SHORT COMMUNICATION

Serum levels of antioxidant vitamins, copper, zinc and magnesium in children with chronic rhinosinusitis

Murat Önal a,*, Lülüfer Tamer b, Yavuz Selim Pata a, Serkan Kilic a, Ulaş Değirmenci b, Yücel Akbaş a, Kemal Görür a, Uğur Atik b

aMerin University School of Medicine, Department of Otorhinolaryngology, Merin, Turkey
bMerin University School of Medicine, Department of Biochemistry, Merin, Turkey

Received 31 January 2004; accepted 30 July 2004

Abstract

Reactive oxygen species including hydroxyl radicals, superoxide anions and hydrogen peroxide which are produced by activated granulocytes play an essential role in many biochemical processes and diseases. Oxidant-mediated tissue damage may be important in the development of chronic sinusitis. The aim of this study was to investigate the serum levels of antioxidant vitamins and elements in 24 children (14 boys and 10 girls, age range: 7–12 years, mean age: 9.2 years) with chronic rhinosinusitis, compared to 20 age and sex matched healthy children. Blood samples were collected in the morning before breakfast and prior to any medication. Vitamin A, E and C levels were determined using reagent kits for high performance liquid chromatography. Cu, Zn and Mg levels were analyzed by atomic absorption spectrometry. Vitamin E, vitamin C, Cu and Zn levels were significantly lower in the patients group than in the control group. However, vitamin A and Mg levels did not differ. In conclusion, serum levels of antioxidant vitamins and elements may be important in the pathogenesis and treatment of chronic rhinosinusitis in children.

Keywords: Chronic rhinosinusitis; Oxidative stress; Vitamin A; Vitamin E; Vitamin C; Copper; Zinc; Magnesium

Introduction

Reactive oxygen species (ROS) including hydroxyl radicals, superoxide anions and hydrogen peroxide which are produced by activated granulocytes play an important role in many biochemical processes such as intracellular messaging in the cell differentiation, apoptosis, immunity and defense against microorganisms [1,2]. However, an overproduction of these reactive species, occurring in inflammation, results in oxidative stress. Oxidative stress may cause severe tissue damage including lipid peroxidation, enzyme inactivation and DNA damage [3]. Aerobic organisms are protected against free radicals by enzymatic and non-enzymatic antioxidant defenses [1]. The defense system includes ascorbic acid (vitamin C), a-tocopherol (vitamin E), vitamin A and elements such as zinc (Zn), copper (Cu) and magnesium (Mg).

Vitamin A is a fat soluble vitamin and essential for the immune system, cellular differentiation, and the maintenance of the respiratory epithelium [4]. Moreover, it has anti-infective, anti-inflammatory and antioxidant activities. Vitamin E is known as one of the most important lipid soluble antioxidants, especially at high oxygen occurrence. ROS are scavenged through
interaction with the ascorbate-tocopherol-glutathione antioxidant system [5]. Vitamin C is one of the strongest naturally occurring reducing agents known. It acts as a specific electron donor for eight enzymes and may also participate as a reducing agent in several non-enzymatic reactions [6]. Trace elements have important functions in the human body. They are required in low concentrations for example as essential components of antioxidative enzymes. The cytoplasmic Cu/Zn-superoxide dismutase (SOD) contains Cu and Zn as cofactors [7]. Mg modulates ion transport pumps, carriers and channels, and the positively charged ion Mg$^{2+}$ is able to bind to the negatively charged groups in membranes, proteins and nucleic acids [8]. Elements have also an important role in primary and secondary T-cell production [9].

The pathogenesis of sinusitis is multifactorial and in some cases associated with a prolonged and excessive state of inflammation rather than a simple bacterial infection. The degree of this inflammation is determined by local microenvironmental factors such as sinus ostial obstruction, excessive local inflammatory responses, ineffective mucosal immunity and disruption of the sinus epithelium and mucociliary clearance mechanism [10].

In the present study we hypothesized that serum levels of antioxidative vitamins and elements may be an important factor in the pathogenesis of chronic rhinosinusitis in children.

