Examination of Oxidative Stress with Thiol-Disulphide Homeostasis in Pediatric Cases with Otitis Media with Effusion: A Case-Control Study

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Objectives: In the literature, various pathologies are accused of affecting the development of chronic otitis media with effusion (OME). However, the relationship between OME and oxidative stress (OS) is not fully understood. To investigate the OS status in OME cases using thiol-disulphide balance. In addition, the presence of OS is exhibited with total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), Ferric Reducing Antioxidant Power (FRAP), Serum lipid hydroperoxide levels (LOOH) and Advanced oxidation protein products (AOPP).

Methods: A total of 150 cases (63 female, 87 male; mean age 5.0±1.3 years; age range: 3-9 years) were included in the study. Patients were divided into two groups according to Sade’s classification: Group A for patients with grade 1 and 2 TM retraction and Group B for patients with grade 3 and 4 TM retraction. Blood parameters of group A, group B and control group were compared.

Results: Native thiol (SH) and total thiol (SH+SS) values in group A and group B were significantly lower than control group (p<0.05). In group B, the %SS/disulphide (SH) value, %SS/SH+SS value, %SH/SH+SS value were significantly higher than group A (all p values <0.05). TOS, OSI, LOOH, and AOPP values were significantly higher in group A and group B than control group (p<0.05).

Conclusion: In this study, decrease of thiol content and increase of disulphide content are observed in the pediatric cases of OME.

Keywords: Otitis media with effusion, oxidative stress, pediatric, thiol-disulphide balance
netic diseases, viral or bacterial infections and local presentations of autoimmune diseases are among such pathologies. Today, it is widely accepted that chronicization of OME is multifactorial [7-9]. Recent studies have been investigating whether oxidative stress is an effective factor in the development of chronic OME [10-11]. In the human body, there is a balance between oxidant and antioxidant molecules. Certain concentrations of oxidant molecules are required for the maintenance of vital functions in the body. The increase in free radicals in the body stimulates antioxidant systems and the body is protected from the harmful effects of these molecules. But the insufficiency of the antioxidant system and the chronic effects of the oxidant molecules lead to oxidative stress (OS). Several studies have shown that OS is associated with the development of some chronic diseases [10-11]. However, the effect of OS in the chronicization of OME has not been fully elucidated. The thiol-disulphide balance is a fairly new marker of OS in the body. The thiol-disulphide levels can be cumulatively or individually calculated with a new and automatic method. Compared to the other markers, the thiol-disulphide balance is an easier and practical marker of the OS [12-14]. The literature has linked the thiol-disulphide balance with several diseases. The relationship between many diseases and thiol-disulphide balance has been investigated in the literature [15-18]. This fact has created the idea to conduct this present study. In this study, the OS status in OME cases was investigated using thiol-disulphide balance. In addition, the presence of OS is exhibited with total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), Ferric Reducing Antioxidant Power (FRAP), Serum lipid hydroperoxide levels (LOOH) and Advanced oxidation protein products (AOPP).

**Methods**

This case-control study was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines. The study was approved by the local ethics committee (16/04/2018-E.15902). This non-randomized, prospective study was conducted in the otolaryngology clinic of our hospital between April 2018 and June 2018. A total of 150 cases (63 female, 87 male; mean age 5.0±1.3 years; age range: 3-9 years) were included in the study. A study group of 100 patients who were followed up for at least three months due to bilateral OME in our clinic, who did not respond to medical treatment and underwent ventilation tube insertion, and a control group of 50 healthy subjects without otitis media history and without any surgical operation were included in the study. Endoscopic nasal and nasopharynx examination, pneumatic otoscopy, pure tone audiometry in children who can co-operate and tympanometry examination (1 kHz) were performed on all patients with a known acute or chronic disease, autoimmune disease, malignancy, history of ear/adeno-tonsillectomy surgery.

**Blood Collection From Cases**

Blood samples were collected from OME cases after the 12 hour fasting before the operation, between 8 and 10 o’clock. Blood samples were collected from the control group after 12 hour fasting, between 8 and 10 o’clock. Blood samples were centrifuged at 4000 rpm for 10 minutes. After waiting for 30-45 minutes; samples were collected and stored at -80°C in Eppendorf tubes until serums separated.

