

PAEDIATRIC OTORHINOLARYNGOLOGY

Advanced oxidation protein product levels as a marker of oxidative stress in paediatric patients with chronic tonsillitis

Ruolo dei prodotti avanzati di ossidazione proteica come marker di stress ossidativo nei pazienti pediatrici affetti da tonsillite cronica

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SUMMARY

We aimed to determine whether advanced oxidation protein product (AOPP) levels can serve as a marker of oxidative stress in paediatric patients with chronic tonsillitis. Thirty children with chronic tonsillitis and 30 healthy children (control group) were recruited from the Otorhinolaryngology (ORL) and Paediatric Surgery departments, respectively, of Dumlupınar University Hospital. In the patient group, blood samples were collected before tonsillectomy, and tonsil tissue was sampled during the operation. Blood samples were also obtained from the control subjects. AOPP levels in the serum and tonsil tissue were measured by the spectrophotometric method. Serum AOPP levels were significantly higher in the patient group (13.1 ± 3.3 ng/ml) than in the control group (11.6 ± 2.3 ng/ml; $P < 0.05$). In addition, the mean AOPP level (41.9 ± 13.5 ng/mg protein) in the tonsil tissue in the patient group was significantly higher than the mean serum AOPP levels in the control and patient groups ($P < 0.05$). AOPP levels are elevated in the tonsil tissue and serum of patients with chronic tonsillitis compared to the serum AOPP levels in healthy controls. AOPPs may represent a novel class of pro-inflammatory molecules that are involved in oxidative stress in chronic tonsillitis. AOPPs may be used as a marker of oxidative stress in paediatric patients with chronic tonsillitis.

KEY WORDS: Chronic tonsillitis • Advanced oxidation protein products (AOPPs) • Oxidative stress

RIASSUNTO

L'obiettivo del presente studio è stato determinare se i livelli plasmatici dei prodotti avanzati di ossidazione proteica (AOPP) rappresentino dei marker di stress ossidativo nei pazienti pediatrici affetti da tonsillite cronica. Per lo studio sono stati arruolati, presso i Dipartimenti di Otorinolaringoiatria e Chirurgia pediatrica dell'Ospedale Universitario di Dumlupınar, trenta bambini sani e trenta affetti da tonsillite cronica. Il gruppo dei pazienti affetti da malattia è stato sottoposto a un prelievo ematico preoperatorio e ad una biopsia intraoperatoria del tessuto tonsillare. Il gruppo dei pazienti sani è stato sottoposto unicamente al prelievo ematico. I livelli plasmatici e tissutali degli AOPP sono quindi stati misurati mediante spettrofotometria. I livelli sierici degli AOPP sono risultati essere più elevati nel gruppo dei pazienti affetti da tonsillite cronica ($13,1 \pm 3,3$ ng/ml) rispetto al gruppo di controllo ($11,6 \pm 2,3$ ng/ml; $P < 0,05$). Il livello tissutale medio degli AOPP nei pazienti malati è risultato essere superiore a quello plasmatico medio sia nel gruppo dei pazienti sani che in quello dei pazienti malati ($41,9 \pm 13,5$ ng/mg; $P < 0,05$). I livelli plasmatici e tissutali degli AOPP sono risultati quindi essere più elevati nei pazienti malati rispetto al gruppo di controllo. Gli AOPP potrebbero quindi rappresentare una nuova classe di molecole pro-infiammatorie coinvolte nello stress ossidativo nella tonsillite cronica e potrebbero avere un ruolo come marker di stress ossidativo nei pazienti pediatrici affetti da tale patologia.

PAROLE CHIAVE: Tonsillite cronica • Prodotti avanzati di ossidazione proteica (AOPPs) • Stress ossidativo

Acta Otorhinolaryngol Ital 2016;36:381-385

Introduction

Tonsil tissue that is part of the Waldeyer ring has a lymphoepithelial structure ¹. Chronic tonsillitis commonly occurs in children. It causes recurrent attacks of throat pain, dysphagia, fever and malaise, and also leads to obstructive sleep apnoea syndrome in children. In addition, it disturbs sleep quality and impairs school success ². An-

tibiotics and diverse drug combinations used to treat this disease cause many side effects in children. Furthermore, the medical and surgical treatment of this disease poses an enormous economic burden.

