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Correlation between Serum Interleukin-17 level and Serum Reactive Oxygen Species levels among Children experiencing Otitis Media with Effusion

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Abstract

Introduction The detection of inflammatory mediators in the serum of children with have otitis media with effusion (OME) and their correspondence with clinical considerations may enable the use of a modern nonsurgical curative treatment for OME.

Objective To determine the relation between interleukin-17 (IL-17) serum level and reactive oxygen species (ROS) serum levels in children suffering from OME and to disclose if any variation occurs in the level of IL-17 Will affect the ROS and antioxidant equilibrium in the serum, which indicates the entire body's reaction to OME.

Methods The present study was a case-control study. A total of 24 children experienced OME, and 24 healthy controls were recruited.

All participants in the study were subjected to a systematic clinical investigation including otoscopic, audiometric, and tympanometric examination. Also, venous blood samples were collected from all children to determine the levels of IL-17 and ROS.

Results The mean $\pm$ standard deviation (SD) age ranges of the patients and the control group were 6.8 ± 2.7 and 6.2 ± 3.4 years, respectively. A stylistically significant difference in the levels of serum nitric oxide (NO), catalase (CT), myeloperoxidase (MPO), and malondialdehyde (MDA) ($p < 0.05$) was detected between OME and control patients. No significant difference was found in serum levels of superoxide dismutase (SOD) and glutathione peroxidase (GPX) between OME and control patients. The serum levels of MDA, NO, and MPO positively correlated with the serum levels of IL-17 in OME patients.

Conclusion In the present study, there is a reasonable role of the IL-17 pathway in OME pathogenesis through an increase in ROS levels.

Keywords
- otitis media
- reactive oxygen species
- interleukins

Introduction

Otitis media with effusion (OME) is considered to be one of the most common childhood illnesses. It is defined as the occurrence of middle ear fluid, with the absence of symptoms and signs of acute ear inflammation, such as acute pain and fever. The formation of this fluid is due to unchangeable pathological alterations to the middle ear mucous membrane.[1] Even though

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the diagnosis of OME is clear, its pathogenesis is not fully acknowledged, and it needs to be elucidated.[2]

Numerous etiological causes can be considered in the pathogenesis of OME; these involve general anatomy, infections, and environmental and physiological causes.[3] In addition, there can also be host-related causes, such as immune responses and allergic predispositions. The most frequently encountered one is Eustachian tube dysfunction caused by nasopharyngeal mucous membrane inflammation. Such inflammation might be the result of bacterial or viral infection, or allergy, which can activate leukocytes to produce proinflammatory cytokines within the middle ear causing chronic inflammation.[4,5]

To realize the pathophysiological aspect of OME, it is important to know the body's defense mechanisms in counteracting pathogens as being innate immunity that involves the production of various cytokines. The latter have been reported as participating in OME's immunologic responses.[6] T-helper (TH-17) cells release a proinflammatory cytokine, which is interleukin-17 (IL- 17); IL- 17has a vital role in the defense against autoimmune diseases and infections. In addition, it controls other chemokines and cytokines, causing mobilization of neutrophils to the inflammatory site.[7]

The study conducted by Moghaddam et al. revealed significantly higher levels of IL-17 in serous fluid than in the serum of patients with OME. In addition, they revealed a higher level of IL-17 in the serum of OME patients than in that of their healthy controls.[8] The study of Wang et al. showed that IL-17 induced middle ear effusion by stimulating the release of myeloperoxidase (MPO) oxidative enzyme, which has a significant function in chronic inflammation.[9]

Reactive oxygen species (ROS) are essential pathological mediators of numerous diseases. Reactive oxygen species, which are created during metabolic and chemical reactions, may contain ingredients that induce toxicity in numerous tissues, such as hydroxyl free radicals, hydrogen peroxide, lipid peroxides, hydroxyl peroxide, and other derivatives. The etiology of ROS oxidative injury is by the destruction of proteins, nucleic acids, cell membranes, and lipids in tissues.[10] The antioxidant defense complex is the initial route of the defense counter to oxygen-derived free radicals. The oxidation produced by free radicals is inhibited by antioxidants through stabilizing and capturing them. As a result, the organisms have initiated systems of enzymatic antioxidants for the protection from those toxic products; these include malondialdehyde (MDA), glutathione peroxidase (GPx), myeloperoxidase (MPO), catalase (CT), superoxide dismutase (SOD), and nitric oxide (NO).[11] Murat et al. established that an elevation in oxygen free radicals' levels and a deficiency of antioxidant defense complex might participate in the formation of OME.[12] Furthermore, Garça et al. advocated that oxidative stress may be a factor involved in the pathogenesis of chronic otitis media (COM).[13]

