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Thiol-disulphide homeostasis in noncomplicated chronic otitis media

Komplikasyonsuz kronik otitis mediada thiol/disülfit dengesi

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Abstract

Introduction: We hypothesized that oxidative stress plays a role in the pathogenesis of chronic otitis media, chronic mucosal inflammatory disease. We aimed to investigate a novel oxidative stress marker in this study.

Methods: Thirty patient with chronic otitis media as the patient group and 30 healthy volunteer subjects as the control group were admitted to the study. Blood samples were taken when they admitted to our clinic before surgical intervention. In healthy volunteers, blood samples were taken when they were admitted to our policlinic. Thiol/disulphide levels were analyzed with a newly developed method by Erel and Neselioglu.

Results: 30 subjects were included in chronic otitis media group (20 females, 10 males). 30 subjects were included in the control group (8 females and 22 males). Sex distribution within the groups was significantly different (p=0.004). There was no significant difference between groups with respect to the age distribution (p=0.072). Measured native thiol, disulphide and total thiol values of the groups were not significantly different from each other.

Discussion and Conclusion: This study demonstrated that although dynamic thiol/disulphide homeostasis was shifted towards disulphide formation as a result of thiol oxidation in patients with chronic otitis media. But we could not find any significant difference between groups.

Keywords: Chronic otitis media; oxidative stress; thiol/disulphide homeostasis

Özet

Amaç: Kronik mukozal inflamatuar bir hastalık olan kronik otitis media patogenezinde oksidatif stresin rol oynayabileceğini düşündük. Bu amaçla yeni bir oksidatif stres belirteci ile bu durumu araştırmayı amaçladık.

Gereç ve Yöntem: Kronik ototis media tanısı konulan 30 hasta ve sağlıklı gönüllülerden oluşan 30 birey kontrol grubu olarak çalışmaya dahil edildi. Kan örnekleri hastaneye yatışta ameliyat öncesinde alındı. Kontrol grubundaki kan örnekleri polikliniğimize başvuru esnasında alındı. Thiol/disülfit düzeyleri Erel ve Neşelioğlu tarafından geliştirilen yeni bir yöntemle analiz edildi.

Bulgular: Kronik otitis media grubuna 30 birey (20 kadın, 10 erkek) dahil edildi. Konrol grubu olarak da 30 birey (8 kadın, 22 erkek) alındı. Gruplar içerindeki cinsiyet dağılımındaki farklılık istatistiksel olarak anlamlı (p=0.004) iken yaş dağılımı açısından anlamlı farklılık yoktu (p=0.072). Ölçülen native thiol disülfit ve total thiol değerleri açısından gruplar arasında anlamlı farlılık yoktu.

Sonuç: Bu çalışma, kronik otitis medialı hastalarda thiol oksidasyonunun bir sonucu olarak dinamik thiol disülfit dengesi disülfit formasyonuna doğru kaydığını göstermesine rğmen gruplar arasında anlamlı bir farklılık bulunmadı.

Anahtar Sözcükler: Kronik otitis media; oxidative stress; thiol/disulphide homeostazisi.

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Chronic otitis media (COM) is the inflammation of the mucosal lining the hollow space in the middle ear and airy spaces of the temporal bone for more than a three-month period with or without perforation of the eardrum. COM with cholesteatoma is characterized by the presence of an expanding growth consisting of keratinizing squamous epithelium in the middle ear and/or mastoid process.[1] It is the leading cause of hearing loss and is associated with significant morbidity.[2,3] Classically the pathology in COM is limited to the mucoperiosteum. Any pathology exceeding this limit can result in complications such as osteitis, bone destruction and meningitis.[4] Many studies have investigated the predisposing factors and pathogenesis of COM. Although COM has been described as a multifactorial disease, its etiopathogenesis has not been fully clarified.[1,5] The pathogenesis of COM is thought to be multifactorial and it includes Eustachian tube dysfunction, allergy, viral/bacterial invasion, and reduced ciliary function of both the middle ear and Eustachian tube mucosa, smoke exposure.[2]