Oxygen deports electrons from other molecules in the cell to create reactive oxygen species (ROS). ROS are controlled by a defence system that relies on the activity of enzymes and non-enzyme substances. An imbalance

between ROS generation and the body's defence system against ROS is termed oxidative stress³. Oxidation products are generated during inflammation and are involved in the tissue injury caused by inflammation. Antioxidants play a role in neutralising oxidation products and limiting tissue injury. Advanced oxidation protein products (AOPPs), a new oxidative stress marker, were first detected in the plasma of uremic patients in 1996⁴. AOPPs are thought to activate mononuclear phagocytes and act as cytokine-like mediators between neutrophils and monocytes⁴. The products of the oxidative modification of proteins are more stable than those of lipids, making AOPPs better marker of oxidative stress⁵. AOPPs are defined as dityrosine-including cross-linked protein products, a description that is significant, as it excludes protein aggregates generated by disulphide bonds created as a result of oxidative stress. Consequently, AOPPs are a good marker of oxidative stress⁶.

Oxidative stress has been linked to chronic tonsillitis^{7,8}. However, no study has yet reported the feasibility of using AOPP levels as a marker of oxidative stress in paediatric patients with chronic tonsillitis. We, therefore, conducted this study to determine whether AOPP concentrations in tonsil tissue and serum are affected by chronic tonsillitis and whether AOPPs play a role in the aetiology of chronic tonsillitis.

Materials and methods

Study design

Our study group consisted of 30 patients who were diagnosed with chronic tonsillitis and scheduled to undergo tonsillectomy in the Department of Otorhinolaryngology (ORL), Dumlupinar University Education and Research Hospital, Kutahya, Turkey. Chronic tonsillitis was diagnosed on the basis of the patient's history and the findings of physical examinations. Chronically inflamed tonsils with white debris arising from the tonsillar crypts and frequent attacks of tonsillitis were considered as indications for tonsillectomy. Patients with chronic tonsillar hypertrophy were included in the study; however, indications such as chronic tonsillitis that did not respond to medical treatment and was associated with persistent sore throat, halitosis or painful cervical adenitis were excluded. In addition, patients with complications were not included in the study. As a control group, 30 healthy individuals with no complaints in the head and neck region and without any infection or other systemic diseases were recruited from the Department of Paediatric Surgery, Dumlupinar University Education and Research Hospital.

Blood and tonsil tissue collection and preparation

Preoperatively in the study group, after overnight fasting, an overall of 10 ml venous blood samples were obtained from each person in addition to the routine preoperative

blood tests immediately before tonsillectomy. In both the patient and control groups, blood samples were collected into evacuated tubes containing a serum separator and clot activator (Vacuette®, Greiner Bio-One, Kremsmunster, Austria). Within 1 h of collection, blood samples were centrifuged at 1500 × g for 15 min to obtain serum samples. After centrifugation, serum samples were stored at -80°C until biochemical analysis. Tonsillectomy was performed under general anaesthesia in all patients. After the tonsillectomy, tonsil tissue samples were immediately rinsed with cold, heparinised, phosphate-buffered saline to remove any red blood cells or clots. Portions of the tonsil samples were placed in Eppendorf tubes and immediately stored at -80°C until biochemical analysis.

Measurement of AOPP levels

Before the measurement of the AOPP levels in the tonsil tissue samples, approximately 10 mg of tonsil tissue was mixed with 100 µl of cold working solution (50 mM phosphate buffer, pH 7.40), and homogenised using a mechanical homogeniser (SpeedMill Plus, Analytik Jena, Germany). The homogenate was then centrifuged at 10,000 × g for 15 min at 4°C, and the resultant supernatant was preserved for biochemical analysis by storing on ice. AOPP levels were measured in tonsil tissue homogenates and serum samples by using commercial enzyme-linked immunosorbent assay kits (Eastbiopharm Co., Ltd., Hangzhou, China) and a microplate reader (BMG Labtech Spectrostar Nano, GmbH, Ortenberg, Germany). Tissue protein concentrations were measured using the Bradford method on a Beckman Coulter AU680 analyser (Beckman Coulter, Miami, FL, USA)¹. AOPP concentrations were expressed as ng/ml for serum samples and ng/ml protein for tonsil tissue samples.

