Group n:164 (%100)[a]	Very Preterm Birth Group n:29 (%100)[a]	Extremely Preterm Birth Group n:15 (%100)[a]
C-section Delivery	2063 (%69,9)	136 (%82,9)	27 (%93,2)	8 (%53,4)
Vaginal Delivery	892 (%30,1)	28 (%17,1)	2 (%6,8)	7 (%46,6)

[a] Column Percentage

The subdivisions of the birth group according to gestional age and infant gender is given, birth weight, height, head circumferance, pondral index and APGAR score were demostrated in Table 4 and 5.

Table 4. Gestational age and infant sexes.

	Term Birth Group	Late Preterm Birth Group	Very Preterm Birth Group	Extremely Preterm Birth Group
Gestational age	273,5±6,8	246,2±10,4	210,5±9,2	170,6±22,4
Female Infant	1423 (%48,1)	80 (%48,7)	18 (%62,1)	5 (%33,3)
Male Infant	1532 (%51,9)	84 (%51,3)	11 (%37,9)	10 (%66,7)

Table 5. Birth weight, height, head circumference, ponderal index and 1st and 5th minute APGAR score.

	Term Birth Group	Late Preterm Birth Group	Very Preterm Birth Group	Extremely Preterm Birth Group
*PI	2,67±0,28	2,54±0,42	2,25±0,41	3,49±1,98
Birth Weight	3323,6±413,2	2483,4±532	1431,7±523	842,6±314,2
Height	49,8±1,6	46±3,5	39,6±5,1	30±6
Head Circumference	35,4±1,9	33±2,6	28,2±3,5	23,6±3,5
APGAR score 1st minute	8,83±0,54	7,86±1,2	6,27±1,48	4,13±1,68
APGAR score 5th minute	9,84±0,49	9,01±1,1	7,65±1,42	6±1,13

**ponderal index*

DISCUSSION

2955 (93,4%) of 3163 pregnancies which resulted in singleton births that were included in the study were detected as term cases and 208 (6,6%) of them were detected as preterm cases. Even though more than 60% of preterm births in the world occur in Africa and South Asia, preterm birth is a global problem. In lower-income countries, preterm birth rate is %12, whereas in higher-income countries the rate is 9%[10]. As income level increases, preterm birth rate decreases. In our study the preterm birth rate was 6.6%. 3163 singleton pregnancies results are divided into term (37-42 weeks), late preterm (32-37 weeks), very preterm (28-32 weeks), extremely preterm (<28 weeks) categories in Graphic 1. 208 preterm birth subcategories are shown in Graphic 2.

In literature the ratio between preterm and term birth is very variable, it is really hard to determine the ratio between extremely preterm and total preterm cases. It is also known that survival in extremely preterm cases is 7%, whereas in very preterm cases it is 52% and in late preterm cases it is 90%. Neonatal deaths occur within the first 3 days and survival rate increases prominently when the birth weight is above 1100 grams[11]. Although the other causes of neonatal death, were reduced significantly in America, prematurity is the leading cause of neonatal deaths with a rate of 25%[12]. Birth mechanism is still a mystery and the cause of preterm birth has not been fully understood yet. 50% of the preterm births do not have a certain cause. Birth is a complicated process in which multiple factors plays role. It is thought that preterm birth has 4 different causes: early fetal endocrine activation, uterus overdistension, decidual bleeding and intrauterine infection[13].

Various maternal factors can cause preterm birth. Very young and advanced maternal age, especially being older than 35 years old, is one of these factors[14,15]. Mother's height and weight also play a role in preterm birth. Short stature of the mother increases the risk by 1,8 times[16]. Preterm birth risk doubles in black mothers[17]. There is no relationship between parity and preterm birth. History of preterm birth is the 2nd most important factor that increases the preterm birth risk by 3,6 times, after cervical fibronectin which increases the risk by 4 times. Maternal age and parity according to birth subdivisions given in Table 1.

2805 (88,6%) pregnancies were spontaneous and 358 (11,4%) of them were conceived with asisted reproductive technics. The subdivision of pregnancies with asisted reproductive technics are shown in Table 2.

All pregnancy complications are more frequent in IVF than spontaneous pregnancies. In IVF pregnancy, preterm birth risk was relatively 1,5 times more. In a double blind study Olivennes and his colleagues conducted, where they examined 73 IVF pregnancies, they reported the rates of birth weight of < 2500 grams as 8,7% for singleton pregnancies and 54,2% for twin pregnancies[18].

In our study 2234 (70,7%) cases were delivered by c-section, and 923 (29,3%) of them were delivered vaginally. This rate is similar to the c-section rate (67,9%) of the private health institutions in 2013 in our country. This high c-section rate may be because of most of the women who admits to our institution and also to the other private health institutions prefers to have c-section. 2063 (69,9%) term labors, and 171 (82,8%) preterm labors were conducted via c-section. 892 (30,1%) term and 37 (17,7%) preterm labors were conducted via vaginally. The division of the delivery methods based on term, late preterm, very preterm, extremely preterm subdivisions is given in Table 3. C-section was preferred in preterm cases in which fetus reached viability limits. Whereas in extremely preterm cases; c-section was less preferred, because most of these cases were admitted to the hospital during the complet cervical dilatation and full cervical effacement.

The subdivisions of the birth group according to gestional age and infant sexes.is given in Table 4.

According to the birth statistics, in 2013, 51,4% of newborn infants were boys and 48,6% of them were girls. These rates are similar that were obtained in our hospital: 51,9% boys, 48,1% girls[3].

Birth weights, heights, head circumferences, ponderal indexes and 1st and 5th minute APGAR scores of 2955 term and 208 preterm infants is given in Table 5.

Ponderal index is another measure that is used for the division of symmetrical and asymmetrical intrauterin growth retardation (IUGR) infants. While this index's results are normal among symmetrical IUGR, the results are low among asymmetrical IUGR. In our study, as it was expected, lower birth weights caused lower APGAR scores.

CONCLUSION

Childbirth in sanitary conditions also reduces perinatal mortality and morbidity. In 2002, the rate of child delivery in health institutions was 75% in our country, and it reached to 98% in 2013. In 2002, in our country, the rate of pregnant women who received antenatal care at least once was 70%

and in 2013, it was raised to 98%. However, in 2013, neonatal death rate was calculated as 0,42%. When compared to the developed countries whose neonatal death rate is 0,4%, this rate is acceptable. In Northeast Anatolia region, the rate of child delivery in health institutions is 89%. Mother's education is an important determinant for the child delivery in health institutions. Mothers who graduated at least primary school are 2,5 times more likely to check for medical aid. Maternal age, parity and patient's insurance status are also important factors. Three-fourths of the mothers who have complications during the postpartum period have not received any medical attention.

With all these results, following the guidelines that have been developed by World Health Organization for maternal and child health will reduce maternal and infant mortality rate. Education of the mothers who gives birth in sanitary conditions is another important factor. İn our country health policy should be changed in a way that everyone can achieve to get medical attention in health institutions if they demand.

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Koru Proceedings

The Appropriate Selection Of Laboratory Animals In Experimental Investigations Through Comparison Of Human Anatomy And Physiology

Deneysel Çalışmalarda İnsan Anatomi ve Fizyolojisi Göz Önünde Bulundurularak Uygun Hayvan Seçimi

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ABSTRACT

Objective: This study aimed to evaluate the relationship between laboratory animals and humans by comparison of genetic, anatomic, physiologic, and developmental features.

Materials and method: Information are collected from English and Turkish literature about laboratory animals by using relevant various keywords. Each organ system and various animals are widely discussed in terms of economics, genetics, organ structure, advantages for selecting specific area studies.

Conclusion: The most appropriate animal is to be chosen according to the subject of the experiment and the organ system that the study is designed for. The number of animals and the economical basis are not to be underestimated.

Key Words: *Laboratory Animals, Rat, Mice, Guinea Pig,*

ÖZET

Amaç: Bu çalışmanın amacı genetik, anatomik, fizyolojik ve gelişimsel özellikler karşılaştırılarak deney hayvanları ve insan arasındaki ilişkiyi değerlendirmektir.

Materyal Metod: Uygun anahtar kelimeler kullanılarak İngilizce ve Türkçe makaleler taranmıştır. Ekonomik, genetik, organ yapısı gibi özellikler göz önünde bulundurularak farklı hayvanlar ve organ sistemleri tartışılmıştır. Çeşitli organ sistemleri bağlamında deney hayvanlarının özellikleri karşılaştırılmıştır.

Sonuç: Deneyin konusu ve çalışılan organ sistemine göre en uygun hayvan seçilmelidir. Kullanılan hayvan sayısı ve deneyin ekonomik boyutu göz önünde bulundurulmalıdır.

Anahtar kelimeler: *Fare, Kobay, Laboratuar Hayvanları, Sıçan*

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INTRODUCTION

Since the beginning of science, animals have been used as models in experiments to understand human anatomy and physiology[1].The examinations spread over a large area with an understanding of the benefits of research on animals. Today, animals including rodents, rabbits, reptiles, dogs, cats, guinea pigs, monkeys, fish and birds are used in fundamental biology studies, biomedical researches, toxicological evaluation, diagnosis of diseases, testing the safety and efficacy of products for human and veterinary medicine, education and training[2].

According to the European Commission reports from 2011, the number of animals used annually in the 27 EU member states is just under 11,5 million. By the way, 80 % of the total number of animals used in the EU is represented by rodents and rabbits. Among them, mice takes first place by accounting for 60,9 % of the total use, followed by rats with 13,9 %. After them, cold-blooded animals (reptiles, fish and amphibians) constitute the second most used group by representing 12,4 %. Birds represent 5,9 %. Horses, donkeys, cross-bred animals, pigs, goats, sheep and cattle represent only 1,2 %. Finally carnivores represent 0,25 % and non-human primates represent 0.05 % of the animals used in 2011[2].

It is now a known fact that most of our knowledge about the human body and pathophysiology of diseases comes from laboratory animals[3]. Mice are used mostly in cancer researches, antibody drug and vaccine developments[4]. Rats are important for administration behavior and toxicity tests. Especially, repeated-dose chronic toxicity tests require their usage[4,5]. Toxicology tests involve also mice, 74 % of the usage of animals in this area are constituted by these two species[6]. Hamsters contribute to investigate reproductive and fetal disorders; guinea pigs are appropriate for immunology studies[4]. With similarities to humans, rabbit may also be used in immunology[7]. Moreover, rabbits are used frequently in eye and skin irritation tests. Cats and dogs are used for cardiovascular diseases and monkeys are often regarded with specific infectious diseases[4].

Considering all these animals and usage areas, appropriate animal selection seems essential. The mismatch between animal and usage area may cause undesirable results, including diagnosis and treatment tests of diseases which are the matter of life and death in some cases. The most important point in the selection of appropriate animal is to characterize the species of animals by comparing the human anatomy and physiology[2].

MATERIALS and METHODS

This study is planned to review, evaluate and compile the information collected from English and Turkish literature about laboratory animals. The gathered information from the literature and books is used for choosing the appropriate laboratory animal for the particular organ and experiment to reach best scientific success. Each organ system and various animals are widely discussed in terms of economics, genetics, organ structure, advantages for selecting specific area studies.

RESULTS

Animal experiments are sensitive working areas. There are considerations to be taken into account including the determination of the subject properly, preparation of study protocol, the adequacy of researchers, controlling alternative options instead of using animals and finally, the necessity of selecting the animal appropriately for the right match with the purpose[4]. By this way, the experiment reaches its goal and the animals sacrificed in experiments would be used for the benefit of humanity. Otherwise, these experiments will just lead to animal waste.

Comparison of the biological characteristics of the laboratory animals and the humans allows us to have basic knowledge about their appropriate usage in experiments[3]. However, choosing the right animal is not only limited by resemblance to humans. Even if they are appropriate in physical and anatomical aspects, there are other factors to be considered. For example, large animals such as equine create feeding and budget problems which are major disadvantages of the equine models among the animal models for cartilage researches[8]. Conversely, rodents - rats, mice, guinea pigs, hamsters- are more preferred animals due to their small size and easy breeding conditions. Also, the use of some mammalian species like canines is restricted due to human's emotional relationship with them. If their usage creates ethical problems, mammals can be replaced with non-mammalian vertebrates, invertebrates or microorganisms according to the type of research. However, due to their general similarities, mammals are accepted as appropriate animals for researches[5]. In addition, the system to be studied is important for correct animal choice.

Nerve System or Behavioral Studies

Lots of different animal types are used in neurological or behavioral studies. For example, rat is commonly used in animal models to study learned helplessness[9,10]. It has been observed that when exposed to unavoidable shocks and stress, rats show similar behavior and activity to clinically depressed patients such as weight loss, decreased libido, elevated motor activity, changes in sleep pattern, activity on HPA (hypothalamic–pituitary–adrenal) axis and a loss of spine synapses in the hippocampal regions. Interestingly, depression therapy that is used on humans, such as tricyclic antidepressants, SSRIs (selective serotonin reuptake inhibitors), monoamine oxidase inhibitors and electroconvulsive therapy, also works on rats[10-15]. Moreover, rats in the learned helplessness model

had elevated levels of glucocorticoids and homocysteine, as is observed in depressed human patients[16].

Rats are preferred over mice for a number of reasons. Their behavioral characteristics are investigated well, their large size allows more precise microsurgical interventions, cell/tissue transplantation, and functional analyses in vivo[17,18]. Stereotaxic injection, a commonly used procedure in neurological studies, is also performed easier with more precision in rats rather than in mice. Therefore, rats are frequently used in animal models for neurodegenerative diseases such as Alzheimer's disease[19]. Invertebrates such as nematode (Caenorhabditis elegans), the zebrafish (Danio rerio), the fruit fly (Drosophila melanogaster), the ascidian (Ciona intestinalis) and the sea urchins (Strongilocentrotus purpuratus and Paracentrotus lividus) are useful models for Alzheimer's Disease and other neurodegerative diseases as well. Their genomes are thoroughly sequenced and interestingly include genes corresponding to the human disease genes. Also, genetic screens performed with the purpose of pinpointing mutations that cause age-dependent neurodegeneration take less time[20].