Thiols are a class of organic compounds, also known as mercaptans, which include the sulfhydryl (ASH) group that has a critical roe in preventing the formation of any oxidative stress situation in cells.[6] Thiol (ASH) groups may be converted into reversible disulphide (SAS) bond structures by being oxidized by oxidant molecules in the environment.[7] The disulphide bond structures thus formed can be reducted into thiol (ASH) groups again, and thus a thioldisulphide homeostasis is maintained. This thiol-disulphide homeostasis, which is a recently defined oxidative stress indicator, is of vital importance.[8] The contribution of the dynamic thiol-disulphide homeostasis to antioxidant protection, detoxification,[9] apoptosis,[10] the regulation of enzymatic activity and cellular signal mechanisms,[11] and also the pathogenesis of various chronic diseases such as diabetes,[12] cancer,[13] chronic renal disease, liver disorders[14] and cardiovascular diseases[15] have also been shown. Currently, there is no method that simultaneously measures the dynamic plasma thiol-disulphide balance by colorimetry. [16] Whereas the double-sided thiol-disulphide balance can only be measured unilaterally since 1979,[17] it can be fully assessed with a new colorimetric method recently developed by Erel&Neselioglu that is easy, reliable, sensitive, cheap, fast, highly accurate and repeatable, and which can be operated both manually and fully automatically.[11]

In this study, we hypothesized that oxidative stress plays a role in the pathogenesis of this chronic mucosal inflammatory disease and we aimed to investigate a novel oxidative stress marker.

Materials And Methods

A total of 60 subjects (30 chronic otitis media and 30 healthy volunteer subjects) who admitted to the Ankara Atatürk Training and Research Hospital between April and September 2016 were included this study. In chronic otitis media group is contained patients who suffer from hearing loss and ear drainage and physical examination revealed an eardrum perforation without cholesteatoma any other invasive complication such as brain abscess, labyrinthine fistulas, and facial paralysis. This study has been designed in accordance with 2013 Brazil version of Helsinki Declaration and was approved by the local Ethics Committee. All participants have provided written consent. The principles of good clinical practices were followed during the study period.

Venous blood samples were taken to measure thiol/disulphide homeostasis parameters of all participants who were included in the study. Blood samples were taken when they admitted to our clinic before surgical intervention. In healthy volunteers, blood samples were taken when they were admitted to our policlinic. After blood samples were quickly centrifuged at 1500 rpm for ten minutes, plasma and serum samples were separated. Serum samples have been stored at −80°C until all samples were obtained.

A new method developed by Erel and Neselioglu was used to measurements of Thiol/disulphide levels. In summary, reducible disulphide bonds were first reduced to form free functional thiol groups. Unused reductant sodium borohydride was consumed and removed with formaldehyde, and all thiol groups including reduced and native ones were detected after reaction with 5,5′-dithiobis- (2-nitrobenzoic) acid. Half of the difference between total and native thiols provided the dynamic disulphide amount (-S-S). After the determination of native thiol (-SH) and disulphide (-S-S) amount, native thiol/disulphide ratio (-S-S-/-SH) was calculated.

Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, USA) program was used for statistical analyses. Kolmogorov-Smirnov test was used to determine the distribution of data. Continuous variables with normal distribution were given as mean±standard deviation and continuous variables without normal distribution were given as median interquartile range [IQR]. Categorical variables were expressed as numbers and percentage. Continuous variables were compared with independent sample -test, ANOVA, Mann Whitney test, and Kruskal-Wallis test where appropriate. Chi-square test and Fisher's exact chi-square test were used to compare categorical variables. The relationship between the numeric parameters was analyzed by Pearson and Spearman correlation analysis.

Results

A total of 60 subjects were evaluated in the study. 30 subjects were included in chronic otitis media group (20 females, 10 males). 30 subjects were included in the control group (8 females and 22 males). Sex distribution within the groups was significantly different (p=0.004) (Table 1).

Mean age was 37 (12-61) in chronic otitis media group whereas it was 31.2 in the control group. There was no significant difference between groups with respect to the age distribution (p=0.072). Measured native thiol, disulphide and total thiol values of the groups were not significantly different from each other (Table 2).

Table 1. Sex distribution within groups*

Groups	Male		Female		p
	n	%	n	%	
COM	20	66.7	10	33.3	0.004
Control	8	26.7	22	73.3	

COM: Chronic otitis media; *Chi square test.

Discussion

Thiols are a class of organic compounds that contain a sulfhydryl group (-SH), which is composed of hydrogen and a sulphur atom attached to a carbon atom.[6] Those disulphide bonds can be reduced back to thiol groups; therefore, thiol/disulphide homeostasis is maintained.[8] Thiols contribute the major portion of the total antioxidants present in the body and play an important role in defense against reactive oxygen species and also play critical roles in programmed cell death, detoxification, antioxidant protection, and regulation of cellular enzymatic activity.[9,10] Recently, it is known that an abnormal thiol/disulfide homeostasis state is involved in the pathogenesis of various acute and chronic diseases.[10] Measuring thiols in serum provides an indirect reflection of the antioxidative defense. The measurement of dynamic thiol/disulphide first started by a new automated method developed by Erel and Neselioglu.[11] Under oxidative stress, disulphide level is expected to increase as thiol level decreases.

