

## ORIGINAL ARTICLE

### Superoxide Dismutase and Glutathione in Otitis Media with Effusion: Relation with Hearing Loss

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**Objective:** To investigate the role of antioxidant defense in otitis media with effusion (OME) and the relation of hearing loss with antioxidant defenses by measuring the concentration of Superoxide Dismutase (SOD) and glutathione (GSH) of both middle ear effusions (MEE) and erythrocytes.

**Materials and Methods:** Twenty-six children with serous otitis media, ages ranging between 4-14 years were prospectively included into the study. A diagnosis of OME was made on the basis of the patient's history and examination, otoscopy, tympanogram and pure tone audiometry. The MEEs were collected via a sterile, disposable aspirator (Juhn Tym-Tap®, Xomed Inc., Jacksonville, FL 32216, USA) after myringotomy under general anesthesia. After an overnight fast in the morning (08-09 am), blood samples were drawn from patients into tubes with ethylenediamine tetraacetic acid (EDTA) for the measurement of GSH and SOD. Reported values of concentration of Superoxide Dismutase (SOD) and glutathione (GSH) of both MEE and erythrocytes in patients with OME were used as outcome measures.

**Results:** Air-bone gap at 1,000 Hz was positively correlated with MEE SOD activity and GSH content ( $r=0.584$   $p<0.01$ ). Air-bone gap were found to be negatively correlated with erythrocyte SOD activity and erythrocyte GSH content ( $r=-0.298$   $p<0.05$ ). MEE and erythrocyte SOD activities were not, but MEE and erythrocyte GSH concentrations were correlated;  $r=0.394$  ( $p<0.01$ ) when the two ears were considered together. Age, which was found to be positively correlated with air bone gap values at right ear and left ear was negatively correlated with SOD activity measured in MEEs of left ear ( $r=-0.628$   $p<0.01$ ).

**Conclusions:** The positive correlation of both SOD activity and GSH concentration of MEE with hearing loss might represent the potentiation of local defense mechanism against oxidative tissue damage; the positive correlation between effusion and blood GSH values in turn, suggests that systemic GSH supplementation might be beneficial in potentiation of local GSH status.

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Otitis media with effusion (OME) is one of the common inflammatory diseases caused by multiple factors such as viral or bacterial infections, eustachian tube dysfunction and allergy<sup>[1]</sup>. It is characterized by secretory transformation of the epithelium lining the middle ear cavity, infiltration of phagocytes, vascular proliferation and hypertrophy/hyperplasia of the connective tissue<sup>[2-4]</sup>. The inflammation ranges from transient, spontaneously healing episodes without sequel to chronic complicated cases associated with several long term effects<sup>[5]</sup>.

Microbial infection is considered to play an important role in the pathogenesis of OME<sup>[6]</sup>. Molecular biological approaches using polymerase chain reaction showed a high incidence of bacteria in middle ear effusions (MEEs)<sup>[7,8]</sup>. Also, B and T lymphocytes, plasma cells, polymorphonuclear cells, macrophages, all classes of immunoglobulins and several cytokines, all have been observed in MEEs<sup>[9]</sup>. Superoxide radicals have been identified in MEEs and have recently attracted the attention of otologists<sup>[10,11]</sup>.

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The superoxide anions are reactive oxygen metabolites generated by leukocytes and other phagocytes whenever necessary. These free oxygen radicals have protective roles directed to the destruction of invading foreign cells. On the other hand they may also have adverse cytotoxic effects on host cells and may contribute to the pathogenesis of some diseases. They cause tissue damage by chemically modifying lipids, proteins, carbohydrates and nucleic acids <sup>[12]</sup>.

To counter the toxicity of these free oxygen radicals a defense system is available in the tissues. In addition to the constituent antioxidant enzymes of the normal tissue cells similar enzymes are released from infiltrating phagocytes. Thus basically the outcome of the inflammatory process might reflect the biochemical balance between the amount of oxygen derived free radicals and the capacity of the defense mechanisms <sup>[6]</sup>.

The antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase and catalase are the three principle antioxidant enzymes that protect tissues from free radical damage. The relative activity of these enzymes in certain tissues may influence the susceptibility of these tissues to injury by free radicals <sup>[6]</sup>.

Conductive hearing loss is one of OME components. When the antioxidant defense system is weakened, the increased free oxygen radicals may contribute to OME formation <sup>[13]</sup>. Superoxide radicals contribute to OME formation, and OME obviously causes conductive hearing loss. Thus, we aimed to investigate the relation between antioxidant defense and conductive hearing loss.