Primates are also important in human nervous system investigations aimed to understand the brain functions. Because their neurological developments and functions relating to cognition is similar to those in humans[6]. Furthermore, they are able to remember their actions and behaviors such as humans and they have memory decrements associated with age-related cognitive decline[5]. These findings point out that primates are candidates to investigate neurological diseases as Alzheimer's[21].

Monkeys were also used on Parkinson's disease which is a neurological disease and the first animal model was created by the scientists who work in the "National Institute of Mental Health" with the injection of "l-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)" into the monkeys. The reason for the success of modelling the monkeys is that the drug affects the human brain in the same way it does the monkeys' brain. At this point, the similarity of "Substantial nigra" which is found in both humans' and monkeys' brains draws the attention[23].

Monkeys are the most commonly used laboratory animal in the investigation of pathogenesis of Huntington's disease (HD). Irregularities in miRNA of the HD monkeys' brains are similar to human patients, thus providing the opportunity of trying alternative treatment on monkeys[22].

Middle ear and ear drum of cats and rabbits are also similar to humans[23]. For auditory tests or hearing researches, rabbits are used as the animal model because their hearing frequency range is approximately 360-42,000 Hz that covers the frequency range of humans[24].

It was found that vitamin A deficiency causes the loss of visual pigments in rats. In this case, adaptation to darkness can be reduced and night blindness may occur[25]. This situation is also seen in humans and therefore rats can be used for researches based on pigmentation and vitamin adequacy.

Binocular vision is a function sensing the depth of the object. Just as in humans, it is also found in monkeys and researches on these animals provide more information about human eye physiology. Physiologists believe that the development of diagnostic and therapeutic methods related to neural disorders is obtainable from the use of monkeys[5].

Genetic - Cancer Studies

All diseases can be affected by genetic factors directly or indirectly, so identifying them is important to prevent or treat diseases. After humans, the genome sequence of mouse was also determined in 2002 and it is found that there are certainly structural and functional differences but ninety-nine percent of the genes in humans and mice are counterpart[1,6]. This was a great benefit for the analysis of the differences between genetic and non-genetic disease applicable to mice. Furthermore, this determination ensures that mice are the most commonly used experimental animals [1].

Mouse is the most frequently used animal in cancer research due to its small size, short gestation period and inexpensive handling. Rodents are easy to manipulate genetically, thus enabling to study molecular pathways and different aspects of cancer in vivo. However, cancer need to be induced genetically or chemically in mouse whereas different types of neoplasms are naturally occuring in dogs.It has been discovered that dog genome is more similar to human than mouse. Same or similar tumor suppresor genes and oncogenes play role in both dogs and humans such asp53, Rb, MDM2, BRCA1, and BRCA2,53,54.This allows dogs to be a more accurate species to model human cancers.[26-29].

Another useful experimental animal for biomedical research is swine. Its morphological and physiological similarities with humans help researchers learn more about the human body and its diseases. For instance, swine is an accepted animal speciesfor organ failure and sepsis. Furthermore, new surgery techniques on liver, intestine, kidney, heart and blood circulation and devices such as laparoscopes are first tried on swines. Despite its relatively expensive and elaborate husbandry compared to smaller laboratory animals, the significance of these studies are realized in the scientific community[30-37].

Immune System Studies

Animals are used also in researches related to the immune system in the human body. Rodents are frequently used in this field. In particular, mice and rats are essential among immune system research models because they have a similar immune

response to humans and human lymphocytes formed after infection can be transmitted to these animals. Genetically modified mice by transmitting human lymphocytes are used in many studies seeking treatment for immune system failure including fatal diseases such as AIDS[6]. Additionally, rabbits have similarities to human immune system[23]. For example, gut microbiota of rabbits is quite similar to those in humans[7].

In the 20th century, monkeys had been subjected to experimental testing for developing a vaccine against the polio virus[6,23]. Through research on animals it has provided a large decrease in poliomyelitis rates[5]. With the researches done on "Rhesus monkeys" and "chimpanzees", a vaccine has been developed against the infections of Hepatitis B virus in humans.[23].

Dermatological Studies

Structure of epidermis in cats and dogs have similarities to humans[38]. In the case of skin, pigs have similar organisms to humans also[4,39]. In a study investigating the morphology of hair follicles and the structure of skin, the pork skin showed the greatest similarity to human skin, while the skin of other animals including cats, dogs and rabbits have significant differences from human skin[39].

The albino rabbit skin is highly sensitive, thus they are preferred in skin irritation tests[3].

Endocrinologic System Studies

Rats are widely used in diabetic wound healing studies due to their similarities with humans[40]. Congestion, hyperemia, necrosis, inflammatory cells, collagen deposition, absence of epithelium, angiogenesis, greater collagen formation induced by fibroblast, fibrin leukocyte crusts are also seen in rats along with absence of necrosis, re-epithelization of the tissues and collagen deposition during healing process[41]. Diabetes complications vary between humans and animals and there is an ongoing debate about whether any animal model represents complications seen in human completely[5]. Glucokinase gene plays an important role in diabetes mellitus and it is known that mutations in that gene cause maturity-onset diabetes of the young (MODY)[42].Type II diabetes in mouse caused by mutations in the glucokinase gene resembles human MODY, thus allowing scientists study the relationship between the gene and the etiology and severity of the disease[43]. It has been discovered that while some mutations can be lethal, some do not affect the phenotype[6]. There is a significant resemblance and homology between rodents' genomes and those that are in humans. Additionally, rodents are practically easy animals to work on. The body weight change is 10 day for a rat but it is almost 1 year for humans. So using rats as obese animal models is quite reasonable[44].

Skeletal System Studies

The skeletal system of pigs has some similarities to those in humans[4]. Deer and sheep are two animal species closest to humans in the case of lumbar spine researches[45]. Rodents, except rats, are the most widely used animal models in osteoporosis.. Bone disorders in rats are seen with osteopenia rather than osteoporosis. In this regard, the female rats are appropriate animals for osteopenia researches, because of the earlier growth plate closing in male rats[25].

In addition, rats are not suitable models for studying the effects of ovariectomy on cortical bone because their bones do not contain Havers channels[25].

Gastrointestinal System Studies

A study comparing digestive systems of laboratory animals and humans showed that, like in many other fields, there is no one animal that represent humans entirely. It has been observed that some animals are more similar than others when digestive system is divided into small topics. For example, dogs share the most resemblance to humans in terms of the morphology of stomach. When looked at the morphology of the colon, dogs have a different structure compared to humans while monkeys and pigs have a sacculated colon like humans[4,7]. Morphology of pig colon shows more similarity to humans than monkeys. Gut microbiota of rabbits show significant similarity to humans microbiologically[7]. Rats are one of the most commonly used animals in studies about digestive system. However, there exist differences that should be taken into consideration. For instance like horse and pigeon, rats don't have gall bladders. Therefore, it would be meaningless to use rat as a model for diseases about the gall bladder[25].

The pancreatic juice secretion rates in humans, pigs, and dogs are similar[7]. Pigs also resemble humans in terms of their pancreatic structure[4].

Liver is a significant organ both for metabolism and construction. It is important to find an appropriate model for the researches of liver diseases. At this point, it would be easy to focus on dogs and cats for the researcher because the liver construction is quite similar to humans compared to the other animals such as pigs and rabbits. Furthermore, mice have resemblances with humans about senile hepatic changes and they are also used in chronic hepatitis investigations[34,35,38].

Cardiovascular System Studies

Biomedical researches on animals also made an important contribution to Coronary Artery Bypass Graft Surgery. For basic studies of principles of circulation or blood pressure; frogs, reptiles, horses, cats, dogs, sheep, and deer were used. For further stages, the choices of animal species became

more special. Dogs, chimpanzees, and finally humans are contributed to these researches[23].

Pigs are also appropriate animals for cardiovascular researches due to their similarity for cardiovascular tree, maturation of blood cells and retinal vessels[4,38]. About embryonic blood circulation, the goats are the most similar organisms to humans[38].

It is a conventional method to use dogs in cardiovascular-renal studies as a model of hypertension or renal function researches due to their large size[5]. Their relevance to the cardiovascular system is also important to evaluate safety in the pharmacological industry[6]. Recently, some mutant rats are accepted as valuable models for investigations of human diseases in this area[5]. For example Brattleboro Rats are used for determining the role of vasopressin, a hormone contributes to the kidney function such as blood pressure regulation, water and ion excretions due to its susceptibility to Diabetes Insipidus as humans[36,37].

Furthermore, rats differ from other animals including dogs, cats, guinea pigs, rabbits, cattle and sheep due to L-amino acid oxidase activity in their kidneys. This suggests that rats could be appropriate animal models for metabolic disorders about this enzyme which plays a significant role in amino acid metabolism in humans. There is also glutamine synthetase in rat kidney. All vertebrate brains with the exception of animals like dog, cat and pig include this enzyme which plays an important role in detoxification by converting ammonium glutamate to glutamine. Therefore, rat should be a candidate for studies about glutamine synthetase activity in kidney[25].

Although there are some differences in Neonatal survival of rodents and primates, their vascular anomalies are were found similar[25]. Also, certain embryological developments such as formation of placenta are similar in rodents and humans[38]. Researchers use these information to see the rats as suitable models for cardiovascular system research. However, all rodents are not appropriate for these studies. For example, the usage of rats in cardiovascular researches as hemodynamic studies, measuring blood flow in the heart is limited because of their small body size[25].

Rats are atherosclerosis resistant animals and have relative resistance against hypercholesterolemia and lesion development. Their lipoprotein profiles do not show similarity to those in humans. For these reasons, rats cannot be used in atherosclerosis studies. Even a single specific event, such as plaque rupture causing vessel occlusion that is found in humans with atherosclerosis cannot be found in rat models[25]. Nevertheless, rabbits are appropriate animals to study atherosclerosis due to their susceptibility to hypecholesterolaemia[46].

As mentioned previously, monkeys are the closest species to humans among laboratory animals. In cardiovascular-renal researches, they are founded appropriate due to their structural make up, just as shown in other researches. "Renin" which is a significant hormone regulation of the blood pressure and the tension, is similar both in humans and monkeys, however there is no such similarity in "non-primates". For this reason, monkeys were used for examining hypertension and the researchers have shown that the hypertension disease may be transformed by the genes[23].

Conclusion

There are no animals completely similar to human anatomy and physiology. The similarities and differences between the human beings and the laboratory animals are to be well known to select the best animal for the particular investigation. During the planning phase of the research, genetic, metabolic, anatomic, physiologic, economic and ergonomic parameters should be overviewed to choose the appropriate animal and method. The validity of the used animal model is always taken into consideration for translational research. Among others especially the predictive validity of the model must be proven to translate animal studies to human diseases.

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Koru Proceedings

Yenidoğan İnfantta Preeklampsinin Etkileri

Effects of Preeclampsia on The Newborn Infant

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ÖZET

Preeklampsi sadece gebelik süresince oluşan bir durumdur. Preeklampsinin, yenidoğan infantların ölümünde birlikte olabileceği bir dizi erken ve geç perinatal/neonatal komplikasyonlarla ilişkili olduğu bulunmuştur. Bunlar çoğunlukla gestasyonel hafta ve doğum ağırlığı ile ilişkili olup preeklamptik tokseminin ciddi veya erken başlamasıyla da ilişkilidir. Güncel olarak preeklampsi tanısında kullanılabilecek optimal antenatal test yöntemi ile ilgili bilgiler kısıtlıdır. Ayrıca preeklampsi intrauterin gelişme geriliği (IUGR) ve premature doğumun önemli sebeplerinden biridir. İntrauterin gelişme geriliğinin derecesi erken ve geç morbiditeler üzerinde negatif etkilidir. Prematür doğan bebeklerin uzun dönem sonuçları da gestasyonel haftaya bağlıdır. Biz bu yazıda preeklapmsinin gelişmekte olan fetüs ve doğum sonrası yenidoğan infant üzerindeki olumsuz etkilerinden bahsetmek istedik.

Anahtar kelimeler: *Preeklampsi, Hipertansiyon, Yenidoğan, Fetüs*

ABSTRACT

Preeclampsia is a condition that occurs only during pregnancy. Preeclampsia is found to be associated with a number of short- and long-term perinatal and neonatal complications, including death. These are mostly related to birth weight and gestational age at delivery, and relevant to severe or early onset pre-eclamptic toxaemia. Currently, little information is available on the optimal antenatal testing modality to be used for pre-eclampsia. Pre-eclampsia is an important cause for intrauterine growth restriction and premature delivery. The degree of intrauterine growth restriction also has negative effects on both early and late morbidities. Longer term outcomes for prematurely born infants are dependent on gestational age. In this article, we aimed to mention about the adverse effects of preeclampsia on the developing fetus and newborn infant.

Keywords: *Preeclampsia, Hypertension, Newborn, Fetus*

Sorumlu Yazar :

Ufuk Çakır

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Preeklampsinin Yenidoğan İnfant Üzerindeki Etkileri

Gebelik sırasında anne karnında gelişmekte olan fetüs doğrudan doruya anneyle ilgili durumlardan etkilenmektedir. Hem anneyi hem de anne karnında gelişmekte olan fetüsü etkileyen önemli hastalıklarda birisi de gebelik boyunca annede görülen hipertansif hastalıklardır. Gebelikle ilişkili hipertansif hastalıklar: preeklampsi/eklampsi, kronik hipertansiyon, kronik hipertansiyon zemininde gelişen preeklampsi/eklampsi ve gestasyonel hipertansiyon olarak sıralanabilmektedir[1,2]. Biz bu yazıda fetus ve yenidoğanda, fetal büyüme kısıtlanması ve erken doğuma neden olması gibi nedenlerle ciddi morbidite ve mortaliteye neden olan preeklapmsinin fetüs ve yenidoğan üzerine olan etkilerinden bahsetmek istedik[3].