The present study aims to estimate the level of IL-17 as well as ROS levels in the serum of patients with middle ear effusions and healthy control and also to disclose if any variation occurs in the level of IL-17 Will affect the ROS and antioxidant equilibrium in the serum, which indicates the entire body's reaction to OME.

Materials and Methods

Subjects

A case-control study was conducted in the otorhinolaryngology department of a teaching hospital. The present study assessed 24 cases complaining of OME aged 3 to 11 years, and 24 age and sex-matched healthy controls. The case group involved 15 girls and 9 boys, whereas the healthy control involved 17 girls and 7 boys. Children who were distrusted of having acute otitis media; frequent adenotonsillitis; craniofacial anomaly, such as cleft palate; systemic diseases; and acquired or congenital immunodeficiency diseases were excluded from the study.

The diagnosis was established by inclusive history taking and a systematic clinical examination involving otoscopic, audiometric, and tympanometric examination.

Inclusive History Taking

Children were diagnosed as OME when they showed no improvement throughout the 3 months' interval of follow-up and after obtaining medical treatment for 2 to 3 weeks.

Otoscopic Examination

The presence of OME is established when there is a defined air-fluid level or presence of obviously visible bubbles in the middle ear cavity. Also, the presence of a hardly retracted tympanic membrane with visible foreshortening of the malleus's handle and restriction of the mobility of the tympanic membrane. Infrequently, the tympanic membrane might be thickened or dull and have an amber shade.[14]

Tympanometric Examination

Impedence audiometry result shows type-B tympanogram.

Audiometric Examination

An audiometric examination was performed in children who showed progression in conductive hearing loss as diagnosed by elevated pure tone threshold with more than 25 dB.

Samples

Serum was collected from 24 patients with OME and 24 control patients. Venous blood samples were collected from all children after an overnight fasting period. Within 1 hour of collection of blood samples, the blood was centrifuged (Eppendorf centrifuge 5810R, Germany) at 3,000 rpm for 10 minutes, and the serum was stored at − 70°C until time of analysis. The concentration of IL-17 was measured using a quantitative immunoassay technique by commercially available enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, Inc., San Diego, CA, USA) according to the manufacturer's protocols. Samples were analyzed in duplicates, and mean cytokine levels were reported in pg/ml in each group.[15]

The serum was analyzed to measure MPO, GPx, CT, NO, MDA, and SOD activity. The reagents used in this study were obtained from Sigma-Aldrich GmbH, Seelze-Germany, and Merck KGaA Darmstadt-Germany.

Myeloperoxidase activity was determined using a modification of the O-dianisidine method. Specific activity was

given in IU/L. The Beutler's method was used for GPx activity measurement. Catalase activity was assayed by measuring the degradation rate of H2O2 using Beutler's method. Catalase activity in the serum was expressed in U/ml. Serum NO levels were measured with the use of Griess reagent, NO results were reported in µM/L. The concentration of serum lipid peroxidation (total MDA) was determined using the Ohkawa method with slight modifications. Malondialdehyde results were expressed in nanomoles per milliliter (nmol/ml). Superoxide dismutase activity was determined as described by Fridovich.[16]

Statistical Analysis

The data obtained were analyzed using the SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA). The normality of the data was determined using the Shapiro-Wilk test. All the variables showed a normal distribution. Normally distributed variables were compared using a one-way analysis of variance (ANOVA) test, followed by Tukey's posthoc test for paired comparison. Independent *t*-test for numerical data were used. The Pearson correlation was used to establish a correlation between different variables. values < 0.05 were accepted as statistically significant.

Results

The mean ± SD age ranges of the patient and control groups were 6.8 ± 2.7 and 6.2 ± 3.4 years, respectively. There was no statistically significant difference between the ages of the patients and the controls.