COM is characterized by the persistent infection and inflammation of the middle ear and mastoid air cells. This condition typically involves a perforation of the tympanic membrane, with intermittent or continuous logorrhea.[18] As chronic otomastoiditis and Eustachian tube dysfunction persist, the tympanic membrane is weakened, which increases the likelihood of an atelectatic ear or cholesteatoma formation. The presence of mucin prevents the transmission of sound waves from the middle ear to the inner ear, leading to conductive hearing loss.[2–5] Nevertheless, the etiopathogenesis of the disease has not been fully clarified.[1–5] Environmental and immunologic risk factors have been implied in the pathogenesis. Recurrent upper respiratory tract infections, presence of immunosuppressive disease, malnutrition, allergy, nasopharyngeal lymphatic tissue hypertrophy and craniofacial malformations all play a role in the pathogenesis. Many risk factors have been identified for the chronicity of the inflammation. In COM, an inflammation develops in the mucosal lining of spaces as a result of the immune responses to various stimuli. This persistent inflammatory stimulation causes pathologic changes in the tissues and inhibits healthy tissue recovery. Moreover, oxidative stress can damage ciliary structure by damaging cellular DNA and protein,[19, 20] thus leading to increased damage in the Eustachian tube and middle ear. In summary, the peroxidation of phospholipids in the cell membranes of the middle ear can prolong the duration of inflammation and thus lead to chronicity. Previously, Garca et al found significantly elevated serum and tissue sample levels of malondialdehyde which was a marker of lipid peroxidation and decreased levels of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase.[20] To the best of our knowledge, this is the first study that investigated thiol/disulphide homeostasis as a novel marker of oxidative stress in patients with COM and compared the results with healthy controls.

Although the disulphide values of the chronic otitis media patients were higher (as we approve shift to the disulphide formation was an indicator for he oxidative stress of the diseased tissue) we could not find any statistically significant difference in serum thiol and disulphide levels between control group and chronic otitis media groups.

There are several limitations in this study that should be taken into consideration. First, this was a pilot study representing an initial investigation into the relationship between chronic otitis media and thiol/disulphide homeostasis parameters. Second is inclusion of relatively small number of patients who were admitted to a single center. And inclusion criteria were not strict so groups were not homogenous both in sex and age.

Table 2. Sex distribution within groups*

Variables	COM		Control		Test statistics	
	Median (Min.; Max.)	Mean±SD	Median (Min.; Max.)	Mean±SD	Z; t	p
Age	35.5 (12.0; 61.0)	37.0±14.3	30.0 (18.0; 56.0)	31.2±9.9	1.797*	0.072
Native thiol	496.7 (380.9; 619.5)	498.2±51.8	505.6 (453.2; 634.0)	515.8±46.1	1.190*	0.234
Disulphide	19.4 (1.1; 34.2)	18.8±10.3	17.1 (5.4; 39.2)	18.5±9.4	0.192*	0.848
Total thiol	536.3 (389.0; 631.4)	535.8±56.6	541.9 (484.3; 672.0)	552.2±52.6	0.621*	0.535
Disulphide/total thiol	3.71 (0.17; 6.53)	3.46±1.84	3.09 (1.03; 7.29)	3.30±1.57	0.532*	0.595
Native thiol/total thiol	92.58 (86.94; 99.66)	93.08±3.68	93.91 (85.42; 97.94)	93.51±3.28	t=0.478**	0.634
Disulphide/native thiol	4.01 (0.17; 7.51)	3.80±2.12	3.29 (1.05; 8.53)	3.59±1.85	0.547*	0.584

COM: Chronic otitis media; Min.: Minimum; Max.: Maximum; SD: Standard deviation; *Mann- Whitney U test; **Independent samples' t test.

Conclusion

This study demonstrated that although dynamic thiol/disulphide homeostasis was shifted towards disulphide formation as a result of thiol oxidation in patients with chronic otitis media. But we could not find any significant difference between groups. Prospective and randomized controlled trials are necessary to confirm the pathophysiologic role of thiol/disulphide homeostasis in chronic otitis media. Further studies are required to optimize the use of this novel oxidative stress marker in conjunction with other established approaches.

Conflict of interest: There are no relevant conflicts of interest to disclose.

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