Oxidative stress and enzymic–non-enzymic antioxidant responses in children with acute pneumonia

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In this article, oxidative stress and enzymic–non-enzymic antioxidant status were investigated in children with acute pneumonia. Our study included 28 children with acute pneumonia and 29 control subjects. The age ranged from 2 to 11 years (4.57 ± 2.13 years) and 2 to 12 years (4.89 ± 2.22 years) in the study and control groups, respectively. Whole blood malondialdehyde (MDA) and reduced glutathione (GSH), serum β-carotene, retinol, vitamin C, vitamin E, catalase (CAT), ceruloplasmin (CLP), total bilirubin, erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels were studied in all subjects. There was a statistically significant difference between the groups for all parameters except for serum CAT. Whole blood MDA, serum CLP and total bilirubin levels were higher in the study group than those of the control group. However, SOD, GPx, β-carotene, retinol, vitamin C, vitamin E and GSH levels were lower in the study group compared with the control group. All antioxidant vitamin activities were decreased in children with acute pneumonia. Our study demonstrated that oxidative stress was increased whereas enzymic and non-enzymic antioxidant activities were significantly decreased in children with acute pneumonia. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS—antioxidant; oxidative stress; pneumonia; child; reactive oxygen species

INTRODUCTION

Toxicity from oxygen metabolites released by stimulated neutrophils, macrophages, and other cells has been proposed as one of the significant mechanisms of lung injury.1 Reactive oxygen species (ROS) in the form of the O2−, H2O2 and the hydroxyl radical (OH•) cause damage to DNA, lipids and proteins. These species are produced by bacteria during aerobic respiration and by phagocytic cells when they encounter bacteria as part of the host defence against infection. It is known that the development of many inflammatory processes is accompanied by an increased activity of free radicals.2 Oxidative stress results from an oxidant/antioxidant imbalance, an excess of oxidants or a depletion of antioxidants. Oxidative stress is thought to play an important role in the pathogenesis of a number of lung diseases, not only through direct injurious effects, but by involvement in the molecular mechanisms that control lung inflammation.4 By-products of lipid peroxidation (LPO) formed in various biochemical reactions are normally scavenged by antioxidants. Antioxidants are compounds that are involved in effective scavenging of free radicals and in suppressing the actions of reactive oxygen substances.4 Pulmonary antioxidant defences are widely distributed and include both enzymic and non-enzymic systems. The major enzymic antioxidants are superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). β-Carotene, vitamin C, vitamin E, reduced glutathione (GSH), ceruloplasmin (CLP), and bilirubin are some of the non-enzymic factors that may function as antioxidants.7

Acute lower respiratory tract infections (ALRI) present as important public health problems in many developing countries. In these countries, ALRI are among the most important causes of morbidity and

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mortality in children, particularly those younger than 5 years. Each year an estimated 3.8 million children die from ALRI, principally pneumonia, worldwide. Differences in the population demographics of developed and developing countries further affect the spectrum and burden of pediatric respiratory tract infections. Children younger than 15 years account for approximately 45% of the total population in developing countries, compared with 22% in developed countries. 6,8

In this article, oxidative stress and enzymic–nonenzymic antioxidant status were investigated in children with acute pneumonia to determine the possible role of their status in the disease. Even though there is a large body of literature regarding the status of individual antioxidants in pneumonia, there is, to our knowledge, no study which evaluates the enzymic and non-enzymic antioxidants status in the disease. We considered that evaluation of the status of these two antioxidants together in the patients with pneumonia may be valuable in order to comprehend the overall antioxidant status in the disease.

SUBJECTS AND METHODS

The study included 28 children with acute pneumonia and 29 control subjects, who were admitted to Yüzüncü Yıl University, Faculty of Medicine Department of Pediatrics. The diagnosis of pneumonia was made by a pediatrician after taking a detailed history and examining the children for signs of respiratory infection. Pneumonia was defined as fever, cough, chest pain, crackling sounds, or dullness to percussion and at least two respiratory distress signs, including the following: tachypnoea (respiratory rate>60, 50, and 40 breaths min⁻¹ in children<2 months of age, between 2 and 12 months of age, and>12 months of age, respectively), and nasal flaring or intercostal retractions within less than 72h of evolution. 9 Chest radiograms were used to confirm the clinical diagnosis. The control group consisted of healthy children with normal physical examination. None had a history of recurrent or recent infection.