The average test results of serum level of oxidant/antioxidant enzymes are presented in ►**Table 1**. There was a statistically significant increase in the levels of serum NO, CT, MPO, and MDA ($p < 0.05$), and there was a statistically significant difference between OME patients and the control group ($P < 0.05$). No significant difference was found in SOD and GPX between OME patients and controls ($P > 0.05$).

There was also a statistically significant increase in levels of IL-17 in OME patients than in the controls ($P < 0.05$), as shown in ►**Table 2**.

The serum levels of MDA, NO, and MPO positively correlated with the serum levels of IL-17 in OME patients, as shown in ►**Table 3**.

Table 1 Comparison of the parameters of the otitis media with effusion and control groups (mean ± standard deviation) and *p*-value of statistically significant difference between the two groups

	OME ($N = 24$)	Control ($N = 24$)	*P*-value
MDA	5.5 ± 2.2	5 ± 1.8	0.04*
GPX	9.7 ± 7.6	8 ± 5.8	0.31
NO	0.8 ± 0.05	0.6 ± 0.2	0.02*
CT	9.1 ± 3.5	6.9 ± 3.4	0.04*
MPO	50.1 ± 17.6	34 ± 16	0.002*
SOD	184 ± 76	137 ± 89	0.44

Abbreviations: CT, catalase; GPS, glutathione peroxidase; MDA, malondialdehyde; MPO, myeloperoxidase; NO, nitric oxide; OME, otitis media with effusion; SOD, superoxide dismutase.
*$p < 0.05$: significant. $P \geq 0.05$: nonsignificant.

Table 2 Mean of interleukin-17 in otitis media with effusion patients and their controls

	IL-17 (pg/ml) OME	IL-17 (pg/ml) control
Mean	9.166667	5.5
Variance	13.36232	4.608696
Observations	24	24

Abbreviations: IL-17, interleukin 17; OME, otitis media with effusion.
*Df 37, t Stat 4.237315, P (T ≤ t) one-tail 7.21E-05, t critical one-tail 1.687094, P (T ≤ t) two-tail 0.000144.
t critical two-tail 2.026192.

Table 3 Correlation between IL-17 and (MDA, NO, and MPO) in the serum of patients with otitis media with effusion

		Correlation
IL-17	MDA	R = 0.4
	NO	R = 0.5
	MPO	R = 0.6

Abbreviations: IL-17, interleukin-17; MDA, malondialdehyde; MPO, myeloperoxidase; NO, nitric oxide.

Discussion

Otitis media with effusion is a frequently occurring, multifactorial, and silent disease. Even though its clinical significance is well-known, the exact pathogenesis of its progression is obscure.[17] As far as we know, there is no literature studying the role of IL-17 in the pathogenesis of OME through disturbance of anti-oxidative/oxidative stress biomarkers in patients with OME.

The present study revealed a significantly higher level of serum IL-17 in OME patients than in their healthy controls (►**Table 2**). The result of this study corresponded to the study by Moghaddam et al. (2017), in which they detected a significantly higher serous fluid and serum IL-17 level in patients with OME.[8] It is important to note that the IL-17 pathway has a significant role in internal immunology as opposed to pathogens, and chronic inflammatory conditions. Previous studies have established the involvement of the IL-17 pathway in the pathogenesis of OME.[9]

While IL-17 exerts its local proinflammatory effects, it is systemically produced and adjusts many types of cells, including fibroblasts, macrophages, endothelial cells, and epithelial cells to mobilize the immunological cells to the inflammatory site.[18]

The present study suggests that IL-17 exacerbates OME by stimulating ROS (MPO, MDA, NO, and CT), through finding a significantly higher level of ROS in patients with OME than in their healthy controls. The study also found a positive correlation between the levels of IL-17 and the levels of MPO, NO, and MDA in the OME patients' serum (►**Table 3**).

The result of the current study is compatible with the proposition that IL-17 promotes neutrophils to raise the release of ROS, which exacerbates OME.[19]

The present study concluded that there is a noticeably high MPO level in OME patients. It has the highest values among other oxidative stress markers. This study did not detect an obvious effect of GPX and SOD on OME (►**Table 1**). The MPO value was significantly higher in OME patients. This result was compatible with the result of Sagiroglu et al. (2019) as it stated that the oxidative enzyme MPO contributes to the chronic inflammatory process.[20] The present study noticed that the MPO serum level was affected by oxidative stress levels. Consequently, increased MPO and increased oxidative stress activity may have a significant role in the pathogenesis of OME.[13] Additionally, the study of Kim et al. (2015) displayed that suppression of IL-17 by using the anti-IL-17 antibody significantly minimized MPO activity and diminished the signs of endotoxin-produced inflammation.[21] The present study showed a positive correlation between IL-17 and MPO in the serum of patients with OME (►**Fig. 1**).