Materials and methods

Subjects

The study included 24 children with chronic rhinosinusitis and 20 age and sex matched healthy children. Informed consent was obtained from all participants’ parents. Patients—14 boys and 10 girls, age range: 7–12 years, mean age: 9.2 years—with chronic rhinosinusitis (>3 months) had symptoms of nasal and postnasal discharge, nasal obstruction and headache. Rhinosinusitis was diagnosed on the basis of clinical and X-ray findings. All patients were treated with standard medical therapy including amoxicillin-clavulanic acid, systemic decongestant and acetaminophen. Children with atypical symptoms and history were excluded. None of them suffered from any other known illness or had taken any medication or dietary vitamin supplements for at least 3 months prior to the study. None of the controls had symptoms and signs of rhinosinusitis during 3 months prior to the study.

Determination of vitamins A, E and C

Blood samples were collected in the morning before breakfast and prior to any medication. After centrifugation, the obtained serum was stored at −20 °C and protected from light. Vitamin A, E and C levels were determined using reagent kits—including reagents and columns—for high performance liquid chromatography (HPLC) analysis of vitamin A, E and C in serum/plasma by Chromsystems Instruments & Chemicals GmbH, München, Germany. Analyses were performed with an isocratic HPLC system with UV detector (HP 1100, Agilent Technologies). The parameters for vitamin A and E analysis were: 50 μl injection volume, 1.5 ml/min flow rate, room temperature 25 °C, detection at a wavelength of 325 nm for vitamin A and 295 nm for vitamin E; for vitamin C: 20 μl injection volume, 1.3 ml/min flow rate, room temperature 25 °C, detection at a wavelength of 245 nm.

Determination of Cu, Zn and Mg

Serum was separated from clotted blood and stored in acid-washed tubes at −20 °C until analysis by atomic absorption spectrometry (AAS Solar 920, ATI Unicam, Cambridge, United Kingdom). The samples were diluted 1:1, 1:5, and 1:10 with deionized water for the determination of Cu, Zn, and Mg, respectively. Calibration standards were prepared with deionized water in the concentration ranges of 1–5 μg/dl for Cu, 0.25–1.25 μg/dl for Zn and 0.1–0.5 mg/dl for Mg. Readings for Cu, Zn and Mg were performed against Cu, Zn and Mg standards at wavelengths of 324.8, 213.9 and 285.2 nm, respectively [11].

Statistical analysis

Student’s t-test was used to investigate the statistical significance of the differences between the vitamin and element concentrations determined in the patients and in the control group. The criterion for significance was set at p<0.05.

Results

The results are shown in Table 1. The mean vitamin A level in the patients and in the control group was 35.6 and 35.8 μmol/l, respectively. The difference was not statistically significant (p = 0.9). The mean vitamin E concentration was 21.5 μmol/l in the patients group and 37.7 μmol/l in the control group, and the difference was statistically significant (p<0.001). The mean vitamin C level (1.1 μmol/l) was significantly lower (p = 0.04) in the patients group than in the control group (1.5 μmol/l).

The mean Cu concentration in the serum of the patients and the controls was 87.3 and 103.1 μg/dl, respectively, the difference being statistically significant (p<0.001). Moreover, the mean serum Zn concentration
Table 1. Mean serum levels of vitamins A, E, C, and Cu, Zn and Mg in the patients and in the control group

<table>
<thead>
<tr>
<th></th>
<th>Patients (µmol/l)</th>
<th>Controls (µmol/l)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>35.6</td>
<td>35.8</td>
<td>-0.46</td>
<td>0.9</td>
</tr>
<tr>
<td>E</td>
<td>21.5</td>
<td>37.7</td>
<td>-8.93</td>
<td>0.000</td>
</tr>
<tr>
<td>C</td>
<td>1.1</td>
<td>1.5</td>
<td>-1.8</td>
<td>0.04</td>
</tr>
<tr>
<td>Elements</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>87.3</td>
<td>103.1</td>
<td>-4.61</td>
<td>0.000</td>
</tr>
<tr>
<td>Zn</td>
<td>70.4</td>
<td>90.9</td>
<td>-6.86</td>
<td>0.000</td>
</tr>
<tr>
<td>Mg</td>
<td>20.6</td>
<td>20.75</td>
<td>-0.26</td>
<td>0.8</td>
</tr>
</tbody>
</table>

α statistic value of Student’s t-test; ρ: value of type one error.