Preeklampsi tanımı ve sıklığı

Preeklampsi, yeni başlangıçlı hipertansiyon (>20 gestasyon haftası) ve proteinüri ya da organ hasarıyla karakterize multisistem bir hastalıktır. Preeklampsi maternal ve/veya fetal mortalite ya da ciddi morbidite ile ilişkilidir. Hastalığın sıklığı bölgelere ve toplumlara göre değişmekle birlikte tüm dünyada tüm gebeliklerin %4.6'sı oranında görülmektedir. Özellikle ilk gebeliklerde 1.5-2 kat fazla görüldüğü bildirilmektedir. Geç başlangıçlı hastalık (≥34 gestasyonel hafta) erken başlangıçlı hastalıktan daha sık olarak izlenmektedir[1,4,5]. Tüm dünyada maternal ölümlerin %10-15'nin preeklampsi/eklampsi ile ilişkili olduğu bildirilmektedir. 100.000 canlı doğumda preeklampsi/eklampsiye bağlı maternal ölüm oranının 1 olduğu bildirilmekle beraber, vaka/ölüm oranının 10.000 vakada 6.4 olduğu görülmektedir[6,7].

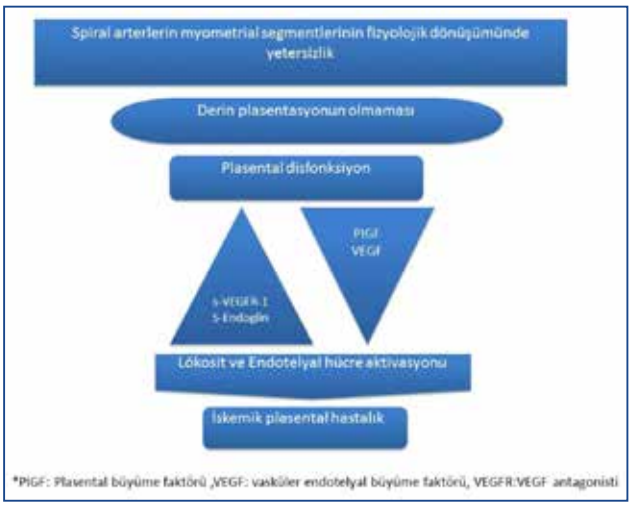
Preeklampsinin risk faktörleri

Preeklampsi oluşumunda değişik risk faktörleri arasında nulliparite, önceki gebelikte preeklampsi olması, anne yaşının >40 ya da <18 yaş olması, preeklampsi aile öyküsü, kronik hipertansiyon, kronik renal hastalık, antifosfolipid antikor sendromu, trombofili, vasküler ya da konnektif doku hastalığı, Diyabetes mellitus (pregestasyonel/gestasyonel), çoğul gebelik, yüksek vücut kitle indeksi, siyah ırk, hidrops fetalis, açıklanamayan fetal büyüme kısıtlılığı, kendisi small gestational age (SGA) olan anne, önceki gebelikte fetal büyüme kısıtlılığı, abruptio plasenta ya da fetal ölüm olması, gebelikler arası intervalin uzun olması, mol hidatiform, genetik faktörler sıralanabilmektedir[8].

Preeklampsinin patofizyolojisine bakış

Preeklampsinin patogenezinde trofoblast migrasyon defektinin önemli bir faktör olduğu bildirilmektedir. Özellikle hücre adezyon moleküllerinin ekspresyonunda ve ekstrasellüler matriks proteinlerinin oluşumunda bozukluk, annenin NK cell KIR-A için homozigot (KIR-AA) olması ve fetusun HLA-C2 genini taşıması gibi genetik nedenler, anjiogenik ve antianjiogenik faktörler arasında dengesizlik olması (vasküler endotelyal büyüme faktörü (VEGF), soluble endoglin, soluble fms- like tyrosine kinase-1 receptors (sFlt-1) ve plasental büyüme faktörü (PIGF) bu duruma yol açmaktadır. Sonuçta spiral arterlerin miyometrium içinde dallanmalarının fizyolojsinde yetersizlik olması, plasenta gelişiminin uygun olmaması, plasental disfonksiyona neden olmaktadır[9-15] (Şekil 1).

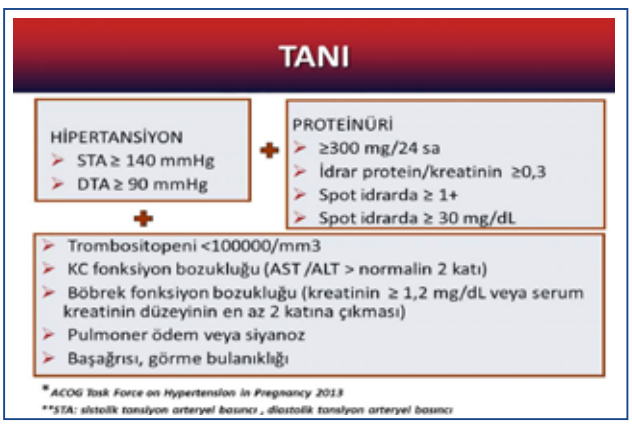
Şekil 1: İskemik plasental hastalık gelişim basamaklar



Preeklampsinin Klinik ve Laboratuar Bulguları (Tablo1)

Preeklampsi klinikte erken ve geç başlangıçlı hastalık, daha az olmak üzere postpartum hastalık görülebilmektedir. En sık geç başlangıçlı (> 34 hafta) hastalık olmakla birlikte, %10 oranında erken başlangıçlı, %5 oranında da postpartum (genellikle ilk 48 saatte) hastalık olarak ortaya çıkabilmektedir. Hastalığın belirti ve bulguları; ağır hipertansiyon sistolik tansiyon arteryel basıncı (STA) ≥160 mmHg ve/veya diastolik tansiyon arteryel basıncı (DTA) ≥110 mmHg), persistan ve/veya ciddi başağrısı, görsel anormallikler (skotom, fotofobi, görme bulanıklığı, temporal körlük gibi), üst abdominal ya da epigastrik ağrı, bulantı, kusma, dispne, retrosternal göğüs ağrısı, mental durum değişikliği şeklinde olabilmektedir[1,2,16,17].

Tablo 1: Preeklampsinin klinik ve laboratuar bulguları



Laboratuvar anormallikleri ise mikroanjiopatik hemolitik anemi (anormal periferik yayma, artmış bilirübin, düşük serum haptoglobulin düzeyi), trombositopeni (<100.000/ microl), artmış serum kreatinin (>1.1 mg/dl), artmış karaciğer enzimleri (normalin üst limitin iki katı) olarak sıralanabilmektedir.

Bazı gebelerde 20. gebelik haftasından önce başlayan hipertansiyon ya da proteinüri, postpartum geç başlangıç ya da alevlenme (postpartum >2 gün, <6 hafta) bulgularından en az birisi ile ortaya çıkabilen atipik preeklampsi vakalarına da rastlanmaktadır. Progresif bir hastalık olan preeklempside çoğu hastada gebeliğin geç dönemlerinde semptomlar gelişmekte ve doğuma kadar kademeli olarak artabilmektedir. Vakaların %25'inde, özellikle erken başlangıçlı olanlarda hipertansiyon hızla ağırlaşabilmekte ve/veya organ hasarı bulguları günler/haftalar içinde gelişebilmektedir.

Daha ileri durumlarda vakaların %2'sinde eklampsi gelişebilmektedir. Preeklampsi hem anne hemde fetus için ciddi sekellerle ilişkilidir (abrupsitio plasenta, KC hematomu/rüptürü, DIC, inme, mekanik ventilasyon, invazif hemodinamik monitorizasyon, transfüzyon, diyaliz gerekliliği; eklampsiye progresyon). Hastalığa eşlik eden göğüs ağrısı, dispne, düşük trombosit sayısının kötü prognozla ilişkili olduğu bildirilmektedir. Plasentanın doğumu hastalığın iyileşmesinde en önemli faktördür. Doğum sonrası bazı semptomlar saatler içinde (örn. başağrısı), bazıları aylar içinde (örn. proteinüri), tipik olarak 3. boşluktaki sıvı mobilizasyonu ve diürez doğum sonrası 48 saatte başlamaktadır. Ancak bazı olgularda hipertansiyon postpartum ilk ve 2. haftada kötüleşebilmekte, genellikle 4 haftada normale dönebilmekte ve nadiren 3 ay sonrasında da devam edebilmektedir[1,2].

Tanı sonrası hastalığın şiddetini değerlendirmek, maternal ve fetal iyilik halini değerlendirerek takip etmek çok önemlidir. Çünkü hastalığın şiddeti doğrultusunda anneye gerekli müdehale yapılacak ve fetüsün gelişim durumuna göre gebeliğin ne zaman sonlandırılacağına karar verilecektir. Şiddetli preeklampsi; STA ≥160 ve/veya DTA ≥110 mmHg olması, trombositopeni <100000/mm3 olması, karaciğer fonksiyon bozukluğu (AST /ALT > normalin 2 katı), epigastrik veya sağ üst kadran ağrısı, serum kreatinin ≥ 1,2 mg/dL veya serum kreatinin düzeyinin artarak en az 2 katına çıkması, pulmoner ödem veya siyanoz, başağrısı, görme bulanıklığı gibi klinik ve laboratuar bulguları ile tanınmaktadır. Ancak proteinüri miktarı, oligüri, IUGR gibi bulgular şiddetli preeklampsi kriterleri olarak kabul edilmemektedir[1].

Preeklampsinin ayrıcı tanısında daha önceden var olan hipertansiyon: <20 haftada başlayan hipertansiyon, proteinüri olmaması(<1gr/gün); superempose preeklampsi: bilinen primer hipertansiyonu olan kadında kan basıncı yüksekliği ve/veya proteinüri ve preeklampsi semptom ve bulguları; böbrek hastalığın alevlenmesi, antifosfolipid sendromu, trombotik trombositopenik purpura (TTP), hemolitik üremik sendrom (HUS), sistemik lupus eritomazus (SLE) gibi hastalıklara dikkat edilmelidir. Ayrıca gebeliğe bağlı hipertansiyonun preeklampsiden ayrımı da yapılmalıdır. Gebeliğe bağlı hipertansiyon, 20. gebelik haftadan sonra ortaya çıkmakta, STA ≥ 140 ve/veya diastolik DTA ≥ 90 mmHg olması, proteinüri veya organ fonksiyon bozukluklarının olmaması ve tansiyonun en geç postpartum 12 hafta içerisinde normale dönmesi ile tanınmaktadır[1,18]. Bazı preeklampsi vakalarında ise kliniğe grand mal nöbetler eklenmektedir. Bu durum artık eklampsi olarak tanımlanmaktadır[19]. HELLP sendromunun ise preeklampsinin ağır formu olabileceği ya da ayrı bir hastalık olabileceği düşünülmektedir. Bu vakaların %15-20'sinde eşlik eden hipertansiyon ya da proteinüri saptanmamaktadır[20].

Preeklampsinin öngörülmesi ve saptanması tabi ki iyi bir gebelik takibiyle olmaktadır. Gebelik takip polikliniğine başvuran hastalara öncelikle iyi bir maternal öykü alınmalı, özgeçmişi ve önceki gebelikler sorgulanmalıdır. Fizik muayene, laboratuvar ve görüntüleme tetkiklerinde uterin arter pulsatilite indeksi, ortalama arter basıncı, PAPP-A, plasental büyüme faktörü gibi bulgular takip edilmelidir. Uygun takiplerle erken başlangıçlı hastalık %95 oranında tanınabilmektedir. Öngörülen vakalarda, riskli gebelerde Aspirin tedavisinin 16. haftadan önce başlanması perinatal ölümde %60 azalma, preeklampside %50 azalma, şiddetli preeklampside %80-90 azalma ve IUGR da %55 azalma ile sonuçlandığını bildireen vaka serileri olduğu görülmektedir[21].

Ancak fetus açısından ilacın yan etkileride göz önünde bulundurulmalıdır. Yine kalsiyum ve heparin/düşük mol ağırlıklı heparin tedavilerinin de yararlı etkileri bildirilmiştir.

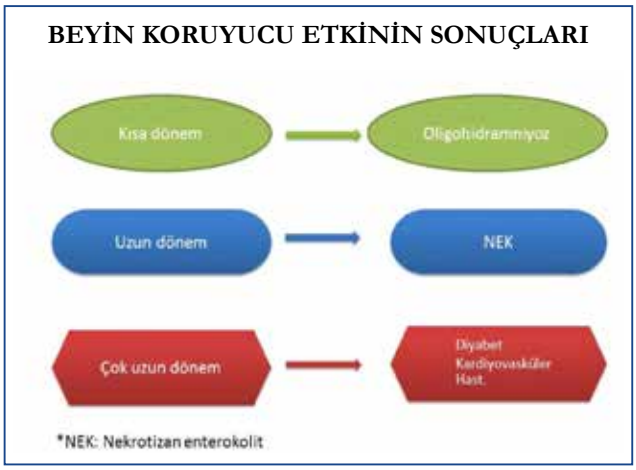
Preeklampsinin asıl tedavisi ise gebeliğin uygun zamanda sonlandırılmasıdır. Bu nedenle hafif ve şiddetli vakaların ayrımının uygun şekilde yapılması gebeliğin sonlandırılması zamanına karar vermede önemlidir. Hafif valarda gebelik 37. gestasyon hastası sonrası rahatlıkla sonlandırılabilmektedir. Şiddetli vakalarda ise fetus yaşam sınırı altında ya da ≥ 34 gestasyon hafta olması, anne veya fetusun durumunda bozulma olması durumunda gebelik sonlandırılmaktadır. Ancak şiddetli vakalarda gestasyon haftası 34 hafta altında olsa da kontrol edilemeyen şiddetli hipertansiyon, eklampsi, annede pulmoner ödem, ablasyo plasenta, tüketim koagülopatisi (DIC), HELLP, fetal distres/fetal ölüm durumları varlığında gebelik sonlandırılmaktadır[1,22].

Plasental Disfonksiyona Fetal Uyum

Fetus metabolik ihtiyaçlarını ve oksijen gibi hayati öneme sahip gereksinimlerini plasenta yoluyla anneden almaktadır. Preeklampsi sonucu gelişen plasental yetersizlik dolayısıyla fetus kendini korumak için değişik yollara başvurmaktadır. Bunlardan en önemlisi beyin gibi hayati organları koruma refleksidir. Beyin koruyucu etki, fetal hipoksemi varlığında,

fetal serebrumda periferik damar direncinde kompansatuvar azalma olması sonucunda; a) santralizasyon: orta serebral arter pulsatilite indeksi azalırken, umblikal arter pulsatilite indeksinde artma olması (oran <1.08 anlamlı), b) redistribisyon: fetal kan akımının yaşamsal önemi fazla olan organlara dağıldığını ve beyin kan akımının korunması gibi kan akımının yeniden düzenlenmesi oluşmaktadır[23] (Şekil 2).