The construction of the MPO in leukocytes can promote an inflammatory reaction that leads to lipid peroxidation. This can activate middle ear oxidative stress, destroying the structure of the cilia by damaging cellular proteins and DNA, which can subsequently cause the tissue damage to deteriorate in the middle ear, including the Eustachian tube. This process may contribute to Eustachian tube dysfunction, the element most attributed to the pathophysiology of COM. Also, obstruction of the Eustachian tube leading to increased middle ear pressure can cause venous stasis, exudation, and local acidosis. Throughout reperfusion, the resettled leukocytes release the MPO-mediated free radicals to eliminate necrotic cells. Free oxygen radicals can cause inflammatory chronicity in the middle ear by phospholipid peroxidation in the mucosal linings' cell membranes with resulting injury to lipoproteins, lipids, DNA, and proteins.[22]

In the present study, there was a statistically significant higher serum level of NO in OME patients than in their healthy controls. As another study[23] verified, COM patients displayed a high serum level of NO, which has prooxidative and antioxidative properties. Also, in the present study, the serum levels of MDA were established to be high in OME and were confirmed to be statistically significant. Another author detected higher MDA levels in OME patients than in their healthy counterparts.[24] Additionally, MDA and lipid peroxidase were found to be high in the study conducted by Yarikta et al.[12] Malondialdehyde, formed from peroxidation of membrane lipids, is an indicator of oxygen free radicals.[24] It can modify enzyme activity and disturb ion transport by affecting the cell membrane permeability. Also, MDA can induce a mutagenic effect by interacting with DNA's nitrogen bases.[25] Similar to this study, Doner et al.[26] recognized that mucosal and serum levels of MDA were elevated in the experimental study of otitis media. In contrast to the present study, Sagiroglu et al. found that MDA level was not significantly increased in patients with OME.[20]

The high CT level in this study was statistically significant. This result is similar to those of Sagiroglu's et al..[20] However, the study by Khakimov et al. revealed low CT serum levels in patients with otitis media.[27] The present study did not disclose an obvious influence of GPx and SOD on OME. Although patients with OME had high GPx and SOD levels, no statistically significant difference in GPx and SOD was observed between OME patients and the control group. As suspected, GPx and SOD may be in a passive status in the OME process.[20] This result aligns with that of the study by Sagirogl et al..[20] In contrast, another study found that the SOD level was significantly elevated in patients with otitis media.[28,29] The studies of Yariktas et al.[17] and Khakimov et al.[27] showed decreasing SOD levels in OME patients.

The limitations of the present study were: First, there is a middle ear complex interaction between the adaptive and innate immunity. Unfortunately, these interactions and

