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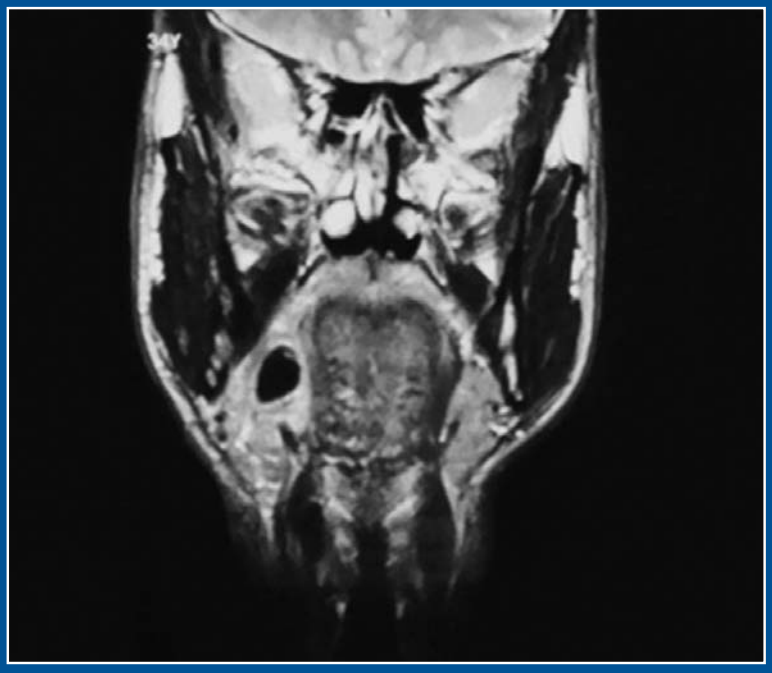

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Description

ENT Updates (formerly Journal of Medical Updates), is a periodical of the Continuing Education, and Scientific Research Association (CESRA), Turkey, which is published in both printed (p-ISSN 2149-7109) and electronic (e-ISSN 2149-6498) versions three times a year on April, August, and December. A peer-reviewed system is used to select manuscripts. The language of the journal is English. The journal is currently indexing and abstracting in Emerging Sources Citation Index (ESCI) by Thomson Reuters, TUBITAK ULAKBIM Turkish Medical Index, Proquest, EBSCO Host, Index Copernicus and Google Scholar.

Aims and Scope

The goal of the journal is to present and improve collective scientific knowledge and the scientific background dealing with otorhinolaryngological disorders and related subjects (allergy, pediatrics, neurology, psychiatry, neurosurgery, radiology, anesthesiology, pulmonology, etc.) via experimental and clinical studies, reviews, case reports, short communications and letters to the editor. The initial aim of this journal is to form a countrywide education platform and to share the recent information and learn about the treatment of various local or rare diseases in aware of the fact that a disease may be rare to a certain region while it is very common to another. The second aim of this journal and Continuous Education and Scientific Research Association (CESRA), a nonprofit organization serving for continuous education, is to represent our country in international arena of science and knowledge with the published papers. We aimed to undertake a novel effort in the international representation and attribution of published articles. That is why we have set an international editorial board from all over the world beside the national board spread to each corner of the country. The target readers of the ENT Updates include otorhinolaryngology specialists and residents as well as all other physicians working in the field of otorhinolaryngology or in related specialities.

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Cetuximab alone has a dose-dependent antitumor effect in oral cavity cancer cells: an in vitro study

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Abstract

Objective: To evaluate the antitumor effect of cetuximab as a single agent for the treatment of oral cavity cancers and to clarify the dose-dependent growth inhibitory effect in oral cavity squamous cell carcinoma cell line (OCSCCCL).

Methods: The OCSCCCL (UPCI-SCC131) were cultured and continuously monitored using the xCELLigence RTCA SP instrument. Thereafter, they were divided into seven groups as: (i) negative control: medium+OCSCCCL, (ii) positive control: medium+OCSCCCL+cisplatin 10 µM/ml, (iii) medium+OCSCCCL+cetuximab 25 µg/ml, (iv) medium+OCSCCCL+cetuximab 50 µg/ml, (v) medium+OCSCCCL+cetuximab 100 µg/ml, (vi) medium+OCSCCCL+cetuximab 200 µg/ml, (vii) medium+OCSCCCL+cetuximab 400 µg/ml. The cell index and viability were statistically analyzed and compared between groups.

Results: The distribution of cell index (mean value) and percentage of viability in groups were as follows: (i) 2.66 (100%), (ii) 0.17 (6.08%), (iii) 2.28 (85.71%), (iv) 2.31 (86.84%), (v) 1.92 (72.18%), (vi) 1.79 (67.29%), (vii) 0.28 (10.53%). The change trend in drug concentration was statistically different in all study groups to which cetuximab was administered (Pillai's trace; p<0.0001). The antitumor effect of cetuximab was initially detected at a dose of 100 µg/mL, when compared with negative control (p=0.01). However, a dose of 400 µg/mL was required in order to have a statistically similar antitumor effect of cisplatin at a dose of 10 µM.

Conclusion: Cetuximab alone is a potentially effective chemotherapeutic agent and has a concentration-dependent growth inhibitory effect in OCSCCCL. The antitumor activity of cetuximab was initially detected at a dose of 100 µg/mL. However, significant antitumor effect was determined at a dose of 400 µg/mL.

Keywords: Antitumor, cetuximab, oral cavity, cancer.

Özet: Tek bir ajan olarak setuksimab oral kavite kanser hücrelerinde doza bağımlı etkiye sahiptir: Bir in vitro çalışma

Amaç: Tek bir ajan olarak setuksimabın oral kavite kanserlerinin tedavisindeki antitümöral etkisini değerlendirmek ve oral kavite yassı epitel hücreli karsinom dizininde (OCSCCCL) doza bağımlı büyümeyi inhibe etme etkisini açıklığa kavuşturmak.

Yöntem: OCSCCCL (UPCI-SCC131) kültürü elde edildi ve xCELLigence RTCA SP cihazı kullanılarak izlendi. Daha sonra yedi gruba bölüştürüldü: (i) negatif kontrol: besiyeri+OCSCCCL, (ii) pozitif kontrol: besiyeri+OCSCCCL+sisplatin 10 µM/ml, (iii) besiyeri+OCSCCCL+setuksimab 25 µg/ml, (iv) besiyeri+OCSCCCL+setuksimab 50 µg/ml, (v) besiyeri+OCSCCCL+setuksimab 100 µg/ml, (vi) besiyeri+OCSCCCL+setuksimab 200 µg/ml, (vii) besiyeri+OCSCCCL+setuksimab 400 µg/ml. Hücre indeksi ve viyabilite istatistiksel açıdan incelendi ve gruplar arasında karşılaştırıldı.

Bulgular: Hücre indeksinin (ortalama değer) ve viyabilite yüzdesinin dağılımı şu şekilde bulundu: (i) 2.66 (%100), (ii) 0.17 (%6.08), (iii) 2.28 (%85.71), (iv) 2.31 (%86.84), (v) 1.92 (%72.18), (vi) 1.79 (%67.29), (vii) 0.28 (%10.53). İlaç konsantrasyonundaki değişiklik eğilimi setuksimabın uygulandığı çalışma gruplarının tümünde istatistiksel açıdan anlamlı idi (Pillai trasesi; p<0.0001). Negatif kontrolle karşılaştırıldığında setuksimabın antitümöral etkisi ilk olarak 100 µg/mL dozda saptandı (p=0.01). Ancak 10 µM sisplatinin etkisine istatistiksel açıdan benzer antitümöral etki için 400 µg/mL doz gerekliydi.

Sonuç: Tek başına setuksimab potansiyel olarak etkili bir kemoterapötik ajan olup OCSCCL'de konsantrasyona bağımlı büyümeyi inhibe edici etkiye sahiptir. Setuksimabın antitümöral aktivitesi başlangıçta 100 µg/mL dozda saptanmıştır. Ancak 400 µg/mL dozda anlamlı bir antitümöral etki belirlenmiştir.

Anahtar sözcükler: Antitümöral etki, setuksimab, oral kavite, karsinom.

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Epidermal growth factor receptor (EGFR) or ErbB1/human epidermal growth factor receptor-1, a transmembrane glycoprotein, is one of the members of ErbB/HER family of receptor tyrosine kinase.[1] The ErbB/HER receptors are normally inactive monomers which are dimerized just after the binding of their ligands. The dimerization of the receptors leads to activation of intracellular tyrosine kinase. Epidermal growth factor receptor and its ligands play an essential role in embryological development of several tissue and organs such as eye, mammary gland, lung and gut.[2–4] Unfortunately, they also promote mechanisms of cancer by activating intracellular signaling pathways such as Ras/mitogen activated protein (MAP or ERK) kinase cascade, phosphatidylinsositol-3-kinase (PI3K)/protein kinase B (AKT) and mTOR pathways. Thereby trigger cellular proliferation, differentiation, survival, invasion, angiogenesis and metastasis.[5,6] Recently, the active role of EGFR and its ligands have been demonstrated in several cancers such as breast, colorectal, lung, esophageal, bladder, gastric, ovarian, head and neck.[7] Therefore, the idea of EGFR targeting strategy was evolved in in the armamentarium of cancer treatment and several therapeutic agents have been introduced thereafter. Currently, there are two main categories of EGFR targeting therapies: (i) monoclonal antibodies that affect by targeting the extracellular domain of EGFR; thereby preventing the activation of ligand-dependent EGFR tyrosine kinase, and (ii) tyrosine kinase inhibitors which exert by blocking the intracellular tyrosine kinase domain of EGFR.[8] Although several members of both categories have been applied in treatment of different cancers; cetuximab, an anti-EGFR monoclonal antibody, is the first and only targered chemotherapeutic agent that has already been approved by Food and Drug Administration (FDA) for the application in head and neck cancers.

Cetuximab, a chimeric monoclonal antibody of EGFR, competitively binds to the extracellular domain of EGFR. Its affinity is 5–10 times higher when compared with the ligands of EGFR.[9–11] As cetuximab binds to EGFR, antibody-receptor complex is internalized and degraded before activation and/or phosphorylation of the receptor.[12] Thereby, the amount of EGFR is down-regulated and EGFR related signaling pathways active in cancer cells can be blocked.[13]

In 2000, initial clinical trial in which cetuximab was applied as a single or adjunctive therapeutic agent reported promising outcomes in patients with several recurrent or metastatic cancers such as head and neck, lung, prostate, breast, pancreas, ovarian, kidney and bladder cancers.[14] Thereafter, a phase Ib clinical trial, particularly focused on head and neck cancers, which examined the effectiveness of cetuximab in selected patients with recurrent and/or metastatic squamous cell carcinoma (SCC) and whose tumor had EGFR overexpression, was conducted. This study demonstrated significant responses with high tolerability and mild to moderate degrees of skin reactions.[15] As a result of this study, authors recommended a loading dose of cetuximab at 400 mg/m^2 with a maintenance dose at 250 mg/m^2. In 2006, FDA approved cetuximab as a single agent in patients with cisplatin-resistant head and neck cancers and as a combination agent with radiotherapy in patients with locally advanced head and neck cancers. Even though, clinical effectiveness of cetuximab in different regions of head and neck cancers has been demonstrated in literature, the efficacy and effective dose of cetuximab in oral cavity cancers has not been reported yet. Therefore, the aim of this in vitro study is to investigate the effectiveness and antitumor dose of cetuximab alone in oral cavity squamous cell carcinoma cell line (OCSCCCL).

Materials and Methods

Cell culture

The human oral cavity squamous cell carcinoma cells (UPCI-SCC131; DSMZ, Braunschweig, Germany), were cultured in MEM Earle's medium (Biochrom GmbH, Berlin, Germany) supplemented with 10% fetal bovine serum (Biochrom GmbH), 2 mM L-glutamine (Biochrom GmbH), penicillin/streptomycin 100 IU/100 µg/ml (Biochrom GmbH) at 37°C in a 5% CO_2 cell incubator (Thermo Fisher Scientific, Waltham, MA, USA).

Real-time cell growth and cytotoxicity analysis

Real-time assessment of cell growth and cytotoxicity were performed using the xCELLigence Real-Time Cell Analyzer (RTCA) Single-Plate (SP) instrument (Roche Diagnostics GmbH, Freiburg i. B., Germany). The basic principle of the RTCA system consisting of RTCA Analyzer, RTCA SP station and capillary gold electrodes coated E-plate 96, is to detect the impedance changes by the interaction of adherent cells and the gold microelectrodes. The cell number and viability of adherent cells will affect the level of electrode impedance, which is presented as the cell index (CI). Cells (10^4 cells/well) were cultured on an electrode-containing 96-well plate for 24 hours. Test compounds were added to the growth medium after 24 hours of seeding and monitored for 72 hours to see the effect of chemotherapeutics.

Study groups are formed as follows:

i. Negative control: Medium + UPCI-SCC 131 cell line.
ii. Positive control: Medium + UPCI-SCC 131 cell line + cisplatin 10 µM
iii. Study group I: Medium + UPCI-SCC 131 cell line + cetuximab 25 µg/ml
iv. Study group II: Medium + UPCI-SCC 131 cell line + cetuximab 50 µg/ml
v. Study group III: Medium + UPCI-SCC 131 cell line + cetuximab 100 µg/ml
vi. Study group IV: Medium + UPCI-SCC 131 cell line + cetuximab 200 µg/ml
vii. Study group V: Medium + UPCI-SCC 131 cell line + cetuximab 400 µg/ml

After the administration of chemotherapeutic agents, impedance was measured automatically for the following 48-hour in every 60 minutes. The alterations on impedance signal were analyzed by normalizing the data of each well to the first read after starting the treatment. Graphical result were represented as normalized CI.

Statistical analysis

Data were collected by software provided with the RTCA system and were analyzed using SPSS v.20.0 (SPSS Inc., Chicago, IL, USA). The changing trends of cells in all groups were analyzed by Mauchly's sphericity test and multivariate analysis was performed by Pillai's trace test. The change in CI according to cetuximab and cetuximab-cisplatin relationship was analyzed using Mann-Whitney U test.

Results

Cetuximab alone has an antitumor activity on OCSCCCL

The analysis of cell viability demonstrated a decrease in CI in all study groups after the application of cetuximab (Figs. 1a and b; Table 1). However, when a comparison between all study groups and negative control was performed according to the growth inhibitory effect of cetuximab, the minimum concentration of cetuximab at which statistically significant difference detected was 100 µg/ml (72.18%, p=0.01).

Cetuximab has a dose-dependent antitumor effect on OCSCCCL

The growth inhibitory effect of cetuximab was initially determined in study group III (cetuximab, 100 µg/ml). However, when study group III was compared with positive control (cisplatin, 10 µM) according to CI, a statistically significant difference in the favor of positive control was determined (p=0.024). Thereby, when all study groups were compared between positive controls according to the inhibition of cell proliferation, similar antitumor effects were detected at a concentration of 400 µg/ml (Figs. 1a and b; Table 1).

Discussion

In head and neck cancers, monoclonal antibody therapies targeting EGFR, such as cetuximab, has been popularized recently. However, evidence regarding the effectiveness and efficacy of cetuximab in oral cavity cancers is inadequate; although the dose-dependent pharmacokinetic effect of cetuximab has been demonstrated previously in different cancer cell lines.[14,16,17] This in vitro study obviously demonstrated that cetuximab alone has a concentration dependent antitumor effect on OCSCCCL. The minimum dose of cetuximab that provided a statistically significant difference in growth inhibitory effect, was 100 µg/mL (72.8%, p=0.001); even though an antitumor effect was detected in every study group. Similarly, Zhang et al. reported the antitumor effect of cetuximab in two different OCSCCCLs in vitro, although they did not examine the growth inhibitory and/or cytotoxic effect of cetuximab as a single agent.[18] In their study, a supra-additive effect was determined, when a combination of chemoradiotherapy (radiation and cisplatin) with cetuximab was administered. In addition, Bussu et al. examined the effectiveness of cetuximab as a single agent and combination with cisplatin in Hep-2 laryngeal cancer cell line and similarly demonstrated the time-dependent effect of cetuximab.[19] They also mentioned the growth inhibitory effect of cetuximab as a single agent at a concentration of 100 µg/mL; however, they were not able to detect its cytotoxic effect. On the other hand, a synergistic effect in growth

Table 1. The cell index and viability of control and study groups at 48th hour, and statistical comparison between study groups and negative and positive controls.

Group	Cell index (median) (min–max)	Cell viability (%)	Negative control (p value)	Positive control (p value)
Negative control	2.74 (2.15–3.04)	100	-	0.004
Positive control	0.17 (0.17–0.18)	6.08	0.004	-
Study group I	2.29 (2.24–2.33)	85.71	0.233	0.100
Study group II	2.33 (2.11–2.48)	86.84	0.053	0.024
Study group III	1.91 (1.35–2.53)	72.18	0.010	0.024
Study group IV	1.79 (1.75–1.82)	67.29	<0.0001	0.024
Study group V	0.28 (0.16–0.41)	10.53	<0.0001	1.000

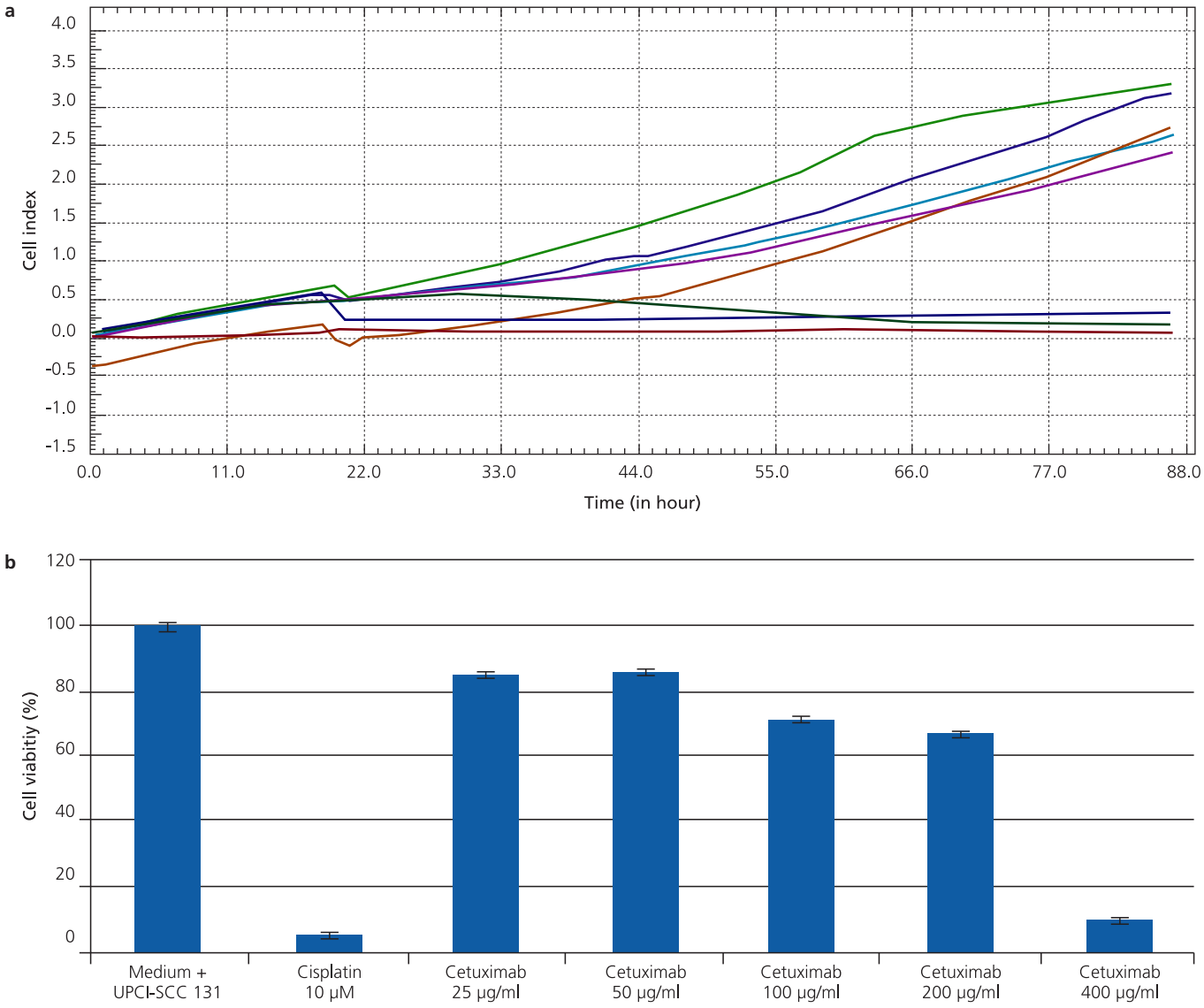

Fig. 1. The growth inhibition curves of both negative and positive controls, and all study groups were presented in panel **a**. UPCI-SCC 131 cell lines were plated onto a 96-well E-Plate and allowed to grow for 24 hours. Thereafter, test compounds were added to the culture. Cells were grown in media alone (**light green**), media alone without cell (**red**) or were treated with cisplatin, 10 µM (**dark green**), cetuximab, 25 µg/ml (**orange**), 50 µg/ml (**purple**), 100 µg/ml (**blue**), 200 µg/ml (**pink**) and 400 µg/ml (**dark blue**). The change in cell index was measured every 60 min for the following 48 hours until the end of the experiment using the RTCA system. The cell viability (%) in all study groups and negative and positive controls were presented in panel **b**.

inhibition and cytotoxicity was determined when a combination of cetuximab and cisplatin was administered.

