The role of free oxygen radicals on the development of otitis media with effusion

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Effusion;
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Summary  Objective: The purpose of this study was to determine if free oxygen radicals (FORs) and antioxidant enzyme activities have some role in pathogenesis of otitis media with effusion (OME) in children with adenoid hyperplasia. Methods: Seventy-four patients were enrolled in three groups of this study. The study group (Group I) included 26 patients who had adenoidectomy with ventilation tube placement due to chronic OME. The control adenoid group (Group II) consisted of 28 age-matched patients who had adenoidectomy without ventilation tube insertion. Twenty children were included in the healthy control group (Group III). Erythrocyte malondialdehyde (MDA) levels, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) enzyme activities were investigated in the venous blood sample. Results: Erythrocyte MDA level and GSH-Px enzyme activity in the blood samples of study group (Group I) were significantly higher than those of Group II and Group III (P < 0.05). SOD enzyme activity in the blood samples of Group I was significantly lower than Group II (P < 0.05), and were significantly higher than Group III (P < 0.05). CAT enzyme activity of Group I was significantly lower than that of Group III (P < 0.05). However, there was no statistically significant difference between Group I and Group II regarding CAT antioxidant enzyme activity (P > 0.05). Conclusions: The inflammation of the middle ear increases the level of FORs in erythrocyte. FOR level is normally maintained at a steady state by antioxidant enzymes. When the antioxidant defense system is weakened, the increased FORs may contribute to OME formation. We supposed that, antioxidant vitamins C and E, and scavenger enzymes such as CAT, SOD and GSH-Px may be added in the management of OME. © 2004 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Free oxygen radicals (FORs) are very reactive molecules because of their unpaired electrons. FORs are also produced in many physiologic con-

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2. Materials and methods

Seventy-four patients were enrolled to this study in the Department of Otorhinolaryngology, School of Medicine, Süleyman Demirel University. The study group (Group I) included 26 patients who had adenoïdectomy with ventilation tube placement (15 bilateral and 11 unilateral) due to chronic OME. The control adenoid group (Group II) consisted of 28 age-matched patients who had adenoïdectomy without ventilation tube insertion. Twenty children (circumcision or screening for vaccination) were included in the healthy control group (Group III). Patients were excluded if they had adenoïdectomy with tonsillectomy, acute otitis media, acute upper respiratory tract infection within the last 3 months, nasal polyp, craniofacial problems such as cleft palate, repeated VT insertion and dry tap at operation. Total IgE levels and history of patients revealed no atopy in all groups.

Diagnosis was based on at least three successive monthly examinations with otomicroscopy, tympanometry and if possible, audiometry. Chronic OME was defined as persistent evidence of effusion by otoscopy, and/or tympanogram with a persistent conductive hearing loss, after 3 months of adequate medical therapy including amoxicillin-clavulanate, systemic decongestants and anti-inflammatory agents. All the patients from both groups (Groups I and II) were operated under general anesthesia. The study group (Group I) comprised of the patients undergone ventilation tube (Paparella type, 1.27 mm i.d., silicone) insertion to tympanic membrane (into the antero-inferior quadrant of tympanic membrane) after the fluid aspiration from myringotomy ostium. Indications for the adenoïdectomy in the control adenoid group (Group II) were obstructed nasal airway and snoring. In this group, there was no suspected OME finding by otoscopic, otomicroscopic and tympanometric evaluation. The healthy control group (Group III) was consisted of children who admitted to the hospital for circumcision or screening for vaccination. These completely healthy subjects were also evaluated by otoscopic, otomicroscopic and tympanometric methods.

The study was approved by faculty ethical committee, and informed consents were taken from parents of each patient.

2.1. Blood study

Venous blood samples of all subjects were taken 5 days before operation for routine preoperative investigations (in the morning, following 12 h fasting). All the blood samples were collected in vacuum containers containing ethyleneaminetetraacetic acid (EDTA). Erythrocyte pellets were obtained immediately from the blood by centrifuging at 3000 x g for 10min at +4°C. Plasma and buffy coat were then removed and the erythrocytes were washed three times in 5 ml of cold 0.9% NaCl solution. Erythrocytes were hemolysed by adding cold distilled water. Erythrocyte hemolysate samples were stored at −80°C until assay.

MDA, as a marker for lipid hydroperoxide, was determined by the double heating method of Draper

GSH-Px (EC 1.11.1.9). GSH-Px also reduces organic peroxides into their corresponding alcohols [2]. When the balance between FORS production and the antioxidative defense mechanism is disturbed, the level of FORs increases, which finally leads to tissue damage. Radiation, oxygen toxicity, ischemia-reperfusion damage, infections and inflammation are some of the causes of increased FORs production [3,4]. The increased production of FORs in inflammation is due to the presence of leukocytes in the affected area [5].