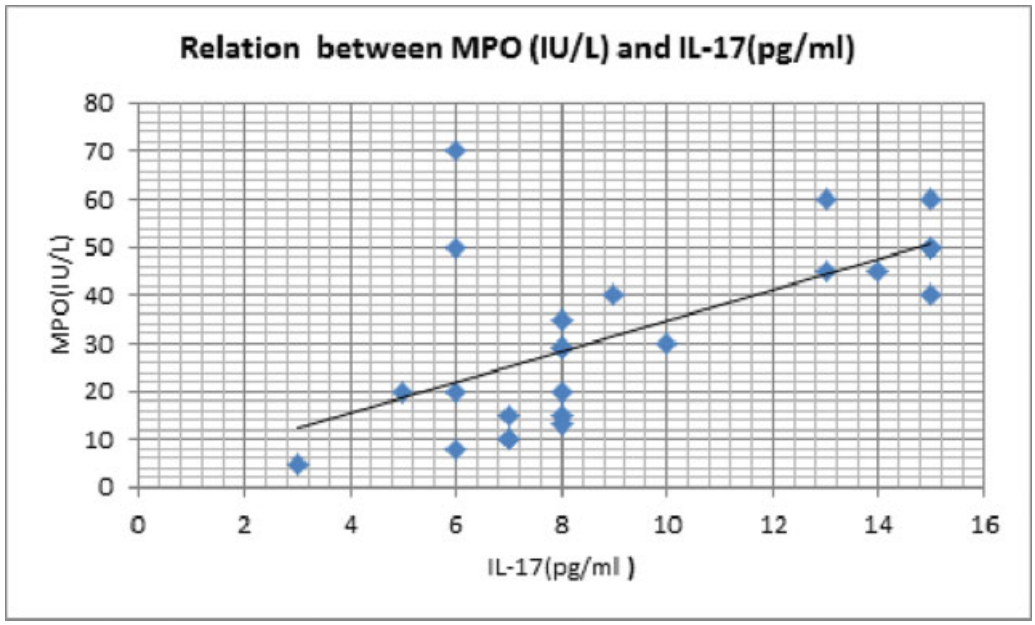

Fig. 1 Correlation between interleukin-17 and myeloperoxidase in the serum of patients with otitis media with effusion.

responses could not be monitored or assessed during the early stages of the disease. Second, immune responses within the middle ear cavity could not be assessed due to the presence of a control group to compare it with OME patients. Further, this study aimed to reveal the entire body's reaction during OME, and not just the local reaction.

Conclusion

The IL-17 pathway plays a significant role in the pathogenesis of OME through increasing ROS (MPO, MDA, NO, and CT), which is crucial in diagnosing and treating patients with OME. Considering this importance, a modern nonsurgical curative treatment of OME may be established. Additionally, the administration of antioxidant vitamins, such as vitamin E, A, and C, together with traditional OME medications is expected to have beneficial effects in the treatment of OME. Hence, appraising perpetual treatment for OME and diminishing the use of destructive treatments (as tympanostomy) is prescribed for refractory OME.

Ethical Considerations

In the present study, all the testing procedures were performed using non-invasive techniques and adhering to the conditions of the ethical approval committee of the institute. Agreement with written knowledgeable consent was gained from the children's parents or caretakers for using children samples, and the aim of the study was also clarified to them as well as to the control group.

Conflict of Interests

The authors have no conflict of interests to report.