To date, the antitumor activity of cetuximab, either as a single agent or in combination with cytotoxic or chemotherapeutic agents and/or radiation, has been demonstrated in a variety of head and neck cancer cell lines and xenografts in several in vitro and in vivo studies. However, we were unable to detect a study in which the similar growth inhibitory and/or cytotoxic effects between cisplatin and cetuximab was investigated for OCSSSCL. Therefore, this is the first study which demonstrated that cetuximab at a concentration of 400 µg/mL (0.28, 10.53%) had a similar antitumor and growth inhibitory effect when compared with cisplatin, a well-known cytotoxic agent, at

a dose of 10 µM (0.17, 6.08%). However, further preclinical and clinical studies are required in order to identify the cetuximab related tumor control and toxicities in oral cavity cancers.

Conclusion

Cetuximab has a dose-dependent antitumor activity in OCSCCCL. Cetuximab alone provides a growth inhibitory effect at a dose of 100 µg/mL, eventhough significant antitumor effect was determined at a dose of 400 µg/mL.

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Conflict of Interest: No conflicts declared.

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The effect of coenzyme Q10 on cisplatin-induced ototoxicity in rats

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Abstract

Objective: To determine the efficacy of systemic administration of coenzyme Q10 at low and high doses on cisplatin-induced ototoxicity in rats.

Methods: Our study was performed with 40 Sprague-Dawley rats. They were divided randomly into five groups: Cis, Cis+Q10$_{30}$, Cis+Q10$_{10}$, Q10, and control. Cis (n=8) group was administered cisplatin [a single intraperitoneal (i.p.) injection of 14 mg/kg], Cis+Q10$_{30}$ (n=8) group was administered cisplatin (a single i.p. injection of 14 mg/kg) and coenzyme Q10 (30 mg/kg/day, i.p.) for 3 days, Cis+Q10$_{10}$ (n=8) group was given cisplatin (a single dose of 14 mg/kg/day, i.p.) and coenzyme Q10 (10 mg/kg/day, i.p.) for 3 days, Q10 (n=8) group was administered coenzyme Q10 (10 mg/kg/day, i.p.) for 3 days and Group C (n=8) (control group) was administered saline solution (1 mL/day, i.p.) once daily for 3 days. Pretreatment and posttreatment hearing levels were evaluated with distortion product otoacoustic emissions (DPOAEs).

Results: There was no statistically significant difference in the results of measurements of 4004, 4358, 4761 and 5188 Hz at end of the study in comparison to baseline ($p>0.05$). On the other hand, there was a significant difference at the measurements of 5652, 6165, 7336 and 7996 Hz (p=0.002, p=0.037, p=0.001, p=0.001, respectively). The rate of change at 5652 Hz revealed that Cis group was different from Cis+Q10$_{10}$, control and Q10 groups ($p<0.01$); measurements at 6165 Hz revealed that change at Cis group was significantly different from control and Q10 groups ($p<0.01$, $p<0.05$). Final measurements of decrease in Cis group at 7336 and 7996 Hz were significantly different from baseline ($p<0.05$; $p<0.01$).

Conclusion: The high-dose coenzyme Q10 showed a protective effect on hearing in cisplatin-induced ototoxicity while low-dose coenzyme Q10 protected hearing at low frequencies but did not show protective effect at high frequencies.

Keywords: Ototoxicity, cisplatin, coenzyme Q10, rats.

Özet: Koenzim Q10'un ratlarda sıçanlarda sisplatinin neden olduğu ototoksisiteye etkisi

Amaç: Sıçanlarda düşük ve yüksek dozlarda koenzim Q10'un sistemik uygulamasının sisplatinin neden olduğu ototoksisite üzerine etkinliğini belirlemek.

Yöntem: Çalışmamız 40 Sprague-Dawley sıçanla gerçekleştirildi. Sıçanlar randomize şekilde beş gruba ayrıldı: Cis, Cis+Q10$_{30}$, Cis+Q10$_{10}$, Q10, kontrol. Cis (n=8) grubuna tek bir 14 mg/kg dozda intraperitoneal (i.p.) yolla sisplatin enjekte edildi. Cis+Q10$_{30}$ (n=8) grubuna tek bir 14 mg/kg dozda i.p. sisplatin ve 3 gün boyunca günde 30 mg/kg dozda koenzim Q10 i.p. enjekte edildi. Cis+Q10$_{10}$ (n=8) grubuna tek bir 14 mg/kg dozda i.p. sisplatin ve 3 gün boyunca günde 10 mg/kg dozda koenzim Q10 i.p. enjekte edildi. Q10 (n=8) grubuna 3 gün boyunca günde 10 mg/kg dozda koenzim Q10 i.p. enjekte edildi. Kontrol grubuna (Grup C) (n=8) 3 gün boyunca günde 1 mL dozda i.p. salin enjekte edildi. Tedavi öncesi ve sonrası işitme düzeyleri distorsiyon ürünü otoakustik emisyonlarla (DPOAE) değerlendirildi.

Bulgular: Başlangıca göre çalışma sonunda 4004, 4358, 4761 ve 5188 Hz'deki ölçüm sonuçlarında istatistiksel açıdan anlamlı herhangi bir değişiklik yoktu ($p>0.05$). Diğer taraftan 5652, 6165, 7336 ve 7996 Hz'deki ölçümlerde anlamlı bir farklılık vardı (sırasıyla p=0.002, p=0.037, p=0.001 ve p=0.001). Ayrıca 5652 Hz'deki değişimin hızı Cis grubunun Cis+Q10$_{10}$, kontrol ve Q10 gruplarından farklı olduğunu ortaya koydu ($p<0.01$). Yine 6165 Hz'deki ölçümler Cis grubundaki değişimin kontrol ve Q10 gruplarından anlamlı derecede farklı olduğunu gösterdi ($p<0.01$, $p<0.05$). Cis grubunda 7336, 7996 Hz'deki azalmanın nihai ölçümleri başlangıçtaki ölçümlerden anlamlı derecede farklıydı ($p<0.05$, $p<0.01$).

Sonuç: Yüksek dozda koenzim Q10 sisplatinin neden olduğu ototoksisitede işitme duyusunu koruyucu etki gösterirken düşük doz koenzim Q10 düşük frekansları işitme duyusunu korumuş, yüksek frekanstaki sesleri işitme duyusunu koruyucu etki göstermemiştir.

Anahtar sözcükler: Ototoksisite, sisplatin, koenzim Q10, sıçanlar.

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Cisplatin (cis-diamminedichloroplatinum II, CDDP), a potent alkylating chemotherapeutic agent, is widely used in the treatment of several cancers despite of multiple side effects, including nephrotoxicity and ototoxicity.[1,2] Cisplatin may cause bilateral, progressive, irreversible high frequency sensorineural hearing loss with tinnitus.[3–6] Even though the potential mechanism of cisplatin ototoxicity is not fully understood, it may cause cell death by the production of reactive oxygen species (ROS). Several clinical and experimental studies demonstrated that multiple areas of the cochlea such as outer hair cells particularly at the basal turn, spiral ganglion cells, and stria vascularis can be damaged after cisplatin treatment; thereby leading to hearing loss. The outer hair cells in the basal turn of the cochlea are initially affected, then the apical turn, and finally the inner hair cells are affected. The formation of free radicals is believed to decrease intracellular glutathione levels and impair the activities of antioxidant enzyme activities. The derangement in antioxidant mechanism may lead to an increase in lipid peroxidation and cause apoptosis of hair and support cells, stria vascularis, and cochlear nerves.[7]

Coenzyme Q10 (CoQ10) terclatrate (Q-Ter) is a moving electron carrier in the mitochondrial electron transport chain and a major source of ATP. Ubiquinone is reduced by the respiratory chain to its active ubiquinol form, an effective antioxidant which prevents lipid peroxidation and mitochondrial damage.[8]

Based on this mechanism, several antioxidants have been reported in the literature;[9–12] however, none of the medicinal products with protective effects against cisplatin ototoxicity has been approved by the FDA. Therefore, the aim of this study is to determine the protective effect of CoQ10 on cisplatin-induced ototoxicity in rats.

Materials and Methods

International Review Board approval was taken from Animal Research Ethics Committee. This study was performed at the Experimental Animal Research Laboratory.

Our study has been performed with a total of 80 ears of 40 male Sprague-Dawley albino rats. Weights of the rats ranged from 200±20 g. Rats were accommodated in an environment under 12 hours light and 12 hours dark where the background noise level was below 50 dB, temperature was 21 °C, and the rats could get free food and water.

In all groups, Sprague-Dawley albino rats were anesthetized by ketamine hydrochloride (JHP Pharmaceuticals, Parsippany, NJ, USA) (0.45 mg/kg) and xylazine (Bayer, Leverkusen, Germany) (5 mg/kg).

Experimental design

Sprague-Dawley rats (n=40) were randomly divided into following groups: (i) Cis, (ii) Cis+Q10$_{30}$, (iii) Cis+Q10$_{10}$, (iv) Q10 and (iv) control. Cis (n=8) group was administered cisplatin [a single intraperitoneal (i.p.) injection of 14 mg/kg], Cis+Q10$_{30}$ (n = 8) group was administered cisplatin (a single i.p. injection of 14 mg/kg) and coenzyme Q10 (30 mg/kg/day, i.p.) for 3 days, Cis+Q10$_{10}$ (n=8) group was given cisplatin (a single dose of 14 mg/kg/day, i.p.) and coenzyme Q10 (10 mg/kg/day, i.p.) for 3 days, Q10 (n=8) group was administered coenzyme Q10 (10 mg/kg/day, i.p.) for 3 days and Group C (n=8) (control group) was administered saline solution (1 mL/day, i.p) once daily for 3 days. 3 rats at Cis group and another 3 at Cis+Q10$_{30}$ group died so 34 rats were able to complete the study.

The DPOAE recordings

All rats underwent the distortion product otoacoustic emission (DPOAE) measurements on days 0 and 4. Otomicroscopic examinations of all of the ears of the rats were performed before DPOAE examination, and rats with middle ear pathologies were excluded. "ILO Cochlear Emission Analyzer" (Otodynamics, London, UK) was used for the measurement of the DPOAEs. Distortion product grams (DPgram) were measured at 80 dB (L1=L2). Two different frequencies (f1 and f2) that might be the most powerful responses were organized as f2/f1=1.22. DPgram measurements were performed and noted at 1001, 1501, 2002, 3003, 4004, 4358, 4761, 5188, 5652, 6165, 6726, 7336 and 7996 Hz frequencies. The noise levels for both DPgram and I/O functions were measured at frequencies 50 Hz above the DPOAE frequencies. During measurements at 2f1-f2 frequency, the OAEs ?3 dB above the noise intensity were considered positive. Emission values were under the noise threshold at 1001, 1501, 2002, and 3003 Hz and above it at the other frequencies. Therefore, statistical analyses were applied to the results obtained at 4004, 4358, 4761, 5188, 5652, 6165, 6726, 7336 and 7996 Hz.

Statistical analysis

An intra- and intergroup comparisons of measurements that were taken before and after experiment were performed. In order to evaluate the results of the study, IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. Descriptive statistics were presented as means and standard deviations. Kolmogorov-Smirnov test demonstrated that values were not normally distributed. Therefore, intergroup comparisons were per-

Table 1. Pretreatment and posttreatment evaluations of 5652 Hz DPOAEs measurements in the groups.

	Baseline measurement	Final measurement	Baseline-final difference
5652 Hz	**Mean±SD (median)**	**Mean±SD (median)**	**p‡**
Cis	32.43±8.82 (32.45)	20.48±5.41 (21.6)	**0.005***
Cis+Q10$_{30}$	38.98±6.57 (38.8)	32.18±13.82 (37.55)	**0.093**
Cis+Q10$_{10}$	29.17±7.63 (28.1)	29.58±8.24 (29)	**0.796**
Q10	40.41±5.54 (41.95)	38.38±8.91 (38.7)	**0.589**
C	40.41±5.54 (41.95)	40.74±13.76 (43.3)	**0.767**
p†	**0.001***	**0.001***	

*p<0.1. †Kruskal-Wallis test, ‡Wilcoxon signed ranks test. **C:** control, **Cis:** cisplatin, **Q10:** coenzyme Q10, **SD:** standard deviation.

Table 2. Pretreatment and posttreatment evaluations of 6165 Hz DPOAEs measurements in the groups.

	Baseline measurement	Final measurement	Baseline-final difference
6165 Hz	**Mean±SD (median)**	**Mean±SD (median)**	**p‡**
Cis	32.91±4.72 (33.2)	21.68±6.78 (22.35)	**0.005***
Cis+Q10$_{30}$	35.53±6.55 (34)	30.32±13.32 (32.7)	**0.333**
Cis+Q10$_{10}$	32.86±6.1 (32.5)	27.76±8.01 (29.5)	**0.066**
Q10	39.47±5.3 (40.45)	35.66±8.8 (36.1)	**0.179**
C	39.47±5.3 (40.45)	38.35±8.85 (40.9)	**0.575**
p†	**0.002***	**0.001***	

*p<0.01. †Kruskal-Wallis test, ‡Wilcoxon signed ranks test. **C:** control, **Cis:** cisplatin, **Q10:** coenzyme Q10, **SD:** standard deviation.

formed using Kruskal-Wallis test, and the Mann-Whitney U test was used in order to determine from which group the difference arose. The Wilcoxon signed-ranks test was applied for intra-group comparisons. All results were evaluated at 95% confidence interval, and a p value of less than 0.05 was considered statistically significant.

Results

No statistically significant difference was determined among groups at baseline measurements (p>0.05). Cis group had remarkably low results at the end of the study when compared to all other groups (p<0.01). There was no significant difference at the final measurement of 4004, 4358, 4761 and 5188 Hz in comparison to baseline values (p>0.05). On the other hand, there was a significant change at final result of 5652 Hz compared to baseline (p=0.002; p<0.01). Mann-Whitney U test revealed that the change in Cis+Q10$_{10}$ (p=0.001) group had significantly higher values than control (p=0.001) and Q10 groups (p=0.003). Change at Cis+Q10$_{30}$ group was significantly higher than the control group at 5652 Hz (p=0.049; p<0.05). There was no significant difference among the changes of other groups at the end of the study in comparison to baseline (p>0.05) (Table 1).

Final measurement showed that there was a significant change in 6165 Hz compared to baseline values. Mann-Whitney U test revealed that the change in Cis group (p=0.037; p<0.05) was significantly higher than control (p=0.001) and Q10 groups (p=0.035; p<0.01; p<0.05). There was no significant difference among the changes of other groups at the end of the study in comparison to baseline (p>0.05) (Table 2).

Final measurement showed that there was a significant change in 7336 Hz compared to baseline value (p=0.001; p<0.01). Mann-Whitney U test revealed that the decrease in Cis group was higher than Cis+Q10$_{30}$, (p=0.023), Cis +Q1010 (p=0.031), control (p=0.001) and Q10 (p=0.001) groups (p<0.05; p<0.01). Cis+Q10$_{30}$ (p=0.017) and Cis+Q10$_{10}$ (p=0.011) groups had significantly higher decrease rates than control groups (p<0.05). There was no significant difference among the changes of other groups at the end of the study in comparison to baseline (p>0.05). (Table 3)

Final measurement showed that there was a significant change in 7996 Hz compared to baseline value (p=0.001; p<0.01). Mann-Whitney U test demonstrated that the decrease in Cis group was higher than Cis+Q10$_{30}$, (p=0.028), Cis+Q10$_{10}$ (p=0.006), control (p=0.001) and Q10 (p=0.001) groups (p<0.05; p<0.01). Decreases in Cis+Q10$_{30}$ and Cis +Q1010 groups were remarkably higher than control group

Table 3. Pretreatment and posttreatment evaluations of 7336 Hz DPOAEs measurements in the groups.

	Baseline measurement	Final measurement	Baseline-final difference
7336 Hz	**Mean±SD (median)**	**Mean±SD (median)**	**p‡**
Cis	34.26±6.04 (35.9)	12.75±11.37 (11.95)	**0.005***
Cis+Q10$_{30}$	39.6±3.98 (40.1)	30.43±14.77 (35.5)	**0.014****
Cis+Q10$_{10}$	37.08±10.22 (36.05)	26.36±13.24 (32.85)	**0.006***
Q10	38.71±2.58 (38.3)	34.49±10.44 (38.25)	**0.469**
C	38.71±2.58 (38.3)	37.91±5.62 (37.85)	**0.674**
p†	**0.005***	**0.001***	

*p<0.05, **p<0.01. †Kruskal-Wallis test, ‡Wilcoxon signed ranks test. **C:** control, **Cis:** cisplatin, **Q10:** coenzyme Q10, **SD:** standard deviation.

(p=0.021; p=0.014 respectively). At the end of the study, no statistically significant difference among the changes of other groups was found when compared with baseline results (p>0.05) (Table 4, Fig. 1).

Discussion

Cisplatin administration can cause hearing loss due to functional and structural changes in the cochlea. Cisplatin may reduce endocochlear potentials, and cause structural damages at several regions of cochlea; thereby, leading to hearing impairment.[4,7] Although the mechanism of cisplatin ototoxicity is not fully understood, it appears to involve the formation of ROS that trigger cell death.[4,7]

As cisplatin damages the organ of Corti, particularly the basal cochlear turn, hearing loss starts at higher frequencies, which may then progress to involve all frequencies.[2,13] In our study, hearing loss involved all frequencies in cisplatin-treated rats. Hence, we saw that single dose of cisplatin caused ototoxicity.