FORS that have short life span can be neutralized with antioxidative defense mechanism in the site produced. Serious effects of FORs on tissues occur by their interactions with macromolecules. The most important mechanism of tissue damage caused by FORs is the production of lipid peroxides produced in the cell membrane [3,5]. Starting with an attack of FORs on polyunsaturated fatty acids, lipid peroxidation occurs through all membrane as a chain reaction. This, in turn, impairs the membrane permeability and fluidity, and results in functional and structural disorders and even cell death [6,7]. Vitamin E localized in cell membrane plays an important role to break the chain reaction. For this reason vitamins C and E administration in infections can be useful for preventing the FORs damage [3,8]. The determination of malondialdehyde (MDA), a lipid peroxidation product, is considered a sign of tissue damage [5].

It has been shown that FORs are important mediators of the inflammatory processes such as otitis media, maxillary sinusitis, degenerative joint disease and destructive lung disease [9–12]. Although it is known that adenoid hyperplasia affects the development of otitis media with effusion (OME), why does it not develop in some patients with adenoid hyperplasia? Do the antioxidative mechanisms such as SOD, CAT and GSH-Px in the blood of these patients affect this process?

The purpose of this study was to determine if FORs and antioxidant enzyme activities have some role in pathogenesis of OME in children with adenoid hyperplasia.
and Hadley [13]. The principle of the method was spectrophotometric measurement of the color produced during the reaction of thiobarbituric acid (TBA) with MDA. For this purpose, 2.5 ml of 100 g/l trichloroacetic acid (TCA) solution was added to 0.5 ml erythrocytes in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling under tap water, the mixture was centrifuged at 1000 x g for 10 min, and 2 ml of the supernatant was added to 1 ml of 6.7 g/l TBA solution in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled with tap water and its absorbance was recorded using a spectrophotometer (Shimadzu UV-1601, Shimadzu Corporation, Kyoto, Japan) at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex (1.58 x 10^5 cm^-1 M^-1).

The measurement of SOD was based on the method of Woolliams et al. [14]. It was based on the principle in which xanthine reacts with xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction.

CAT activity was measured according to the method of Aebi [15]. The principle of the assay is based on the determination of the rate constant (k (s^-1)) of hydrogen peroxide decomposition by catalase enzyme. The rate constant was calculated from the following formula: k = (2.3/A2t1/2b)(logA1/A2).

This formula, A1 and A2 are the absorbance values of hydrogen peroxide at t1 (0ths) and t2 (15th s) times, a is the dilution factor, and b is the hemoglobin content of erythrocytes.

The determination of GSH-Px activity was based on the method of Paglia and Valentine [16]. The principle of the method was as follows: GSH-Px catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance of NADPH was measured at 340 nm.

2.2. Statistical analysis

Statistical analysis of data was performed by Mann–Whitney U-test.

3. Results

The study group (Group I) consisted of 26 patients (17 males and 9 females) ranging in age from 4 to 14 years (the mean age was 7.7 ± 2.7). The control adenoid group (Group II) consisted of 28 patients (12 males and 16 females) ranging in age from 3 to 11 years (the mean age was 6.8 ± 2.6). The healthy control group (Group III) consisted of 20 subjects (12 males and 8 females) ranging in age from 4 to 11 years (the mean age was 7.4 ± 2.3). There was no statistically significant difference among all three groups with respect to the age and gender (P > 0.05).

The mean, standard deviations, median and minimum–maximum values of erythrocyte MDA level, SOD, CAT and GSH-Px enzyme activities in the blood samples of all groups and statistical results have been shown in Tables 1 and 2. Erythrocyte MDA level and GSH-Px enzyme activity in the blood samples of study group (Group I) were significantly higher than those of the control adenoid (Group II) and the healthy control group (Group III) (P < 0.05). SOD enzyme activity in the blood samples of the study group (Group I) was significantly lower than those of the control adenoid group (Group II) (P < 0.05), and were significantly higher than the healthy control group (Group III) (P < 0.05). CAT enzyme activity of the study group (Group I) was significantly lower than those of the healthy control group (Group III) (P < 0.05). However, there was no statistically significant difference between Group I and Group II regarding CAT antioxidant enzyme activity (P > 0.05).

4. Discussion

OME is a multi-factorial, common, asymptomatic and silent disease, especially during infancy [17]. Although the clinical importance of OME is well recognized, the precise pathogenesis of the disease process is unknown and the condition has provoked considerable controversy [18]. Adenoid hyperplasia plays an important role in the etiology of OME [19]. Adenoidectomy has been shown to be effective in improving the resolution rate of OME [20,21], but whether this is because it removes a physical obstruction of the Eustachian tube, or a source of ascending infection is a matter for debate.

