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Is otitis media with effusion associated with oxidative stress? Evaluation of thiol/disulfide homeostasis☆

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ABSTRACT

Background: We evaluated the relationship between otitis media with effusion and thiol/disulfide homeostasis using a novel marker of oxidative stress.
Methods: The study group consisted of 30 patients (mean age 8.33 ± 3.30 years) with bilateral otitis media with effusion admitted to our hospital. The control group consisted of 35 (mean age 7.40 ± 3.97 years) age-, sex-, and body mass index-matched healthy subjects. Thiol/disulfide homeostasis was measured using a newly developed method.
Results: Native and total thiol levels were lower in the study than the control group (native thiols 421.37 ± 72 μmol/L vs. 464.46 ± 46.42 μmol/L, $p < 0.05$; total thiols 468.42 ± 77.89 μmol/L vs. 501.32 ± 50.30 μmol/L, respectively). Disulfide levels and the disulfide/native thiol and disulfide/total thiol ratios were higher in the study group (disulfides 23.56 ± 4.68 μmol/L vs. 18.43 ± 4.94 μmol/L; disulfide/native thiol ratio 5.65 ± 1.05 vs. 3.97 ± 1.03%; disulfide/total thiol ratio 5.06 ± 0.83 vs. 3.66 ± 0.88%, respectively).
Conclusion: Oxidative stress may be the major cause of the increase in oxidized thiols in patients with bilateral otitis media with effusion, however, this relationship requires further investigation.

1. Introduction

Otitis media with effusion (OME) is a common disease in childhood and a very important cause of hearing problems. OME is an inflammatory condition of the middle ear space, either acute or chronic, featuring fluid collection in the presence of an intact tympanic membrane. Although various environmental, genetic, allergic, mechanical, and immunological factors have been investigated in terms of OME etiology, no definitively proven etiological agent has yet been identified [1,2]. Oxidative stress has been implicated in the etiology of OME, and has been the subject of several studies [2–4]. Thiol organic compounds react with free radicals to protect against tissue and cellular injury caused by reactive oxygen products; thiols contain sulfhydryl (-SH) groups bound to carbon atoms. During oxidative stress, the sulfhydryl groups are converted into disulfide bridges that are subsequently reconverted into thiols; the cycle continues in the balanced state. The thiol/disulfide balance plays a critical role in oxidative stress, apoptosis, detoxification, protection against antioxidants, cellular signal transmission, and enzymatic activity [5–7]. Serum levels of thiol/disulfide were formerly analyzed indirectly; direct measurement became possible when a fully automatic colorimetric method was described by Erel and Neşelioğlu in 2014 [5,8,9].

In this study, we evaluated the relationship between OME and the thiol/disulfide balance (a marker of oxidative stress) using the novel technique. To the best of our knowledge, this is the first work to measure this important biomarker in OME patient.

2. Materials and methods

The study group consisted of 30 patients (mean age

☆ The authors declare that they have no conflict of interest. Informed consent was obtained from all individual participants included in the study.

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8.33 ± 3.30 years) with bilateral OME admitted to our hospital. The control group consisted of 35 (mean age 7.40 ± 3.97 years) age-, sex-, and body mass index (BMI)-matched healthy subjects.

Patients with the following were excluded: those receiving systemic steroid therapy; with allergic rhinitis, adenoid hypertrophy, or diabetes mellitus; with cardiovascular, cerebrovascular, acute/chronic kidney, or liver disease; with acute or chronic systemic disease such as malignancy; taking antioxidants or vitamins; with acute-chronic infectious diseases; and exposed to smoking at home. Patients were diagnosed on the basis of otoendoscopic examinations and odio-tympanometric analyses. Adenoid hypertrophy was diagnosed via exams using a flexible endoscope (Karl-Storz GmBH & Co., Tuttlingen, Germany). The study was approved by our local ethics committee. Signed consent forms were received from the parents of all patients and healthy controls. Blood specimens were collected between 8 and 10 a.m. after 8 h of fasting and immediately centrifuged for 10 min at 1500 rpm. Serum specimens were stored at −80 °C prior to analyses. Thiol/disulfide measurements were subsequently made using the method of Erel and Neşelioğlu [8]. We measured total and native thiols, disulfides, and the disulfide/total thiol, disulfide/native thiol, and native thiol/total thiol ratios.

Venous blood was collected from the antecubital vein following a 12 h overnight fast. Blood glucose and other biochemical data (kidney and liver parameters) were measured with an autoanalyzer using standard methods (Beckman Coulter AU 2700) employing commercial kits. BMI was calculated by dividing the weight in kilograms by the square of the height in meters.

3. Statistical analyses

All statistical analyses were performed using SPSS for Windows, version 17.0. Unless otherwise stated, the results are expressed as means ± SDs. We used the Mann–Whitney *U* test or the independent samples *t*-test for between-group comparisons, and the Pearson or Spearman correlation test, as appropriate. Multiple regression analyses were used to exclude possible confounding effects of other variables during correlation analyses. A p-value < 0.05 was considered to reflect statistical significance.