Animal studies have demonstrated that cisplatin administration may elevate the ABR thresholds. In addition, cisplatin induced ototoxicity may occur as a result of inner ear hair cell degeneration due to oxidative stress. In the literature, a variety of antioxidant agents have been suggested to prevent ototoxicity, including dexamethasone,[3] alpha-tocopherol, tiopronin,[6] sodium salicylate,[12] amifostine,[13] d-methionine,[14] vitamin E,[15] pentoxifylline,[16] neurotropines,[17] flunarizine,[18] and melatonin.[19] In cisplatin ototoxicity, the use of protective agents may prevent hearing

Table 4. Pretreatment and posttreatment evaluations of 7996 Hz DPOAEs measurements in the groups.

	Baseline measurement	Final measurement	Baseline-final difference
7996 Hz	**Mean±SD (median)**	**Mean±SD (median)**	**p‡**
Cis	36.38±6.36 (37.85)	6.82±9.48 (1.5)	**0.005***
Cis+Q10$_{30}$	40.19±5.31 (41.15)	27.82±17.61 (32)	**0.037****
Cis+Q10$_{10}$	35.02±6.11 (35.15)	24.02±15.52 (21.95)	**0.023****
Q10	39.7±3.78 (40.4)	36.58±11.77 (41.2)	**0.776**
C	39.7±3.78 (40.4)	38.44±4.2 (38.45)	**0.069**
p†	**0.026****	**0.001***	

*p<0.05, **p<0.01. †Kruskal-Wallis test, ‡Wilcoxon signed ranks test. **C:** control, **Cis:** cisplatin, **Q10:** coenzyme Q10, **SD:** standard deviation.

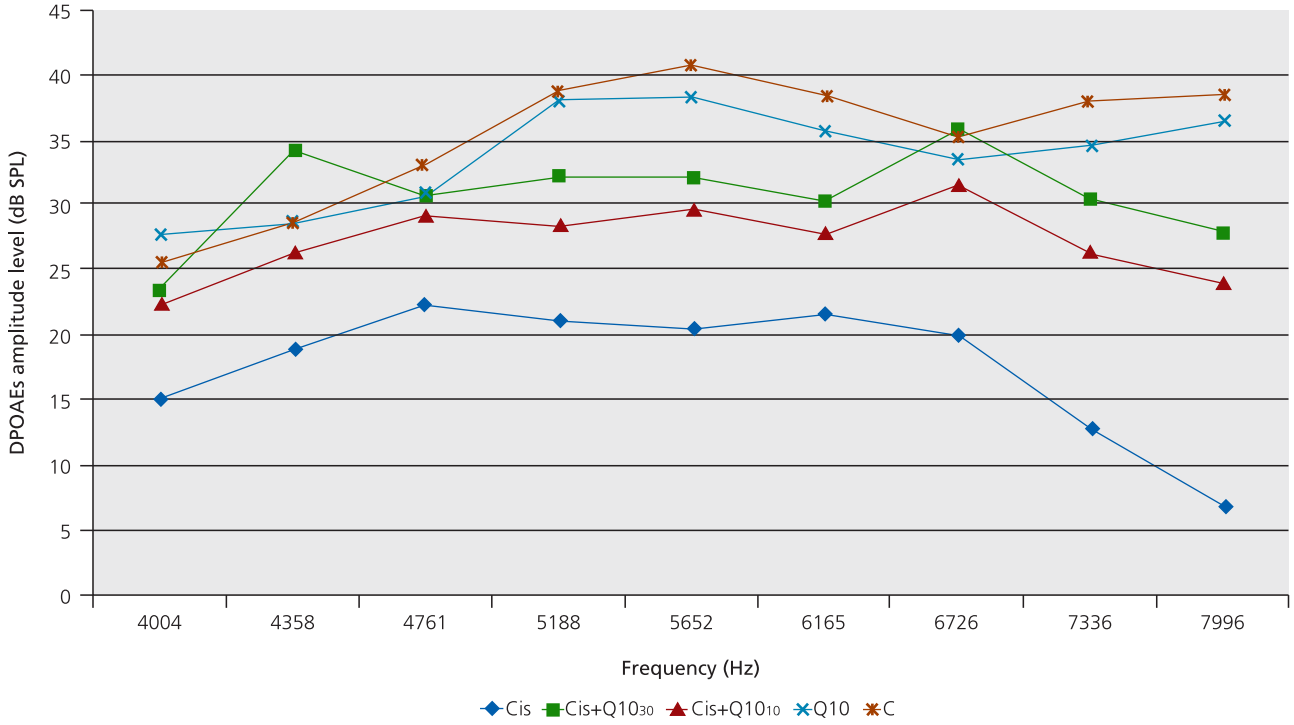

Fig. 1. Post-treatment DPOAE amplitudes (dB SPL) of the groups by frequencies. **C:** control, **Cis:** cisplatin, **Q10:** coenzyme Q10 (10 mg/kg/day), **Q10$_{10}$:** coenzyme Q10 (10 mg/kg/day), **Q10$_{30}$:** coenzyme Q10 (30 mg/kg/day).

loss and lipid peroxidation. In addition, clinical application of these protective agents may reduce or prevent the cisplatin related damage to the inner ear in patients who were administered chemotherapy for cancer without causing an alteration in antitumor effect of cisplatin.[20,21] Cisplation administration may lead to a significant elevation in superoxide dismutase, catalase activities, and malondialdehyde levels; on the other hand, cochlear GSH-peroxidase and GSH reductase activities are decreased.[2] CoQ10, with its known antioxidant properties, has been popularized recently, and has been investigated for the treatment of diseases related to oxidative stress. Within mitochondria, ubiquinone is reduced by the respiratory chain to its active ubiquinol form, which is an effective antioxidant that prevents lipid peroxidation and mitochondrial damage.[22] Some studies have demonstrated that CoQ10 is effective for the treatment of noise-induced hearing loss, presbyacusis, and sudden sensorineural hearing loss.[9,21–23]

Idebenone, a synthetic analogue of CoQ10, reduces noise-induced hearing loss. CoQ10, with its antioxidant properties, shows protective effects against gentamicin ototoxicity both in ABR as well as histopathologically.[24]

This study was designed in order to evaluate the protective role of CoQ10 based on dose. We detected that although it did not protect hearing totally, it kept it at better level. There was no difference between high and low dose.

Several recent studies have evaluated the functional changes in the cochlea in cisplatin ototoxicity. Guinea pig may be the most sensitive animal for studies of cisplatin ototoxicity; transient-evoked otoacoustic emissions (TEOAEs) and DPOAEs are sensitive techniques for assessing the functional status of the outer hair cells.[25] In this study, we particularly selected DPOAEs for the assessment of cochlear function because it is a noninvasive, objective, and highly sensitive technique for the assessment of outer hair cell function and cochlear damage. It is a useful technique for monitoring drug-induced ototoxicity. Fetoni monitored the protective effects of Q-Ter® and reported that DPOAEs represent a sensitive test for monitoring the effects of noise in preclinical conditions and under pharmacological treatment.[26]

There are several limitations in this study. As cisplatin is administered in humans for several months at intervals of 2 to 4 weeks, typically no hearing loss is induced with a single dose. However, as in previous animal studies, we evaluated the effects of a single dose of cisplatin in animals for effort- and cost-related reasons. Different cisplatin doses were used in previous studies to evaluate cisplatin ototoxicity; it was reported that no significant hearing loss occurred at doses below 14 mg/kg/day, while hearing loss could occur at doses over 14 mg/kg/day, but the mortality rate also increased.[2,13] In our study, we administered cisplatin at the dose of 14 mg/kg/day, and there was a marked impairment in the general condition of the rats.

Conclusion

Although Q10 did not protect hearing, it kept it at better levels. There was no difference in protecting function of high and low.

Conflict of Interest: No conflicts declared.

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Topical dexpanthenol application improves healing of acute tympanic membrane perforations: an experimental study

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Abstract

Objective: To investigate the healing effects of topical dexpanthenol on acute tympanic membrane (TM) perforations in rats through observations of healing time and histopathological changes.

Methods: A total of 20 Sprague-Dawley rats were included in the study. Every perforation was formed at the pars tensa of TMs with a size 2 mm in diameter. The right TM of each rat was treated with topical dexpanthenol for 2 days (treatment group); on the other hand, no topical agent was applied on the left TMs of rats (sham group). All TMs were examined under otomicroscopy at the third, fifth, and seventh days to determine the healing of TM perforations. Moreover, TMs were histopathologically examined to assess neovascularization, collagenization, fibroblastic activity, inflammatory cell positivity at the lamina propria (LP) layer of TMs.

Results: The TM perforations in the treatment group healed significantly earlier ($p<0.05$). The collagenization at LP was significantly higher in the treatment group ($p<0.05$), while neovascularization and inflammatory cell positivity were significantly higher in the sham group ($p<0.05$). The fibroblastic activity was higher in the treatment group although no statistically significant difference was determined.

Conclusion: The findings of the current study suggest that dexpanthenol may accelerate the healing of acute TM perforation.

Keywords: Dexpanthenol, tympanic membrane perforation, rat, wound healing.

Özet: Topikal dekspantenol uygulaması akut kulak zarı perforasyonlarının iyileşmesini hızlandırmaktadır: Deneysel bir çalışma

Amaç: Bu çalışmanın amacı, sıçanlarda akut kulak zarı (KZ) perforasyonunda topikal dekspantenol uygulamasının iyileştirici etkilerini iyileşme süresi ve histopatolojik değişikliklerin gözlenmesi ile araştırmaktır.

Yöntem: Yirmi adet Sprague-Dawley cinsi sıçanın her iki KZ'nin pars tensa bölgesinde 2 mm genişliğinde perforasyon oluşturuldu. Sıçanların sağ kulaklarındaki perforasyonlar (tedavi grubu) 2 gün boyunca topikal dekspantenol ile tedavi edildi. Sol kulaklara (sham grubu) hiçbir topikal tedavi verilmedi. Kulak zarı perforasyonlarının kapanma durumunu değerlendirmek için sıçanlar üçüncü, beşinci ve yedinci günlerde otomikroskopi ile değerlendirildi. Histopatolojik olarak kulak zarları, lamina propria tabakasındaki neovaskülarizasyonu, fibroblastik aktiviteyi, inflamatuar hücre pozitifliğini ve kollajenizasyonu değerlendirmek için incelendi.

Bulgular: Tedavi grubunda KZ perforasyonları istatistiksel olarak anlamlı düzeyde erken iyileşti ($p<0.05$). Lamina propria kollajenizasyonu tedavi grubunda anlamlı derecede yüksek bulunurken ($p<0.05$), neovaskülarizasyon ve inflamatuar hücre pozitifliği tedavi verilmeyen grupta anlamlı derecede yüksekti ($p<0.05$). Fibroblastik aktivite tedavi grubunda daha yüksek bulunmasına rağmen fark istatistiksel olarak anlamlı değildi.

Sonuç: Bu çalışmanın bulguları, akut kulak zarı perforasyonun iyileşmesini hızlandırmada dekspantenolun etkin olduğunu göstermektedir.

Anahtar sözcükler: Dekspantenol, kulak zarı perforasyonu, sıçan, yara iyileşmesi.

Tympanic membrane (TM) perforation is a relatively common problem. Its incidence is not known exactly; however, estimated to be less than 1%.[1]

The leading cause of acute TM perforations is middle ear infections, followed by trauma. Various types of traumatic insults (e.g., insertion of objects into the ear canal,

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concussion caused by an explosion or open-handed slap, head trauma, barotrauma and iatrogenic trauma due to myringotomy, irrigation, or foreign body removal) can cause acute TM perforations. Most TM perforations, especially those caused by trauma, heal spontaneously. However, in a small group of patients, the TM perforations remain open and surgical intervention is needed.[2]

Complex biological mechanisms play a role in the healing process of acute TM perforations including epithelial proliferation, migration, fibroblast proliferation, angiogenesis, and tissue remodeling.[3] Various agents and treatment methods have been used with the aim of accelerating the spontaneous healing process and aiding the completeness of TM closure. The effects of topical agents, such as hyaluronic acid, heparin, epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF), on the closure of TM perforations have been investigated.[4–7] Although positive results were found with these agents, limited availability and high costs currently prevent their widespread use.

Dexpanthenol is an alcoholic analog of panthotenic acid (vitamin B5), which is a component of coenzyme A. In the body, it is converted to the active form, panthotenic acid, an essential molecule for human epithelial cells. Topical dexpanthenol has high tissue penetration, and due to its prominent effects such as stimulation of epithelization and granulation, its efficacy in wound healing is well established.[8]

Therefore, through examination of the closure time of TM perforations and of the histopathological changes in regenerated membranes, we aimed to investigate the efficacy of topical dexpanthenol in the healing of acute TM perforations in a rat model.

Materials and Methods

This study was performed in the Laboratory of Experimental Studies of Karadeniz Technical University School of Medicine and complied with the guidelines for the care and use of experimental animals. The approval of the local ethics committee of Karadeniz Technical University was obtained before the study was conducted. Twenty-two adult male Sprague-Dawley rats, each weighing between 250 and 300 g, were involved in the study. Animals were housed in 50±10% humidity at 22±1 oC on a 12-hour light-dark cycle and had free access to water and standard dry pellets.

Four ears (2 rats) were used as the control group. The other 20 animals were anesthetized with intraperitoneal (IP) ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (10 mg/kg). A 2 mm perforation was created by a myringotomy knife at the posterosuperior quadrant of the pars tensa in each TM of 20 rats using an otomicroscope (Deca 21; Inami Corp., Tokyo, Japan). During the procedure, 3 rats were observed to have serious middle ear effusion in one side and were excluded from the study. The study was completed with a total of 19 rats. The right TM perforations were treated with 5 drops of dexpanthenol immediately after perforation, and additional applications of 5 drops were administered after 24 hours and after 48 hours. No treatment was given to the left ears (sham group), and the left TMs were allowed to spontaneously heal. The four TMs of the 2 rats that were not perforated were not treated, serving as a reference for the comparison of histopathological changes evaluated in the dexpanthenol and sham groups.

Otomicroscopic examination under IP ketamine anesthesia was performed on the third, fifth, and seventh days to check the status of the myringotomy patency on each side. The healing of TM perforations was evaluated as total or partial closure.

On the fourteenth day of the study, the rats were sacrificed by decapitation under anesthesia with IP ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (10 mg/kg). The tympanic bullae were opened, and the right and left TMs were removed. The specimens were kept in 10% formaldehyde solution. After 24 hours, the 38 specimens were decalcified in formic acid and sodium citrate. For both treatment and sham groups, the TM specimens were bisected through the center of the healed perforation and embedded in paraffin blocks. The TMs of the controls were bisected medially. For histopathological examination, 5 µm thick sections were stained in hematoxylin and eosin and examined under light microscopy at a 40× magnification (Olympus BX51; Olympus Corp., Tokyo, Japan). The changes in LP, including neovascularization of the lamina propria (LP), fibroblastic activity, collagenization, and inflammatory cell presence were evaluated, referencing the findings to the control TMs. The LP changes were evaluated as positive if they were prominent and as negative if there were no differences compared to the control TM specimens. All specimens were evaluated by the same pathologist.

The statistical analysis of the data regarding the histopathological changes in the LP and the TM-perforation healing time was conducted using Fischer's exact chi-square test. A p value of less than 0.05 was considered statistically significant.

Results

All TMs in the treatment and sham groups were found to be completely closed on the seventh day. The mean perforation closure time was 5.6±1.4 days in the sham group and 4.4±1.2 days in the dexpanthenol treatment group (Fig. 1). The difference in healing time between two groups was statistically significant ($p<0.05$).

A comparison of the LP changes in the treatment and sham groups is shown in Table 1. The LP collagenization in the treatment group was significantly higher than sham group ($p<0.05$) (Table 1). In the sham group, LP neovascularization and inflammatory cell positivity were significantly higher than in the treatment group ($p<0.05$). The LP fibroblastic activity was higher in the treatment group, however the difference between both groups was not statistically significant ($p>0.05$).

Table 1. The comparison of LP changes in the treatment and sham groups.

		Sham group		Treatment group		Chi square	p
		n	(%)	n	(%)		
LP neovascularization	-	2	11.8	9	52.9	4.838	0.028
	+	15	88.2	8	47.1		
LP fibroblastic activity	-	5	29.4	1	5.9	1.821	0.177
	+	12	70.6	16	94.1		
LP inflammatory cells	-	6	35.3	16	94.1	10.432	0.001
	+	11	64.7	1	5.9		
LP collagenization	-	14	82.4	4	23.5	9.563	0.002
	+	3	17.6	13	76.5		

Discussion

This study showed that the healing time of the TM perforations in the dexpanthenol-treated ears was significantly shorter than in the untreated ears. The LP collagenization was also significantly higher in the treatment group, while neovascularization and inflammatory cell positivity were significantly higher in the sham group.

The healing of a traumatic perforation of the TM is a complex process that requires epithelial proliferation and migration, fibroblast proliferation, neovascularization, and tissue remodeling.[9,10] In the typical wound healing of soft tissues, the formation of granulation tissue precedes epithelization. On the contrary, the key mechanism in the healing of TM perforations is the initial closure of the epithelial layer by increased mitotic activity of epithelial cells, followed by the regeneration of the LP.

In experimental studies, it has been reported that various topical agents may accelerate the healing of acute TM perforation. Among these agents, polypeptide growth factors have been widely studied with promising results in TM healing. In guinea pigs, bFGF was reported to promote healing of TM perforations by inducing neovascularization, fibroblast proliferation, and matrix deposition compared with controls.[7] In other studies investigating experimental models of acute TM perforations, a shorter healing time was reported in bFGF-treated groups.[11–13] EGF and transforming growth factor-β1 (TGF-β1) were found to stimulate TM-perforation healing in animal models.[14,15]

Dexpanthenol is known for promoting wound healing and epithelization, especially in dermatological conditions, such as epidermal wounds, burn injuries, and various skin irritations (e.g., scaling, pruritus, fissures, erythema). Pantothenic acid, the active form of dexpanthenol, is an essential ingredient for epithelial function.[8] In vivo and in vitro studies with dexpanthenol have shown that it has a key role in wound-healing by activating the fibroblast proliferation.[8] The prominent effects of dexpanthenol formulations that accelerate healing processes are the stimulation of epithelization and granulation.

In the field of otorhinolaryngology, beneficial effects of dexpanthenol were found for rhinitis sicca treatment and nasal mucosa regeneration after nasal surgeries.[16,17] To the best of our knowledge, this is the first study in which the role

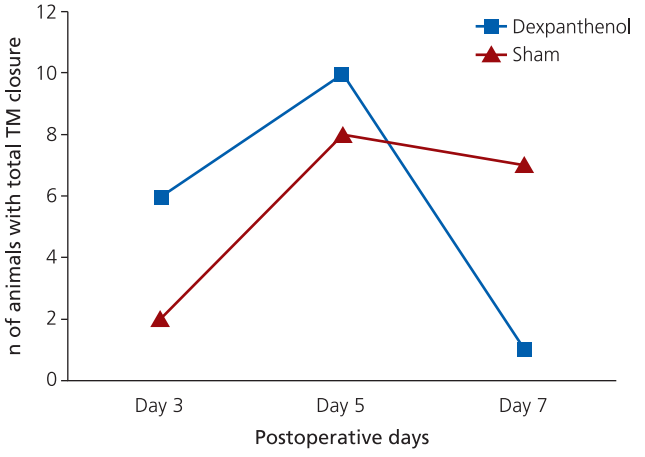

Fig. 1. The closure time (days) of TM perforations in dexpanthenol treatment and sham groups.

of topical dexpanthenol application on healing of TM perforation and LP changes was evaluated. Our findings demonstrate the beneficial effects of dexpanthenol for TM-perforation healing, as indicated by significantly shorter TM-perforation closure time and higher LP fibroblastic activity and collagenization following dexpanthenol treatment.

