Evaluation of the Serum Antioxidant Status in Asthmatic Children

MEN-FEN LIAO1, CHUN-CHUAN CHEN2, MING-HUI HSU3

Many studies have shown the balance between the oxygen reactive species (ROS) and the antioxidant capacities, and that the massive ROS generation could lead to cell damage and diseases such as atherosclerosis, aging and cancer. Changes in antioxidant capacity like free radicals scavenging antioxidant agents such as vitamin E, C contents, serum concentrations of bilirubin, uric acid, albumin and antioxidant enzyme systems like SOD, and GPx activities have been described to be related to many diseases. However, the research on chronic airway inflammatory disease and the antioxidant defense system is still not enough. Understanding of the antioxidant status and antioxidant enzymes in asthmatic patients is still unclear.

In the present controlled study, we investigated the total antioxidant status (TAS) in serum and the antioxidant enzyme (total SOD and GPx) activities in 46 asthmatic children and 32 normal controls. The serum level of TAS in asthmatic children was significantly lower than the controls. The SOD concentration in asthmatic children was higher than the control, however the GPx was much lower than the control children, though it was not statistical significance.

In conclusion, these results suggested the existence of higher oxidative stress and reactive oxygen species (ROS) in asthmatic children, and that the antioxidant capacities in asthmatic children were altered. If the production of ROS was persistent, it would result in chronic inflammation and the imbalance of oxidative-reductive status in those patients. (Acta Paediatr Tw 2004; 45:213-7)

Key words: asthma, total antioxidant status

INTRODUCTION

The free radicals theory is one of the hypotheses to explain cell apoptosis. Free radicals are quite unstable chemical species carrying unpaired electrons that perform strong oxidation and bring the organism into the state of oxidative stress. Oxidative stress has been implicated in a large number of diseases, especially cardiovascular diseases such as coronary heart disease, atherosclerosis, rheumatoid arthritis, lupus, multiple sclerosis, allergy and chronic respiratory disease, and even cancer and aging.1 However, the human possesses efficient antioxidant defense mechanisms against the toxic effects of free radicals. These include the enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione reductase reduced glutathione, and nonenzymatic antioxidant capacity in serum, including α-tocopherol, ascorbic acid, selenium, bilirubin, serum albumin and pigments, etc.2

Asthma is a worldwide disease. Its prevalence varies among countries, from less than 3% to as high as 30%. In past years, the prevalence and severity been noticed to be increasing in Taiwan. According to a survey among the newly enrolled primary school children in Taipei city, the prevalence of asthmatic children is about 19.8% (Chang Chung Hospital 2001) compared with 10.7% reported in 1994 and 14.3% in 1996, respectively, by Professor Shin (Dr. Shin).3 Asthma is increasing recognized as a chronic airway inflammatory disease, a chronic, eosinophilic bronchiolitis and mediator-driven inflammatory process of the lungs. These mediators; such as prostaglandins, thromboxanes, leukotrienes etc., produce effects that are characteristic of asthma: bronchoconstriction, increased endothelial membrane permeability leading to airway edema and enhanced secretion of thick and viscous mucus. Asthma has a 50% to 60% familial heritability. Its pathogenesis is the development of chronic inflammation leading to
bronchial hyperresponsiveness and airway remodeling which is characterized by smooth muscle hypertrophy, submucosal edema, subepithelial fibrosis, basement membrane thickening and disruption of epithelial layer. 56 The remodeling process of airway in asthmatic patients is reported to be related to superoxide O₂⁻ production. Few articles mention that intrinsic nitric oxide (iNOS) are increased in asthmatic patients. Although nitric oxide (NO) is a relatively unreactive molecule, it may trigger asthmatic induction once its concentration overwhelms its neurotransmitter and second messenger functions, particularly in activated macrophage. NO reacts with superoxide (O₂⁻) generating peroxynitrite (ONOO⁻). Under oxidative stress, it activates the releasing of prostaglandins PGE and thromboxanes TXA2, and has a close relationship with airway inflammation and cell division.

The aim of this case-controlled study was to attempt to evaluate the influence of chronic inflammation on the activity of primary intracellular antioxidant enzymes SOD and GPx, serum macroantioxidants and the total antioxidant status (TAS) in asthmatic children and matched controls.

MATERIALS AND METHODS

Subjects

Our study comprised 98 children divided into two groups. Patients referred for the management of asthma at the outpatient clinic of Han-Ming Hospital were recruited for this study during November 2001 and December 2002. The experimental group consisted of 46 children aged from 5 to 9 years; they were diagnosed to be asthmatic by pediatricians on the basis of histories, symptoms and risk factors. According to the GINA (Global Initiative for Asthma Guidelines, 1998) classification of asthma severity, all these 46 asthmatic children were classified to be in step 2, mild persistent asthma classification. Before the study, children signed a subject consent by their parents. Volunteers with clinical and historical evidence of renal, hepatic, cardiac, and endocrine disease, hypertension, common cold, and respiratory disease beyond asthma were excluded. Subjects were not permitted to take medicines before the blood sampling including antihistamines, leukotriene antagonists, glucocorticoids, aspirin, antivitamins, any herbal drugs and antibiotics. The control group consisted of 52 newly enrolled primary schoolchildren, aging 6-9 years of age. Under the same exclusion criteria, they were clinically free from any allergic and hypersensitive diseases and no atopic family histories could be traced.