4. Results

The groups did not differ significantly in terms of sex, age, BMI, or biochemical and hematological results (all p > 0.05). Renal and hepatic test results were similar between the two groups. Native thiol and total thiol levels were lower in the study group than in controls (native thiols 421.37 ± 72 μmol/L vs. 464.46 ± 46.42 μmol/L, p < 0.05; total thiols 468.42 ± 77.89 μmol/L vs. 501.32 ± 50.30 μmol/L, p < 0.05, respectively). Disulfide levels and the disulfide/native thiol and disulfide/total thiol ratios were higher in the study group (disulfides 23.56 ± 4.68 μmol/L vs. 18.43 ± 4.94 μmol/L, p < 0.05; disulfide/native thiol ratio 5.65 ± 1.05 vs. 3.97 ± 1.03%, p < 0.05; disulfide/total thiol ratio 5.06 ± 0.83 vs. 3.66 ± 0.88%, p < 0.05, respectively) (Table 1 and Graphs 1–2).

The thiol/disulfide ratio and other risk factors were subjected to multiple regression analyses. The low thiol and high disulfide levels in OME patients were independent of sex, age, and BMI.

5. Discussion

OME is more common in childhood, with accumulation of middle ear cavity effusion without the clinical findings of acute OM. The rates vary, but an average of about 2 million new patients are diagnosed annually; most are about 5 years of age [1,10,11]. As the etiology is poorly understood, treatment varies and may include systemic antibiotherapy, the use of decongestants and antihistamines, and insertion of a ventilation tube [12]. In untreated chronic patients, hearing is significantly affected, triggering speech and language problems; poor mental, social, and behavioral development; and problems at school [10]. We commenced with medical treatment and performed surgery only if necessary.

Table 1
Thiol/disulphide homeostatic parameters in the EOM and control groups Parameters were expressed as mean ± SD. *p<0.05 was considered significant for statistical analyses.

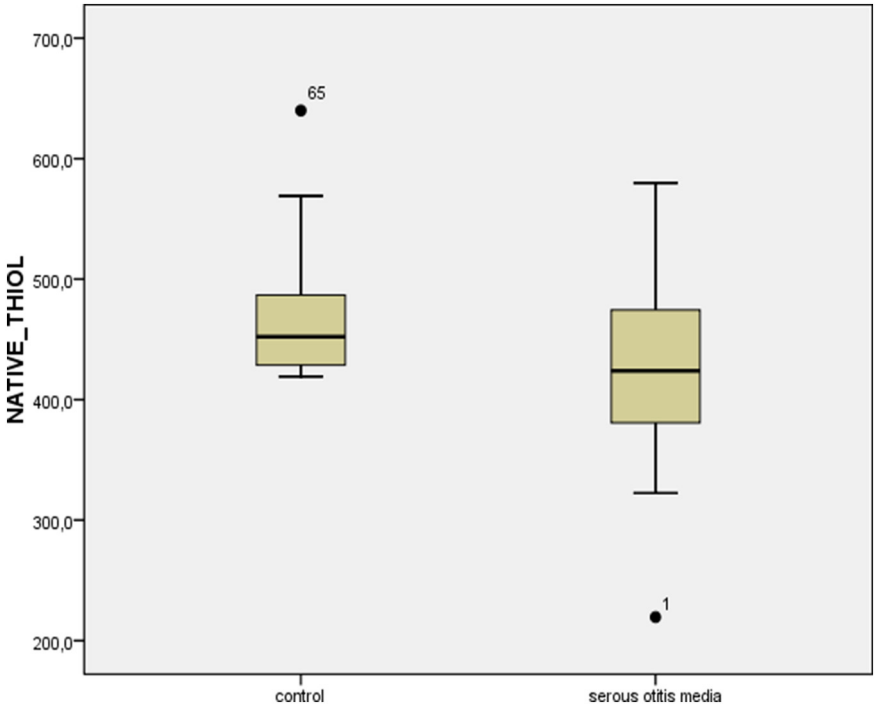
	Group	N	Mean	Std. deviation	P value
Age	Control	35	74,048	397,943	0,31
	EOM	30	83,333	330,447	0,30
NATIVE_THIOL	Control	35	464,469	46,4223	0,005*
	EOM	30	421,377	72,0006	0,007*
TOTAL_THIOL	Control	35	501,329	50,3071	0,044*
	EOM	30	468,420	77,8959	0,053*
DISULPHIDE	Control	35	18,430	49,466	0,00*
	EOM	30	23,567	46,807	0,00*
SSSH	Control	35	39,750	103,173	0,00*
	EOM	30	56,539	105,503	0,00*
SSTOTALTHIOL	Control	35	36,659	,88,145	0,00*
	EOM	30	50,657	,83,183	0,00*
SHTOTALTHIOL	Control	35	92,6682	176,289	0,00*
	EOM	30	89,8944	165,541	0,00*