In the only study using dexpanthenol in a TM-perforation model, Özel et al.[18] investigated the effects of dexpanthenol, hydrogen peroxide (H_2O_2), and erdosteine on myringosclerosis development and TM healing time. The authors found significantly less myringosclerosis in the dexpanthenol and erdosteine groups than in the control, isotonic, and H_2O_2 groups. Consistent with our findings, they also showed that TM-perforation healing time was significantly shorter in the dexpanthenol and erdosteine treatment groups.

In the current study, the effects of dexpanthenol on healingof acute TM perforation were investigated. The finding shows that the TM-perforation closure time was significantly shorter in the dexpanthenol-treated group which is consistent with the findings in previous literature[18] and can be attributed to the stimulation of epithelization by dexpanthenol. Consistent with previous studies,[7–10] the significant collagenization and higher fibroblastic activity in the LP in the dexpanthenol group demonstrate the wound-healing promoting effect of the molecule. Unexpectedly, we found that the LP neovascularization and inflammatory cell positivity was significantly higher in the sham group. This finding is in contrast with the findings of previous studies that revealed increased neovascularization and inflammatory cell infiltration with dexpanthenol treatment.[8,19] However, these dexpanthenol studies were performed in wound healing models in different tissues. The healing of the TM, unlike that of other tissues, is characterized by an initial epithelial migration followed by LP regeneration. This migration and regeneration may contribute to the different healing pattern found in our study. Additionally, the anti-inflammatory effects of dexpanthenol described in previous studies[8,20] could contribute to decreased neovascularization and inflammatory cell infiltration. However, it is controversial whether dexpanthenol, due to its anti-inflammatory action, inhibited neovascularization and inflammatory cell infiltration in the LP in our experimental model or not.

Conclusion

The clinical and histopathological findings of this study demonstrated that topical dexpanthenol application may promote the healing of TM perforations. We suggest that topical dexpanthenol, as a readily available and simple agent, may be a reasonable alternative to surgery in treating acute traumatic TM perforations.

Conflict of Interest: No conflicts declared.

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The roles of endothelial nitric oxide synthase (eNOS) and myeloperoxidase (MPO) genes in microtia

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Abstract

Objective: The aim of this study was to determine the relationship between polymorphisms of endothelial nitric oxide synthase (eNOS) and myeloperoxidase (MPO) genes and development of microtia.

Methods: Nineteen (11 males, 8 females) unrelated cases with microtia and 40 healthy controls were enrolled in the present study. The study focused on three functional variants; a variant in exon 7 (G894T) and a variable number of 27 bp tandem repeats in intron 4 (VNTR) of eNOS gene and a variant in the promoter region (G463A) of MPO gene. We genotyped these variants using the polymerase chain reaction (PCR) and/or PCR-restriction fragment length polymorphism (RFLP) method. The distribution of allele and genotype in eNOS and MPO genes were compared between cases with microtia and healthy controls using chi-square test.

Results: With regard to the eNOS (G894T) variant, there was a significant difference in genotype distribution between cases with microtia and healthy controls (OR: 1.267, 95% CI: 1.004–1.598; p=0.009). Our study demonstrated that cases with eNOS (G894T) TT genotype had increased risk of microtia. The allele frequencies of eNOS (VNTR) variant showed statistically significant difference between cases with microtia and healthy controls (OR: 2.947, 95% CI: 1.188–7.311; p=0.028). eNOS (VNTR) B allele was higher in the cases. However, there was no significant difference for MPO (G463A) variant according to genotype distribution and allele frequency between cases with microtia and healthy controls.

Conclusion: To the best of our knowledge, this is the first analysis of the eNOS (G894T and VNTR) and MPO (G463A) variants in cases with microtia. Our data demonstrate that eNOS gene variants might play crucial role on the etiopathogenesis of microtia in Turkish population. The findings of the current study highlight the necessity for prospective longitudinal studies in elucidating the relative contributions of various factors in diseases with a multifactorial etiology where there is interplay among genetic susceptibility and exogenous factors.

Keywords: Microtia, endothelial nitric oxide synthase, myeloperoxidase, PCR, RFLP.

Özet: Endotelyal nitrik oksit sentaz (eNOS) ve miyeloperoksidaz (MPO) genlerin mikrotiyadaki rolü

Amaç: Bu çalışmanın amacı endotelyal nitrik oksit sentaz (eNOS) polimorfizmleriyle miyeloperoksidaz (MPO) genleri ve mikrotiya gelişimi arasındaki ilişkiyi belirlemekti.

Yöntem: Çalışmaya akraba olmayan 19 (11 erkek, 8 kadın) mikrotiyalı olgu ve 40 sağlıklı kontrol alındı. Çalışma, ekson 7'nin bir varyantı (G894T), eNOS geninin 4. nitronunda (VNTR) değişken sayıda 27 bp ardışık tekrarlar ve MPO geninin promoter bölgesinde (G463A) bir varyant olmak üzere üç fonksiyonel varyant üzerine odaklandı. Polimeraz zincir reaksiyonu (PCR) ve/veya PCR-restriksiyon parça uzunluk polimorfizm (RFLP) yöntemi kullanarak bu varyantların genotiplerini çıkardık. Ki-kare testi kullanarak mikrotiya olgularıyla sağlıklı kontroller arasında eNOS ve MPO genlerinde alel ve genotip dağılımını karşılaştırdık.

Bulgular: eNOS (G894T) varyantı açısından, mikrotiya olgularıyla sağlıklı kontroller arasında genotip dağılımı açısından önemli bir farklılık vardı (OR: 1.267, %95 GA: 1.004–1.598; p=0.009). Çalışmamız eNOS (G894T) TT genotipli olgularda mikrotiya riskinin arttığını gösterdi. eNOS (VNTR) varyanının alel sıklıkları mikrotiya olgularıyla sağlıklı kontroller arasında istatistiksel açıdan anlamlı farklılık olduğunu gösterdi (OR: 2.947, %95 GA: 1.188–7.311; p=0.028). Olgularda eNOS (VNTR) B aleli daha yüksek sıklıkta görülmüştür. Ancak genotip dağılımına göre MPO (G463A) varyantı, genotip dağılımı ve alel sıklığı açısından mikrotiya olguları ve sağlıklı kontroller arasında anlamlı bir farklılık yoktu.

Sonuç: Bildiğimiz kadarıyla bu çalışma ile mikrotiya olgularında eNOS (G894T ve VNTR) ve MPO (G463A) varyantları ilk kez incelenmiştir. Verilerimiz eNOS gen varyantları Türk halkındaki mikrotiyanın etyopatogenezinde kritik rol oynayabildiğini göstermektedir. Güncel çalışmanın bulguları genetik yatkınlık ve dışsal etmenlerin etkileşimde olduğu bir multifaktöryel etiyolojili hastalıkta değişik faktörlerin göreceli katkılarını aydınlatmada prospektif uzunlamasına çalışmaların gerekliliğini vurgulamaktadır.

Anahtar sözcükler: Mikrotiya, endotelyal nitrik oksit sentaz, miyeloperoksidaz, PCR, RFLP.

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Microtia is a congenital deformity affecting the outer ear, characterized by a small, abnormally shaped auricle. External ear canal is commonly narrowed, blocked or absent and middle ear is underdeveloped because the outer ear and the middle ear have common embryologic origin.[1] The prevalence of microtia varies between 0.83 and 17.4 per 10,000 births.[2] It is also reported that microtia is more common in males, and most cases are unilateral, predominantly being on the right side.[3] The pathogenesis of microtia remains unclear. Several risk factors including prenatal exposure to drugs, paternal age, high parity, maternal diabetes, high maternal age, multiple births have been implicated with this deformity.[2–5] The hereditary factors are most likely associated with microtia because it is generally seen some specific syndromes with chromosomal abnormalities, including Goldenhar syndrome, Treacher Collins syndrome, trisomy 21 and trisomy 18.[3]

Nitric oxide (NO) acts as an essential molecular mediator in many physiologic processes that are important for organogenesis, such as gene expression, cell growth, matrix remolding, proliferation, differentiation and apoptosis.[6] In embryonic tissues, the expression of NO sythase isoforms is temporally and spatially regulated, and impairment of endogenous NO secretion can result in developmental defects. The catalyst in endothelial NO synthesis is endothelial nitric oxide synthase (eNOS). A functional variant in exon 7 of human eNOS is related to a Glu-Asp change at codon 298 (Glu298Asp, also called G894T) (rs1799983). The GG ancestral genotype of the eNOS G894T variant, located in exon 7 of the eNOS on chromosome 7, has been claimed to cause increased protein expression and activity.[7] Other functional variant is a variable number of tandem repeats (VNTR, 27 nt) in intron 4, which accounts for >25% of basal plasma NO production.

Myeloperoxidase (MPO) is a lysosomal hemoprotein enzyme related with oxidative stress, located in polymorphonuclear neutrophils and monocytes. This enzyme catalyzes production of hypochloric acid (HOCl), which in turn may lead to damage in host DNA and result in the mutation of homeobox, oncogenes and tumor supressor genes.[8] The MPO gene has a common variant within the gene promoter. This guanin 463 adenine (G463A) (rs2333227) base transition has been described at the SP1 binding site, where the variant A allele is linked with reduced messenger RNA (mRNA) expression, leading to approximately 25 times less transcription activity compared to the G allele.[9]

In this study, we examined the relationship between eNOS (G894T and VNTR), MPO (G463A) gene variants and microtia risk.

Materials and Methods

Study population

Nineteen (11 males, 8 females) nonsyndromic, unrelated cases with microtia and 40 healthy controls were examined in the study. We genotyped the eNOS (G894T and VNTR) and MPO (G463A) variants. Informed consent was obtained from each participant before blood sampling, and the study was approved by the local Ethical Committee.

Genotyping procedure

DNA isolation: Peripheral blood samples were collected from the cases with microtia and healthy controls. Genomic DNA was extracted from EDTA (ethylenediamine tetraacetate)-treated peripheral venous blood using salting out method and stored at -20 °C until analysis.[10]

eNOS (G894T) variant genotyping: The eNOS variant was analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assays. The segment amplification of exon 7 with the flanking intronic primers 5'-CATGAGGCTCAGCCCCAGAAC-3' (sense) and 5'-AGTCAATCCCTTTGGTGCTCAC-3' (antisense) followed by MboI restriction endonuclease (Invitrogen CA, USA) digestion for 16 hours at 37 °C. Digestion was resolved on 3% agarose gel and visualized using ultraviolet light. The 206 bp PCR products had a consistent restriction site resulting in 119 bp and 87 bp fragments. Twenty percent of the samples were duplicated as internal quality control to avoid sample or reading errors.[11]

eNOS (VNTR) variant genotyping: eNOS intron 4 variant was analyzed by PCR using following primer: F: 5'-AGGCCCTATGGTAGTGCCTTT-3', and R: 5'-TCTCTTAGTGCTGTGGTCAC-3'. The PCR product (393 bp and/or 420 bp) was obtained. The products were then separated on 4% NuSieve GTG agarose gel. The experimental process was repeated twice for each sample.[12]

MPO (G463A) variant genotyping: The region with G463A variant which is located at promoter of MPO gene was multiplied with PCR by using MPO-F 5'-CGG TAT AGG CAC AAT GGT GAG and R: 5' GCA ATG GTT CAA GCG ATT CTT C primary chains and amplification control was done with 2% agarose gel electrophoresis. Amplified region was incubated for 16 hours with 5 units of *AciI* enzyme at 37 °C and analyzed with 3% agarose gel electrophoresis.[13]

Statistical analysis

All data were analyzed using software SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL, USA). The statistically significant differences between cases with microtia and healthy controls were estimated by logistic regression analysis. Odds ratio (OR) and 95% confidence interval (CI) were also calculated. The differences in eNOS (G894T and VNTR) and MPO (G463A) variants genotype frequencies between cases with microtia and healthy controls were compared with chi-square test, and Fisher's exact test was used when needed (http://ihg.gsf.de/cgi-bin/hw/hwa2.pl). All analyses were two-tailed, and differences were interpreted as statistically significant when <0.05.

Results

The genotype and allele distributions of the eNOS (G894T and VNTR) and MPO (G463A) variants were presented in Table 1.

eNOS (G894T) variant: The distribution of GG, GT and TT genotypes for eNOS (G894T) were observed in 65%, 35%, 0% of healthy controls and in 52.6%, 26.3% and 21.1% of cases with microtia, respectively. The allele frequency of G and T were 82.5% and 17.5% in healthy controls, and 65.8% and 34.2% in cases with microtia. A statistically significant difference between eNOS variant and microtia was determined (OR: 1.267, 95% CI: 1.004–1.598; p=0.009). The cases with eNOS TT genotype had increased risk of microtia.

eNOS (VNTR) variant: The distribution of AA, AB and BB genotypes for eNOS3 (VNTR) variant were observed in 72.5%, 25% and 2.5% of healthy controls and in 47.4 %, 36.8% and 15.8% of cases with microtia, respectively. While the allele frequency of A and B were 85%, 15% in healthy controls and 65.8%, 34.2% in cases with microtia. The allele frequencies of eNOS variant showed statistically significant difference between cases with microtia and healthy controls (OR: 2.947, 95% CI: 1.188–7.311; p=0.028). eNOS B allele was higher in cases with microtia.

MPO (G463A) variant: The distribution of GG, GA and AA genotypes for MPO (G463A) variant were 65%, 30% and 5% in healthy controls and 47.4%, 42.1% and 10.5% in cases with microtia, respectively. We were unable to determine statistically significant difference in any genotype or allele frequency of MPO when cases with microtia and healthy controls were compared.

Discussion

Nitric oxide modulates the growth of smooth muscle and regulates blood flow through smooth muscle cells, decreases endothelial permeability and influences leukocyte adhe-

Table 1. The genotype distribution and allele frequencies of the eNOS (G894T), (VNTR) and MPO (G463A) variants in cases with microtia and healthy controls.*

	Genotype/Allele	Patients n† (%)	Controls n§ (%)	OR (95% CI)	p
eNOS (G894T)	GG	10 (52.6)	26 (65)	0.598 (0.197–1.816)	0.403
	GT	5 (26.3)	14 (35)	0.633 (0.198–2.225)	0.565
	TT	4 (21.1)	0 (0)	1.267 (1.004–1.598)	**0.009**
	G allele	25 (65.8)	66 (82.5)		
	T allele	13 (34.2)	14 (17.5)	2.451 (1.013–5.935)	0.060
eNOS (VNTR)	AA	9 (47.4)	29 (72.5)	0.341 (0.110–1.064)	0.083
	AB	7 (36.8)	10 (25)	1.750 (0.540–5.668)	0.372
	BB	3 (15.8)	1 (2.5)	7.313 (0.707–75.669)	0.094
	A allele	25 (65.8)	68 (85)		
	B allele	13 (34.2)	12 (15)	2.947 (1.188–7.311)	**0.028**
MPO (G463A)	GG	9 (47.4)	26 (65)	0.485 (0.160–1.471)	0.260
	GA	8 (42.1)	12 (30)	1.697 (0.546–5.276)	0.390
	AA	2 (10.5)	2 (5)	2.235 (0.290–17.220)	0.588
	G allele	26 (68.4)	64 (80)		
	A allelle	12 (31.6)	16 (20)	1.846 (0.769–4.435)	0.174

*Fisher's exact test. †n=19, §n=40

sion to vascular endothelium.[14] The impairments in NO production promote thrombogenesis through platelet adhesion and aggregation, and production of cytokines and adhesion molecules.[15] During embryonic growth, the cell numbers were determined by the balance between cell proliferation, differentiation, migration and apoptosis. Nitric oxide acts on a variety of physiological and pathological pathways such as the regulation of the balance between apoptosis and mitosis, and have an inhibitory on cell proliferation.[16] In embryogenesis of Drosophila, it regulates the balance between cell proliferation and differentiation.[17] The deficiency of NO generation leads to endothelial dysfunction, which in turn facilitates the development of several disorders such as type II diabetes mellitus, insulin resistance, and cardiovascular events. NO is produced from L-arginine by 3 nitric oxide synthase isoenzymes and eNOS gene is one of them. eNOS is mainly produced by vascular endothelial cells and has a key role in the modulation of vascular tonus and angiogenesis. It is also expressed in several cell types, including bronciholar and renal epithelial cells, cardiomyocytes, and neutrophils.[18] The relations of eNOS with the actin cytoskeleton, microtubules, and intermediate filaments were studied with great interest.[18]

The changes in NO generation caused by cytoskeletal reorganization play a significant role in numerous physiological and pathophysiological conditions. The G894T variant located in exon 7 causes an amino acid substitution at position 298 (Glu298Asp) which may result in proteolytic cleavage of the eNOS protein and may diminish NO bioavailability, rather than altering generation of NO, in subjects with the GT and TT genotypes compared to those with GG genotype in a dose-dependent manner.[19] VNTR variant of eNOS gene is associated with plasma concentrations of NO.[20] In repeats of a 27-bp consensus sequence, two alleles, a common large allele and a smaller allele, were found. It is noteworthy that the larger allele, designated "b-insertion", has five tandem repeats, and the smaller allele "a-deletion" has four repeats.

Both endogenous processes and exogenous exposures are likely to generate reactive oxygen species (ROS). Reactive oxygen species may cause oxidative damage to DNA and other macromolecules, thereby leading to genetic alterations, a process modulated by several antioxidant systems which may change the balance between prooxidant cellular activity and antioxidant defense system.[21] Reactive oxygen species act as primary or secondary messengers in processes related to cellular growth or death. A variety of examples demonstrate the crucial role of ROS in development as redox status is one of the major regulator of the basic transcription factors that affect cell signaling pathways related to proliferation, differentiation, and apoptosis. Thus, oxidative stress may modify several reactions that have an impact on embryonic development both positively and/or negatively.[22] MPO produces ROS endogenously by behaving like an antimicrobial enzyme, catalyzing hydrogen peroxide-dependent oxidation of chloride to generate a strong oxidizing agent, HOCl. HOCl contributes to generation of secondary oxidation products by reacting with other biological molecules.[21] The variant of MPO G463A within the MPO-463 gene promoter has been studied extensively and an association between high activity of G463A G allele and increased MPO activity was reported for various diseases. The lower activity A allele, which is associated with lower levels of polycyclic aromatic hyrocarbons and ROS production, implicated lower risk for relevant diseases.[23]

In the current study, the distribution of the eNOS (G894T and VNTR), MPO (G463A) genotypes between the cases with microtia and healthy controls were evaluated. We found that eNOS (G894T) and (VNTR) variants were statistically different between these two groups. The cases with eNOS (+894) TT genotype had increased risk of microtia (p=0.009) (Table 1). Also, eNOS (VNTR) B allele was higher in the cases (p=0.028) (Table 1). However, there was no significant difference for MPO (G463A) variant according to genotype distribution and allele frequency between the cases with microtia and healthy controls.

Conclusions

To the best of our knowledge, this is the first study in which the relationship between eNOS, MPO gene variants and microtia was evaluated. Our data suggest that eNOS gene variants may play a role in the etiopathogenesis of microtia in Turkish population. Although etiopathology of microtia is still unclear, we thought that genetic variations might influence development of embryologic phase. Therefore, prospective longitudinal studies, mainly focusing on to reveal the contributions of genetic susceptibility and exogenous factors in microtia is required.

Conflict of Interest: No conflicts declared.

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Can neutrophil-lymphocyte ratio, platelet-lymphocyte ratio and mean platelet volume be used as inflammation markers in patient selection for tonsillotomy?

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Abstract

Objective: The aim of this study was to investigate whether mean platelet volume (MPV), neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) can be used as inflammation markers in selection of pediatric patients, who were planned to undergo tonsillotomy for sleep apnea, or not.

Methods: The tonsillotomy group consisted of pediatric patients who had undergone tonsillotomy for sleep apnea between 2013–2015 years. The control group consisted of children who had presented to the Well-Child Outpatient Clinics. The patient charts were reviewed retrospectively. MPV, NLR and PLR values were recorded and analyzed.