**Measurement of Parameters**

1. **Measurement of Thiol/Disulphide**

The total thiol content of the blood sample was measured using a modified Ellman’s reagent. Half of the difference between total and native thiols results in the amount of dynamic disulphide. After determining the natural thiol and disulphide levels, the disulphide/thiol ratio was calculated as previously described by Erel and Neselioğlu [20].

2. **Total Antioxidant Status Level**

Total antioxidant status levels of the samples (TAS) were measured using Rel Assay brand commercial kits (Rel Assay Kit Diagnostics, Turkey) with spectrophotometric method (Thermo Scientific Multiskan GO, ThermoFisher Scientific, Vartaa, Finland). Trolox, a water-soluble analog of vitamin E, was used as the calibrator. The results are expressed in mmol Trolox equiv./lt.

3. **Total Oxidant Status (TOS) Level**

Total oxidant status levels of the samples (TAS) were measured using Rel Assay brand commercial kits (Rel Assay Kit Diagnostics, Turkey) with spectrophotometric method (Thermo Scientific Multiskan GO, ThermoFisher Scientific, Vartaa, Finland). Hydrogen peroxide was used as the calibrator. The results are expressed in μmol H₂O₂ equiv./lt.
4. Calculation of Oxidative Stress Index

While calculating OSI, which is defined as the percentile ratio of TOS levels to TAS levels, mmol value in the TAS test was converted to μmol units as in the TOS test. The results were expressed as "arbitrary unit" (AU) and calculated according to the following formula.

\[
OSI = \frac{\text{TOS, μmol H}_{2}\text{O}_{2} \text{ equiv./lt}}{\text{TAS, mmol Trolox equiv./lt} \times 10}
\]

5. Serum Lipid Hydroperoxide Level

Serum lipid hydroperoxide concentrations were measured spectrophotometrically using ferrous oxidation-xylenol orange (FOX-2) test.

6. Ferric Reducing Antioxidant Power (FRAP) Assay

A simple, automated test measuring the ferric reducing ability of plasma, the FRAP assay, is presented as a novel method for assessing "antioxidant power" Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex to form \[21\]. The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method employing an easily reduced oxidant, Fe (III). Reduction of a ferric tripyridyltriazine complex to ferrous-(2,4,6-tripyridyl-s-triazine)2 ie: Ferric (III) colorless to Ferrous (II) blue can be monitored by measuring absorbance at 593 nm. The absorption readings are related to the reducing power of the electron-donating antioxidants present in the test compound. Hence the FRAP assay can rank the reducing power and the antioxidant potential of a wide range of test compounds.

FRAP value μM=[Abs. (sample)×FRAP value of Std (μM)}/Abs. of Std.

7. Assay of Advanced Oxidation Protein Products

Spectrophotometric determination of advanced oxidation protein products (AOPP) was performed by a modification of Witko, Nguyen, and Descamps-Latscha's method \[22\]. Samples were prepared in the following procedure: 100 μL of supernatant was diluted 1:5 in phosphate buffered saline (PBS), 5 μL of 1.16 M potassium iodide was then added to each tube, followed by 10 μL acetic acid two minutes later. The absorbance of the newly formed mixture was immediately measured at 340 nm by using a blank as reference containing 1000 μL of PBS, 50 μL of Potassium Iyodide (KI), and 100 μL of acetic acid. The chloramine-T absorbance at 340 nm was observed to be linear within the range of 0 μmol/L to 100 μmol/L. AOPP concentrations were expressed as micromoles per liter of chloramine-T equivalent.

Statistical Analysis

Mean, standard deviation, median lowest, highest, frequency and ratio values were used in the descriptive statistics of the data. Independent sample t test of Kruskal-Wallis and Mann-Whitney U test were used in the analysis of quantitative independent data. Chi-squared test was used in the analysis of qualitative independent data. SPSS 22.0 software package was used for analyses.

Results

There was no statistically significant difference in age and gender distribution among the groups (p values of 0.214 and 0.782, respectively).