Şekil 2: **Beyin koruyucu etkinin uzun ve kısa süreli etkileri**



Plasental yetmezlik sonrası fetüste şiddetli hipoksi, metabolik bozukluklar, yüksek enerjili fosfatların yıkımı, beyin ödemi gelişmesi, kompansatuar serebral vazodilatasyonun kaybı oluşmaktadır. Son evrede ise hipoksemik/ iskemik miyokardiyal disfonksiyon, kardiyak dilatasyon ve holosistolik atrioventriküler kapak regürjitasyonu, duktus venozusta tersine akım, umblikal arterde pulsatil akım meydana gelmektedir[23,24]. Olayların erken dönemde umblikal arterde direnç artışı, midserebral arter direncinde azalma, umblikal arterde end diyastolik akım yokluğu görülebilir. Doğum kararı ise non reaktif nonstres test (NST), variyabilite 3 vuru/dk altındaysa verilebilmektedir[1,2,23].

Öncelikle plasentayı etkileyen kronik plasental hipoperfüzyon fetuste de fetal büyüme kısıtlılığı, oligohidramniyoz gibi olumsuz etkilere neden olmaktadır. Ağır ve erken başlangıçlı preeklampside doğum ağırlığında düşüklüğün derecesi en fazladır (%11 ve %23). Geç başlangıçlı preeklampside plasental perfüzyonun ve fonksiyonun sonradan bozulması nedeniyle doğum ağırlığı ortalamanın üzerinde olabilir[25].

Preeklampsi ilişkili neonatal olumsuz sonuçlar

Preeklampsi, iyatrojenik prematüre doğumun en sık nedeni olmakla birlikte preeklampsinin en önemli morbiditesi prematüritedir. Ayrıca preeklamptik anne bebeklerinde inrtauterin gelişme geriliği ve fetal kayıp riski artmıştır[26,27]. Erken başlangıçlı preeklampsi fetal ölüm ve perinatal ölüm/ciddi neonatal mortalite ile ilişkili saptanmıştır. Geç başlangıçlı preeklampside ise fetal kayıp riski daha az bulunmuştur[28,29]. Ayrıca IUGR ve SGA riskinin de erken başlangıçlıda 7 kat, geç başlangıçlıda 3 kat arttığı bildirilmektedir[30,31].

Preeklamptik anne bebeklerinde respiratuvar distress sendromu (RDS) gelişimi ile ilgili çelişkili sonuçlar bulunmaktadır.

Bir çalışmada 34-37 gestasyonel haftada bebeklerde RDS sıklığının azaldığı bildirilmektedir[32]. Ancak diğer bir çalışmada 34- 37 gestasyonel haftadaki preeklamptik anne bebeklerinde RDS sıklığı arttığı bildirilmektedir. Preeklampsinin fetal akciğer üzerindeki etkisi kesin olarak bilinmemekle birlikte preeklampsinin prematürelerde RDS için risk faktörü olabildiği, term ve pretermlerde artmış solunumsal morbiditeler ile ilişkili olabileceği bildirilmektedir[33]. Preeklampsinin patofizyolojisinde meydana gelen anormal plasenta oluşumu nedeniyle plasental yetmezlik ve uterin kan akımının yetersizliği fetüsün gelişimini olumsuz etkilemektedir[34]. Bu nedenle oluşan hipoksi ve iskemi fetal anjiogenezisi de bozmaktadır[35]. Anne karnında fetuste meydana gelen damarsal yapıların gelişiminin sürekliliğinin korunması fetal akciğerde alveollerin gelişimi için kritik öneme sahiptir. Ancak preeklapmtik anne bebeklerinde normal akciğer gelişimi için kritik öneme sahip olan akciğer damar gelişiminin bozulduğu düşünülmektedir[36,37]. Ayrıca preeklamptik gebelerde VEGF antagonisti soluble VEGFR1 düzeyi arttığı ve bu nedenle anjiogenezisin bozulduğu ve VEGF sinyalinde bozulma akciğer gelişimini etkileyerek bronkopulmoner displaziye (BPD) yol açabileceği bildirilmektedir[38]. Güncel çalışmalar annede intrauterin fetal gelişme geriliğine neden olabilecek kadar şiddetli preeklampsinin BPD gelişiminde rol oynadığını da desteklemektedir[39].

Preeklampsinin yenidoğan döneminde sepsis sıklığını arttırdığı bildirilmektedir. Nötropeni (absolu nötrofil sayısı <500) preeklamptik anne bebeklerinde sıkılıkla görülmekte ve büyük ölçüde intrauterin başlamaktadır. Yapılan çalışmalarda annesi preeklamptik olan düşük doğum ağırlıklı bebeklerinin %47-%50 sinde yaşamlarının ilk 12 saatinde nötropeni olduğu izlemektedir. Yine ciddi preeklampsi olan annelerin bebeklerinde nötropeninin daha sık olduğu ve doğum ağırlıklarının daha düşük olduğu bildirilmektedir.

Bu nedenlerle preeklamptik anneden doğan, intrauterin gelişme geriliği ve nötropenisi olan bebeklerde erken (%4,6-6) ve geç başlangıçlı (%24) sepsis oranın normale göre yüsek olduğu görülmektedir. Hastaların takiplerinde nötropenin günler/haftalar içinde düzeldiği ve granülosit-koloni uyarıcı faktor (G-CSF) tedavisine ihtiyaç duyulmadığı görülmektedir[40]. Nötropenin sebebinin ise uteroplasental yetmezlik, plasental inhibitör faktörlere bağlı nötrofil üretiminde azalma ve kemik iliği myeloid seri hücrelerinde baskılanma nedeniyle olduğu düşünülmektedir[41,42,43]. Ayrıca preeklampsili anne bebeklerinde kanlarında koloni forming unit-granulosit

makrofajın (CFU-GM) azaldığı ve periferik kanda nötrofil havuzunun azaldığı bildirilmektedir[40].

Preeklampsili anne bebeklerinde trombositler de etkilenmektedir. Trombositopeni (<150,000/uL), özellikle intrauterin gelişme geriliği olan bebeklerde sıklıkla ilk 72 saatte ortaya çıkmakta ve birçok hastada genellikle 10 gün içinde düzelmektedir[40,44]. Trombositopeninin ciddiyeti hastalık şiddetine göre değişmekle birlikte çok küçük oranda da olsa ciddi ya da klinik olarak önemli trombositopeni (<50,000/uL) bazı hastalarda gelişebilmektedir[45]. Preeklamptik anne bebeklerinde trombositopenin nedeni kesin olarak bilinmemekle birlikte; fetal hipoksi nedeni ile baskılanmış megakaryosit proliferasyonu sonucu azalmış üretim, mikroanjiyopatik yıkım ve plasental yıkım nedeniyle oluştuğu düşünülmektedir[46].

Preeklampsinin erken ve geç pretem doğan infantların göz gelişimi üzerine etkilerinin olduğu bildirilmektedir. Çelişkili sonuçların bildirildiği çalışmaların bir kısmında preeklampsinin, artmış premature retinopatisi (ROP) sıklığı ve ROP şiddeti ile ilişkili olduğu, yenidoğanda retinal hipoksi, inflamasyon, oksidatif stres oluşturduğu ve ROP riskini arttırdığı belirtilmektedir[47].

Öte yandan preeklampsi durumunda dolaşımdaki anjiogenik faktörlerin (soluble fms-like tyrosine kinase 1 (sFlt1)[48] ve endoglin (transforming büyüme faktörü-1'in ko-faktörü)[49] arttığı, biyoaktif proanjiogenik faktörlerin (VEGF, plasental büyüme faktörü (PlGF)) azaldığı[50,51] bildirilmektedir. ROP patogenezinde anjiogenezisin özellikle VEGF'in rolü çok iyi bilinmektedir[52]. Bu nedenle relatif olarak proangiogenik olan ancak daha çok antianjiogenik olan annede preeklampsi gibi hipertansiyonun eşlik ettiği durumların ROP gelişiminden koruyabileceği düşünülmektedir[47,53,54].

Preeklampsili anne bebekleri intrauterin plasental yetmezlik nedeniyle hipoksik bir ortama maruz kalmakta ve fetal organlara kan akımı dağılımı öncelikle vital organlara kaymaktadır. Özellikle end- diyastolik umblikal akımı olmayan SGA bebeklerde intrauterin distres oluştuğu bildirilmektedir. Bu durum preeklamptik anne bebeklerinde intestinal dokuların etkilenerek barsağa giden kan akımının azalmasına, sonuç olarak hipoksi-iskemiye neden olabilmektedir. Özellikle preterm ve düşük doğum ağırlıklı bebeklerde prenatal ve postnatal barsak perfüzyon bozuklukları, staz ve immünolojik faktörlerin de etkisi ile intestinal doku perfüzyonunu bozarak ve bakteriyel kolonizasyonu kolaylaştırarak nekrotizan enterokolit (NEK) gelişimine, NEK'in daha erken ortaya çıkıp daha geç sonlanmasına neden olabilmektedir[55]. Ayrıca güncel çalışmalarda maternal preeklampsinin preterm bebeklerde spontan intestinal perforasyon sıklığını arttırdığını (%6,2) ve bağımsız risk faktörü olduğunu desteklemektedir[56].

Preeklampsi heterojen bir hastalık olması nedeniyle bu annelerin bebeklerinde nörolojik etkileniminde değişken olduğu görülmektedir. İntrauterin gelişme geriliği olan ve kronik hipoksiye maruz kalan preterm doğan preeklamptik anne bebeklerinde intrakranial kanama ve periventrüküler lökomalazi riskinin azaldığı bildirilmektedir (%4,8'e karşı %20,5)[57,58].

Ancak preeklamptik anneden term doğan bebeklerde ise ensefalopati riskinin arttığı bildirilmektedir. Preeklamptik annelerde doğumda > 37,5 derece ateş olması preeklampsinin sistemik inflammatuvar bir olay olduğunu düşündürmektedir. Ancak yenidoğan ensefalopatisinin gelişiminin preeklamptik anne bebeklerinde obstetrik müdahaleden bağımsız olduğu, asidemi veya annede ateş ile izah edilemeyiceği, oksidatif strese ikincil olarak fetusta bir sistemik inflamatuvar yanıtın preeklamptik anne bebeklerinin beyninde vazokonstriksiyon meydana gelmesi sonucu neonatal ensefalopati oluşabileceği düşünülmektedir[59]. Yapılan bazı çalışmalarda preeklamptik annelerden doğan gestasyon haftaları 32 altında olan bebeklerin 24 aylık oldularında daha düşük Bayley II gelişim skorlarına sahip oldukları ve kognitif fonksiyonlarda bozukluğunda eşlik ettiği bildirilmekle birlikte[60], bazı güncel çalışmalarda preeklamptik anneden doğan bebeklerin doğum sonrası 18 aylık olduklarında gelişim skorlarının daha yüksek olduğunu bildirmektedir[61]. Bu farklılığın ise preeklampsinin farklı şiddetteki tiplerinin farklı etkileri nedeniyle olabileceği düşünülmektedir. Diğer çalışmalarda ise preeklampsinin kognitif fonksiyonlarda bozulma olmadan serebral palsiden koruyucu etkisinin olduğu belirtilmektedir[62].

Preeklamptik anneden doğan bebeklerde yaşamlarının erken dönemlerinde olduğu gibi ileriki dönemlerinde de değişik sistemlere ait bazı problemler olduğu görülmektedir. Yapılan çalışmalarda preeklapmtik anneden doğan intrauterin gelişme geriliği olan bebeklerin yaşamlarının ilk günlerinde tansiyon değerlerinin normal bebeklere oranla daha yüksek olduğu bildirilmiştir[63]. Preeklamptik anneden doğan bebeklerin ileride çocuk ya da adelosan döneminde hipertansiyon gibi kardiyovasküler morbiditeler, diyabetes mellitus ve inme riskinin arttığı bildirilmiştir[64,65]. Ciddi preeklamptik anneden doğan çocuklar üzerinde yapılan bir çalışmada (ortalama yaşları 12,8 yıl) serum spesifik IgE düzeyinin arttığı, allerjik duyarlılık, allerjik rinokonjunktivit ile ilişki olduğu saptanmıştır[66].

Sonuç olarak; preeklampsi sadece gebelik süresince oluşan bir durumdur. Preeklampsinin, yenidoğan infantlarda ölümünde birlikte olabileceği ve birçok sistemin etkilendiği bir dizi erken ve geç perinatal/neonatal komplikasyonlarla ilişkili olduğu görülmektedir. Bunlar çoğunlukla gestasyonel hafta ve doğum ağırlığı ile ilişkili olup preeklamptik tokseminin ciddi veya erken başlamasıyla da ilişkilidir. Ayrıca preeklampsi intrauterin gelişme geriliği ve prematüre doğumun önemli sebeplerinden biridir. İntrauterin gelişme geriliğinin derecesi erken ve geç morbiditeler üzerinde negatif etkilidir. Prematür doğan bebeklerin uzun dönem sonuçları da gestasyonel haftaya

bağlıdır. Güncel olarak preeklampsi tanısında kullanılabilecek optimal antenatal test yöntemi ile ilgili bilgiler kısıtlıdır. Preeklampsinin erken dönemde tanınması ve dikkatli takip edilmesi anne ve doğacak bebeği için gelişebilecek olumsuz sonuçların engellenebilmesi açısından önemli olacaktır.