Results: In the tonsillotomy group, there were 23 patients whereas the control group consisted of 31 healthy children. The median age was 5 in the tonsillotomy group and 6 in the control group. MPV, NLR and PLR values did not have statistically significant differences between the tonsillectomy and control groups (p=0.838, p=0.314 and p=0.896, respectively).

Conclusion: MPV, NLR and PLR values are not inflammation markers that can be used in selection of patients to undergo tonsillotomy for sleep apnea.

Keywords: Tonsillotomy, tonsillar hypertrophy, sleep apnea, mean platelet volume.

Özet: Tonsillotomi hasta seçiminde nötrofil-lenfosit oranı, trombosit-lenfosit oranı ve MPV inflamasyon belirteci olarak kullanılabilir mi?

Amaç: Bu çalışmanın amacı uyku apnesi nedeni ile tonsillotomi yapılması planlanan pediatrik hastaların seçiminde ortalama trombosit hacmi (OTH), nötrofil-lenfosit oranı (NLO) ve trombosit-lenfosit oranının (TLO) inflamasyon belirteci olarak kullanılıp kullanılmayacağını araştırmaktır.

Yöntem: 2013–2015 yılları arasında uyku apnesi nedeni ile tonsillotomi yapılan pediatrik hastalar tonsillotomi grubunu oluşturdu. Sağlam çocuk polikliniğine başvuran pediatrik hastalar ise kontrol grubunu oluşturdu. Hastaların dosyaları retrospektif olarak incelendi. OTH, NLO ve TLO değerleri kaydedildi ve analiz edildi.

Bulgular: Tonsillotomi grubunda 23, kontrol grubunda 31 hasta mevcuttu. Tonsillotomi grubunda medyan yaş ortalaması 5, kontrol grubunda 6 idi. OTH, NLO VE TLO değeri tonsillotomi ve kontrol grubunda istatistiksel olarak anlamlı farklılık göstermiyordu (p=0.838, p=0.314 ve p=0.896).

Sonuç: OTH, NLO ve TLO değerleri, uyku apnesi nedeni ile tonsillotomi yapılması planlanan hastaların seçiminde kullanılabilecek inflamasyon belirteçleri değildir.

Anahtar sözcükler: Tonsillotomi, tonsil hipertrofisi, uyku apnesi, ortalama trombosit hacmi.

The most common indication for tonsillar surgery is tonsillar hypertrophy, which leads to sleep apnea, in the pediatric age group.[1] In recent years, tonsillotomy has frequently been used in the treatment of tonsillar hypertrophy, due to its advantages such as creating less surgical trauma, less bleeding and pain together with faster recovery.[2–4] Polysomnography is not routinely used for diagnosis in pediatric patients. An indication of tonsillectomy is

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based on findings in medical history and physical examination. There has been no available marker reported for decision-making on the indication for tonsillotomy.

Recently, mean platelet volume (MPV), the neutrophil-lymphocyte ratio (NLR) and the platelet-lymphocyte ratio (PLR), which are prominent inflammation markers, have been studied on patient groups having sleep apnea. Besides the reports showing a correlation of these values with the disorder, there have also been publications that revealed no relationship.[5-7]

We reviewed the pediatric patients who had undergone tonsillotomy with the indication of sleep apnea and investigated whether MPV, PLR, and NLR values can be used as inflammation markers.

Materials and Methods

The charts of 23 pediatric cases, who had been admitted to the Outpatient Clinics of Department of Otorhinolaryngology between May 2013 and June 2015 with symptoms of sleep apnea such as sleeping with the mouth open, snoring, nocturnal enuresis, attention deficit, witnessed apnea and problems in learning, in whom grade 4 tonsillar hypertrophy had been identified, and tonsillotomy had been performed by cold knife-bipolar cautery, were reviewed retrospectively. In tonsillotomy cases, polysomnography was not performed during decision-making for indication. These patients constituted the tonsillotomy group. For the NLR, PLR and MPV values of the tonsillotomy group, the complete blood counts (hemogram) used for anesthetic evaluation during the preoperative period were used. The MPV, NLR, and PLR values of all patients were measured by using the same hematology analyzer.

For the control group, non-obese children, who were admitted to the Well-Child Outpatient Clinic, with no cardiac or acute infectious disease, in whom medical history revealed no infection in the last 2 months, no antibiotic use within the last month, no snoring, absence of sleeping with the mouth open, witnessed apnea or nocturnal enuresis, no attention deficit, who did not have a chronic inflammatory disorder and in whom tonsillar size was grade 1–2 in physical examination, were randomly selected. The charts of the subjects in the control group were reviewed retrospectively. The complete blood counts during their admissions were used for NLR, PLR, and MPV values. The MPV, NLR, and PLR values of all subjects in the control group were measured by using the same hematology analyzer. NLR value was calculated by dividing neutrophil count to lymphocyte count. PLR value was obtained by dividing platelet count to lymphocyte count. The age, gender, MPV, NLR and PLR values were recorded, and the data were statistically analyzed.

Statistical analysis

Compliance of quantitative variables with normal distribution was analyzed with the Kolmogorov-Smirnov test. Since MPV variable was consistent with normal distribution, t-test for independent groups was used for comparisons between groups and descriptive statistics were shown as a mean ± standard deviation. Since the variables of age, NLR and PLR did not comply with the assumption of normal distribution; the analysis was performed by Mann-Whitney U test for intergroup comparisons, and the descriptive statistics were shown as median (25–75 percentiles). For comparison of groups regarding gender, the chi-square test was used. A value of $p<0.05$ was considered statistically significant.

Results

There were 23 patients in the tonsillectomy group and 31 subjects in the control group. The median age was 5 (range: 2–16) years in the tonsillotomy group, whereas 6 (range: 2–15) years in the control group. The female/male ratio was 11/12 in the tonsillectomy group and 16/15 in the control group. There were no statistically significant differences between the two groups regarding age and gender (Table 1). MPV value did not have any statistically significant difference between the tonsillotomy and control groups (p=0.838) (Fig. 1). NLR value did not have any statistically significant difference between the tonsillotomy and control groups (p=0.314) (Fig. 2). PLR value did not have any statistically significant difference between the tonsillotomy and control groups (p=0.896) (Fig. 3).

Discussion

Tonsillectomy and tonsillotomy are commonly used surgical methods in the treatment of tonsillar hypertrophy which

Tablo 1. Demographic characteristics, median MPV, NLR and PLR values in terms of groups, and p values.

	Tonsillotomy group (n=23)	Control group (n=31)	p value
Age (years)	5 (2–16)	6 (2–15)	
Gender (Female/Male)	11/12	16/15	
Mean platelet volume (MPV) (fL)	8.99±0.84	8.93±0.98	0.838
Neutrophil-lymphocyte ratio (NLR)	1.28	1.05	0.314
Platelet-lymphocyte ratio (PLR)	109.4	96.8	0.896

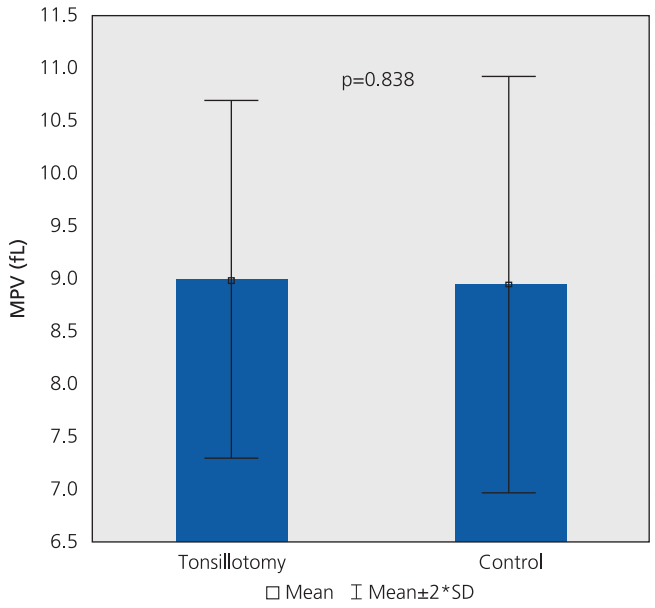

Fig. 1. Statistical difference of mean platelet volume (MPV) value between the groups.

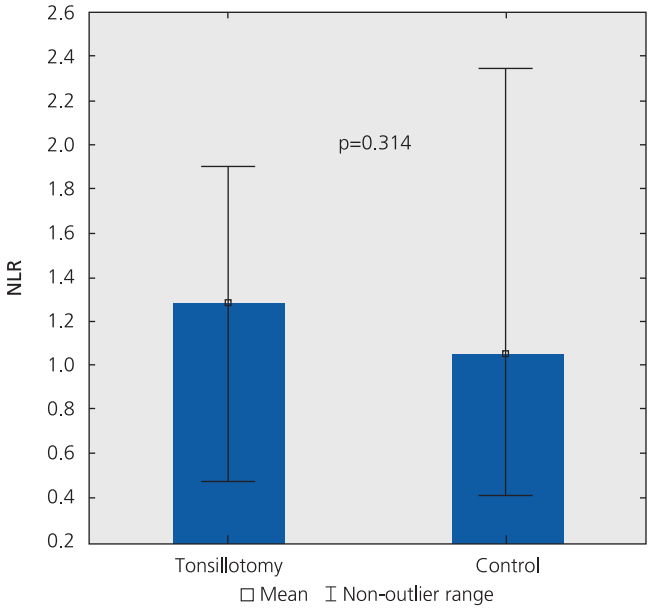

Fig. 2. Statistical difference of neutrophil-lymphocyte ratio (NLR) value between the groups.

causes obstructive sleep apnea in children. According to the report of American Academy of Otolaryngology-Head and Neck Surgery Foundation, tonsillectomy has the primary role in the treatment of sleep apnea related to tonsillar hypertrophy.[8] However, tonsillotomy has started to be used as an alternative method in the treatment of tonsillar hypertrophy in recent years, since it leads to less postoperative pain and bleeding.[9] The high efficacy of tonsillotomy in the treatment of pediatric sleep apnea has been proven by polysomnography.[10]

Windfuhr and Werner have defined two types of operations for reducing the tonsillar size in their study; tonsillotomy and partial intracapsular tonsillectomy.[9] Tonsillotomy can be performed by using a laser, coblator, cold knife, radiofrequency, microdebrider, monopolar needle cautery or bipolar cautery.[11–14] In our study, cold knife and bipolar cautery were used for tonsillotomy.

Regarding tonsillar hypertrophy, the mechanism causing obstructive sleep apnea has not been clearly identified in children.[15] Although the pathogenesis of sleep apnea is not clear yet, increase in the levels of inflammatory markers is well-known.[16] There are many inflammatory pathways and markers which play a role in the pathophysiology of sleep apnea in children.[17] CRP, interleukins, TNF-α, TNF-β, adiponectin, and leptin are some of these markers.[18–20]

The sleep-related respiratory disorder is known to be associated with low-level systemic inflammation, and it is characterized by recurrent collapse of the upper airway.[21] Recently, NLR, PLR and MPV values, which have come to the forefront as inflammation markers, have been studied in patient groups with sleep apnea and varying results have been obtained.[5–7]

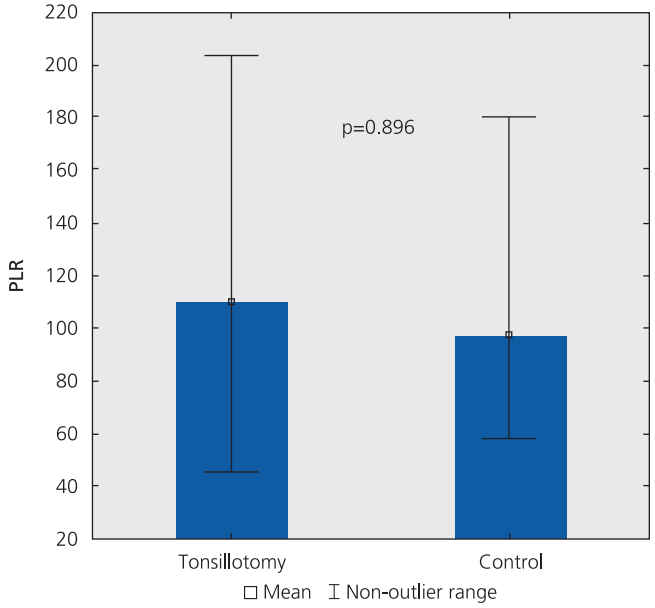

Fig. 3. Statistical difference of platelet-lymphocyte ratio (PLR) value between the groups.

Increased MPV has been shown to be associated with cardiovascular complications and sleep apnea.[7] Increased MPV has also been shown to be associated with upper airway obstruction which is accompanied by snoring.[22] Onder et al. have shown that no correlation was present between obstructive adenoid hypertrophy and MPV.[7] According to our study results, MPV does not have a diagnostic value as a marker in patient selection for tonsillotomy.

Neutrophil-lymphocyte ratio is a marker of systemic inflammation and increased NLR was shown to be correlated with unfavorable prognosis of the cardiac disease.[23,24] In patients with severe apnea, besides the presence of reports showing a relation between NLR and apnea-hypopnea index (AHI), there are also reports which have determined no relation between NLR and sleep apnea.[25,26] NLR has been reported to be usable as a marker to show chronic intermittent hypoxia.[27] According to our study results, NLR does not have a diagnostic value as a marker for patient selection for tonsillotomy.

Platelet lymphocyte ratio increases in peripheral arterial disorders, some malignancies and myocardial infarct with poor prognosis.[28–31] Koseoglu et al. have shown that PLR value was reduced in sleep apnea.[27] In another study, showing that PLR value was increased in patients with severe apnea, it has been suggested that PLR can be used as a marker for diagnosis of cardiovascular disorders in patients with obstructive sleep apnea syndrome (OSAS).[32] According to our study results, PLR does not have a diagnostic value as a marker for patient selection for tonsillotomy.

Numerous studies in which NLR, PLR, and MPV values have been investigated in patients with sleep apnea and having different results are present in the medical literature. In this study, we were not able to determine any relationship of pediatric sleep apnea with NLR, PLR, and MPV values. This result may be explained either by the relation of sleep-related respiratory disorder with low-level systemic inflammation or rarity of cardiovascular complications due to sleep apnea in this age group.

Conclusion

As a conclusion, we consider that NLR, PLR, and MPV are not markers which can be used in patient selection for tonsillotomy with the indication of sleep apnea and do not have any diagnostic value for this patient group.

Conflict of Interest: No conflicts declared.

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The effect of parotid gland examination and massage on serum amylase levels in patients with acute parotitis

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Abstract

Objective: Acute infection of the parotid gland is common in the clinical practice of ear-nose-throat medicine. The present study aims to demonstrate the effect of parotid gland massage on serum amylase levels.

Methods: The study included 30 patients with acute parotitis presenting to our clinic and 14 healthy volunteers. The correlation between the serum samples collected before and after parotid gland massage was compared.

Results: A significant difference was observed in the amylase levels before and after massage in the acute parotitis group. Amylase levels did not differ significantly between measurements before and after massage in healthy subjects.

Conclusion: Patients should be evaluated with consideration to the effect of parotid gland massage performed to determine suppuration of parotid gland on amylase levels.

Keywords: Amylase, parotid gland, acute parotitis, parotid massage.

Özet: Akut parotit hastalarında parotis muayenesi ve masajın serum amilaz düzeylerine etkisi

Amaç: Kulak-burun-boğaz tıbbı klinik uygulamasında akut parotis bezi enfeksiyonu sık görülmektedir. Bu çalışma parotiz bezi masajının serum amilaz düzeylerine etkisini göstermeyi amaçlamaktadır.

Yöntem: Çalışma kliniğimize başvurmuş 30 akut parotit hastasını ve 14 sağlıklı gönüllüyü içermekteydi. Parotis masajı öncesi ve sonrası toplanan serum örnekleri arasındaki korelasyon karşılaştırıldı.

Bulgular: Akut parotis grubunda masaj öncesi ve sonrası amilaz düzeyleri arasında anlamlı bir fark gözlenmiştir. Sağlıklı kişilerde ise masaj öncesi ve sonrası ölçümler birbirlerinden anlamlı derecede farklı değildi.

Sonuç: Parotis bezi süpürasyonunun amilaz düzeylerine etkisini belirlemek için uygulanmış parotis bezi masajının etkisi açısından hastaların değerlendirilmeleri gerekir.

Anahtar sözcükler: Amilaz, parotid bezi, akut parotit, parotis masajı.

Parotid gland is one of the major salivary glands, releasing 25–30% of saliva and 80% of amylase. In ear-nose-throat (ENT) practice, where parotid gland pathologies are suspected, parotid massage is performed in cases of parotid grand palpation, Stensen's duct inspection on the oral cavity and saliva secretion to observe suppuration. Examining sensitivity with palpation helps to understand if there are any masses within the parotid gland, if the mass is painful, if there is edema or saliva secretion from the Stensen's duct, and serous or purulent character of the secretion guides diagnosis.

Salivary amylase represents basically 10–20% of all proteins synthesized from the parotid gland and produced in salivary glands. Amylase inhibits both growth of some microbiological agents and their adhesion to intact tissue. Amylase is also used in diagnosis since it is a specific enzyme released by salivary glands.

Serum amylase levels may be elevated in conditions that affect salivary glands such as acute and chronic pancreatitis, perforated peptic ulcer, ectopic pregnancy rupture, pancreatic cyst, parotitis and mumps, but may also be found at increased levels in many other disorders including fluid bal-

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ance disorders, cerebrovascular disease, respiratory and cardiac disorders, hepatobiliary disorders, diabetes mellitus, peptic ulcer, renal-ovarian-gastrointestinal system malignancies, intra- and extra-abdominal surgeries, trauma, dyslipidemia, pheochromocytoma, multiple myeloma, organ transplantations, infections, ethnic hyperamylasemia, chronic pancreatic non-pathological hyperamylasemia and familial hyperamylasemia.[1] Several medicinal products can also lead to elevations in pancreatitis and amylase levels. Hyperamylasemia may develop in cases of reduced metabolic clearance of amylase such as macroamylasemia or renal failure without pancreatic and/or salivary gland involvement.[2–4]

Amylase levels secreted from salivary glands in response to neurotransmitter stimulation of salivary glands with either sympathetic or parasympathetic innervation indicate sympathetic activity.

In acute parotitis, serum amylase values usually guide diagnosis. It is common practice to look at amylase levels following a physical examination in patients who present with swelling of the parotid gland, pain and similar pathologies and for whom acute infection is suspected. Thus, amylase levels have to be measured after the parotid gland has been massaged. This may misguide the clinician if the massage alters amylase values in patients with or without parotid pathology. In our study, we intend to compare amylase levels before and after parotid massage to conclude whether this is the case or not.

Materials and Methods

Our study included patients who presented to our clinic with acute parotitis that had an onset within a few days and healthy volunteers with no complaints. The patients and volunteers were provided with information about the study. Their informed consents were obtained. For the study, the approval was received from Istanbul Medipol University's ethics board for clinical trials. Forty-four subjects were enrolled in the study. This included 30 patients diagnosed with acute parotitis at the ENT outpatient clinic of the Medical Faculty of Istanbul Medipol University. Fourteen individuals with no medical conditions involving the parotid gland at the same period were included as controls. For both groups, serum samples were collected before parotid massage and serum amylase and lipase levels were measured to be compared for pancreatic disorders. Parotid massage was performed afterwards and serum amylase and lipase levels were measured again. For serum amylase determinations in serum samples, Beckman amylase kit on Beckman Coulter analyzer was used and the results were reported after performing relevant analyses. For plasma lipase determinations from the same serum samples, Dade Behring lipase kit on XPand analyzer was used and similarly, the results were reported after performing the required analyses. Patients with history of salivary gland disorders, patients with existing acute or chronic pancreatitis, those taking medicinal product(s) that affect amylase levels (acetyl salicylic acid, thiazide, corticosteroids, asparaginase, azathioprine, cyproheptadine, narcotic analgesics, oral contraceptives, rifampin, sulfasalazine), those with chronic conditions, malignancies, those with prior radiotherapy and those with conditions that affect salivary secretion such as dehydration and malnutrition were excluded from the study.