Methods

A single blood sample was drawn by venipuncture and separated serum stored under light tight conditions at -70°C. The median storage time for sera from both groups was 2 weeks. Albumin, bilirubin and uric acid in serum were assayed on automatic random access biochemistry analyzer (HITACHI 917). Total antioxidant status (TAS) was determined in heparinized plasma with Randox commercial kit, according the method of Miller et al. Incubation of ABTS+ with a peroxidase (memoglobin) resulted in production of the radical cation ABTS·+. This species was blue-green in colour and could be detected at 600 nm. Antioxidants in the added sample cause suppression of this colour production to a degree that was proportional to their concentration. This was a two-reagent assay and might use either serum or plasma. The activity of SOD was determined in hemolysates of heparinized blood with Ransod test (Randox, Antrim, UK). This method is based on the dismutation of toxic superoxide radical (O₂⁻) into hydrogen peroxide and molecular oxygen for this enzyme. The superoxide radicals produced react with p-aminothiophenol or semicarbazide to form a red formazan dye. SOD present in the sample is plated with the INT for superoxide radicals and inhibits the production of formazan dye. SOD was measured by the degree of inhibition of formazan dye formation.

The activity of selenium-dependent GPx was also quantified with Ransel commercial kit (Randox, Antrim, UK). GPx catalyzes the oxidation of glutathione with cumene hydroperoxide, which is transformed into reduced glutathione in the presence of glutathione reductase. 9-10 The concentration of GPx was assayed from the decrease in absorption at 340 nm due to oxidation of NADPH to NADP+. Serum level of albumin and uric acid were expressed as g/dl and mg/dl, respectively. TAS and enzyme activities were documented as mmol/l.

Statistics

The sampling data are presented in Mean±SD, analyzed by ANOVA using SPSS. The correlation and regression analyses of total antioxidant capacity in serum and other serum concentrations were also computed by SPSS. The P-value was obtained by independent paired t-test, and P<0.05 was considered to indicate statistical significance. Correlation between TAS and immunoglobulin E in the asthmatic group was obtained by Pearson correlation and Mann-Whitney test.

RESULTS

The characteristics of cases and controls were similar except the BMI data was higher in the asthmatic group.
(p<0.05) than the controls (Table 1). The final statistical analysis showed no significant differences between the cases and control groups in age, sex, body weight, body length and the living country. The results related to the influence of asthma on serum bilirubin, uric acid, albumin, TAS and on SOD, GPX in healthy and asthmatic children are presented in Table 2. The level of TAS was significantly decreased in the asthmatic group in comparison with the control group (p<0.001). The serum levels of some potent antioxidants such as bilirubin, albumin and uric acid were also lower in the asthmatic children than the controls, but the differences were not statistically significant (p=NS). However, the serum antioxidant enzymes activities such as SOD were significantly higher in asthmatic children than the controls (p<0.05). However, GPX was lower in the asthmatic group compared to the control group, even though that was not statistically significant (p=NS).

The immunological parameters between the patients and the control children, including complete blood count (CBC), eosinophil blood count and immunoglobulin E (IgE) concentration are documented in Table 3. Only serum concentration of immunoglobulin E in the asthmatic group was significantly higher than the control (p<0.05). But we could not find any significant correlation between the serum concentrations of TAS and IgE. TAS and the eosinophil blood count (Fig. 1).

DISCUSSION

Our results indicated the serum level of antioxidant capacity (TAS) in asthmatic children decreased in comparison with that of normal controls. It might result from persistent oxidative stress and lipid peroxidation in chronic inflammatory diseases such as asthma.11

Reactive oxygen species (ROS), free radicals, an integral part of human oxygen metabolism, due to their high potential to damage vital biological systems, have now been related to more than 100 diseases, including

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n=65)</th>
<th>Controls (n=52)</th>
<th>P-value</th>
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<tr>
<td>Age (yr)</td>
<td>6.05</td>
<td>6.70</td>
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<td>Sex (male: female)</td>
<td>25.21</td>
<td>24.28</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>21.35</td>
<td>22.20</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
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<td>121.25</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>15.97</td>
<td>18.58</td>
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Values are medians (interquartile range).

BMI: body mass index.

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<th>Cases (n=65)</th>
<th>Controls (n=52)</th>
<th>P-value</th>
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<tr>
<td>TAS (mmol/l)</td>
<td>1.189±0.093</td>
<td>1.338±0.241</td>
<td>&lt;0.001</td>
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<tr>
<td>Uric acid (g/dl)</td>
<td>4.1±1.103</td>
<td>4.05±1.63</td>
<td>NS</td>
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<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.41±0.17</td>
<td>0.45±0.18</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin (mg/dl)</td>
<td>4.26±0.16</td>
<td>4.35±0.16</td>
<td>NS</td>
</tr>
<tr>
<td>SOD (mmol/l)</td>
<td>978±403</td>
<td>740±275</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GPX (mmol/l)</td>
<td>4876±1932</td>
<td>5679±351</td>
<td>NS</td>
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Values are Mean±SD.