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Derleme / Review

Koru Proceedings

DNA Hasarı Tamiri ve İlişkili İnsan Hastalıkları

DNA Damage Repair and Related Human Diseases

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ÖZET

İnsan vücudu, genetik kararlılığı etkileyen hücre içi ve çevresel stres etmenlerine sürekli olarak maruz durumdadır. Genetik kararlılığın kaybolması Deoksiribonükleik asit (DNA) hasarlarına, mutasyonlara, kanserlere ve gelişimsel bozukluklara sebep olur. Bu nedenle hücreler, DNA hasarına karşı, DNA hasar tamir mekanizması olarak bilinen biyolojik savunma mekanizmaları geliştirmişlerdir. Bu mekanizma, hücre içi DNA hasarını tanıyıp, tamir ederek insan genomunu korur. Farklı tiplerdeki DNA hasarlarını onarmak için farklı biyokimyasal stratejiler kullanan farklı alt DNA tamir mekanizmaları vardır. DNA'da meydana gelen tek zincir kırıkları, tek zincir kırık tamir mekanizması ile düzeltilir. Baz eksizyon onarımı, alkillenmiş, amin grubunu kaybetmiş ve oksitlenmiş bazları düzeltir. Büyük DNA lezyonları nükleotid eksizyon onarımı mekanizması ile onarılırken; yanlış baz eşleşmeleri, baz eklenme ve silinmeleri yanlış eşleşme eksizyon onarım mekanizması tarafından düzeltilir. DNA hasarlarının en ölümcül formu olan çift zincir kırıkları ise, çift zincir tamir mekanizması tarafından düzeltilir.

Anahtar kelimeler : *DNA hasarı, tek zincir kırığı tamir mekanizması, çift zincir kırığı tamir mekanizması, genetik kararsızlık, kanser.*

ABSTRACT

Human body is constantly exposed to various endogenous and enviromental stresses that affect the genetic stability. The loss of genetic stability causes Deoxyribonucleic acid (DNA) damages, mutations, cancers and developmental disorders. Therefore, cells developed biological defence mechanisms against DNA damage, which is known as DNA repair mechanism. This mechanism protects the human genome by recognizing and repairing intracellular DNA lesions. There are different DNA repair submechanisms that use different biochemical strategies to repair different types of DNA damages. Single strand breaks that occur in DNA are repaired by single strand break repair mechanism. Base excision repair corrects alkylated, deaminated and oxidized bases. Bulky lesions are corrected by nucleotide excision repair; whereas base mismatches, base insertions and deletions are repaired by mis-match repair mechanism. The most lethal form of DNA damages, double strand breaks, are corrected by double strand repair mechanism.

Key words: *DNA damage, single strand repair mechanism, double strand repair mechanism, genomic instability, cancer.*

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1. GİRİŞ

İnsanlığın devamı için gerekli olan genetik bilgi genomda saklanır ve nesilden nesile iletilir. Her hücre bölünmesinde, yaklaşık olarak üç milyardan fazla Deoksiribonükleik asit (DNA) baz çifti kopyalanır ve oluşan yeni hücrelere aktarılır. Hücre içi DNA replikasyon mekanizması çok etkili bir biçimde çalışıyor olsa da replikasyon hataları ortaya çıkabilmektedir. Bu hataların bir çoğu zararsızdır; ancak bazı durumlarda hastalıklara neden olabilmektedir.. DNA molekülünün genetik bilginin saklanmasındaki rolü, tartışılamaz seviyede önemlidir. Ancak DNA, sürekli olarak DNA hasarına neden olan endojen ve ekzojen etmenlere maruz kalır ve DNA'nın kimyasal yapısının kararlılığının da bir sınırı vardır[1]. DNA replikasyonu ve rekombinasyonu sırasında meydana gelen hatalara ek olarak, hidroliz ve oksidasyon gibi hücrenin doğal fizyolojik aktiviteleri sonucu ortaya çıkan hücresel metabolitler de endojen kaynaklar olarak, DNA'nın yapısında spontan değişiklikler meydana getirebilmektedir[2]. Ekzojen kaynaklar arasında en yaygın olarak bilinen DNA hasar ajanı, güneşten gelen ultraviyole (UV) ışınlardır[3]. Ozon tabakası her ne kadar UV spektrumunun en tehlikeli olan kısmını (UV-C) absorbe etse de, geriye kalan güneş ışınları içerisindeki UV-A ve UV-B her saat başı her hücre için yaklaşık olarak 100.000 DNA hasarına neden olabilmektedir[4]. Rodon bozunumu sonucu ortaya çıkan iyonize radyasyon (IR) ise çok çeşitli DNA hasarlarına neden olur[3,4]. Bunlar arasında en toksik olanı, DNA çift zincir kırıklarıdır (DSB'ler)[4]. UV ışınları ve IR gibi fiziksel ajanlar dışında, kimyasal ajanlar olarak adlandırılan mantar kaynaklı aflatoksinler, benzopren, kemoterapi ilaçları, alkilleyici ajanlar ve hardal gazları da DNA hasarına neden olan ekzojen etmenler olarak bilinir[2]. Yukarıda bahsi geçen hücresel ve çevresel etmenler, hücrenin genetik kararlılığını bozarak canlı yaşamını tehtit eden DNA hasarlarına, mutasyonlara, kansere ve çeşitli gelişimsel bozukluklara sebep olur[1, 4, 6]. Genetik materyalin, ekzojen ve endojen ajanlar tarafından moleküler bütünlüğünün bozulması 'DNA hasarı' olarak adlandırılmaktadır[7]. Canlı organizmalar, genetik materyalin bütünlüğünü korumak ve DNA hasarlarını düzeltebilmek için, nükleotid eksizyon tamiri (NER) ve baz eksizyon tamiri (BER) gibi farklı biyokimyasal stratejilere dayanan farklı DNA tamir mekanizmalarına sahiplerdir[8, 9]. Günümüzde DNA tamirinde rol oynayan yolakların moleküler mekanizmaları, bu konunun öncülerinden olan Sancar, Lindahl ve Modrich sayesinde ayrıntılı bir şekilde anlaşılmıştır.

Bu derlemede, DNA hasarlarının ortaya çıkış nedenlerinden ve hücrelerin DNA hasarlarını düzeltebilmek için moleküler seviyede nasıl bir yol izlediklerinden bahsedilmektedir. Ayrıca, DNA tamir yanıtının, diğer hücresel mekanizmaları nasıl etkilediğinden, DNA tamirinin biyolojik öneminden ve DNA hasarları sonucu ortaya çıkan kalıtsal ve sporadik hastalıklardan bahsedilmektedir. Son olarak, DNA tamir yanıtının bazı hastalıkların tanı ve tedavisindeki önemi üzerinde durulmaktadır.

2. DNA Onarım Mekanizmaları

DNA hasarı, hücrelerde, öncelikle sensör proteinler olarak bilinen ve içerisinde ATM (ataxia telangiectasia mutated) ve ATR (ATM and Rad3 related) kinazların da bulunduğu proteinler tarafından algılanır; aracı proteinler olan 53BP1 (p53 binding protein) ve g-H2AX ile iletilir[10, 11]. Sensör proteinler tarafından algılanıp aracı moleküllere iletilen bilgi, sonrasında CHK1 (checkpoint kinase 1) ve CHK2 (checkpoint kinase 2) tarafından alınıp effektör proteinler olan TP53 ve CDC25 (cell division cycle 25) proteinlerine aktarılır[11]. Efektör proteinlerin aktivasyonu sonucu hücre döngüsü duraklatılır ve DNA hasarının miktarına bağlı olarak hücre ya apopitoza girer ya da DNA tamir proteinleri hasarlı bölgeye çağrılarak mevcut hasar düzeltilir[3, 8] DNA hasar oluşum şekillerinin ve tiplerinin birbirinden farklı olması nedeniyle memeli hücrelerinde farklı DNA hasarları farklı DNA tamir yolları ile düzeltilmektedir. DNA tamir mekanizmaları; doğrudan onarım (DR), BER, NER, yanlış eşleşme onarımı (MMR) ve çift zincir kırık onarımı (DSBR) şeklinde sınıflandırılmaktadır[8, 9, 12].

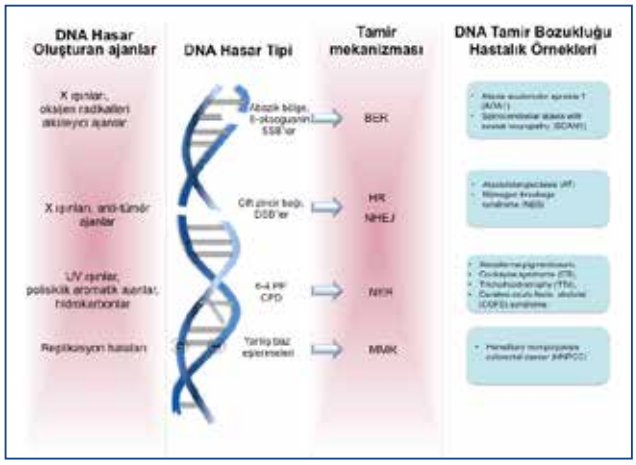
2.1. Doğrudan Hasar Onarımı (Direct Repair)

Doğrudan hasar onarımı mekanizmalarında, hasarlı bölge tek bir enzimle (fotoliyaz veya O-6-Metil-DNA-alkiltransferaz), DNA'nın yapısında herhangi bir zincir kırığı oluşturmadan, uzaklaştırılmaktadır[3, 13]. Her ne kadar 'doğrudan onarım' yoluyla az miktarda DNA hasar çeşidi düzeltiliyor olsa da, kolay ve hatasız olması nedeniyle DR tamir mekanizması hücreler tarafından sıklıkla tercih edilmektedir[3, 13]. Doğrudan onarım mekanizması yoluyla fotoliyaz enzimi kullanılarak iki tip DNA lezyonu düzeltilir: UV ışınları sonucu meydana gelen siklobütan pirimidin dimerleri (CPD'ler) ve primidin-primidonlar (6-4 PP'ler). Fotoliyaz enzimi kullanılarak 300-600 nm dalga boyundaki ışıkla indüklenen DNA üzerindeki timin dimerlerinin birbirinden ayrılmasına 'fotoreaktivasyon' adı verilir[3]. CPD fotoliyazlar prokaryot ve ökaryotların büyük çoğunluğunda bulunurken, plasentalı memelilerde bulunmaz [13].

2.2. Baz Eksizyon Onarımı (Base Excision Repair-BER-)

İyonize edici radyasyon, reaktif oksijen türleri, monofonksiyonel alkilleyici ajanlar ile oluşan DNA baz hasarları ve tek zincir kırıkları (SSB'ler), BER yoluyla tamir edilir (Şek.1) [15, 16]. BER mekanizmasının fonksiyon gösterebilmesi için DNA N-glikozilaz, AP endonükleaz (APE), DNA polimeraz ve DNA ligaz olmak üzere başlıca dört farklı enzime ihtiyaç vardır (16). BER yolağı, DNA hasarlarını dört farklı enzimatik

basamakla düzeltir: i. Yanlış bazın uygun bir DNA-N-glikozilaz kullanılarak uzaklaştırılması ve apürinik/apirimidinik (AP) bölge oluşması, ii. AP endonüklezlar tarından AP bölgesinin 5' tarafından veya 3' tarafından çentik atılması ve AP bölgesine komşu bir 3'OH ucu oluşturulması, iii. AP bölgesini içeren DNA parçasının kesilerek uzaklaştırılması ve bu boşluğun DNA polimeraz I tarafından doldurulması, iv. DNA ligazın kırık ucu (çentik) birleştirerek DNA heliksinin eski haline getirilmesiyle tamir sürecini tamamlanır[17-19].



Şekil 1. DNA hasarına neden olan etmenler, DNA tamir mekanizmaları ve DNA tamir mekanizması bozuklukları sonucu orta çıkan bazı kalıtsal hastalık örnekleri;

X ışınları, oksijen radikalleri ve alkilleyici ajanlar SSB'lere neden olur, BER tamir mekanizması ile düzeltilir ve BER mekanizmasında bozukluk olması durumunda AOA1 ve SCAN1 gibi kalıtsal hastalıklar görülebilir. DSB'ler X ışınlarına ve anti-tümor ajanlara maruz kalınması sonucu oluşur ve HR yada NHEJ ile düzeltilir, AT ve NBS hastalıkları DSBR mekanizması bozukluklarında ortaya çıkabilir. Pürin ve pirimidin dimerleri (6-4 PP ve CPD) UV ışın, polisiklik aromatik ajanlar ve hidrokarbonlar nedeniyle ortaya çıkar; NER ile düzeltilir ve NER mekanizmasında bozuklukluk olması durumunda XP, CS, TTd ve COFs sendromları ortaya çıkabilmektedir. Replikasyon hataları sonucu ortaya çıkan yanlış baz eşleşmeleri ise MMR tamir mekanizmasıyla düzeltilir ve bu mekanizmanın bozukluğunda HNPCC ortaya çıkabilmektedir.

2.2. Nükleotid Eksizyon Onarımı (Nucleotide Excision Repair-NER-)

Güneşten gelen UV ışının etkisinde kalan bir hücrede, komşu pirimidinlerin kovalent bağlanmaları ile pirimidin dimerleri (CPD, 6-4 PP) oluşur (Şek.1); ve bu dimerler DNA polimerazın çalışmasını ve DNA zincirinin replikasyonunu önler[4, 9, 11]. DNA heliks yapısında bozulmalara yol açan hasarlar, genellikle NER sistemleri ile tamir edilir[20-22]. Ayrıca okside edici ve alkilleyici ajanların etkisiyle oluşan küçük baz lezyonlarının tamirinde, BER mekanizmasının yeterli olmadığı hallerde NER mekanizmasının etkili olduğu belirlenmiştir[20]. NER mekanizması genel olarak şu basamakları içerir; i. Hasarın tanınması, ii. Protein kompleksinin hatalı bölgeye bağlanması, iii. Hasarlı bölgeyi içeren ~24-32 nükleotid uzunluğundaki bölgenin kesilip çıkartılarak uzaklaştırılması (BER sırasında hasarlı bazlar serbest baz olarak kesilir ve çıkartılırken, NER'de ise hasarlı bazlar oligonükleotid fragmanları olarak kesilirler), iv. DNA sarmalı üzerinde meydana gelen boşluğun DNA polimeraz tarafından doldurulması ve v. Oluşan çentiğin ligasyonu ile DNA çift zincirinin bütünlüğünün tamamlanması[22]. Bu basamaklar arasında en önemli olanı, hasarın tanınması basamağıdır[20,23]. NER onarım mekanizmasının düzgün bir şekilde işleyebilmesi için 300'den fazla proteinin görev yapması gerekmektedir[22]. NER tamir mekanizmasında rolü olan proteinlerden herhangi birini kodlayan bir gende oluşan mutasyonlar sonucu nadir görülen otozomal resesif geçişli sendromlar görülebilmektedir (şek.1)[20, 23].