The patients with pneumonia and healthy control subjects were recruited into the study after obtaining their parents’ informed consent. Whole blood was collected into heparinized tubes and whole blood malondialdehyde (MDA) and GSH levels were studied on the day of admission. Blood was also collected into a polyethylene microtube and after clotting, this was centrifuged at 4000 r.p.m. for 7 min and the serum was removed using EDTA-washed Pasteur pipettes. The serum was stored in polystyrene plastic tubes at 20°C until the time of analysis. Serum β-carotene, retinol, vitamin C, vitamin E, CAT, CLP, total bilirubin and erythrocyte SOD and GPx levels were studied within 6h of admission. Whole blood MDA concentration was determined by using the method described by Jain et al. 10 and based on thiobarbituric acid reactive substances (TBARS). 10 Erythrocyte SOD and GPx activities were studied on hemolysates by using commercial kits (Randox). 11,12 Serum CAT level was studied according to the methods of Gott. 13 The levels of β-carotene and retinol were detected by the methods of Suzuki and Katoh. 14 Serum vitamin C level was determined after derivatization with 2,4-dinitrophenylhydrazine. 15 Vitamin E was analysed colorimetrically with 2,4,6-tripridyl-s-triazin and ferric chloride after extraction with absolute ethanol and xylene. 16 GSH concentration was measured spectrophotometrically. 17 Serum CLP activity was determined by using a modified Ravin Method. 18 Serum total bilirubin levels were measured by using commercial kits for an automa lysar (Roche). The same parameters were also studied in the control subjects. All values are expressed as mean±SD. The test of significance between the two groups was estimated by Student’s t-test.

RESULTS

Our study included 28 (15 male, 13 female) children with acute pneumonia and 29 (16 male, 13 female) control subjects. The age ranged from 2 to 11 years (4.57±2.13 years) and 2 to 12 years (4.89±2.22 years) in the study and the control group, respectively. There was no statistically significant difference between the groups for sex and age (p>0.05). Table 1 shows the whole blood MDA (indicator of lipid peroxidation), erythrocyte SOD and GPx levels and serum CAT enzyme antioxidant levels in healthy subjects and patients with acute pneumonia. LPO as

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Whole blood MDA (indicator of lipid peroxidation), erythrocyte SOD and GPx and serum CAT (enzymic antioxidants) levels in the patient and control groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute pneumonia (n=28)</td>
</tr>
<tr>
<td>MDA (nmol/mL⁻¹)</td>
<td>1.60±0.6</td>
</tr>
<tr>
<td>SOD (U/mg⁻¹)</td>
<td>6.45±0.4</td>
</tr>
<tr>
<td>GPx (U/mg⁻¹)</td>
<td>3.33±0.4</td>
</tr>
<tr>
<td>CAT (KU⁻¹)</td>
<td>8318±1768</td>
</tr>
</tbody>
</table>

Data are the mean±SD. p values were calculated using Student’s t-test. MDA, malondialdehyde; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase.
Table 2. Serum and whole blood (GSH) non-enzymatic antioxidants levels in the patient and control groups

<table>
<thead>
<tr>
<th></th>
<th>Acute pneumonia (n = 28)</th>
<th>Control (n = 29)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-carotene (ug dl⁻¹)</td>
<td>11.94 ± 3.1</td>
<td>17.44 ± 1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Retinol (ug dl⁻¹)</td>
<td>23.82 ± 4.2</td>
<td>32.81 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ascorbic acid (mg dl⁻¹)</td>
<td>1.19 ± 0.3</td>
<td>1.29 ± 0.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>α-tocopherol (mg dl⁻¹)</td>
<td>0.60 ± 0.2</td>
<td>0.71 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GSH (mg dl⁻¹)</td>
<td>25.19 ± 4.8</td>
<td>34.38 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CLP (mg)</td>
<td>63.76 ± 5.3</td>
<td>39.44 ± 3.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total bilirubin (ug dl⁻¹)</td>
<td>0.47 ± 0.12</td>
<td>0.31 ± 0.2</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Retinol, vitamin A; ascorbic acid, vitamin C; α-tocopherol, vitamin E; GSH, reduced glutathione; CLP, ceruloplasmin.

LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN PNEUMONIA

Evidenced by MDA, was markedly increased in the children with acute pneumonia as compared to the control group (p < 0.001). SOD and GPx enzymatic antioxidants were significantly decreased in children with acute pneumonia as compared to the control group whereas for CAT levels, no significant changes between the groups were detected. Serum β-carotene, retinol, vitamin C, vitamin E, CLP, total bilirubin and whole blood GSH non-enzymatic antioxidant levels in all of the subjects are shown in Table 2. All antioxidant vitamin activities such as β-carotene, retinol, vitamin C and vitamin E were decreased in the study group. Additionally, GSH levels were also found to be decreased in the patient group. Certain other non-enzymatic antioxidants including CLP and total bilirubin levels were elevated in the study group compared to the control group. As seen in the tables, there was a statistically significant difference between the groups for all parameters except for serum CAT.

DISCUSSION

ALRI continues to be a major cause of morbidity and mortality in children in developing countries. ALRI accounts for nearly one-third of all deaths of children under 5 years in many developing countries. Pneumonia is responsible for most of these deaths.