Statistical analysis

Data were analyzed using "Statistical Package for Social Sciences" software (SPSS for Windows 15.0; SPSS Inc., Chicago, IL, USA). Values for continuous variables were given either as mean ± standard deviation or as median, based on the normality of distribution. Student's t-test was used in the comparison of normal and homogeneous distribution of the parametric values. Chi-square and Mann-Whitney U test were used to compare non-parametric values. Student's t-test and Wilcoxon test were used in the comparison of dependent variables for parametric data in dependent samples and for non-parametric data, respectively. Statistical significance was set at $p<0.05$.

Results

Of the patients, 22 (73.3%) were females and 8 (26.7%) were males and their mean age was 27.9±13.3 (range: 14–42) years. In the control group, 10 (71.4%) were females and 4 (28.6%) were males, with a mean age of 30.3±14.2 years. Patient and control groups did not differ significantly by age or gender ($p=0.651$ and $p=0.632$, respectively).

Amylase levels before massage were elevated in all patients with parotitis while lipase levels were normal. Patients' mean amylase level before massage was 409.8±163.1 (range: 101–643) U/L but mean amylase level after massage was 487.1±214.4 (range: 144–886) U/L. Amylase levels were increased in all patients. Mean increase was 77.3±87.5 (range: 13–289) U/L. The increase between the two measurements was significant ($p<0.001$). In the control group, mean amylase level before massage was 70.3±6.6 (range: 60–78) U/L while it was 66.0±6.4 (range: 57–73) U/L after massage. Amylase levels were increased in only 2 (14.3%) of the controls. Mean change was -4.3±7.2 (range: -14–8) U/L. The difference between

the two measurements was not statistically significant (p=0.128) (Table 1).

Patients' mean lipase level before massage was 29.8±8.3 (range: 19–45) U/L and mean amylase level was 28.0±7.0 (range: 19–42) U/L after massage. Lipase levels increased in 12 patients. Mean change was -1.3±4.3 (range: -12–3) U/L. The increase between the two measurements was not statistically significant (p=0.201). In the control group, mean lipase level before massage was 30.7±4.8 (range: 23–35) U/L and 31.0±6.8 (range: 23–43) U/L after massage. Lipase levels increased in only 4 (24.6%) of the controls. Mean change was 0.3±8.1 (range: -12–13) U/L. The difference between the two measurements was not statistically significant (p=0.917) (Table 1).

Table 1. Comparison of serum amylase and lipase levels of patients and healthy volunteers before and after massage.

		Patients (n=30) Mean±SD (U/L)	Controls (n=14) Mean±SD (U/L)
Mean serum amylase	Before massage	409.8±163.1	70.3±6.6
	After massage	487.1±214.4	66.0±6.4
p		**<0.001**	0.128
Mean serum lipase	Before massage	29.8±8.3	30.7±4.8
	After massage	28.0±7.0	31.0±6.8
p		0.201	0.917

SD: standard deviation

Discussion

Serum amylase levels are used in the diagnosis of salivary gland and pancreas disorders but it is released from many tissues including the ovaries, testes and striated muscle. Changes in serum amylase levels are observed in many conditions.[5]

The effect of parotid gland palpation on serum amylase levels is not well known according to the literature.

Ericson et al. found no difference in alpha-amylase levels when they compared children with recurring parotitis and normal children in their study.[6]

It is certain that rectal-digital examination and sonographic probe application affect on serum prostate-specific antigen.[7,8] Likewise, routine parotid gland massage may increase serum amylase levels. So we wanted to investigate the potential effect of parotid gland massage on serum amylase levels. During the half-life period of amylase, we can observe the alterations of serum amylase levels after examination, message or any manipulations. There are two groups in our study: the first group we investigated consists of patients with parotitis and the second group consists of healthy people. In the first group, serum amylase level was significantly higher when measured after massage compared to before massage. Serum lipase levels were not altered significantly in the first group. In the second group, the difference between serum amylase or lipase levels was not statistically significant before and after massage. This study demonstrated that routine parotid gland massage results in increased serum amylase levels in patients with acute parotitis. Based on our results, parotid gland massage may cause a transient increase in serum amylase levels. The false amylase value may lead to inaccurate diagnosis and treatment. Therefore, measuring serum amylase levels before any manipulation will lead to more accurate results.

Toros et al. studied the effect of routine palpation of the thyroid gland on thyroid gland and found statistically significantly higher values for total T3, free T3, free T4 and thyroglobulin in patients who received thyroid palpation compared to those who did not receive palpation. This study indicated that thyroid palpation may lead to a transient increase, though within normal ranges, in total circulating levels of T3, free T3, free T4 and thyroglobulin.[9]

A study by Lever et al. demonstrated that surgical palpation and fine needle aspiration but not external manual palpation increased serum thyroglobulin levels.[10] In a similar study, Luboshitzky et al. demonstrated increased serum levels of thyroglobulin in 4 of the 25 patients who were given thyroid palpation and fine needle aspiration.[11]

There are no studies on the effect of parotid gland massage on serum amylase levels. Based on the results of our study, parotid gland massage does not alter serum amylase levels in patients without parotid gland pathology but results in increased levels in patients with acute parotitis.

Conclusion

Parotid gland examination and massage do not alter serum amylase measurements in individuals without any parotid gland pathology. In patients with acute parotitis, however, parotid gland massage results in a significant increase in serum amylase levels. Evaluating serum amylase levels before any manipulation will lead to more accurate values and will avoid inaccurate diagnosis and treatment.

Conflict of Interest: No conflicts declared.

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How much are the incidental abnormalities on brain MRI clinically significant in otolaryngology practice?

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Abstract

Objective: We aimed to investigate the frequency of incidental diagnosis of paranasal sinus and mastoid abnormalities on brain magnetic resonance imaging (MRI) and its correlation with symptoms of patients.

Methods: We examined 100 patients who underwent brain MRI due to several different complaints other than sinusitis and mastoiditis. The patients who had any nasal or otologic pathology in otolaryngology examination were excluded from the study. Afterwards, a total of 65 patients were included into the study. The questionnaire consisted of otological symptoms and Sino-nasal Outcome Test (SNOT-20), Lund and Mackay scoring system for rhinosinusitis were filled by all patients immediately prior to imaging. The analysis of the MRI scan in terms of rhinosinusitis according to the Lund-Mackay radiological scoring and mastoiditis was performed by the same radiologist.

Results: The mean age of 65 patients was 46.62±17.73 years. Eighteen (27.7%) of these were men and 47 (72.3%) were women. In 26 (40%) of 65 patients, MRI demonstrated mastoiditis. We could not find any statistically significant correlation between mastoiditis and upper respiratory tract infection (p=0.896). There was no statistically significant relationship between radiological scores and total sinus symptom scores (p=0.93). Additionally, we could not find any correlation between radiological scores and SNOT-20 (p=0.923).

Conclusion: Our findings demonstrated that although some of these patients had various symptoms of sinus or mastoid diseases, these symptoms had no statistically significant correlation with the radiological diagnosis. In conclusion, radiologists should advise clinical correlation of their radiologic findings rather than reporting a clinical diagnosis such as sinusitis and mastoiditis.

Keywords: Incidental findings, mastoiditis, MRI, paranasal sinus, rhinosinusitis.

Özet: Beyin MR'ında saptanan insidental bulgular otolarengoloji pratiğinde klinik olarak ne kadar önemlidir?

Amaç: Serebral manyetik rezonans görüntülemede (MRG) saptanan insidental paranazal sinüs ve mastoid anomali tanılarının sıklığı ve bu tanıların hastaların semptomları ile korelasyonunu araştırmayı amaçladık.

Yöntem: Sinüzit ve mastoidit harici başka şikayetler nedeniyle beyin MRG çekilen toplam 100 hasta incelendi. Otolarengoloji muayenesinde herhangi bir nazal veya otolojik patoloji saptananlar çalışmadan çıkartıldıktan sonra çalışmamıza 65 hasta dahil edildi. Tüm hastalara görüntüleme öncesi otolojik semptomlarını ve rinosinüzit şikayetleri için Sinonazal Sonuç Testi (SNOT-20) ve Lund and Mackay skorlama sistemini içeren bir anket formu uygulandı. Mastoidit varlığı ve Lund-Mackay radyolojik skorlaması not edilerek yapılan MRG analizi aynı radyolog tarafından uygulandı.

Bulgular: Çalışmamızdaki 65 hastanın ortalama yaşı 46.62±17.73'tü. Bunların 18'i (27.7%) erkek ve 47'si (72.3%) kadındı. Altmış beş hastanın 26'sında (%40) MRG'de mastoidit saptandı. Üst hava yolu enfeksiyonu ile mastoidit arasında istatistiksel olarak anlamlı bir ilişki bulamadık (p=0.896). Radyolojik skorlar ile total sinüs semptom skoru arasında istatistiksel olarak anlamlı korelasyon yoktu (p=0.93). Ayrıca, radyolojik skorlar ile SNOT-20 arasında korelasyon bulamadık (p=0.923).

Sonuç: Bulgularımız göstermiştir ki; her ne kadar hastaların sinüs veya mastoid hastalıkları ile ilişkili şikayetleri olsa da bu şikayetler ile radyolojik tanılar arasında istatistiksel olarak anlamlı bir korelasyon bulunmamıştır. Sonuç olarak, radyologlar sinüzit ve mastoidit gibi klinik tanı rapor etmek yerine radyolojik bulgularının klinik korelasyonunu önermelidir.

Anahtar sözcükler: İnsidental bulgular, mastoidit, MRG, paranazal sinüs, rinosinüzit.

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There are various definitions for incidental finding. It is defined as "asymptomatic findings that are discovered unintentionally and has potential clinical significance because they may need treatment or cause symptoms unrelated to the purpose of study" in neuroimaging research.[1,2] The overall prevalence rates of incidental findings on brain magnetic resonance imaging (MRI) ranged from 1.7% to 10.2% in numerous studies after exclusion of incidental white matter lesions.[3–7] Additionally, radiologists usually describe mastoiditis and sinusitis as an incidental finding on brain MRI. Reneman et al.[8] found 5.6% of head and neck findings in 180 participants. Sandeman et al.[9] reported 76 varied ENT problems in 700 subjects, particularly 62 of these subjects had sinus problems and 6 had mastoid problems.

Clinical mastoiditis is defined as inflammation of mastoid air cells of temporal bone by otolaryngologists and is mostly caused by middle ear infection. Symptoms include redness and swelling behind the ear. On the other hand, if radiologists detect any increased signal in the mastoid region on MRI, they define this condition as mastoiditis. The frequency of incidental diagnosis of radiological mastoiditis has been reported in a few studies.[10–13] Clinical and radiological mastoidites are different entities as mentioned above and clinical significance of these incidental findings in otolaryngology practice remains controversial.

Another point that differs in clinical and radiological evaluation is the sinus abnormalities on MRI scans. Previous studies showed that the prevalence of abnormal sinus mucosal thickening on MRI ranges from 30% to 50%.[14–16] However, this definition varies between studies because there is no standardized scoring schema of MRI. Lim et al.[17] reported sinus abnormalities in 38% of 60 childrens. They noted 'more than double' scores in the symptomatic group according to the Lund and Mackay scoring system. Similarly, McNeill et al.[18] used the same scoring system in their study evaluating the relationship of symptoms and radiologic findings of sinus pathology in MRI scans.

Recently increased prevalence of incidental findings in the head and neck region which is documented most frequently as mastoiditis and sinus disease is observed due to neurology and other clinics' increased usage of MRI.[19,20] These asymptomatic patients are mostly referred to otolaryngology practice.

In this prospective study, we evaluated the frequency of incidental diagnosis of paranasal sinus and mastoid abnormalities on brain MRI and its correlation with symptoms of patients by using a questionnaire consisted of otological symptoms and additionally using Sino-nasal Outcome Test (SNOT-20), Lund and Mackay scoring system for rhinosinusitis in patients who underwent brain MRI for reasons other than sinusitis and mastoiditis. Additionally, we aimed to determine the differences in the rate of these incidental abnormalities between Turkish people and other populations.

Materials and Methods

Study population

A total of 100 consecutive patients who underwent brain MRI due to several different complaints other than sinusitis and mastoiditis were examined. All patients have performed an otolaryngological examination. The patients who had any nasal or otologic pathology in otolaryngology examination were excluded from the study. According to these criteria, a total of 65 patients were enrolled in this study. The study was approved by the Institutional Review Board and Ethics Committee of Başkent University and all patients signed the agreement to participate in the study.

Data collection

A questionnaire was filled by all patients immediately before imaging. Age, gender, detailed history of patients' consumption of tobacco, allergy, asthma, and upper respiratory tract infection (within the past two weeks) were recorded. Additionally, the questionnaire consisted of Lund-Mackay symptom scores,[21] SNOT-20, and questions about otologic symptoms. Patients were asked to record the average intensity of each symptom of rhinosinusitis by using a visual analog scale (VAS) method ranging from 0 (no symptom) to 10 (severe symptom) and scoring was done by Lund-Mackay staging system. Furthermore, we also used SNOT-20 for evaluation of the symptom scores of rhinosinusitis in this study.

All the analysis of the MRI scan regarding rhinosinusitis according to the Lund-Mackay radiological scoring and mastoiditis was performed by the same radiologist. The radiologist was blinded about the patients' otologic symptoms and SNOT-20 scores and Lund-Mackay symptom scores to avoid interobserver bias.

MR imaging parameters

Indications of MRI scans performed to the patients were various including mostly dizziness, headache, brain lesion, etc. All patients underwent MRI on a 1.5-T scanner (Siemens, Avanto, Erlangen, Germany). MR imaging of patients during the axial, coronal, and sagittal T2W sequences was

included in the evaluation. Coronal and sagittal plans specifically used to evaluate the sinuses of upper and lower walls. MRI readings of the sinuses were done blinded to all participant data by a head and neck radiologist. In the paranasal sinus wall, T2WI signal more than 1 mm was defined as mucosal thickening. The Lund-Mackay scoring system was used for evaluation. According to this, paranasal sinuses are divided into six sections; maxillary, sphenoid, frontal sinus, osteomeatal complex, anterior and posterior ethmoid cells. The severity of sinus mucosal inflammation was scored as 0 (less than 1 mm), 1 (partial) or 2 (complete). Ostiomeatal complex obstruction categorized as: 0 (not obstructed), 1 (mild) or 2 (obstructed). The mastoid cells were scored as either 1 (aerated mastoid cells) or 2 (presence of fluid in mastoid cells).

Statistical analysis

The data were presented as means ± standard deviation. The analysis was performed using SPSS v.20.0 for Mac (SPSS Inc., Chicago, IL, USA). For the statistical analyses of data, chi-square test, and Spearman's correlation coefficient test were performed. Differences with a p-value <0.05 were considered to be statistically significant.

Results

We examined 100 consecutive patients and 65 of these with a mean age of 46.62±17.73 years (between 16 and 80 years of age) were included in this study. Eighteen (27.7%) of these were men, and 47 (72.3%) were women. Six subjects (9.2%) had allergic rhinitis and asthma. Three (4.6%) subjects had symptoms of allergic rhinitis, and 12 (18.5%) subjects had a history of upper respiratory tract infection during last 15 days.

In 26 (40%) of 65 participants, MRI demonstrated mastoiditis. Four subjects had the sensation of fullness of ear, 1 had ear discharge, 6 had otalgia, 6 had hearing loss, 9 had tinnitus, and 19 had vertigo. Out of participants who had mastoiditis, five subjects had a history of upper respiratory tract infection and no statistically significant correlation was found between mastoiditis and upper respiratory tract infection (p=0.896). Furthermore out of participants who had mastoiditis, only one subject had allergic rhinitis. We could not find any significant relationship between the presence of allergic rhinitis and mastoiditis (p=0.221).

Thirty-five had Lund and Mackay score of 2 or more, and moreover, 10 of them had a score of 5 or more. There was not any mucosal abnormality on MRI of 21 subjects. Furthermore, we could not find any statistically significant correlation between Lund and Mackay scores and total sinus symptom scores (p=0.93) and there was no relationship between these scores and SNOT-20 (p=0.923).

Discussion

In recent years, asymptomatic patients with incidental findings in the head and neck region have been mostly referred to otolaryngology clinics or general practitioners due to neurology and other clinics' increased usage of MRI.[19,20] We generally observe the radiological diagnosis of mastoiditis and sinus disease in our otolaryngology practice. The reported prevalence of these incidental findings was 5.6% in a study of 180 participants.[8]

The middle ear and mastoid region diseases are one of the most common conditions in otolaryngology practice. Though they are a clinical diagnosis, imaging is usually performed if the otolaryngologist should observe the relationship of surrounding tissue to rule out other complications or if advanced treatment modalities such as surgery of ear are planned.[22] Mostly computed tomography of the temporal bone is used to confirm a clinical diagnosis of mastoiditis by the finding of the destruction of bony septa or secretions in mastoid air cells.[22,23] However, if radiologists detect any increased signal in the mastoid region on MRI, they define this situation as mastoiditis in clinical practice.

The prevalence of radiological mastoiditis varies among studies.[5,12,13] Orhan et al.[12] found radiological mastoiditis in only ten patients of a series of 2700 temporomandibular joint MRIs, Mirza et al.[13] found 5% incidence. However, concordant with these studies, a higher rate of subjects had radiological mastoiditis (40%) in our study. The reason for this difference might be the description variety of radiologic mastoiditis.

Polat et al. investigated 406 patients who have radiological mastoiditis on MRI and showed that 82% of patients did not have clinical mastoiditis and did not suggest MRI as an effective tool for mastoiditis.[10] In our study, none of 26 (40%) subjects of mastoiditis, who were screened on brain MRI, demonstrated any otoscopic or otomicroscopic abnormality in clinical examination.

There are numerous studies about the prevalence of incidental findings, whereas there have been a few studies about the clinical significance.[24,25] Von Kalle et al. evaluated the mucosal thickening in mastoid cells and paranasal sinuses in 147 pediatric patients' brain MRI. They concluded that 25% of patients had mucosal swelling in their mastoid cells, and 48% had in paranasal sinuses, but they found no correlation of these pathologies with a history of a headache, asth-

ma and allergies.[24] Our results were similar with that study as we also did not find any correlation between mastoiditis and allergic rhinitis. Additionally, statistically significant correlation between mastoiditis and upper respiratory tract infection was not obtained. However, in contrast with these findings, Lee et al. evaluated 100 adults and 30 children and detected statistically significant correlation between MRI findings and clinical examination of the middle ear and a mastoid cavity in adults.[25]

Similar to mastoid and middle ear diseases, CT is the significant imaging modality for evaluating paranasal sinuses, but otolaryngologists come across frequent consultations of patients with sinusitis due to brain MRI in clinical practice. The reported prevalence of incidental sinus problems on brain MRI varies between 31.7% to 55% in the literature.[15,17,26,27] Lund and Mackay scoring system are usually used as a rhinological staging system of paranasal sinus CT scan, but there is no standardized scoring schema of MRI. Lim et al.[17] used this scoring system in evaluation of the sinus abnormalities on MRI scans of children, and McNeill et al.[18] investigated the relationship of symptoms and sinus abnormalities in MRI scans of patients by using the same scoring system. Similarly, we have also used the Lund and Mackay scoring system in the present study. Lim et al.[17] evaluated the MRI of 60 children and observed sinus abnormalities in 38% of them. They noted 'more than double' scores in symptomatic group. In concordant with this study, McNeill et al.[18] did not obtain a correlation between radiological scores and symptom scores which were similar to our results. Furthermore, we also investigated the correlation between SNOT-20 and radiological score and we could not find any significance. Some studies suggest the association with upper respiratory tract infections and sinus abnormalities on MRI,[26,27] but we did not find out any association in our study.