2.3 Yanlış Eşleşme Onarımı (Mismatch Repair-MMR-)

MMR mekanizması, DNA replikasyonu sırasında meydana gelen DNA yanlış baz eşleşmelerini düzeltir ve ortaya çıkabilecek mutasyonların bölünen hücrelerde kalıcı olmasını engeller (şek.1) [23]. Çünkü MMR, replikasyon esnasında meydana gelen hata yüzdesini düşürür[23]. MMR mekanizmasının çeşitli mutasyonlar sonucu inaktif hale gelmesiyle kendiliğinden oluşabilecek mutasyonların oluşum sıklığının artar ve bunun sonucunda, insan hücrelerinde birçok kalıtsal ve sporadik kanserler ortaya çıkmaktadır[24]. MMR mekanizması E. coli üzerinde çok ayrıntılı bir biçimde çalışılmıştır[21,23]. E. coli'de, hatalı eşleşme tamiri için MutS, MutL, MutH, DNA helikaz II (MutU/UvrD), 4 ekzonükleaz protein (ExoI, ExoVII, ExoX, and RecJ), SSB proteinler, DNA polymeraz III holoenzim, ve DNA ligaz enzimlerine ihtiyaç vardır[24]. E. coli DNA'sında, 5'-GATC-3' dizisindeki adeninler metillenmiştir ve prokaryotlarda yanlış DNA eşleşmeleri daima kalıp zincirdeki bilgi baz alınarak tamir edilir[24, 25]. DNA sentezi esnasında sentezlenen yeni zincir, kısa bir süre için metillenmemiş yapıya sahiptir[24, 25]. Tamir sistemine ait proteinler metillenmeye göre kalıp zinciri ve yeni sentez edilen zinciri ayırt edebilir ve yeni zincirdeki yanlış eşleşmeleri düzeltebilir[25]. Ökaryotlarda, E. coli' de bulunan proteinlere homolog proteinler vardır; fakat yanlış eşleşme hataları DNA çift zincirindeki metilasyon durumuna bakılmaksızın düzeltilir[24, 25].

2.4. Çift Zincir Kırık Onarımı (Double Strand Break Repair-DSBR)

DNA hasarlarının en ölümcül formu DSB'lerdir[26]. İyonize radyasyon, anti-tümör ilaçları, ve topoizomeraz inhibitörleri DSB'lere neden olan ekzojen ajanlar arasında sayılabilir[27]. Oksidatif metabolizma faaliyeti sonucu oluşan serbest radikaller ve V(D)J rekombinasyonları çift zincir kırıklarına neden olan endojen kaynaklara örnek olarak verilebilir. DSB'ler, DSBR mekanizması tarafından tamir edilir[26]. Eğer DSB'ler düzeltilmezse, kromozom kırılmalarına ve hücre ölümüne neden olur[1, 27]; eğer yanlış onarılırlarsa da kromozom translokasyonlarına ve kanser oluşumuna neden olurlar[1, 27]. DSB'ler iki şekilde düzeltilirler: Homolog rekombinasyon (HR) veya homolog olmayan uçların bağlanması (NHEJ) mekanizmalarıyla[27]. HR'de çift zincir DNA kırıkları homolog DNA ile rekombinasyon yoluyla tamir edilirler ve bu mekanizmada genetik bilginin korunarak tamir edilmesi esastır[26, 27]; ancak NHEJ mekanizması ile kırık olan uçlar genetik bilginin korunmasına bakılmaksızın birbirine yapıştırılarak tamir edilir[26, 28].

3. DNA TAMİR BOZUKLUĞU HASTALIKLARI

3.1 DNA Onarımı ve Kanser

Birçok kanserojen, genom üzerinde mutasyonlar oluşturarak DNA hasarlarına neden olur[1, 28]. Hem kalıtsal hem de çevresel faktörlerin etkisiyle meydana gelen DNA hasarlarının düzeltilemediği durumlarda genetik kararsızlık ortaya çıkar[28]. Genetik kararsızlıklar, kanserin karakteristik özelliklerinden biridir ve kanserlerin çoğu genetik kararsızlığa neden olan tamir edilmemiş mutasyonların birikimiyle meydana gelir[29]. Onarım sisteminde rol alan tamir mekanizmalarındaki bozukluklar ve enzimlerdeki mutasyonlar direkt olarak kalıtsal kanser oluşumlarına neden olurlar (şek.1)[5, 6]. Sporadik kolon ve endometrium kanserlerinin büyük çoğunluğu MMR hataları sonucu meydana gelen mikrosatelit karasızlıklarından (MIN) kaynaklanır[30]. Kalıtsal non-polipozal kolerektal kanserler yine MMR hatalarından kaynaklanırken, kolerektal kanser ise BER mekanizmasındaki bozukluktan kaynaklanır (şek.1)[31]. DNA onarım mekanizmaları arasında NER mekanizması, bilinen en genel ve etkili onarım mekanizmasıdır[21, 22]. NER mekanizmasındaki bozukluklar, güneşe duyarlılığı arttırır ve UV kaynaklı cilt kanserlerinin ortaya çıkmasına neden olur[5, 6, 23]. Meme kanserleri kalıtsal olmasının yanında iyonize radyasyon etkisiyle meydana gelen DSB'ler sonucuyla da ortaya çıkar[32]. Tüm bunlara ek olarak kötü huylu prostat kanserli hücrelerde, DNA onarım genlerinin ifadeleri ile fonksiyonları arasındaki farklılık, prostat kanseri gelişiminde, hatalı DNA onarımının rolü olabileceğini göstermektedir[1] .

3.2. Nörodejeneratif Bozukluklar

Nöronlarda DNA hasarlarının birikimi, Alzheimer, Huntington ve Parkinson gibi birçok nörodejeneratif hastalıkla ilişkilidir[33, 34]. Bunun nedenlerinden bir tanesi nöronların yüksek oranda mitokondriyal solunum yapması ve bunun sonucunda ortaya çıkan reaktif oksijen türlerinin (ROS) mitokondrial ve nükleer DNA'ya zarar vermesiyle ilişkilidir[35]. Bu tip DNA hasarları BER ve SSBR mekanizmalarıyla düzeltilirler ve bu mekanizmalardaki bozukluklar nöronların zarar görmesine ve fonksiyon bozukluklarına neden olurlar[34, 36]. Nöral sistemin DNA hasarlarına yatkın olmasının diğer nedeni de, bu hücrelerin bölünerek kendilerini yenileme kapasitelerinin sınırlı olmasıdır[1]. Bu durum, DNA hasarlarının birikimine ve sonucunda nöronların geri dönüşümsüz bir şekilde farklılaşmasına neden olur[1]. Ayrıca, bölünmesi durmuş G0 aşamasındaki hücreler, çift zincir kırıklarını HR yolağıyla tamir edemezler[1]. Bu durum, hücreleri hata oranı yüksek olan NHEJ yoluyla tamir mekanizmasını kullanmaya zorlar ve sonucunda hücre defektleri ortaya çıkar[1]. Bu sebeple, DSB tamir mekanizması bozukluğu sonucu ortaya çıkan Cockayne sendromu (CS) gibi hastalıklarda nörodejeneratif bozukluklar sıklıkla görülür[34-36].

3.3. Kalıtsal DNA Tamir Bozuklukları

DNA tamir mekanizmasındaki yetersizlik veya eksiklikler, insanda önemli kalıtsal hastalıklara yol açar[1, 5, 6]. Tamir mekanizmasında rol oynayan enzim veya proteinlerin gen defektlerine bağlı olarak insanda otozomal resesif kalıtım gösteren DNA tamir sendromları ortaya çıkmaktadır[1, 5, 6]. NER tamir bozukluklarının neden olduğu kalıtsal hastalıklar arasında en bilinenleri: Xeroderma Pigmentosum (XP), CS ve Trikotiyodistrofi'dir (TTD)[36-38] (şek.1) . XP ve TTD hastalık fenotiplerinde güneşe aşırı hassasiyet ve UV'den etkilenen bölgelerde çeşitli deri kanserlerinin oluşumuna yatkınlık gözlenir[1, 37]. Fanconi anemi (FA), Bloom sendromu (BS), Werner sendrom (WS), Ataxia telangiectasia (AT), Hereditary non-polipozal kolorektal kanser (HNPCC) ve kalıtsal meme kanserleri DNA hasar tamir mekanizması ve genleri ile ilgili bilinen diğer kalıtsal sendromlara örnek olarak verilebilir (şek.1)[1]. Tüm bunlara ek olarak, mitokondri DNA'sında meydana gelen mutasyonlar bir çok kalıtsal hastalığa sebep olurlar ve tamir edilmezlerse; Amyotrofik lateral skleroz (ALS), Mitokondrial ensefalopati (MELAS), Leigh sendromu, myoklonik epilepsi, Leber'in Herediter Optik Nöropatisi, ve nöro-miyopatilere neden olmaktadır[1, 40].

3.4. İmmün Bozuklar ve İnfertilite

DNA hasar yanıtında yer alan bazı enzim ve proteinler, rekombinasyon yoluyla genomu yeniden düzenleyerek immün sistem gelişimine katkıda bulunurlar[1, 6]. Bunun anlamı şudur;

DDR'de ortaya çıkabilecek hatalar aynı zamanda immün çeşitliliği oluşturan V(D)J rekombinasyon mekanizmasını (antijen tanıma bölgelerini kodlayan ekzon V, D, J şeklinde üç segmentten oluşur ve bu segmentlerin birçoğu farklı kombinasyonlarla bir araya gelir) etkileyerek, immün rahatsızlıkların ortaya çıkmasına neden olmaktadır[40]. Örneğin, NHEJ faktörlerinde B ve T hücrelerinde immün bozukluklara neden olan mutasyonlar sonucu, bazı AT hastalarında ölümle sonuçlanan enfeksiyonlara yatkınlık vardır[1, 6]. Benzer şekilde B ve T hücre kaynaklı V(D)J rekombinasyon hatalarının lenfoma ve lösemi hastalıklarına sebep olduğu da bilinmektedir[1, 6]. Batı ülkelerinde yaşayan erkelerin yaklaşık olarak %20'si infertilite sorunu yaşamaktadır[1]. Mayoz bölünme esnasında rekombinasyon gerçekleşirken DSB'ler meydana gelir ve DNA hasar yanıtındaki bozukluklar nedeniyle spermatogenez gerçekleşirken rekombinasyonlar olması gerektiği gibi gerçekleşmezse insanlarda infertilite sorunlarına neden olabileceği düşünülmektedir[1]. Yapılan çalışmalarda insan spermatogenezi sırasında DDR sinyal yolağının aktivitesi gösterilmiştir[42,43].

3.5 Yaşlanma ve Metabolik Hastalıklar: Yaşlanma ve DNA hasarı birikimi arasındaki ilişki bir çok çalışmada gösterilmiştir[1, 6, 43]. Kendiliğinde meydana gelen DNA hasarları yaşla birlikte sağlıklı bireylerin nükleus ve mitokondri genomlarında birikmektedir[1, 6, 44]. Bu durum sadece DNA hasarlarının artışını yansıtmakla kalmayıp, ayrıca DNA tamir kapasitesinin zamanla azalmasıyla da ilişkilidir[1, 44]. Kalıtsal olarak DNA hasar tamir mekanizmasında bozukluklarına sahip hastalarda, sıklıkla erken yaşlanma belirtileri gözlenmektedir[44]. Büyüme hormonu ve insülin benzeri büyüme faktörü ile ilgi yapılan çalışmalarda bu hormonların ömür uzunluğu ile olan ilişkisi gösterilmiştir ve DNA hasarı durumlarında bu hormonların etkilediği sinyal yollarında aktivite bozuklukları tespit edilmiştir[1]. Ateroskleroz gibi metabolik sendromların bir çoğu anormal glikoz metobolizması ve insülin direnci durumlarıyla karakterize edilmektedir[1, 45]. İlginç bir şekilde DNA hasar tamiri genlerinden ATM mutasyonu olan hastalarda insülin direnci ve glikoz intölaransı ortaya çıkmaktadır ve ATM homozigot ve heterozigot mutasyonu olan farelerde yapılan benzer çalışmalarda aterosikleroz gibi bir çok metabolik hastalığa yatkınlık gözlemlenmiştir[45]. Glikoz metabolizması ve insülin-AKT yolağında bulunan proteinler, DDR ilişkili kinazlar tarafından hedef molekül olarak kullanılmaktadırlar[1, 45]. Her ne kadar DDR ve metabolik sendromlar arasındaki ilişki bazı durumlarda indirekt olsa da[1, 45, 46], DDR direkt olarak metabolik sendromlarla ilişkili enerji metabolizması ve vasküler fizyolojiyi etkiliyor olabilir.

4. TARTIŞMA

İnsan genomunda dış ve iç etkenler sonucunda DNA hasarları ortaya çıkmaktadır. Bu hasarlar çeşitli onarım mekanizmaları ile düzeltilmektedirler[1, 9]. DNA hasar tamir mekanizmasının düzgün bir şekilde çalışmaması ya da bu tamir yolağında işlev gören proteinlerin çeşitli mutasyonlarla aktivitesini kaybetmesi sonucunda canlı yaşamının etkileyen kanserler, nörodejeneratif bozukluklar, immün hastalıklar, erken yaşlanma ve bazı kalıtsal genetik hastalıklar ortaya çıkmaktadır[1, 6, 45]. Yeni tedavi yöntemlerinin geliştirilebilmesi amacıyla gerçekleştirilen temel bilimsel araştırmalar, tamir mekanizmaları sonucu ortaya çıkan bu hastalıkların moleküler mekanizmalarının anlaşılmasına önemli katkılar sağlamaktadır[46]. Bu sebeplerle, DNA onarım mekanizmalarında yer alan gen ve proteinlere yönelik çalışmalardan elde edilecek sonuçlar doğrultusunda bazı genetik ve metabolik hastalıkların tanı ve tedavisi ile ilgili klinik açıdan kullanılabilecek yeni çözüm yollarının ortaya çıkartılabilmesi mümkündür.