To investigate the relation between antioxidant defense and conductive hearing loss in OME, the activity of SOD and the concentration of glutathione (GSH) of both MEE and erythrocytes were measured, and the relation between the clinical and biochemical data was evaluated.

## **Materials and Methods**

26 patients (14 male, 12 female) were enrolled in the study. All had persisted middle ear effusion for 3 months or longer and were unresponsive to repeated

courses of concurrent antibiotics. All had documented hearing loss and flat tympanograms. Hearing loss expressed as air-bone gap was determined by pure tone audiometry, measuring air and bone conduction at 500, 1,000, 2,000 and 4,000 Hz. All patients had hearing loss. Patients who had acute upper respiratory tract diseases, history of ventilation tube (VT) insertion or adenoidectomy, received antibiotics in the preceding month, cooperation problem with pure tone audiometry were excluded. A diagnosis of OME was made on the basis of the patient's history and examination, otoscopy, and pure tone audiometry. The mean age was 8.69 (ranging from 4 to 14 years).

The MEEs were collected by aspiration with Juhn-Tym-Tap after myringotomy under general anesthesia. Samples of MEE could be obtained bilaterally from 22 patients (85%) and unilaterally from 4 patients (15%). In order to avoid contamination with blood during the collection of MEE samples, an equal mixture of 0.1% epinephrine and 4% lidocaine hydrochloride was applied in the surgery under general anesthesia. This agent was washed out with sterile saline solution from the external ear canal before myringotomy. The patients whose samples were apparently contaminated with blood were omitted from the study. The samples were diluted 10 fold with 0.01mol/L sterile phosphate-buffered saline (pH 7.4) and homogenized with Potter type homogenizer. Each sample was divided into two aliquots for the measurement of GSH and SOD.

After an overnight fast in the morning (08-09 am), blood samples were drawn from patients into tubes with ethylenediamine tetraacetic acid (EDTA). Blood plasma was immediately separated from whole blood by centrifugation at 3000 rpm at 4°C. For erythrocyte lysate preparation, erythrocytes were separated and washed 3 times in 5 ml of sterile 9 g/L NaCl solution, hemolyzed by diluting four-fold with water.

GSH concentration in erythrocytes and effusion was determined according to the method of Beutler et al. <sup>[14]</sup>, using metaphosphoric acid for protein precipitation and 5,5'-dithiobis 2-nitrobenzoic acid for color development; the molar absorption coefficient

( $1.36 \times 10^4$  M $\cdot$ lcm $^{-1}$ ) was used in calculations. Erythrocyte GSH concentration was expressed as mg/g Hb; hemoglobin concentration was determined by the cyanomethemoglobin method<sup>[15]</sup>. GSH concentration in effusion was expressed as mg/dl.

SOD activity in erythrocytes and effusions was measured using colorimetric of assay kit (Randox Laboratories Ltd., Antrim, UK). The assay involves inhibition of INT reduction with superoxide radical which is produced by xanthine-xanthine oxidase. Erythrocyte SOD activity was expressed as U/g Hb. SOD activity in effusion was expressed as U/ml.

### Statistics

All data are presented as means  $\pm$  SD. Correlation between the variables were studied by Spearman's rank correlation coefficient. The  $p < 0.05$  level was selected as the point of minimal statistical significance.

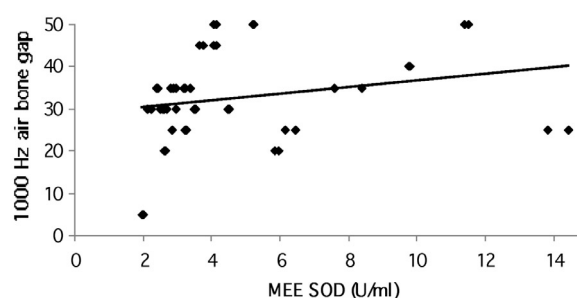
### Results

SOD activity was measured as  $1017.51 \pm 196.36$  U/g Hb in the erythrocytes. SOD activity was  $4.73 \pm 3.10$  U/ml in the effusions when the left and right ears were considered together (Table 1). Erythrocyte and MEEs SOD activity values were not found to be correlated.

GSH concentration was measured as  $1.49 \pm 0.39$  mg/g Hb in the erythrocytes and  $74.79 \pm 41.66$  mg/dl in MEEs when the left and right ears were considered together (Table 1). GSH concentration values of erythrocyte and MEEs were found to be correlated. The correlation coefficient was 0.394 ( $p < 0.01$ ) when the two ears were considered together, and was 0.686 ( $p < 0.001$ ) when only the right ear was considered.