Native thiol (SH) and total thiol (SH+SS) values in group A and group B were significantly lower than control group (p<0.05). Native thiol and total thiol values in group B were significantly lower than in group A (p=0.0001). In group A and group B, the disulphide (SS) values were significantly higher than the control group (p=0.0001). In group A, disulphide values were significantly higher than group B (p=0.0001).

In group A and group B, %SS/SH value, %SS/total thiol value, %SH/total thiol value were significantly higher than control group (all p values <0.05). In group B, the %SS/SH value, %SS/total thiol value, %SH/total thiol value were significantly higher than group A (all p values <0.05).

In group A and group B, TAS and the FRAP values were significantly lower than control group (all p values <0.05). In group A and group B, LOOH, and AOPP values were significantly higher in group A and group B than control group (p<0.05). There was no significant difference between TAS, FRAP, LOOH and AOPP between group A and group B (all p values <0.05) (Table 1).

Discussion

In this study, OS presence in pediatric cases of chronic OME was investigated using thiol-disulphide balance. TAS, FRAP, LOOH and AOPP parameters were assessed in pediatric cases of chronic OME and the presence of OS was proved. According to our literature review, this is the first study to investigate the presence of OS in the chronicity of OME with thiol-disulphide balance in pediatric settings.

Recent studies have reported that OS plays role in OME development and chronicity \[23, 24\]. However, the relationship between OME and OS is not fully understood. The use of different parameters in the studies carried out and the relatively small number of such studies have prevented this issue from being clarified. Our limited information on OME seems to negatively affect the OME treatment and follow-up process. Elucidation of this subject will bring about pre-
This article has been retracted.
membrane retraction grade 1 and 2, while grade 3 and 4 cases were in group B. Typanic membrane retraction may be considered a clinical finding of the chronic process of OME. The purpose of this classification is to be able to demonstrate the relationship between the OME chronicity process and OS. In the group A cases, the duration of OME is shorter and in group B cases, the duration of OME is longer. OS parameters in both groups were higher than the control group’s. When the amount of thiol is higher than that of the control group, there is a further decrease in the amount of thiol in group B than group A. In group A and B, OS parameters (TAS, FRAP, LOOH, AOPP) were found to be higher than control group, whereas antioxidant system parameters (TAS, FRAP) were lower. There was no statistically significant difference in these parameters between groups A and B. In this study, the presence of OS was evidenced in the development of chronic OME in pediatric cases. The amount of thiol was found to be more sensitive to the presence and level of OS. With the prolongation of the chronic process of OME it was observed that there was a further decrease in the amount of thiol.

As several diseases result from the prolonged exposure to OS, it is suggested that these diseases are preventable. The elimination and prevention of the cause of the OS may prevent the development of many diseases and improve the quality of life. Testa et al. [29] reported that the number of OS molecules increases in OME cases and the majority of the cases recover with antioxidant therapy.

The strengths of this study are that it is prospective and that it includes a control group. Using twelve parameters to prove the OS’s presence is another strength. Clinically, the results obtained from this study data suggest that the presence of OS in OME cases may lead to many diseases and that the treatment should be administered without delay in these cases. There is a need to investigate the efficacy of antioxidant therapy in treatment of OME cases.

Although this work provides valuable information, there are some restrictive factors. Relatively small number of cases and impossibility of randomization are main limiting factors. In addition, the inability to assess thiol-disulphide balance after the treatment in pediatric cases of OME is another limiting factor. More studies assessing a greater number of cases are needed to investigate thiol-disulphide balance after treatment.

Conclusion

In this study, decrease of thiol content and increase of disulphide content are observed in the pediatric cases of OME. Long-term OS exposure of OME in pediatric settings can lead to many chronic diseases. In such cases, treatment should be administered without delay. Thiol-disulphide balance is a practical method that can be used to demonstrate OS presence in these conditions. There is a need for prospective studies with more patients to support the results of this study.

Disclosures

Ethics Committee Approval: The study was approved by the local ethics committee (16/04/2018-E.15902).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

References

This article has been retracted.