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Derleme / Review

Koru Proceedings

Facial and Hypoglossal Paresis Following Dental Local Anesthesia: A Case Report

Dental Lokal Anesteziyi Takiben Gelişen Fasial ve Hipoglossus Parezisi: Bir Olgu Sunumu

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ABSTRACT

Neurological complications following local anesthesia in dental procedures are rare. Although peripheral facial paralysis is more commonly reported, the number of cases is limited. Review of the english literature revealed no intraoral injection of anesthetic agent-induced isolated hypoglossal paresis or 7th and 12th cranial nerve paresis in the same patient. Herein, we report an 18-year old woman with left facial and right hypoglossal paresis following local anesthesia during tooth extraction.

Key words: *Dental procedures, Facial paralysis, Facial Paresis, Hypoglossus Palsy, Intraoral anesthesia, Local anesthesia*

ÖZET

Dental girişimlerde kullanılan lokal anesteziyi takiben nadiren nörolojik komplikasyonlar görülebilmektedir. En sık görülen komplikasyon periferik fasial parezi olmasına rağmen bildirilmiş birkaç adet olgu bulunmaktadır. İngilizce literatürde intraoral anestezik madde enjeksiyonuna bağlı izole hipoglossus parezisi veya aynı hastada gelişen 7. ve 12. kranial sinir parezisi bildirilmemiştir. Bu makalede diş çekimi sırasında yapılan lokal anesteziyi takiben sol fasial ve sağ hipoglossus parezisi gelişen 18 yaşında bir kadın hasta sunulmuştur.

Anahtar Kelimeler: *Dental girişim, Fasial Paralizi, Fasial Parezi, Hipoglossus Felci, İntraoral anestezi, Lokal anestezi*

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INTRODUCTION

In cases operated under office conditions for most tooth and nasal procedures, the frequency of resulting neurological complications enhances with increased frequency of using local anesthetic agents. In maxillary anesthesia applied before dental procedures, blockage of the posterior superior alveolar nerve and infraorbital nerve is targeted, whereas blockage of alveolar, lingual and mental nerve is targeted in mandibular anesthesia[1]. However, rare neurological complications can be observed due to direct trauma during anesthesia or to the toxic effect of anesthetic agent[2]. Although facial nerve paresis is rarely seen among these complications, it is the most frequently encountered neurological complication. It often develops following blockage of inferior alveolar nerve and is thought to emerge as a result of the impact on peripheral facial nerve branches in the parotis log[1].

Dental infections and procedures-related hypoglossal nerve paresis is rare and is related to hypoglossal nerve impression due to the infection or edema spreading into the pharyngeal cavity[3]. To the best of our knowledge, no patient was reported with hypoglossal nerve paresis following intraoral injection of the anesthetic agent.

Herein, we report the first case of facial and hypoglossal serve paresis developed in the same patient during tooth extraction in the light of literature data.

CASE REPORT

An 18-year old woman was admitted with complaints of movement restriction on the left corner of the mouth, limitated tongue movements, and difficult swallowing. She had no history of trauma, disease, allergy and medication. Her complaints started after syncope following local anesthesia (Citanest; Zenica medical, Paris, France) administered 10 days ago (for approximately 6 hours later) during extraction of her inferior teeth on both sides, and her complaints regressed minimally after treatment with oral steroid administered at an external center. The right frontal side of the tongue lost its sense of taste. On her physical examination, movements of the left angulus labialis were limited and the nasolabial sulcus, palpebra and eyebrow movements were intact. Examination of the tongue also demonstrated that the tongue was rotated to the left within the mouth and to right outside the mouth. Other cranial nerve functions were normal. During examination of the oral cavity, the right 3rd mandibular molar tooth and the left 2nd mandibular molar teeth were observed to have been extracted. Routine blood tests were within normal limits. No pathologic values were found in immunological or serological markers suggesting a possibility of viral infection or autoimmune disease. In the radiological examination of head-neck site with computed tomography, no intracranial and extracranial finding was found which could explain facial and hypoglossal nerve pathology (Figure 1).

Figure 1

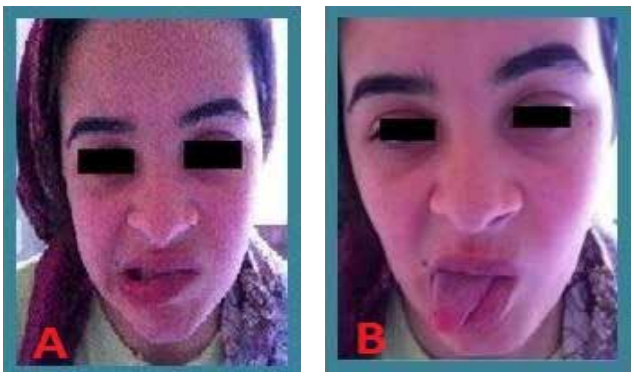

Figure 1A: An image after local anesthesia performed by dentist showing marginal mandibular branch of facial nerve paresis

Figure 1B: An image after local anesthesia performed by dentist showing hypoglossal nerve paresis

The patient was admitted to our service. High dose 'pulse' i.v. prednisolone (Prednol; Mustafa Nevzat İlaç Sanayii A.Ş, İstanbul, Turkey) treatment was initiated. On Day 3, movements of tongue was recorded to have recovered completely and the sense of taste was re-gained. A nearly complete recovery was recorded in facial nerve functions on Day 10 and the medication was gradually reduced. The patient was discharged with no sequel on the mouth corner except for minimal asymmetry (Figure 2).

Figure 2

Figure 2A: An image showing the progression of marginal mandibular branch of facial nerve after medical treatment

Figure 2B: An image showing the progression of hypoglossal nerve after medical treatment

as informed and has given a consent to be published as a case report in literature.

DISCUSSION

Facial nerve paresis is the most frequently seen complication of dental local anesthesia and it usually occurs during inferior alveolar nerve blockage[1]. It is divided into two groups: immediate or delayed paresis. Immediate paresis often develops within the first three hours. It is related to a variation in facial nerve anatomy or direct contact of local anesthetic agent with facial nerve branches passing through its gland capsule. However, chorda tympani involvement cannot be explained by these hypotheses[2]. Delayed paresis, on the other hand, develops hours following administration of the anesthetic agent. Three hypotheses are suggested to explain this condition: (i) stimulation of the sympathetic plexus around the external carotid artery by anesthetic agent or degradation products and stimulation of the sympathetic plexus around the stilomastoid artery by these plexus fibers. It is considered that stimulation of the stilomastoid sympathetic plexus leads to reflex spasm in the facial nerve vasa nervosms; (ii) stimulation of the sympathetic plexus associated with external carotid artery by local anesthetic agent; (iii) activation of a latent viral infection remaining after trauma. There is no case of facial nerve paresis reported after blockage of the posterior superior alveolar nerve. Herein, the reason for paralysis is considered to be the transfer of local anesthetic agent from the posterior superior alveolar artery to the middle meningeal and petrosal veins[1,4].

In the English literature, there are 12 facial paresis cases developing after dental procedures[4-7] (Table 1). It was reported that eight of these cases resulted from local anesthesia, three from intraoral infections and one from impression during chewing after tooth extraction[4-7] (Table 1). Six of the cases developed after molar tooth extraction, one after premolar tooth, one after canine tooth, one after primary tooth extraction and two after other dental procedures[4-7] (Table 1).

F: Female, M: Male, LAA: Local Anesthesic Agent, H&B: House & Brackman, i.v. : intravenous, p.o.: peroral

While two patients had sudden (<3 hours) facial paresis, ten patients were reported to have a history of delayed (>3 hours) facial paresis[4-7] (Table 1). A full peripheral facial paresis was detected in eigth of the cases, involvement of the buccal branch in one case and buccal and marginal mandibular branch in one case, and decrease in the sense of taste due to chorda tympani involvement in two cases[4-7] (Table 1). After treatment, seven of the patients showed nearly complete recovery, four patients showed partial recovery, while one patient could not be followed up[4-7] (Table 1). Steroid treatment was applied to nine of the cases[4,5,7] (Table 1). In a patient who was not administered steroid, but given vitamin B and cytidine-uridine complex treatment, a regression was recorded from Grade 4 to Grade 2 according to House&Brackman staging; whereas in another patient who could not receive treatment due to pregnancy, a regression was recorded from Grade 6 to Grade 2[5] (Table 1). Another patient with facial paresis after submandibular abscess was reported to recover completely within 24 hours after drainage of the abscess[6] (Table 1). Recovery was detected in patients with facial paresis due to local anesthesia between two and 10 weeks[4,6,7] (Table 1). The patient who was unable to be treated due to pregnancy showed a partial recovery within eigth months[5] (Table 1). This article presents an 18-year old female patient who developed left marginal mandibular and chorda tympani, right hypoglossal paresis following local anesthesia administered during extraction of the right 3rd and 2nd mandibular molar teeth and who recovered completely on the 10th day of i.v. steroid treatment.

There are rare reported cases developing hearing loss, visual loss, Horner syndrome, n. abducens palsy and combined nerve paralyses (3,4,6. cranial nerves) based on intraoral local anesthetic applications[8].

However, no isolated hypoglossal paresis has been reported[3,9]. Pathologies in the carotid region or lingual segment all along the hypoglossal nerve trace may lead to isolated paresis. Neoplasia, trauma, infection, autoimmune and vascular reasons can be listed in the differential diagnosis. In the literature, two hypoglossal nerve paresis cases developing after tooth infection were reported. Patients with infections spreading over the floor of the mouth and deep pharyngeal cavity showed relief after tooth extraction and regression during paresis[3,9]. In our case, there was no predisposing factor other than local anesthesia. We believe that different paralyses developing after anesthesia applied to both sides can be affected by vasospasm developing after access of anesthetic agent to different regions.

In conclusion, the use of local anesthetics has increased with enhanced dental approaches and procedures in recent years. Neurological complications following injection of anesthetic agents are rare; however, these complications may lead to extremely difficult problems for both the patient and dentist. Such complications can be avoided by keeping the concentration levels of local anesthetic agent low, not injecting the agent without aspiration, directing the tip of syringe to the regions with anatomically less veins and nerves.

TABLE 1: Reported facial paresis cases secondary to dental procedures

Number of Patients	Age / Gender	Local anesthetic agent (LAA) or / secondary to infection	Affected tooth	Duration of facial paresis	Grade of Facial Paresis	Treatment	Time of Recovery	Complete recovery/ sequel
1	38, M	LAA	Right inferior 2nd molar tooth	6 hours	H&B Grade II, loss of sense of taste in frontal 2/3 part of tongue	i.v. prednisolone	3 weeks	Complete recovery
2	29, M	LAA	Right superior third molar tooth	8 hours	H&B Grade IV	i.v. prednisolone	Several weeks	Complete recovery
3	45, F	LAA	Left inferior 1st premolar tooth	24 hours	H&B Grade II	p.o. prednisolone	9 weeks	H&B Grade II
4	45, M	LAA	Left inferior 1st molar tooth	14 hours	H&B Grade II	i.v. prednisolone	Several weeks	Complete recovery
5	49, M	LAA	Dental injection	18 hours	H&B Grade I	Prednisolone	-	-
6	37, F	Apisectomy (effect of direct impression due to chewing)	Left superior lateral and incisive and canine tooth	2 weeks	H&B Grade II involvement in facial nerve buccal branch	i.v. prednisolone	2 weeks	Complete recovery
7	35, F	LAA	Bimaxillary osteotomy	2 weeks	H&B Grade IV	Dexamethasone, depomedrone, prednisolone	2 weeks	Partial recovery
8	21, F	Infection	Right inferior 3rd molar tooth complex	3 hours	H&B Grade IV	Vitamin B, cytidine-uridine	3 weeks Grade II	H&B
9	35, F	LAA	Left inferior 2nd molar tooth	2 hours	H&B Grade VI	No treatment due to pregnancy	8 months	H&B Grade II
10	35, M	Infection (abscess in submandibular site)	Left inferior 3rd molar tooth	Several hours	Involvement in facial nerve buccal and marginal mandibular branch, loss of sense of taste in frontal 2/3 part of tongue	Drainage of abscess, antibiotherapy	24 hours	Complete recovery
11	20, F	LAA	Left inferior 1st molar tooth	24 hours	H&B Grade V	p.o. prednisolone	8 weeks recovery	Complete
12	8, M	LAA, infection	Primary tooth	7 days	H&B Grade IV	p.o. prednisolone, acyclovir	70 days	Complete recovery

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Derleme / Review

Koru Proceedings

Severe Amiodarone-Induced Pulmonary Toxicity: A Case Report and Review of Literature

Amiodarona Bağlı Ciddi Pulmoner Toksisite: Bir Olgu Sunumu ve Literatür Derlemesi

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ABSTRACT

Amiodarone is one of the most frequently prescribed antiarrhythmic agents worldwide. Although it is extensively used in the treatment of life-threatening arrhythmias, it can act as a double-edged sword considering its potentially serious side effects which warrants careful patient selection and follow-up. In this paper, we present a case with severe amiodarone-induced pulmonary toxicity and a review of amiodarone induced pulmonary toxicity, its associated risk factors, pathogenesis, diagnosis, treatment and prognosis.

Key words: *Amiodarone, Pulmonary toxicity*

ÖZET

Amiodaron dünya üzerinde en sık reçetelenen antiaritmik ilaçlardan biridir. Hayatı tehdit eden ciddi aritmilerin tedavisinde yaygın olarak kullanılmasına rağmen ciddi yan etkileri nedeniyle iki ucu keskin bir kılıç olarak düşünülmeli ve bu nedenle dikkatli hasta seçimi ve takibi gerekmektedir. Bu yazıda, amiodarona bağlı ciddi pulmoner toksisite gelişen bir hasta sunulmuş ve amiodarona bağlı pulmoner toksisite, risk faktörleri, patogenez, tanı, tedavi ve seyri anlatılmıştır.