Hearing loss of the patients were measured and classified as air-bone gap at 500, 1,000, 2,000, and 4,000 Hz.

Air-bone gap of right ear at both 1,000 Hz and 2,000 Hz was found to be correlated with MEE SOD activity ( $r = 0.621$   $p < 0.01$  and  $r = 0.418$   $p < 0.05$  respectively). When the left and right ears were considered together, the correlation of air-bone gap at 1,000 Hz was found to be 0.304 ( $p < 0.05$ ) (Figure 1). MEE GSH content, similar to MEE SOD activity, was found to be positively correlated with air-bone gap of left ear at 1,000 Hz ( $r = 0.584$   $p < 0.01$ ).



**Figure 1.** Correlation between MEE SOD activity and air-bone gap at 1,000 Hz in OME patients (correlation coefficient  $r = 0.304$ ,  $p < 0.05$ ) when the values measured for left and right ears were considered together. (MEE: middle ear effusions).

Air-bone gap of right ear at both 500 Hz and 4,000 Hz were found to be negatively correlated with erythrocyte SOD activity ( $r = -0.398$ ,  $p < 0.05$  and  $r = -0.634$   $p < 0.01$  respectively). Air-bone gap of left ear at 500 Hz, 1,000 Hz and 2,000 Hz were all found to be negatively correlated with erythrocyte SOD activity ( $r = -0.437$   $p < 0.05$ ,  $r = -0.524$   $p < 0.01$ , and  $-0.744$   $p < 0.001$  respectively).

When the values measured for left and right ears were considered together, air-bone gap at 500 Hz, 1,000 Hz, 2,000 Hz, and 4,000 Hz were all found to be negatively correlated with erythrocyte SOD activity ( $r = -0.433$

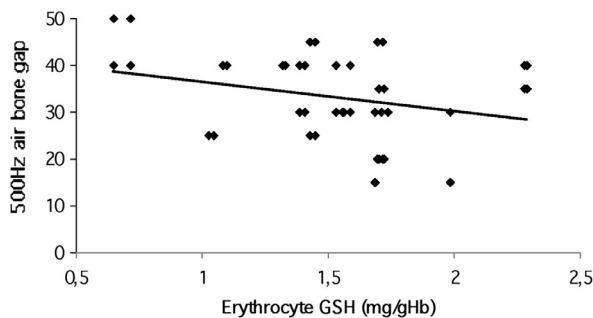
**Table 1.** SOD levels and GSH concentrations of MEEs and erythrocyte in OME patients

SOD		GSH	
MEE (n=48)	Erythrocyte (n=26)	MEE (n=48)	Erythrocyte (n=26)
$4.73 \pm 3.10$ U/ml	$1017.51 \pm 196.32$ U/g Hb	$74.79 \pm 41.66$ mg/dl	$1.49 \pm 0.39$ mg/g Hb

MEE: indicates middle ear effusions; (Values are means (SD)).

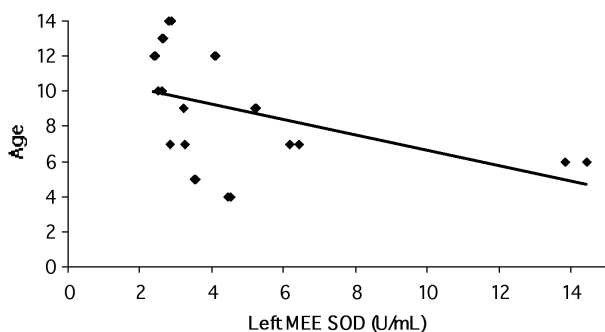
$p < 0.01$ ,  $r = -0.313$   $p < 0.05$ ,  $r = -0.391$   $p < 0.01$ , and  $r = -0.512$   $p < 0.001$ , respectively).

When the values measured for left and right ears were considered together, air-bone gap at 500 Hz was found to be negatively correlated with erythrocyte GSH content ( $r = -0.298$   $p < 0.05$ ) (Figure 2).



**Figure 2.** Correlation between erythrocyte GSH content and air-bone gap at 500 Hz in OME patients ( $r = -0.298$   $p < 0.05$ ) when the values measured for left and right ears were considered together.