The etiology of OME remains unknown; suggestions have included eustachian tube dysfunction, adenoid hypertrophy, viral and bacterial infections, exposure to cigarette smoke, reflux disease, allergic rhinitis, and oxidative stress [2–4,13,14]. Histological and pathophysiological data suggest that the mechanism is multifactorial. Oxidative stress and free oxygen radicals may play roles [2,4,15]. Excessive redox activity is evident in the middle ear mucosa, caused by various types of inflammatory cells, cytokines, and other mediators associated with chronic inflammation. Oxidative stress involves activation of redox signaling pathways by various mediators (including transcription factors) as inflammation progresses [2,3,16]. The thiol/disulfide balance is a pincipal protective mechanism, playing an important role in modulation of intracellular enzymatic reactions, programmed cell death, detoxification, and antioxidant protection [5,6,17]. Increased formation of disulfide triggers functional and structural pathologies in many organs and systems by adversely impacting protection against oxidation and redox products [5,6]. Investigations have been performed in patients with hypertension [6], diabetes mellitus [18] inflammatory bowel disease [5], cardiovascular diseases [6,19], gynecological and obstetric diseases, and infectious diseases such as brucellosis, tonsillitis, and nasal polyposis [7,9,17,18], and close associations with oxidative stress have been reported.

Several studies have explored the relationship between OME and oxidative stress, employing various markers including lipid hydroperoxide levels and oxidation of proteins in general, paraoxonase, and arylesterase [2,16,20]. Some studies have found close relationships between OME and oxidative stress; others have not [16]. We found that the thiol/disulfide balance, an important marker of oxidative stress, significantly changed, with a decrease in thiols and an increase in disulfides in OME patients. Multiple regression analyses revealed that the decrease in native thiols was independent of age, BMI, and sex, which indicates that antioxidant capacity may be involved in the etiology of OME.

OME compromises quality of life, being associated with hearing and speech problems. This is particularly important in children. The increase in disulfide products of oxidative stress, and the decrease in antioxidant thiols, may explain treatment resistance. Because our study group consisted of treatment-resistant and chronic OME patients.

In particular, the determination of significant relationship with oxidative stress in patients with refractory OME suggests the importance of antioxidant treatment that can have a positive effect on the pathological process. Further studies investigating the benefits of



Graph 1. Native thiol levels in EOM and control group. Control children had higher native thiol values than EOM patients.

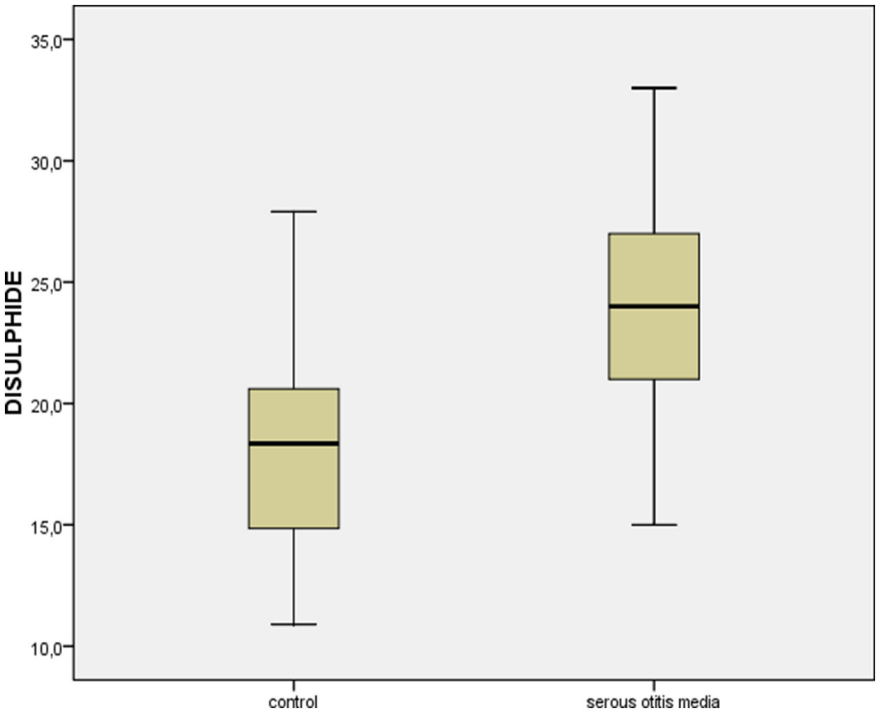
antioxidant use in chronic OME patients may lead to new horizons for improving the quality of life of patients.

6. Limitations

Our patient numbers were low. Further studies are required.

7. Conclusion

We examined the thiol/disulfide balance in OME patients using a new technique. To the best of our knowledge, this is the first study to employ this approach to evaluate oxidative stress in OME. We found that oxidative stress may play a role in OME development. We think that further, wide-ranging studies are now needed to assess the benefits of antioxidant therapy by raising thiol and reducing disulfide levels.



Graph 2. Disulphide levels in EOM and control group. Control children had lower disulphide values than EOM patients.

Declaration of interest

No author has any conflicts of interest. The authors alone were responsible for the content and preparation of the paper.

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