Ethical considerations

The study protocol was approved by the local ethics committee. The parents of all included subjects provided written and oral informed consent, and subjects were enrolled only after their parents had agreed to participate in the study and signed an informed consent form.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 6.05 (GraphPad Software, Inc., CA, USA). All data sets were tested for normality using Shapiro-Wilk test. Since the values were normally distributed and the sample size was ≥ 30, data were presented as mean ± standard deviation (SD) and parametric statistical tests were used. The comparison of AOPP values between groups were analysed using unpaired t-test. The comparison of AOPP values between serum samples and tonsil tissue samples in patients with chronic tonsillitis were analysed using unpaired t-test. A P value < 0.05 was considered as statistically significant.

Results

The study group consisted of 30 patients (17 males, 13 females, mean age 7.9 ± 3.4) undergoing tonsillectomy who fulfilled the inclusion criteria. The control group consisted of 30 subjects (17 males, 13 females, mean age 7.7 ± 3.2).

When the serum AOPP levels measured in study group and control group were compared, since the 95% confidence interval (CI) did not include zero and the P value was < 0.05 , we concluded that there was a statistically significant difference between the two groups [$t(57) = -1.47$, $P = 0.048$, 95% CI: -2.92 to -0.01]. Our results revealed that serum AOPP levels were higher in study group compared to control group (Table I; Fig. 1).

When serum AOPP levels and tonsil tissue AOPP levels in the study group were compared, significant difference was found between AOPP levels [$t(55) = -28.83$, $P < 0.001$, 95% CI: -33.99 to -23.67]. Tissue AOPP levels were significantly higher in tonsil tissue samples compared to serum samples (Table II; Fig. 2).

Discussion

Chronic tonsillitis is characterised by episodes of local infections with diverse pathogens, which are associated with the activation of lymphoid cells and episodes of excessive hypoxia/reoxygenation⁷. Free oxygen radicals (FORs), which are part of the defence mechanism, are profusely generated by neutrophils, monocytes, eosinophils and macrophages to kill bacteria during tonsillar inflammation^{2,8}. Yılmaz et al.² concluded that tonsillectomy diminishes the total oxidative stress by eliminating a microbial source and thereby strengthens the immune system. FORs negatively impact the immune system by reducing the proliferation capacity of defensive cells through DNA damage and by decreasing the synthesis of some critical factors, which decreases antioxidant levels and thereby increases the susceptibility to upper respiratory tract infections. Cvetkovic et al.⁸ claimed that the antioxidant defence system may help prevent the recurrence of tonsillitis; however, tonsillectomy alone cannot supply this conservation.

Oxidative stress is involved in the pathogenesis of many diseases. Antioxidant therapy, which decreases oxidative stress, is a potential treatment for a number of pathologies. Whether oxidative stress is the reason or the result of disease is unclear. The in vivo quantification of oxidative stress is complicated, and practical and easy methods of measurement are still being developed. During oxidative stress, AOPPs are generated as a result of myeloperoxidase activity against hypochloric acid and chloramines in activated neutrophils; thus, AOPPs are a reliable marker to measure the oxidative modification of proteins⁹. FORs are molecules that contain one or more unpaired electrons,

Table I. Comparison of the levels of advanced oxidation protein products (AOPPs) between chronic tonsillitis patients and control subjects.

AOPP	CT Group (mean \pm SD) N = 30	Control Group (mean \pm SD) N = 30	95% CI	P
Serum (ng/mL)	13.1 ± 3.3	11.6 ± 2.3	-2.92 to -0.01	0.048*

CT: Chronic tonsillitis, AOPP: Advanced oxidation protein product, SD: Standard deviation, CI: Confidence interval. Data are presented as mean \pm SD for each group. Data were tested using the unpaired t test. A P value of less than 0.05 was considered as statistically significant. Since the 95% CI did not include zero, there was a statistically significant difference between the two groups.