P-values are compared by independent paired t-test.

NS means not significant.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Controls (n=52)</th>
<th>P-value</th>
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<tr>
<td>CBC</td>
<td>8.28±3.81</td>
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<td>Eosinophil count</td>
<td>378.3±323.9</td>
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<tr>
<td>IgE (IU/L)</td>
<td>471.6±886.7</td>
<td>105.6±135.2</td>
<td>&lt;0.05</td>
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Antioxidant status in asthmatic children

Fig. 1. The correlation between TAS and serum IgE in asthmatic children.

Allergic disease. Change in lipid peroxidation production reactions and antioxidant defence systems were associated with changes in a variety of biochemical pathways. The human body has developed complex antioxidant systems to counteract free radicals and reduce their damage. These antioxidant systems included primary defenses in plasma such as vitamin E, vitamin C and β-carotene, bilirubin, uric acid, etc. Macromolecules such as albumin, ferritin, transferrin, ceruloplasmin, and the iron-scavenging proteins contribute to the total antioxidant capacity in plasma too. Various antioxidant scavenging enzymes in plasma such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) located in cytosol and mitochondrial matrix and selenium-dependent GPx associated with membranes reported by Ursini and colleagues acted as another defensive mechanism in humans against free radical-induced damage. Although it was well known that the lipid peroxidative product MDA (Malondialdehyde) correlated with oxidative state of cell membrane, acting as a biomarker of oxidative stress. Because all antioxidants acted separately and because of the interactions among different antioxidants species, several methods have been developed to assess the total antioxidant capacity of human serum or plasma. In this study, the total antioxidant status (TAS) was measured based on the absorbance of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ABTS under 600 nm light, proposed by Miller et al. in 1993. TAS represents various antioxidant activities in human plasma, albumin and urine being more efficient and albumin less active. Ascorbate, α-tocopherol, glutathione, cysteine, β-carotene and bilirubin also act as antioxidants in plasma. Applying this method, our study clearly indicates the plasma level of TAS in asthmatic children decreased in comparison with those of the healthy controls. We also measured some potent plasma antioxidant activities such as albumin, uric acid and bilirubin, and those antioxidants were both lower in the asthmatic group than the controls (Table 2). But the level of TAS was not significantly correlated with the immunoglobulin E (p = 0.084) in asthmatic children, perhaps due to their huge IgE variations.

The RBC’s SOD is often called the primary antioxidant defence; it dismutates of O2– to H2O2 and this enzyme prevents the further generation of free radicals. The higher concentration of SOD in asthmatic children may be reactive to the increasing production of superoxide O2–, resulted from persistent oxidative stress. Besides that, the concentration of SOD is usually regulated by the level of H2O2. However glutathione peroxidase (GPx), intracellularly located in the cytosol, mitochondrial matrix and membranes catalyzes the reduction of H2O2 and organic hydroperoxides. Both types of GSH-PX enzymes, selenium-dependent and selenium-independent, have been shown to protect against radical damage by reducing peroxides and effectively blocking lipid peroxidation in the presence of vitamin E. The activity of GPx in asthmatic children was lower, which indicated the activity of antioxidant enzymes in degradation of phospholipid of cell membrane was impaired. That suggested might be due to the damage from the free radicals resulted from persistent oxidative stress and tissue ischemia. The respiratory burst shown by activated granulocytes and production of O2– has already been alluded too. Granulocytes are capable of chemotaxis, and they congregate at sites of injury or infection. A large fraction of the O2– produced during the respiratory burst escapes from granulocytes; we might anticipated that a collection of activated phagocytes would damage each other as well as surrounding cells and connective tissues. The O2– produced at activated phagocytes, presumably to facilitate killing of engulfed bacteria, could exacerbate and prolong the inflammatory process. The remodeling process of airway in asthmatic patients was proposed to be related to chronic inflammation and superoxide O2– production. Injection of antioxidant enzyme such as superoxide dismutase (SOD) having an anti-inflammatory effect has been reported. Under this basis, many antioxidants, including vitamin E, ascorbic acid, β-carotenes, catechin and polyphenol compounds, and biosulfavoids should have free radical-scavenging and anti-inflammatory effects on the process of airway remodeling and the management of asthmatic control.

Many herb drugs and food are widely accepted by asthmatic and allergic patients in Taiwan, such as gingo, propolis etc. The main composition of these herb drugs is their polyphenol compounds, including isoflavonoids,
catechin, isocoumarins and their polymers. They were recognized to be antioxidants acting as free radical scavengers.21 The further clinical trial of these antioxidants on the management of asthmatic patients and the relationship of their TAS are needed before the conclusion.

REFERENCES

18. The biology of oxygen radicals: the superoxide radical is an agent of oxygen toxicity: superoxide dismutases provide an important defence. 1978; 201.