TBARS and free radicals have been reported to be increased in inflammatory lung disorders such as pneumonia. Kohli et al. investigated the effect of LPO after antioxidant therapy in patients with acute pneumonia. Whole blood LPO levels were found to be increased in the acute and subacute periods, whereas they were reported to be decreased in the clinical recovery period. TBARS (mainly MDA) are recognized as end-products of polyunsaturated fatty acid peroxidation; however, they are also formed during oxidative injury of DNA, proteins, or carbohydrates. In the present study, we found increased TBARS concentrations that indicate oxidative stress in children with acute pneumonia. Our findings also suggest that oxidative stress occurs in children with acute pneumonia.

Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems, leading to cell membrane impairment or DNA damage. Antioxidant depletion or deficiency may contribute to oxidative stress. Antioxidants protect cells against oxidative injury and prevent the production of oxidation products, such as 4-hydroxy-2-nonenal or MDA, which are able to induce protein damage, apoptosis or release of pro-inflammatory mediators, such as cytokines. In our study, enzymic and non-enzymic antioxidants were decreased except for serum CLP and total bilirubin in the study group compared to the control group. Therefore, it is suggested that the antioxidant defences are compromised, and the tissues become very susceptible to the oxidative damage as the ROS levels increase.

Many investigators have measured the major enzymic or non-enzymic antioxidants in patients with pneumonia. All of these studies show a depletion of enzymic and non-enzymic antioxidants.

Three major antioxidant enzymes that are involved in the degradation of ROS are SOD, which degrades superoxide, and GPx, and CAT, which degrade hydrogen peroxide. Several authors have reported decreased blood SOD and GPx activity in patients with pneumonia. Cui et al. measured plasma GPx level and white blood cell count in 57 patients with pneumonia and in 85 control children under 10 years old. They reported decreased GPx levels in the pneumonia group. In the present study, decreased activities of SOD and GPx were found whereas CAT levels remained unchanged following oxidative stress. Overexpression of SOD and GPx could be used protectively against exacerbation of oxidative stress. From the unchanged CAT levels, it can be speculated that CAT is not as effective as the GPx since the latter enzyme is much more active than the CAT in most antioxidant reactions. Even though some investigators and our present study revealed decreased SOD and GPx levels in pneumonia, our two previous studies and those of Shukla et al. showed increased SOD, GPx and CAT levels in several infectious diseases such as meningitis, encephalitis, otitis media and tonsillitis. Similarly to Park et al., our present study showed that CAT levels remained unchanged in diseases such as experimental otitis media and pneumonia.
these findings, we speculate that the reason for these controversies may be the varying conditions such as whether the onset of disease occurs spontaneously or is induced experimentally, whether it develops acutely or chronically, its degree of severity and the types of diseases. Having seen the different responses depending on the varying conditions, it is tempting to say that SOD and GPX initially react against the oxidative products while CAT remains unchanged and, following this depletion in SOD-GPx, CAT comes into play for coping with the oxidants. During the CAT-active period meanwhile, the prime enzymes (SOD and GPx) regain their activities to play their roles in oxidative stress.

Vijayamalini and Manoharan reported that plasma vitamin C, vitamin E, and GSH activities were greatly decreased in patients with pulmonary tuberculosis, but were accompanied by increased concentrations of TBARS. They suggest that the elevated LPO and decreased vitamin C, vitamin E, and GSH levels indicate the potential of oxidative damage. Kohil et al. measured serum vitamin E and CLP levels in patients with acute pneumonia. They reported a decrease in vitamin E level and an increase in CLP level. Reves et al. investigated vitamin A deficiency in children. They identified vitamin A deficiency in 17.8% of children. Velasquez-Melendez and colleagues studied plasma vitamin A level, carotenoids and retinol binding protein in children with acute upper respiratory infection, pneumonia and diarrhoeal diseases. They reported that plasma vitamin A and retinol binding protein levels were decreased in diarrhoea and pneumonia groups and plasma carotenoids levels were lower in all groups than in the control groups. In our study, after having measured all antioxidant vitamin activities, it has been found that not only a few antioxidant vitamins but almost all vitamins were affected by the oxidative challenge of the disease. Moreover antioxidant vitamin deficiency can result in a decreased immune response, phagocytosis and killing of bacteria. In this study we found that GSH levels were lower in patients with acute pneumonia than among the control subjects. GSH depletion may be due to free radicals and ROS generation. Sobol and Pyda investigated the levels of copper and CLP in infants with pneumonia. They showed that copper and CLP levels were significantly higher in the study group than in the controls. In the present study, the CLP level was found to be increased, which may be due to the scavenging action on peroxyl radical. In this study, we determined that serum the total bilirubin level was increased. We hypothesized that elevated serum bilirubin levels might be the result of increased oxidative stress.

In conclusion, a significant decrease in the enzymic non-enzymic antioxidant capacity of serum and erythrocytes, and an increase in whole blood LPO products was detected in children with acute pneumonia. It is suggested that oxidant/antioxidant balance is abnormal in pneumonia and oxidative stress plays a role in worsening the inflammation.

REFERENCES