Anahtar Kelimeler: *Amiodaron, Pulmoner toksisite*

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INTRODUCTION

Amiodarone is a class III anti-arrhythmic agent structurally related to thyroxine. Although originally developed as a new class of anti-anginal vasodilator, is derivated, desethylamiodarone, found to be a potent anti-arrhythmic agent with atypical class III Vaughan - Williams properies[1]. It is one of the most frequently prescribed specific antiarrhythmic drugs in the World[1,2]. However, physicians should be cautious about toxicities associated with this drug. Amiodarone-induced pulmonary toxicity (AIPT) is the most serious side effect and potentially fatal. It remains underdiagnosed and can have a variable presentation[3]. The aim of this paper is to describe the case of a patient who developed severe AIPT and died because of this potential side effect.

CASE REPORT

A 66-year-old male patient admitted to our clinics with increased dyspnea for 3 days. In his past medical history, he was diagnosed to dilated cardiomyopathy and implanted an implantable-cardioverter defibrillator due to ventricular tachycardia attacks 1 year ago. He was underwent catheter ablation for ventricular tachycardia but because of recurrences amiodarone was given. He was on treatment with amiodarone (400 mg/day for at least 6 months). Other medications included bisoprolol (5 mg/day), trandolapril (4 mg/day), spironolactone (25 mg/day). There was no history for any pulmonary disease or smoking for at least 10 years.

Moreover, his chest x-ray was noted as normal in another center 3 months before admission. At admission, he was in acute distress and vital signs were as follows: respirator rate 35 breaths/min, heart rate 110 beats/min, blood pressure 110/70 mmHg. Pulmonary examination revealed bilateral diffuse crackles. Cardiac examination revealed sinusal tachycardia, grade 2/6 systolic murmurs at the apex and mesocardiac area, with jugular venous distention but no peripheral edema. Electrocardiogram demonstrated pacemaker rhythm. Chest x-ray showed enlarged cardiac silhuette, extensive bilateral alveolar and interstitial infiltrates (Figure 1). Transthoracic echocardiography detected a dilated left ventricle and impaired systolic functions (Ejection fraction about 38%), moderate pulmonary hypertension. Computed tomography (CT) demonstrated extensive ground-glass opacities and airspace consolidations suggesting diffuse interstitial pneumonitis with bilateral pleural effusions (Figure 2). No significant adenopathy was found. Workups for infectious etiology, emboli and vasculitis were negative. Lung function test demonstrated a marked decline in the diffusion capacity for carbon monoxide (DLCO) (6.9, 28% of predicted). Patient settled with diuretics, nebulazation with bronchodilators and empiric broad-spectrum antibiotic treatment. Since clinically patient was diagnosed as amiodarone induced pulmonary fibrosis/toxicity, amiodarone was discontinued, patient treated with intra-venous steroids (500 mg IV methylprednisolone bolus followed by 1mg/kg/day maintenance treatment). However, due to severe respiratory failure and progressive ameloriation in blood gases, the patient deserved intubation and mechanical ventilation with sedation on the 2nd day. After entubation we got broncho-alveolar lavage that revealed vacoulizations in histiocytes and no findings for malignancy. Despite all respiratory support and medical treatment, the patient died on the 5th day of admission due to cardiopulmonary insufficiency and acute respiratory distress.

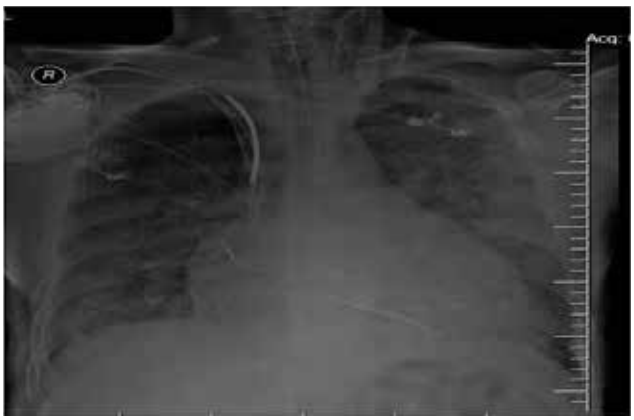

Figure 1. Chest roentgenogram: Inhomogenous opacities in both lung fields.

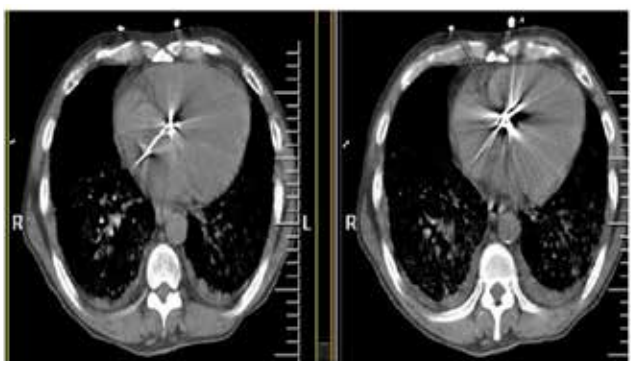

Figure 2. Thorax BT revealed bilateral areas of consolidation and extensive ground glass opacities.

DISCUSSION

The popularity of amiodarone is explained by its efficacy and usefulness in reducing and preventing several arrhythmias such as recurrent ventricular tachycardia and atrial fibrilation[1,2]. Amiodarone is an amphiphilic compound which can result in long elimination half life of approximatry 30-108 days with a volume of distribution close to 50,000 li-

tres[3]. Therefore, amiodarone and monodesethyl-amiodarone accumulate in peripheral tissues over long periods. It tends to accumulate extensively in adipose tissue and highly perfuseed organs such as liver, lung and spleen. Increase duration and dose of the therapy tends to increase the prevalance of drug-related side effects[1-3]. Amiodarone-induced pulmonary toxicity (AIPT) is the most serious side effect and potentially fatal[3].

Prevalance: The incidence of pulmonary toxicity from amiodarone is not exactly known[3]. Amiodarone pulmonary toxicity will develope in approximately 0.1% to 0.5% of patients that take up to 200 mg per day and 5 to 15% of patients that consume 500 mg or more per day.[1,3]. Recent studies have reported that the overall incidence is between 5 and 13% and 1% per year[4,5].

Risk factors: Potential risk factors for developing pulmonary toxicity are a high cumulative dose, a daily dose greater than 400 mg/day, duration of therapy exceeding 2 months, increased patient age, male gender, pre-existing lung disese or an abnormal chest x –ray before the commencement of treatment with amiodarone and thoracic or non-thoracic surgery, high dose supplemental oxygen therapy with or without mechanical ventilation[4,6]. Age and duration of therapy are the two most significant risk factors for AIPT. Toxicity can occurr at any point during treatment and or even after discontinuation of therapy[6]. Recently, it is shown that low dose of amiodarone may also cause pulmonary toxicity[7].

Time course and presentation[8]: There are several APT clinical presentations but four clinical forms are the most common.

i. Chronical interstitial pneumonitis is the most common presentation. Sub-acute attacks begin with nonproductive cough, dyspnea, and weight loss; these generally occur after two or more months of therapy. Chest radiography demonstrates focal or diffuse interstitial thickening.

ii. Organizational pneumonia with or without bronchiolitis obliterans pneumonia (BOOP) accounts for about 25% of cases. It presents with more acute findings initially: non-productive cough, crackles and signs of pleurisy. There are irregular interstitial, alveolar or mixed infiltrates on chest X-ray. The clinical presentation mimics infectious pneumonitis.

iii. Acute respiratory distress syndrome (ARDS) is a potentially deadly form. It occurs rarely, and it is of particular interest to anesthesiologists because it is characterized by fulminant course, especially in patients who have undergone surgery or pulmonary angiography. The incidence of ARDS after lung surgery is 11% in patients treated with amiodarone as compared with the 1.8% of those not so treated. Acute lung damage develops one to four days after lung surgery. It is characterized by diffuse alveolar damage, showing signs of acute interstitial pneumonitis with hyaline membranes. Due to possible development of ARDS after surgery in patients receiving amiodarone, thoracoscopy and open lung biopsy are performed only after all other diagnostic modalities have been exhausted.

iv. A solitary or multiple pulmonary masses are typically located in upper lobes and may suggest lung neoplasm.

Pathogenesis and mechanisms: Several mechanisms have been proposed by which amiodarone results in pulmonary toxicity[3,9].

Amiodarone has potent inhibitory effects on lysosomal phospholipase leading to accumulation of phospholipid bound during in membrane- rich structures[10]. Drug induced phospholidiosis leads to formation of lipid-laden macrophages, known as foam cells in all organs. In the lungs, accumulation occures in type II pneumocytes , intraalveolar cells and interestitial inflammatory cells. There have been several suggested mechanisms of AIPT , but the two most likely hypotheses are cytotoxic effects to type II pneumocytes and lung paranchyme and an immune – mediated mechanism in genetically predisposed patients[10].

Diagnosis: Diagnosing APT is based on a combination of strong clinical suspicion, history, radiographic and clinical evidence, and the rigorous exclusion of alternative etiologies. On examination, bilateral crackles could be heard on inspiration. Clubbing was absent[3,6,9]. Radiology plays a key role in diagnosis. Chest roentogenography shows bilateral diffuse or patchy infiltrates, more commonly in the right lobe. Early in the course of disease, ground -glass opacities are also common. Pleural thickening and/or effusion, and multiple pulmonary nodules in the upper lobes secondary to iodine accumulation in type II pneumocytes has been described. Pulmonary function tests typically reveal either a restrictive or mixed obstructive/restrictive pattern with a decreased diffusion lung capacity of 15-20%[11]. The earliest abnormality in APT is a decrease in the diffusion capacity for carbon monoxide. Gallium scanning, though nonspecific, is useful to demonstrate increased parenchymal activity early in the course of disease. Gallium scanning may also help differentiate AIPT from CHF, one of the common comorbidities that often confounds diagnosis[12]. Bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial biopsy are helpful in diagnosis[12].

BAL findings suggestive of APT include a CD8+ predominant lymphocytic alveolitis, and increased phospholipid content. In difficult cases, open lung biopsy may be considered, however the known risk for worsening APT after thoracic surgery can become a potential deterrent. Physical insult to lung parenchyma is known to increase susceptibility to toxic-

ity even if low dose Amiodarone therapy was used. It is also for this reason that cardiac surgery patients are at increased risk, given the frequent perioperative use of amiodarone for the control of postoperative tachyarrhythmia[13]. Pathologic findings also include alveolar sepal widening with inflammatory infiltrate, type II pneumocyte hyperplasia, interstitial fibrosis, and diffuse interstitial pneumonitis. A classic finding is the aforementioned lipid-laden macrophages in alveolar spaces, as are cytoplasmic lamellar bodies[13]. Given that these findings have also been reported in nontoxic patients undergoing chronic treatment with amiodarone, their existence is suggestive of amiodarone exposure, and less indicative of amiodarone toxicity[11].

Conversely, the corollary of their absence makes diagnosis of AIPT unlikely. Other pathologic manifestations are patchy bronchiolitis obliterans organizing pneumonia or diffuse alveolar damage with hyaline membrane formation. Plasma levels of amiodarone are nondiagnostic ,but elevated levels of its metabolite, desethylamiodarone, might be more frequent in patients with pulmonary toxicity[13]. Sirsikar et al.[14] proposed a diagnostic criteria for amiodarone pulmonary toxicity are as follows. Two out of seven must be there to label it.

1. New onset of pulmonary symptoms such as dyspnoea, cough, or pleuritic chest pain

2. A decrease in the diffusing capacity of the lungs for carbon monoxide (DLCO) of 20% from the pretreatment value, or if none is available, a value less than 80% of predicted

3. New chest radiographic abnormality such as an interstitial or alveolar infiltrate;

4. Abnorma l lung uptake with gallium-67 radioisotope

5. Improvement in symptoms with drug discontinuation

6. CD8+ lymphocytosis on bronchoalveolar lavage

7. Lung biopsy with interstitial pneumonia, bronchitis obliterans orginizing pneumonia or fibrosis

Treatment and Prognosis: Once the diagnosis of amiodarone pulmonary toxicity is considered likely, the drug should be discontinued. Due to amiodarone accumulation in fatty tissues and long elimination half-life, pulmonary toxicity may initially progress despite drug discontinuation and may recur after steroid withdrawal[13]. Discontinuation of Amiodarone as sole therapy may be sufficient if the extent of the disease is limited. Corticosteroids should be administered in patients who show substantial involvement of the lung parenchyma on imaging studies with or without concomitant hypoxemia in the attempt to speed up the recovery process and perhaps to minimize the likelihood of lung fibrosis[3,6-9]. Systemic corticosteroids are recommended for at least 4-12 months to avoid relapse. Prednisone at 40 to 60 mg per day with a tapering dose over 2 to 6 months has been suggested as an appropriate regimen[3,6,8].

If the patient's pulmonary toxicity is not life threatening and amiodarone cannot be discontinued because it is the only or is the optimal therapy for a patient, lowering the dosage of amiodarone as much as possible along with administering low-dose steroids may be a option[15].

Evidence has shown benefit from this treatment strategy as demonstrated by earlier recovery and decreased parenchymal fibrosis. Chest roentgenogram findings can take up to 18 months to completely resolve[13]. When treated early, most AIPT cases are considered reversible with good prognosis[12]. More precipitous presentations can lead to worse outcomes, including pulmonary fibrosis and/or death, particularly in cases that are complicated by ARDS[3,6-9,13].

CONCLUSION

Patients who should benefit from amiodarone should be carefully selected and the lowest effective dosage of amiodarone should be taken. Pulmonary evaluation with chest X-ray and pulmonary function testing, including diffusion capacity for carbon monoxide is recommended when amiodarone is started. The increase in dyspnea may be attributable to deterioration of heart failure since most of the patients using this durg have structural heart disease such as heart failure but amiodarone toxicity must be considered in the differential diagnosis of all patients on this medication admitted with progressive or acute respiratory symptoms, especially those with history of chronic lung disease, supplemental oxygen therapy and post cardiac surgery. Radiology plays a key role in diagnosis.

Pulmonary function tests especially a decreased diffusion lung capacity of 15-20 is highly indicative for toxicity. Bronchoscopy with BAL and transbronchial biopsy may be useful in diagnosis. Early aggressive corticosteroid therapy should be employed as a viable treatment strategy. AIPT resolution may be gradual, and supportive care for patients is critical for successful outcome.

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