References

1 Lieberthal AS, Carroll AE, Chonmaitree T, et al. The diagnosis and management of acute otitis media. Pediatrics 2013;131(03): e964–e999
2 Zielnik-Jurkiewicz B, Stankiewicz-Szymczak W. Pro-inflammatory interleukins in middle ear effusions from atopic and non-atopic children with chronic otitis media with effusion. Eur Arch Otorhinolaryngol 2016;273(06):1369–1378
3 Qureishi A, Lee Y, Belfield K, Birchall JP, Daniel M. Update on otitis media - prevention and treatment. Infect Drug Resist 2014;7:15–24
4 Bernstein JM, Doyle WJ. Role of IgE-mediated hypersensitivity in otitis media with effusion: pathophysiologic considerations. Ann Otol Rhinol Laryngol Suppl 1994;163:15–19
5 Bluestone CD, Hebda PA, Alper CM, et al. Recent advances in otitis media. 2. Eustachian tube, middle ear, and mastoid anatomy; physiology, pathophysiology, and pathogenesis. Ann Otol Rhinol Laryngol Suppl 2005;194:16–30
6 Jin W, Dong C. IL-17 cytokines in immunity and inflammation. Emerg Microbes Infect 2013;2(09):e60
7 Isailovic N, Daigo K, Mantovani A, Selmi C. Interleukin-17 and innate immunity in infections and chronic inflammation. J Autoimmun 2015;60:1–11
8 Yeghaneh Moghaddam A, Talaei R, Nikoueinejad H, Akbari H. Studying the Serum as Well as Serous Level of IL-17 and IL-23 in Patients with Serous Otitis Media. Iran J Allergy Asthma Immunol 2017;16(06):520–524
9 Wang W, Liu W, Liu J, et al. Interleukin-17A aggravates middle ear injury induced by Streptococcus pneumoniae through the p38 mitogen-activated protein kinase signaling pathway. Infect Immun 2017;85(10):e00438-17
10 Yilmaz T, Koçan EG, Besler HT. The role of oxidants and antioxidants in chronic tonsillitis and adenoid hypertrophy in children. Int J Pediatr Otorhinolaryngol 2004;68(08):1053–1058
11 Kalyanaraman B. Teaching the basics of redox biology to medical and graduate students: Oxidants, antioxidants and disease mechanisms. Redox Biol 2013;1(01):244–257
12 Yariktas M, Doner F, Dogru H, Yasan H, Delibas N. The role of free oxygen radicals on the development of otitis media with effusion. Int J Pediatr Otorhinolaryngol 2004;68(07):889–894
13 Garça MF, Aslan M, Tuna B, Kozan A, Cankaya H. Serum myeloperoxidase activity, total antioxidant capacity and nitric oxide levels in patients with chronic otitis media. J Membr Biol 2013;246(07): 519–524
14 Ballenger JJ, Snow JB. Ballenger's otorhinolaryngology: head and neck surgery. Pmph-usa2003
15 Jiang Z, Hennein L, Tao Y, Tao L. Interleukin-23 receptor gene polymorphism may enhance expression of the IL-23 receptor, IL-17, TNF-α and IL-6 in Behcet's disease. PLoS One 2015;10(07):e0134632
16 Kwon OE, Park SH, Kim SS, et al. Increased IL-17 and 22 mRNA expression in pediatric patients with otitis media with effusion. Int J Pediatr Otorhinolaryngol 2016;90:188–192
17 Engel J, Anteunis L, Volovics A, Hendriks J, Marres E. Risk factors of otitis media with effusion during infancy. Int J Pediatr Otorhinolaryngol 1999;48(03):239–249
18 McInnes IB, Kavanaugh A, Gottlieb ABPSUMMIT 1 Study Group. , et al; . Efficacy and safety of ustekinumab in patients with active psoriatic arthritis: 1 year results of the phase 3, multicentre, double-blind, placebo-controlled PSUMMIT 1 trial. Lancet 2013; 382(9894):780–789
19 Ye X, Zhang L, Wang H, et al. The role of IL-23/Th17 pathway in patients with primary immune thrombocytopenia. PLoS One 2015;10(01):e0117704
20 Sagiroglu S, Ates S, Tolun FI, Oztarakci H. Evaluation of oxidative stress and antioxidants effect on turning process acute otitis media to chronic otitis media with effusion. Niger J Clin Pract 2019;22(03):375–379
21 Kim SR, Kim HJ, Kim DI, et al. Blockade of interplay between IL-17A and endoplasmic reticulum stress attenuates LPS-induced lung injury. Theranostics 2015;5(12):1343–1362
22 Yilmaz T, Koçan EG, Besler HT, Yilmaz G, Gürsel B. The role of oxidants and antioxidants in otitis media with effusion in children. Otolaryngol Head Neck Surg 2004;131(06):797–803
23 Podrez EA, Abu-Soud HM, Hazen SL. Myeloperoxidase-generated oxidants and atherosclerosis. Free Radic Biol Med 2000;28(12): 1717–1725
24 Cemek M, Dede S, Bayiroğlu F, Çaksen H, Cemek F, Yuca K. Oxidant and antioxidant levels in children with acute otitis media and tonsillitis: a comparative study. Int J Pediatr Otorhinolaryngol 2005;69(06):823–827
25 Kalender S, Kalender Y, Ogutcu A, Uzunhisarcikli M, Durak D, Açikgoz F. Endosulfan-induced cardiotoxicity and free radical metabolism in rats: the protective effect of vitamin E. Toxicology 2004;202(03):227–235
26 Döner F, Delibaş N, Dogru H, Yariktaş M, Demirci M. The role of free oxygen radicals in experimental otitis media. J Basic Clin Physiol Pharmacol 2002;13(01):33–40
27 Khakimov AM, Arifov SS, Faĭzulaeva FN. [Efficacy of antioxidant therapy in patients with acute and chronic purulent otitis media]. Vestn Otorinolaringol 1997;(05):16–19
28 Shigemi H, Egashira T, Kurono Y, Mogi G. Role of superoxide dismutase in otitis media with effusion. Ann Otol Rhinol Laryngol 1998;107(04):327–331
29 Aktan B, Taysi S, Gumustekin K, Bakan N, Sutbeyaz Y. Evaluation of oxidative stress in erythrocytes of guinea pigs with experimental otitis media and effusion. Ann Clin Lab Sci 2003;33(02):232–236