In the present study, we found a higher rate of radiological mastoiditis concordant with other studies. Furthermore, we did not observe a correlation between radiological scores and total sinus symptom scores. The major limitation of our study was small sample size. Besides, we did not have a study group of patients whose radiological findings correlated with clinical diagnosis. Thus, further studies with larger sample size should be performed.

Conclusion

Although there have been some reports about the prevalence of radiological diagnosis of mastoiditis and sinusitis on brain MRI, clinical significance remains controversial. Our findings demonstrated that although some of these patients had various symptoms of sinus or mastoid diseases, these symptoms had no statistically significant correlation with the radiological diagnosis. On the other hand, the occurrence of symptoms might be related to other diseases. In conclusion, radiologists should point out radiologic findings on brain MRI and advise clinical correlation of them rather than reporting a clinical diagnosis such as sinusitis and mastoiditis.

Conflict of Interest: No conflicts declared.

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Evaluation of hearing in patients with psoriasis considering the disease severity

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Abstract

Objective: Nowadays, psoriasis is accepted to be an autoinflammatory/autoimmune disease. As a result of chronic inflammation, psoriasis is widely investigated for associated diseases and comorbidities. However, there are limited data about the effects of psoriasis on hearing functions. The aim of the study was to investigate prospectively if patients with psoriasis have sensorineural hearing loss and the effect of the disease severity on hearing levels.

Methods: Overall, 50 patients (100 ears) with psoriasis and 45 healthy controls (90 ears) were included in the study. After otoscopic examination, pure tone air and bone conduction including high frequencies (500, 1000, 2000, 4000, 8000, and 16,000 Hz) and speech audiometry were performed to all participants.

Results: Median pure tone average of the patients was significantly different than controls. Moreover, the frequency levels of patients with psoriasis for both of the ears were all significantly different from the control group. As the limitation of the study, patients were not investigated for psoriatic arthritis and sera from patients were also not investigated for anti-bodies for inner ear antigens such as anti-connexin 26, anti-DEP/CD148 and anti 68K.

Conclusion: The possibility of inner ear involvement should be kept in mind in psoriasis as a result of chronic systemic inflammation. Patients with psoriasis may be evaluated with audiometry periodically even if they do not exhibit any hearing problems.

Keywords: Psoriasis, hearing loss, disease severity.

Özet: Psoriasis hastalarında işitmenin hastalığın şiddetine göre değerlendirilmesi

Amaç: Günümüzde psoriasis otoinflamatuar/otoimmün bir hastalık olarak kabul görmektedir. Kronik inflamasyonun sonucu olarak psoriasis eşlik eden hastalıklar ve komorbiditeler için yaygın araştırılmaktadır. Ancak psoriasisin işitme fonksiyonları üzerine etkisi hakkında sınırlı veri bulunmaktadır. Çalışmanın amacı psoriasisli hastalarda sensörinöral işitme kaybı ve hastalık şiddetinin işitme düzeylerine etkisini prospektif olarak araştırmaktı.

Yöntem: Çalışmaya toplamda 50 psoriasisli hasta (100 kulak) ve 45 sağlıklı kontrol (90 kulak) dahil edildi. Katılımcılara otoskopik muayene sonrası yüksek frekansları da içeren hava ve kemik iletimi (500, 1000, 2000, 4000, 8000, 16.000 Hz) ve konuşma odyometrisi uygulandı.

Bulgular: Hastaların ortanca saf ses ortalaması kontrollerden anlamlı düzeyde farklıydı. Ayrıca psoriasisli hastaların her iki kulak için tüm frekans düzeyleri kontrol grubundakilerden anlamlı derecede farklıydı. Çalışmanın kısıtlılığı olarak; hastalar psoriatik artrit açısından incelenmedi ve hastaların serumlarında anti-konneksin 26, anti-DEP/CD148 ve anti 68K gibi içkulak antijenleri için antikorlar da araştırılmadı.

Sonuç: Kronik sistemik inflamasyonun bir sonucu olarak psoriasiste içkulak tutulumu olasılığı akılda tutulmalıdır. Psoriasisli hastalar işitme problemleri olmasa bile periyodik olarak odyometriyle değerlendirilebilirler.

Anahtar sözcükler: Psoriasis, işitme kaybı, hastalık şiddeti.

Psoriasis is a chronic inflammatory immune-mediated skin disease accompanied by various disorders.[1] Although the exact cause remains unknown, it is clear that both genetic and environmental factors play a role in these possible associations. The more common comorbidities include psoriatic arthritis, inflammatory bowel disease, cardiovascular diseases, hypertension, obesity, diabetes, and dyslipidemia which are thought to be the results of chronic inflammation in patients

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with psoriasis.[2] A better understanding for the pathophysiology of psoriasis resulted in a shift from a hyperkeratotic disorder to a cytokine mediated malfunction of the immune system. Psoriasis has been classified as a T-cell mediated disease rather than a skin disease.[3] The role of T cells in pathogenesis is the determinant of the extent of systemic involvement. Especially, T-helper (Th)-1, Th-17, and Th-22 cell subpopulations proliferate and trigger the secretion of inflammatory cytokines.[4] There are also several studies investigating the association between psoriasis and autoimmune disorders. This systemic inflammation is involved in the pathogenesis of various autoimmune disorders like multiple sclerosis and systemic lupus erythematosus.[5]

Sensorineural hearing loss can occur during autoimmune diseases and was first described as autoimmune sensorineural ear disease (ASED).[6] Since then it has been reported in many inflammatory disorders such as rheumatoid arthritis,[7] ankylosing spondylitis,[8] Behçet's disease.[9] Since the underlying mechanism in psoriasis is a systemic inflammation effecting multiple organ systems, we decided to investigate if patients with psoriasis have sensorineural hearing loss, and then to compare audiometric results according to age, sex, disease severity, disease duration, localization of the disease, and treatment modalities.

Materials and Methods

Study design

Overall, 50 patients with psoriasis who were diagnosed at the dermatology department of a tertiary care center and 45 healthy controls were included in the study. The diagnosis of psoriasis in all patients was done by the help of the clinical findings. The disease severity was calculated by Psoriasis Area Severity Index (PASI). Informed consent was taken from all of the participants. A detailed history including disease duration, medical history, and distribution of the lesions as well as possible etiological factors leading to hearing loss (including chronic otitis media, trauma, and drug use) were taken. In addition to dermatological examination, otoscopic examination of all the patients was performed by a single clinician at Hacettepe University Hospital Department of Otorhinolaryngology. Patients with tympanic membrane perforation were excluded. As a control group, 45 healthy people without any systemic disorders or any cochleovestibular disease associated with hearing loss were selected.

Audiological evaluation

All patients underwent pure tone audiogram at frequencies of 500–16,000 Hz, and pure tone averages (PTA) were noted (average of 500, 1000, 2000, 4000 Hz). Also speech audiometry was performed to all participants in a sound-proof chamber (MAICO 41, MAICO Diagnostics, Berlin, Germany). The hearing level ≤20 dB was accepted as normal.

Statistical analysis

The statistical analyses were performed using SPSS 16.0 program (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered as significant. Chi-square test was used to compare the gender of the patients and controls and Mann-Whitney U test was used to compare the age of the patients. Spearman's rho was calculated for the correlations between pure tone audiometric results and PASI scores, duration of the disease. For overall comparisons of audiological data of two groups, Mann-Whitney U test was used.

Results

Demographic data (Table 1)

In the study group, there were 50 patients with psoriasis (31 female, 19 male) with a median age of 34 (range: 18–74) years. The control group was composed of 45 healthy subjects (22 female, 23 male) with a median age of 29 (range: 20–56) years. There was no significant difference between patients and control group according to age and sex.

Table 1. Demographic data of the patients and controls.

No. of the patients / controls	50 (31 F, 19 M) / 45(22 F, 23 M)
Median age of the patients / controls	34 (min 18, max 74) years / 29 (min 20, max 56) years
Median duration of the disease	114 (min 6, max 7200) months
Previous treatment modalities	Topical treatments 98% (n=48) Oral cyclosporin 24% (n=12) Oral/subcutaneous methotrexate 24% (n=12) Phototherapy 12% (n=6) Acitretin 12% (n=6) Biologics 2% (n=1)
Median value of PASI score	17 (min 1, max 47)
Localizations of the lesions	Scalp 78% (n=38) Earlobe 70% (n=34) Face 36% (n=17) Upper extremities 72% (n=35) Palmoplantar 16% (n=8) Lower extremities 88% (n=43) Trunk 74% (n=36) Back 66% (n=32) Gluteus 73% (n=36) Nail 29% (n=14)

F: female; M: male

The median duration of the disease in patients with psoriasis was 114 (range: 6–720) months. According to medical history of patients with psoriasis, 98% (n=48) of the patients were treated with topical treatment modalities, 24% (n=12) with oral cyclosporin, 24% (n=12) with oral and/or subcutaneous methotrexate, 12% (n=6) with phototherapy, 12% with (n=6) acitretin, and with 2% (n=1) biological treatment modalities, respectively. Scalp involvement was observed in 78% (n=38) of the patients, earlobe involvement in 70% (n=34), face involvement in 36% (n=17), upper extremities in 72% (n=35), palmoplantar in 16% (n=8), lower extremities in 88% (n=43), trunk in 74% (n=36), back in 66% (n=32), gluteus in 73% (n=36), and nail in 29% (n=14) of the patients. Median value of the PASI score of the patients was 17 (range: 1–47).

Audiometric data

All of the patients with psoriasis and the control group had normal hearing levels (<20 dB) according to PTA. The median PTA of the right ear was 10.6 (range: 0–78) dB while the left ear was 10 (range: 1.25–106) dB in patients with psoriasis. The median PTA of the right ear was 5 (range: 0–28) dB while the left ear was 5 (range: 0–125) dB in the control group. Overall, median PTA of the patients with psoriasis was 10 (range: 1–92) dB and 6 (range: 0–26) dB in the control group. There was significant difference between patients with psoriasis and controls according to median PTA variables (p=0.00).

When the effect of disease severity on PTA variables was taken into consideration, we observed no correlation between PTA of the patients and PASI scores (Spearman's rho=0.629). The patients were also divided into 2 subgroups according to PASI scores. Group 1: patients who had PASI scores ≥10 (n=39, 78%), Group 2: patients who had PASI scores <10 (n=11, 22%). There were no significant differences according to PTA scores between above two groups (p=0.122). Median PTA variables of the patients with psoriasis and controls are shown in Table 2.

The differences between PTA variables of the patients with psoriasis were also investigated according to distribution of the lesions. There were no significant differences between median PTA variables according to distribution of the lesions such as face, ear, scalp, trunk, back, upper extremities, lower extremities, gluteus, nail and palmoplantar region. Median PTA scores of the patients with psoriasis were also investigated according to the previous systemic medications such as oral cyclosporine, systemic methotrexate, oral acitretine, and phototherapy. No significant differences were observed according to previous medications of the patients with psoriasis between PTA variables. There was also no correlation between disease duration and median PTA variables of the patients with psoriasis (Spearsman's rho=0.852).

Table 2. Median PTA variables of the patients with psoriasis and controls.

	Median PTA (dB)	p value
Patients Controls	10 (min 0, max 93) 6 (min 0, max 26)	0.00
Group 1 Group 2	9.3 (min 4, max 16) 11.2 (min 0, max 95)	1.22

PTA: pure tone average, **Group 1:** patients who had PASI scores ≥10, **Group 2:** patients who had PASI scores <10

When the hearing levels of the patients with psoriasis according to specific hearing frequencies (500, 1000, 2000, 4000, 8000, and 16,000 Hz) were taken into consideration, there was a significant difference between patients with psoriasis and control group, as shown in Table 3. However, the statistical analysis revealed significance in all frequencies, the median of hearing level at frequences 500–4000 Hz, were not lower than 20 decibels. But at the hearing level of high frequencies such as 8000 and 16,000 Hz, patients with psoriasis had hearing levels lower than 20 decibels, which can be accepted as hearing loss at high frequencies.

Discussion

In a review of the English and Turkish literatures, we found only two original articles, investigating the effects of psoriasis on ear functions. In a study by Karabulut et al.,[10] 42 patients (mean age 36.1 years; range: 13–71 years) with psoriasis and 60 controls were investigated for hearing and cochlear function. Hearing examination included complete

Table 3. Median frequency values of the patients and controls.

Median frequencies (Hz)	Patients		Controls		p value
	Right ear (dB)	Left ear (dB)	Right ear (dB)	Left ear (dB)	
500	10	10	5	5	0.00
1000	10	10	5	5	0.00
2000	10	10	5	5	0.03
4000	10	10	5	5	0.02
8000	20	5	17	10	0.00
16,000	37	40	10	10	0.00

audiological evaluation as well as distortion product otoacoustic emission. The mean PASI score of the patients was 5.7±3.2. Unlike our study, there was no statistically significant difference between patients and controls in audiometric values as well as distortion product otoacoustic emission values.[8] As the PASI score of the patients in the study above is smaller than our study, we think that they have less systemic inflammation, and less immune impairment causing no effects on hearing functions. Moreover, the audiometric data of the patients were not investigated according to demographic findings, disease severity, treatment modalities apart from our study. In the Turkish literature, there is one more study investigating the hearing functions in psoriasis. In this study by Guvenc et al.,[11] 51 patients with psoriasis and 51 controls were examined for hearing loss with pure tone audiometry. Mean PASI score of the patients were 9.6±6.4 which is also less than our PASI scores. Similar to the study by Karabulut et al.,[10] there was no significant difference between patients and controls according to median PTA values. However, the bone and hearing thresholds were statistically different at all frequencies except 1000 Hz for right ear and 500 and 1000 Hz for left ear in patients than controls. The seek for a correlation between PASI scores and hearing frequencies yielded a significant link between PASI scores and hearing loss at medium and high frequencies. In contrast to the study stated above, we could not find any correlation between mean frequency (4000, 8000, 16,000 Hz) variables of the patients and PASI scores, disease duration, previous systemic treatment modalities, and distribution of the lesions. However, similar to the study by Guvenc et al.[11] the hearing levels of the patients with psoriasis according to specific hearing frequencies (500, 1000, 2000, 4000, 8000, and 16,000 Hz) were significantly different than controls.

Moreover, there are two case reports about sensorineural hearing loss associated with psoriatic arthritis in the English literature. Giani et al.[12] described a 12-year-old girl who had sensorineurol hearing loss during etanercept therapy. She was successfully treated with oral prednisolon without any recurrence in spite of etanercept administrations. Srikumar et al.[13] also described a 62-year-old man with psoriatic arthritis presented with sudden-onset hearing loss on medication with methotrexate. The patient recovered on oral corticosteroids. The authors above stated that patient with psoriatic arthritis may be prone to sensorineural hearing loss. Although a clear relationship is still uncertain, immune impairment in arthritis may increase susceptibility to an associated autoimmune disease.[10,11]

Since the original description of ASED by McCabe et al.,[6] there had been several studies trying to explain the exact mechanism of the hearing loss. The disease was defined as a progressive hearing loss that deteriorates in the course of weeks and months and it may improve with immunosuppressive therapy. The incidence of ASED is not well-established as it has no definitive diagnostic test. Similar with other autoimmune disorders, it occurs more frequently in women. The diagnosis of ASED is based primarily on clinical evaluation and audiological test. Serological tests which can detect specific antibodies were described and heat shock protein 70 (HSP-70) is particularly noteworthy. Unfortunately, HSP-70 is expressed in many inner ear diseases as an indicator of early cell damage, and it is not specific for ASED.[14]

The pathogenesis of immune-mediated hearing loss in autoimmune diseases other than psoriasis is still vague. The autoimmune theory is the most popular idea and the term autoimmune inner disease encompasses the cases of cochleovestibular dysfunction related to various systemic immune-mediated diseases. In this entity, the inner ear may not be a target of an immune attack but is vulnerable to indirect injury by deposition of immune complexes or other mechanisms.[15] In recent years, psoriasis was classified not as only a skin disease but also as a T-cell mediated systemic disorder. T cells lines such as T-helper (Th)-1, Th-17, Th-22 populations may be expanded and trigger the release of inflammatory cyctokines including tumor necrosis factor-α, and interleukins.[4,16] In this perspective, the systemic inflammation associated with psoriasis may have a role in the development of immune-mediated hearing loss.

One of the limitations of our study is that the patients were not investigated by a rheumatologist for arthritis. However, they had been asked if they had any symptoms of active arthritis during dermatology visits. Sera from patients were also not investigated for antibodies for inner ear antigens such as anti-connexin26, anti-DEP/CD148 and anti 68 kD. Patients who had lesions in external ear meatus were not noted. However, audiological tests were evaluated after local treatments for patients who had severe psoriatic lesions in external meatus.

In conclusion, psoriasis is defined as a chronic, immune-modulated inflammatory disease that affects many systems. The range and nature of the conditions that are associated with psoriasis suggest a possible link between the underlying systemic inflammatory pathways that drive psoriasis and ASED. Consequently, the possibility of inner ear involvement should be kept in mind and psoriasis patients

with high PASI scores should be monitored with audiometry regularly even if they do not admit with hearing problems directly.

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Is mean platelet volume a predictive marker for sudden sensorineural hearing loss?

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Abstract

Objective: There are many factors considered to be related with the etiology of sudden sensorineural hearing loss (SSNHL) such as viral infections, microvascular diseases and inflammation. Increased mean platelet volume (MPV) levels are assumed to represent more active enzymatic process and thrombotic predisposition. Our aim in this study is to show whether MPV could be a predictive value in SSNHL or not.

Methods: The medical records of a total of 93 patients with SSNHL and 93 healthy controls were reviewed retrospectively in this study. Peripheral blood MPV values of both groups were compared statistically.

Results: Mean platelet volume levels did not show a statistically significant difference between these groups.

Conclusion: As a result of our study, we consider MPV is not a predictive parameter in idiopathic SSNHL.

Keywords: Mean platelet volume, sudden deafness, thrombosis.

Özet: Ortalama trombosit hacmi ani işitme kaybı için prediktif bir değer midir?

Amaç: İdiyopatik ani sensörinörinal işitme kaybının (AİK) etiyolojisinde viral enfeksiyonlar, mikrovasküler hasar ve inflamasyon başta olmak üzere birçok etken suçlanmıştır. Artmış ortalama trombosit hacmi (MPV) değerleri daha aktif enzimatik süreç ve trombosit predispozisyonu ile ilişkilidir. Çalışmanın amacı MPV ile AİK arasındaki ilişkiyi araştırmaktır.

Yöntem: Çalışma retrospektif olarak hasta kayıtlarının taranması ile yapıldı. AİK tanısı konulan 93 hasta ve 93 sağlıklı kontrol grubu çalışmaya dahil edildi. Grupların periferik kan MPV değerleri istatistiksel olarak karşılaştırıldı.

Bulgular: Her iki grup arasında MPV değerleri açısından istatistiksel olarak anlamlı farklılık bulunmadı.

Sonuç: Çalışmamızın sonuçlarına göre MPV, idiyopatik AİK için prediktif bir parametre değildir.

Anahtar sözcükler: Ani işitme kaybı, MPV, tromboz.