Adenoid hyperplasia may contribute to the formation of OME in several ways. Two of them are mechanical obstruction due to increased adenoid mass or local inflammatory reaction in the eustachian tubes and the middle ear caused by release of inflammatory mediators [22,23]. The studies of eustachian tube function on OME patients reported that abnormalities of eustachian tube function are not confined to children with OME [24,25].
<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Median (min–max)</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>Age</td>
<td>2.7 ± 2.7</td>
<td>7.0 (4.0–14.0)</td>
<td>6.8 ± 2.6</td>
</tr>
<tr>
<td>MDA (nmol/g Hb)</td>
<td>145.1 ± 59.8</td>
<td>163.8 (45.0–253.5)</td>
<td>104.6 ± 69.8</td>
</tr>
<tr>
<td>SOD (U/g Hb)</td>
<td>3973.2 ± 163.6</td>
<td>4021.7 (3433.3–4106.7)</td>
<td>3976.0 ± 64.2</td>
</tr>
<tr>
<td>CAT (k/g Hb)</td>
<td>192.1 ± 66.4</td>
<td>175.0 (95.0–320.0)</td>
<td>213.9 ± 88.8</td>
</tr>
<tr>
<td>GSH-Px (U/g Hb)</td>
<td>50.4 ± 12.9</td>
<td>51.3 (31.4–69.7)</td>
<td>43.1 ± 12.2</td>
</tr>
</tbody>
</table>
Table 2  Statistical results of FOR level, antioxidant enzyme activities and ages of all groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>i–II</th>
<th>I–III</th>
<th>II–III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.421</td>
<td>0.884</td>
<td>0.553</td>
</tr>
<tr>
<td>MDA (mmol/g Hb)</td>
<td>0.004*</td>
<td>0.002*</td>
<td>0.754</td>
</tr>
<tr>
<td>SOD (U/g Hb)</td>
<td>0.048*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>CAT (U/g Hb)</td>
<td>0.267</td>
<td>0.035*</td>
<td>0.516</td>
</tr>
<tr>
<td>GSH-Px (U/g Hb)</td>
<td>0.028*</td>
<td>0.019*</td>
<td>0.966</td>
</tr>
</tbody>
</table>

*Mann–Whitney U-test (P < 0.05 was significant).

Some patients suffering from adenoid hyperplasia are known to develop OME in contrary to the others who never develop OME even suffering from large adenoid hyperplasia. Gates et al. [26–28] reported a series of articles in which adenoidlectomy has been considered in the initial surgical management of children with OME that is refractory to medical management. In those studies, it has been stated that the size of the adenoid cannot be used as a criterion for adenoidectomy in these patients. They suggested that reduction of the adenoidal bacterial reservoir might be the mechanism whereby adenoidectomy is effective. Inoue et al. [29] reported that adenoid tissue of patients with OME seem to be infectious foci, aggravating immune reaction which might attack the middle ear through an ascending route. According to these studies, for the formation of OME, what is important is the inflammation of adenoid tissue not its volume. This could also partially explain why some patients with adenoid develop OME while others do not.

Besides the well-known ways of formation of OME, we consider that FORs may influence the formation of OME. Higher levels of FORs and antioxidant enzyme activity disorder in patients with adenoid hyperplasia may contribute to formation of OME in these patients.

Some studies with a guinea pig model of otitis media have demonstrated evidence of FOR damage to the middle ear mucosa. Parks et al. [3] reported that lipid hydroperoxide and mucosal MDA levels increased in experimental otitis media and FORs in the mucosa might cause tissue damage by lipid peroxidation. Doner et al. [11] established that serum and mucosa MDA levels increased in experimental otitis media. Takoules and Haddad [30] demonstrated the presence of lipid hydroperoxide in middle ear fluid from children with chronic otitis media, and FORs may contribute to inflammatory damage in human otitis media. Our pilot study was conducted to examine erythrocyte MDA levels for peroxidation of lipids as evidence of free radical damage. This is the first study to document the blood levels of free radicals on pathogenesis of OME. In our study, erythrocyte MDA levels and GSH-Px enzyme activities in the blood samples of study group (Group I) were significantly higher than those of the control adenoid group (Group II) (P < 0.05).

SOD enzyme activities in the blood samples of study group (Group I) were significantly lower than those of the control adenoid group (Group II) (P < 0.05). There was no statistically significant difference between Group II and Group III regarding erythrocyte MDA levels, CAT and GSH-Px antioxidant enzyme activities (P > 0.05). These results showed that antioxidant enzyme activity disorder tend to produce FORs. Increased levels of FORs may be responsible for the damage occurring in middle ear and partly for the late morbidity.

Whatever the cause, the inflammation of the middle ear increases the level of FORs in erythrocyte. FOR level is normally maintained at a steady state by antioxidant enzymes. When the antioxidant defense system is weakened, the increased FORs may contribute to OME formation. Although antibiotics and ventilation tubes are mainstays for treatment of OME, they do not specifically treat tissue damage due to FORs. We supposed that, antioxidant vitamins C and E and scavenger enzymes such as CAT, SOD and GSH-Px may be added in the management of OME. Further investigations into the role of therapy directed against FOR need to be conducted.

References