Age was found to be correlated with air bone gap at right ear at both 500 Hz and 1,000 Hz ( $r = 0.404$   $p < 0.05$  and  $r = 0.462$   $p < 0.05$  respectively); at left ear at 500 Hz, 1,000 Hz, 2000 Hz and 4,000 Hz ( $r = 0.678$   $p < 0.001$ ;  $r = 0.405$   $p < 0.05$ ;  $r = 0.580$   $p < 0.01$  and  $r = 0.617$   $p < 0.001$  respectively). Age was found to be negatively correlated with SOD activity of left ear MEE ( $r = -0.628$   $p < 0.001$ ) (Figure 3).



**Figure 3.** Correlation between SOD activity of left ear MEE and age in OME patients ( $r = -0.628$   $p < 0.01$ ).

## Discussion

Middle ear mucosa originates from the same ectoderm as upper airway mucosa<sup>[16]</sup>. OME may be an extension

of upper respiratory disease such as adenoiditis, rhinosinusitis<sup>[17, 18]</sup>. Pathophysiological characteristics and immunologic behaviour are very similar. The immune defense system of middle ear mucosa produces various chemical entities during the inflammation. Among these soluble and cellular immunological elements, oxygen-derived free radicals with strong bactericidal activity, have recently attracted the attention of otologists. The protective roles of these destructive free oxygen radicals are limited by the antioxidant components of the host cell whose functions are to avoid the oxidative tissue damage. The imbalance of reciprocal activity might be related to the prognosis of OME.

The presence of oxygen derived free radicals in experimental OME has been described before Kanawa et al.<sup>[19]</sup> measured the concentration of superoxide anions in middle ear effusions in an animal model of pneumococcal otitis media. Parks et al.<sup>[20]</sup> reported that two different markers of lipoxidative tissue damage, lipid hydroperoxide and malondialdehyde, were significantly higher in pneumococci infected middle ear mucosa of guinea pigs when compared to normal middle ear mucosa.

Ovensen and Borglum<sup>[5]</sup> demonstrated that the SOD activity of left and right ears of the same individual displayed significant differences. Shigemi et al.<sup>[6]</sup> showed higher values for SOD activity in MEEs than in plasma and found no statistical correlation between them. We in this study report data on both SOD and GSH concentrations measured in both MEEs and erythrocytes together with the recorded hearing loss values. We observed that both SOD and GSH were related with hearing loss in OME patients. We noted a positive correlation of hearing loss with MEE SOD activity whereas a negative correlation with both erythrocyte SOD and GSH values. These findings reflect the potential of the antioxidant defense at the site of inflammation whereas attenuation in the erythrocytes. The correlation was strong at 500Hz, 1,000Hz and 2,000Hz. Considering the topographic organization of the cochlear spiral ganglion, the

correlation between function of cochlear apex and concentrations of the two antioxidant parameters at MEE and erythrocyte was found. We believe further research is required to understand the correlation better.

We observed that hearing loss was positively correlated with age in OME patients and also noted a significant decline with age in MEE SOD activity. Insertion of ventilation tube (VT) is the main surgical treatment for persistent middle ear effusions. As oxygen-derived free radicals are increased in hyperoxic conditions <sup>[5,21]</sup> VT treatment might be ineffective for MEEs which have low SOD activity.

We observed a positive correlation between GSH concentrations measured in MEEs and erythrocytes, and a negative correlation of hearing loss with erythrocyte GSH values. The former correlation was considered important because it implies that GSH treatment could be considered for the management of chronic OME. Testa et al. <sup>[1]</sup> reported that GSH-treated OME patient group which received 600 mg glutathione in 4 mL saline per day subdivided into five 2-minute administrations given by nasal aerosol every 3 or 4 waking hours for 2 weeks, showed otoscopic, tympanometric and audiometric improvement (66.6%) after a 3-months follow up when compared to saline-treated patient group (8%). N-acetylcysteine is known as a mucolytic drug with antioxidant properties, increasing the concentrations of GSH in plasma and the airways <sup>[22,23]</sup>. Antioxidant capacity improvement by using N-acetylcysteine for bronchoalveolar diseases have been described by Bridgeman et al. <sup>[22]</sup>.

Clearly, the association of hearing loss in OME with SOD activity warrants further research for better understanding and thus the treatment of the disease. We plan future studies questioning the clinical significance of measuring SOD activity in the effusions for assessing the risk of oxidative stress subsequent to hyperoxia when considering insertion of ventilation tube. According to our study we can suggest using N-acetylcysteine to treat OME especially in the elderly patients.

It is hoped that the study will provide a useful step for treatment of OME. Well defined prospective clinical studies are needed.

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