Discussion and conclusions

The precise incidence of chronic rhinosinusitis in children is not known. It has been estimated that 0.5–5% of children suffering from an upper respiratory tract infection have sinusitis [12]. Opinions regarding the treatment of sinusitis vary from no therapy to extensive surgical interventions, but an antimicrobial therapy is advised in chronic sinusitis for at least 10–14 days.

The role of oxygen radicals in microbial killing, as described by Babior, is now well known [13]. Neutrophils, eosinophils and mononuclear phagocytes possess a membrane-bound flavoprotein cytochrome b-245 NADPH oxidase system [14]. In humans, the chronic granulomatous disease develops when this enzyme is absent or does not function normally, and patients suffer from persistent bacterial infections, generally from staphylococcus [14]. During phagocytosis, cells consume increased amounts of oxygen, and reactive oxygen species are generated [14]. Those are capable of damaging cell membranes and various biomolecules. Oxidant-mediated ciliary dysfunction of human nasal epithelium and toxic effects of human eosinophil peroxidase to cultured nasal epithelial cells have been described [15]. Glutathiones, vitamin C and uric acid are found in nasal secretions [16]. Dönker et al. observed an increased ROS production in experimentally induced maxillary sinusitis [17]. Thus, oxidant-mediated tissue damage may play a role in the development of chronic rhinosinusitis.

Westerveld et al. reported that reduced glutathione and uric acid levels in mucous samples obtained from patients with chronic rhinosinusitis were significantly lower than in healthy controls [2]. However, Kassim et al. did not find such a relation between reduced glutathione and uric acid levels and chronic rhinosinusitis, but they showed a low superoxide dismutase activity in patients with chronic rhinosinusitis [5]. They concluded that this low activity may be due to a possible Zn deficiency in these patients. Matsunaga et al. previously reported a lower SOD activity associated with Zn deficiency in sera of patients with small dysfunction caused by chronic rhinosinusitis or common cold, compared to healthy controls [18]. SOD is an antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O₂ and to the less reactive peroxide H₂O₂ [1]. In this context, there are three forms of SOD: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD. Cu/Zn-SOD has two subunits, containing each a metal cluster, the active site, constituted by a Cu and a Zn atom bridged by a histamine residue [1]. Cu/Zn-SOD is believed to play a major role in the first line of antioxidant defense. For example, calves that were fed milk supplemented with 25 mg/l Cu and 100 mg/l Zn showed a stronger immune response and a higher SOD activity [19]. Thus, it may be possible that supplementation with Cu and Zn enhances the activity of Cu/Zn-SOD in children with chronic rhinosinusitis.

Friedman et al. compared the lipid peroxide levels in healthy and diseased tissue in 13 patients with chronic rhinosinusitis, and did not find a statistically significant difference [20]. They speculated that lipid peroxidation may have occurred during the acute phase of sinusitis, then mucosal tissue recovered from the initial injury, and free radical induced damage decreased as well. Önerci and Koş found a significant decrease in mean serum Cu concentration, but an increase in Mg concentration in patients with chronic rhinosinusitis [9]. They did not find any difference in mean serum Zn levels between the patients and the control group. In the present study, there was a significant difference in serum Cu and Zn levels between the patients and the controls. However, the mean Mg levels did not differ.

Westerveld et al. investigated the tissue levels of vitamin E in patients with chronic rhinosinusitis, and did not find a statistically significant difference compared to the control group [2]. On the contrary, Kassim et al. reported a significant decrease in tissue vitamin E in the patients group [5]. In the present study, we have found a significant decrease in serum levels of vitamins E and C in the patients, but vitamin A levels did not differ.

The controversial results found in different studies may be due to variations in the number and age of the patients or the severity of the disease. The present study is the first comprehensive investigation on alterations in serum levels of antioxidant vitamins and elements in children with chronic rhinosinusitis. Further studies are needed to investigate their exact role in the pathogenesis and treatment of chronic rhinosinusitis.
References