Sudden sensorineural hearing loss (SSNHL) is the loss of hearing in three or more contiguous frequencies at least 30 dB or more, over three days or less. SSNHL makes up 1% of all sensorineural hearing loss cases with an incidence reported between 5–20/100,000.[1] While certain etiology is still unknown, viral infections, autoimmune diseases and vascular pathologies are assumed to be involved.[1,2] Vascular pathologies are believed to cause SSNHL via hypoxia or free radical damage like pathways.[3] It has been recently reported that hypercoagulopathy and increased fibrinogen levels may be associated with SSNHL.[4]

Platelets are the small components of peripheral blood cells, which function in hemostasis and vascular integrity. These cells secrete chemical mediators and play parts in inflammation, coagulation, thrombosis and atherosclerosis-like processes.[5] Platelets differ in size, volume and hemostatic activities. Mean platelet volume (MPV) is an objective marker that can easily be measured in peripheral blood. This ratio also represents platelet activities with increased MPV levels reflecting enzymatic and metabolically active platelets that are more effective in thrombus formation.[6]

The aim of the present study was to figure out whether MPV is a predictive value for SSNHL or not.

Materials and Methods

This study was held retrospectively via patient records. A total of 93 patients diagnosed with SSNHL in our clinic

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between January 2008 and May 2016 and 93 healthy controls who applied for routine health check and had audiometric evaluation, similar to the study group in terms of age and gender, were enrolled in this study. The study got the approval from the local ethic committee of the hospital. The patients who had 30 dB or more loss in 3 contiguous frequencies in pure-tone audiometry within 3 days had the diagnosis of SSNHL. Exclusion criteria were having active inflammation, suspicion of an autoimmune inner ear disease, a discovered etiology for SSNHL, history of otologic surgery or head and neck trauma, previously diagnosed cardiovascular disease, chronic disease like diabetes mellitus, hypertension, hyperlipidemia, obesity, antiaggregant or anticoagulant drug use, chronic alcohol consumption and smoking. MPV levels were recorded via the retrospective analysis of full blood counts. Cell-Dyn Ruby® multi-parameter cell counter device was used for measurements (Abbott Laboratories, Abbott Park, IL, USA).

In descriptive statistics of the data; mean, standard deviation, median minimum, maximum, frequency and ratio values were used. Distribution of variables was assessed by Kolmogorov-Smirnov test. Mann-Whitney U test was used in the analysis of quantitative data. SPSS 22.0 (PASW for Windows®; SPSS Inc., Chicago, IL, USA) program was used in the analyses.

Results

A total of 186 individuals (93 patients and 93 healthy controls) were enrolled in this study. Female to male ratio was 52/41 in the study group and 47/46 in the control group. Mean age was 32.3±7.9 years in the study group and 31.4±8.1 in the control group. Mean MPV in the study group was 8.2±2.2 (range: 6.9–16) fL and it was 8.7±1.3 fL in the control group (Fig. 1). There was no statistically significant difference between these groups (p=0.465).

Discussion

Sudden sensorineural hearing loss is a disease without a known etiopathology and generally thought to occur as a result of idiopathic and multifactorial subjects. SSNHL was tried to be explained by various hypotheses. The most highlighted causes are viral infections, inflammation and hypoxia. As cochlear artery is a terminal branch of anterior inferior cerebellar artery (AICA), any pathology in this location may damage cochlea and cause hearing loss.[6]

Platelets are heterogeneous cell groups in peripheral blood and big platelets with more granules are associated with more secretion of mediators, more glycoprotein receptor expression, and they are associated with a faster aggregation as a result.[7] MPV is an objective marker that can easily be measured in peripheral blood and its correlation with several diseases is reported in literature. Increased MPV has been associated with vascular endothelial damage and some cardiovascular diseases.[8–10] Also inflammatory bowel diseases like ulcerative colitis and Crohn's disease is reported as associated with high MPV values.[11] Today, its clinical significance has not been accepted yet.

In our study, we tried to evaluate the association between MPV levels and SSNHL in which vascular pathologies are considered to involve in the etiopathogenesis. We did not find a significant difference in MPV levels, between healthy controls and SSNHL patients group. Ulu et al.[12] reported that MPV levels were higher in SSNHL patients, compared to controls, with 40 patients, and stated that due to low number of patients, it should be supported by other studies. Similarly, Sagit et al.,[3] in a study of 31 patients, they found a positive correlation between SSNHL and MPV and they claimed that this might support the vascular pathology hypothesis in SSNHL etiology. By contrast with them, as supporting our results, Blaha et al. could not find a correlation between SSNHL and MPV.[13] Additionally, Karli et al. also stated that MPV is not a predictive factor in SSNHL in their study with 46 patients.[6]

If the correlation of MPV and cardiovascular diseases is assumed to be true, theoretically it could be reasonable to expect higher levels of MPV in SSNHL, as hypoxia is thought to lie behind the pathophysiology and the benefits of

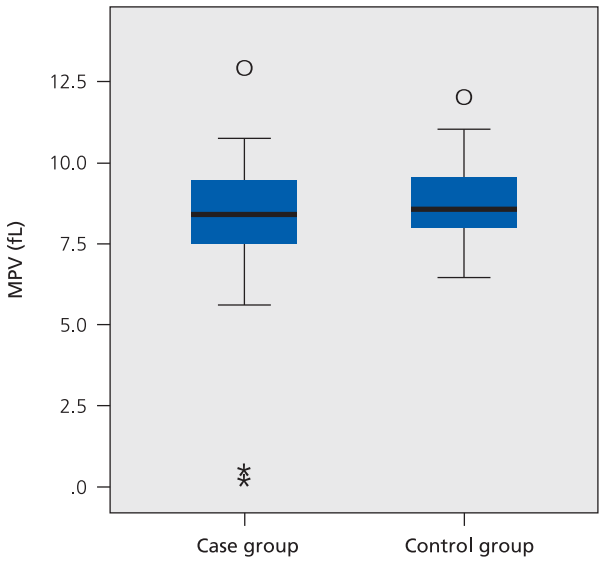

Fig. 1. Mean platelet volume (MPV) values of two groups.

hyperbaric oxygen treatment are known for it.[14] However, we could not find such a correlation in our study.

The inconsistency of the results in literature may be a result of differences in blood samples and laboratory evaluation techniques or a nonstandard measuring time. It is reported that up to 40% of difference in results is due to measuring time and different materials.[15]

The reason that we could not find any correlation between MPV and SSNHL might be our exclusion of patients having diseases like cardiovascular risk factors, smoking, hyperlipidemia, hypertension, chronic diseases which are shown to be correlated with MPV in literature. Our aim of exclusion was to show the possible correlation with idiopathic SSNHL and MPV but, in daily practice, co-morbidities are frequently observed in patients with SSNHL.[16–18] Whether we showed idiopathic SSNHL was unrelated with MPV or not, we still consider that increased MPV can be indirectly associated with SSNHL due its close correlation with the diseases that are usually coming along with SSNHL.

Conclusion

Sudden sensorineural hearing loss is still an undiscovered clinic entity. There are conflicting results on the correlation of MPV and SSNHL. Further prospective studies on larger patient populations via standardized MPV measuring techniques are needed to highlight this relation.

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Case Report

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Rare coexistence of sialolithiasis and actinomycosis in the submandibular gland

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Abstract

Sialolithiasis is a condition characterized by the obstruction of salivary gland or its excretory duct by a calculus or sialolith. This condition provokes swelling, pain, and infection of affected gland leading to salivary ectasia and even causing the subsequent dilatation of the salivary gland. The aim of this case report is to present a rare condition of sialolithiasis of the submandibular gland with actinomycosis. In this report, we presented a 35-year-old male patient having coexistence of submandibular sialolithiasis and actinomycosis with a literature review. Patient underwent excision of the right submandibular gland due to siaololithiasis. Pathologic examination revealed chronic sialadenitis, sialolithiasis, actinomyces which all necessitate the excision of right submandibular gland with stones with 1.5 cm in diameter. It should be keep in mind that sialolithiasis may be a predisposing factor for submandibular actinomycosis and removal of the sialolith or the entire gland is of particular importance.

Keywords: Submandibular, sialolithiasis, actinomycosis.

Özet: Submandibüler bezde siyalolityaz ve aktinomikozun seyrek görülen birlikteliği

Siyalolityaz, tükürük bezi veya boşaltım kanalının bir taş veya siyalolit ile tıkanması ile karakterizedir. Bu durum etkilenmiş bezin şişmesi, ağrıması ve enfeksiyonunu teşvik ederek tükürük bezi ektazisine, hatta daha sonra tükürük bezinin dilatasyonuna neden olmaktadır. Bu olgu raporunun amacı submandibüler bezde siyalolitle birlikte aktinomikozun görüldüğü nadir bir siyalolityaz olgusunu sunmaktır. Bu raporda submandibüler siyalolit ve aktinomikozu olan 35 yaşında erkek hasta literatür taraması eşliğinde raporlanmıştır. Siyalolityaz nedeniyle sağ submandibüler bez eksize edilmiştir. Patolojik incelemede gözlemlenen kronik siyaladenit, siyalolityaz ve aktinomiçes nedeniyle çapı 1.5 cm taşlarla dolu sağ submandibüler bezin eksizyonunun gerektiği görülmüştür. Siyalolityazın submandibüler aktinomikozun predispozan faktörü olabildiği, siyalolit veya bezin tümüyle çıkartılmasının son derece önemi olduğu akıldan çıkartılmamalıdır.

Anahtar sözcükler: Submandibüler, siyalolityaz, aktinomikoz.

Actinomycosis is a chronic suppurative infection. This infection is characterized by formation of multiple abscesses, draining sinuses, and granulation tissue. The three major clinical presentations of actinomycosis include the cervicofacial region, pulmothoracic region, and abdominopelvic regions.[1] In cervicofacial actinomycosis, the submandibular area, parotid gland and buccal space are affected. Primary involvement of the submandibular gland is not frequent. Actinomycosis with sialolithiasis of the submandibular gland is a rare situation.[1] Actinomyces are found as commensal organisms in the human oral cavity, respiratory and digestive tracts. The bacteria become invasive when a mucosal lesion gains access to the subcutaneous tissue.[2]

Sialolithiasis usually appears between the age of 30 and 60 years.[3] It most commonly involves the submandibular gland (80% to 95%) and less frequently the parotid (5% to 20%). The sublingual gland and the minor salivary glands rarely (1% to 2%) have sialolithiasis.[4] The stones may reach from 0.1 to 30 mm.[5] While 85% of submandibular gland stones are located in Wharton's duct, the remaining 15% are in gland parenchyma.[6] However, giant sialoliths (>15 mm) in the submandibular duct have rarely been reported.[7]

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Different aetiological hypotheses have been put about salivary gland calculi. These are mechanical, inflammatory, chemical, neurogenic, infectious, strange bodies, etc. Submandibular gland is more susceptible to the development of the stone compared to parotid gland. Wharton's duct is longer and wider than the Stensen's duct. Submandibular salivary flow is against gravity; and salivary pH is more alkaline; and mucin proteins, calcium and phosphates are contained in greater amount than serous secretion of parotid saliva.[8]

In this report, we presented a 35-year-old male patient having coexistence of submandibular sialolithiasis and actinomycosis with a literature survey.

Case Report

Thirty-five-year-old male patient admitted to Şehit Kamil Government Hospital complaining of swelling in the right submandibular region. He had a history of episodes of right submandibular swelling occurring during meals and gradually decreased by resting. Physical examination showed increased sensation and pain on the right submandibular gland. By intraoral examination, saliva flow from the right Wharton's duct was not observed. The ear, nose and throat examination findings were normal. Submandibular neck ultrasonography was reported as "The right submandibular gland diameter increased; ducts dilated in gland; and right sialoadenitis was present". Neck

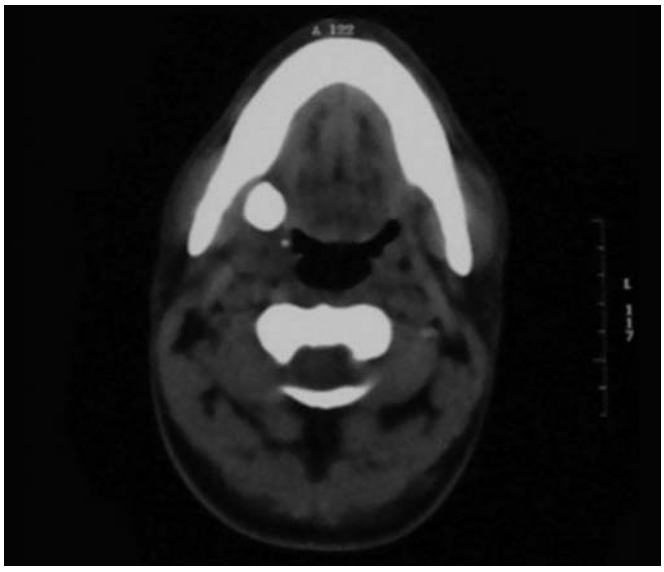

Fig. 1. Computed tomography scan of the right submandibular gland showing calculus.

Computerized Tomography examination showed bone density structure which was 1.5 cm of diameter in the right submandibular gland parenchyma (Fig. 1). Magnetic resonance imaging (MRI) of the neck also demonstrated 1.5 cm structure in the parenchyma (Figs. 2 and 3).

Patient underwent excision of the right submandibular gland. Cephalosporin antibiotic was administered and the

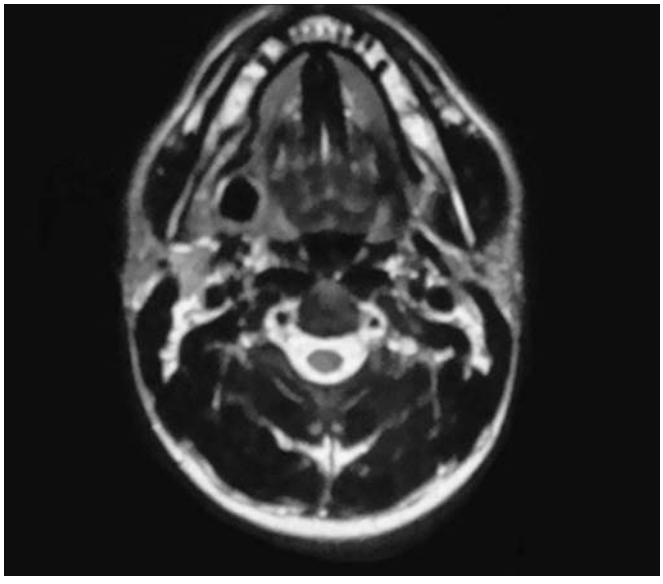

Fig. 2. Axial MRI of the right submandibular gland.

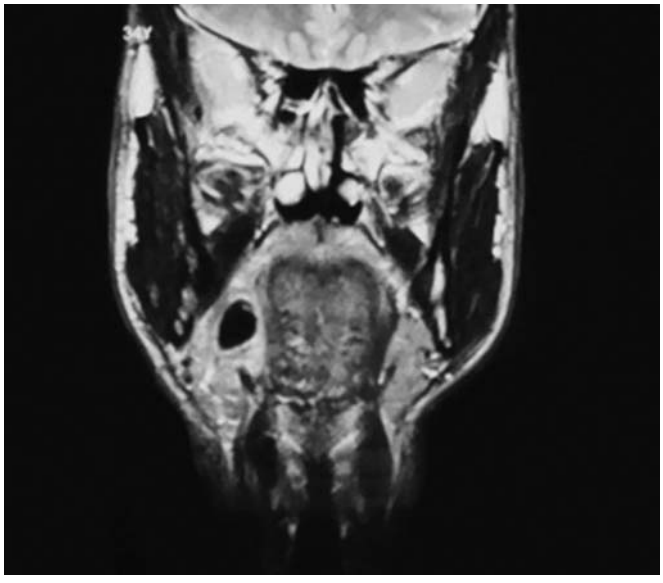

Fig. 3. Coronal MRI of the right submandibular gland.

patient was discharged without problems. On the third day of the patient's discharge, he admitted to the hospital with complaints of pain, swelling and discharge at the operation region. He was hospitalized, and pathology result of the operation was reported simultaneously as "Chronic sialadenitis, sialolithiasis and actinomyces necessitate the right submandibular gland excision with stones with 1.5 cm in diameter". Postoperative infection was thought to be due to infection with Actinomyces. The patient was administered amoxicillin and clavulanic acid 1 g i.v (twice a day) for 10 days; and later, 1 g p.o (twice a day) for 21 days. The patient's symptoms improved without the need for another surgical intervention. The patient gave informed consent for using his data for scientific purposes.

Discussion

Actinomycosis is an *Actinomyces israeli* infection. This commensal organism exists at high amounts in tonsil tissue and carious teeth. Poor oral hygiene, diabetes, immune suppression, malnutrition and local tissue damage are predisposing factors. These conditions may lead to infection and subsequent invasion of subcutaneous tissues.[8] Actinomycosis infection usually spreads into nearby soft tissues without regard for tissue planes or lymphatic drainage.[2]

Cervicofacial actinomycosis is frequently the result of oro-maxillofacial trauma, dental manipulation or dental caries. Cervicofacial actinomycosis mostly presents as a firm, painless, slow-growing swelling and a multiloculated abscess which frequently progresses to multiple discharging sinuses.[3] The infection, most commonly, presents as a chronic, fluctuant mass, commonly positioned at the border of the mandible, becoming progressively larger within weeks or months. The colonies may be visible to the naked eye as 'sulphur granules', which are pathognomonic. When this is identified, the appropriate therapy should be initiated.[9]

Diagnosis of actinomycosis infection is difficult, as it is hard to isolate *A. israeli.* When actinomycosis is suspected, fine needle aspiration, smears of freshly obtained pus, standard culture swabs, and special stains for fungi are required.[3] Furthermore, imaging techniques of CT and MRI usually provide non-specific findings.[4] In this case, the patient's diagnosis was established by the presence of clusters in pathology specimen, but actinomycosis did not grow in culture. Additionally, preoperative CT and MR images did not comply with actinomyces.

Disease is more rarely seen in the submandibular region. Actinomycosis and salivary gland stones rarely occur. It should be keep in mind that sialolithiasis may be a predisposing factor for submandibular actinomycosis, and removal of the sialolith or the entire gland is of particular importance. In our case, we thought that actinomyces infection was present with the submandibular stone, and to be induced by surgery.

Actinomycosis in the head and neck has a better prognosis than other sites since this region is more responsible to surgery and antibiotic therapy. Surgery plays an important role in diagnosis and treatment. High-dose intravenous antibiotics for 2–4 weeks are a fundamental part of treatment and 3–6 months of treatment with oral antibiotics is recommended. Tetracycline and erythromycin are used in patients with penicillin allergy.[10] In our case, infection has been treated with proper therapy after the diagnosis without invasive surgery.

The instrumental diagnosis of sialolithiasis is based on several imaging techniques. Ultrasonography represents an excellent first-level diagnostic technique because it reveals ductal and highly mineralized stones with a diameter of at least 1.5 mm with an accuracy of 99%.[11] In this case, USG examination did not give definitive diagnosis but CT established a definitive diagnosis.

The ultimate aim of giant sialolith treatment is to restore a normal salivary flow. When the stone can be palpated intraorally, the best option is to remove it through an intraoral approach. The choice of a surgical approach to access the sialolith with submandibular gland preservation requires careful imaging evaluation, minimal invasive removal and transoral sialolithotomy. After surgical calculi removal, the patients show asymptomatic and normally functioning glands in a short time.[12] In our case, we removed the huge stone in parenchyma with salivary gland.

Our case showed that giant stone in submandibular gland may be coexistent with actinomycosis. The appropriate use of antibiotics should be recommended to treat these infections.

Conflict of Interest: No conflicts declared.

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