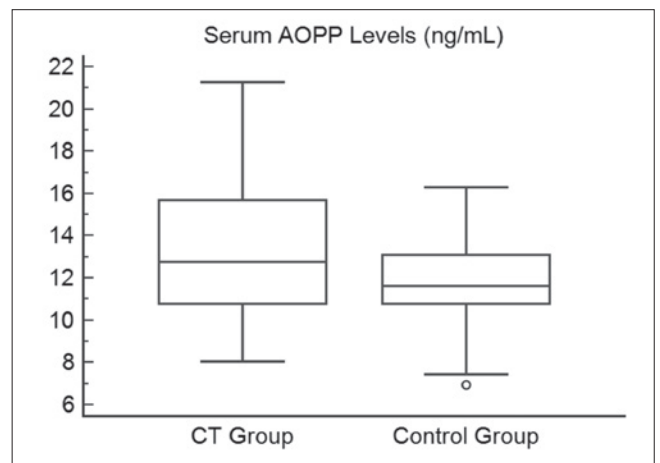


Fig. 1. Comparison of the serum levels of advanced oxidation protein products (AOPPs) between patients with chronic tonsillitis and control subjects.

and can accumulate to high levels in living systems¹⁰. These molecules are very reactive, and in particular, react with lipids, proteins, nucleotides and carbohydrates, causing tissue injury¹¹. Under normal conditions, the potential deleterious effects of free radicals are precluded by antioxidants¹¹, which consist of enzymes such as superoxide dismutase (SOD), glutathione peroxidase, catalase and glucose-6-phosphate dehydrogenase and non-enzymatic factors such as ascorbic acid, α -tocopherol, retinol and β -carotene^{12,13}. Antioxidants within cells, cell membranes and extracellular fluids preclude extreme free radical for-

Table II. Comparison of the levels of advanced oxidation protein products (AOPPs) between serum samples and tonsil tissue samples in patients with chronic tonsillitis.

Serum AOPP (ng/mL) (mean \pm SD) N = 30	Tonsil Tissue AOPP (ng/mg protein) (mean \pm SD) N = 30	95% CI	P
13.1 ± 3.3	41.9 ± 13.5	-33.99 to -23.67	$< 0.001^*$

AOPP: Advanced oxidation protein product, SD: Standard deviation, CI: Confidence interval. Data are presented as mean \pm SD for each group. Data were tested using the unpaired t test. A P value of less than 0.05 was considered as statistically significant. Since the 95% CI did not include zero, there was a statistically significant difference between the two groups.

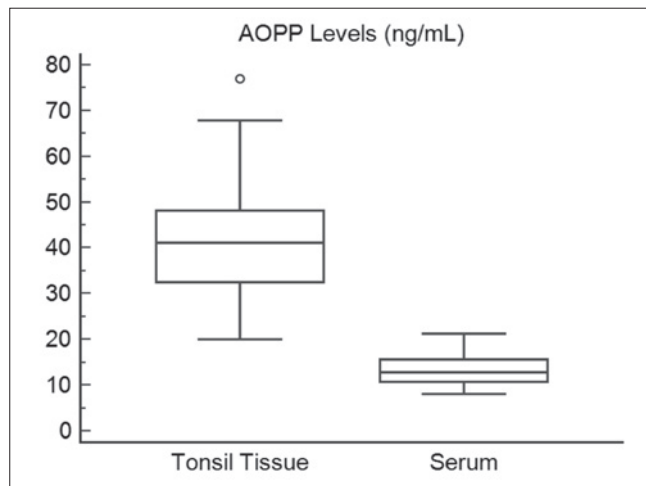


Fig. 2. Comparison of the levels of advanced oxidation protein products (AOPPs) between serum samples and tonsil tissue samples in patients with chronic tonsillitis.

mation and maintain a balance¹². Blood plays an important role in the maintenance of the equilibrium between antioxidants and oxidants, as it facilitates the distribution of antioxidants throughout the body^{10,11}.

During chronic inflammation, the antioxidant level slowly declines until the level of oxidative stress exceeds the capacity of the body to neutralise it. Low antioxidant levels may be the result of chronic illnesses. Free radical-mediated damage to lipids in the membranes of leucocytes augments the permeability of these cells and diminishes their immune function. In addition, DNA damage caused by free radicals inhibits the synthesis of certain important factors by leucocytes and decreases the proliferation capacity of leucocytes. Low antioxidant levels in blood may predispose children to frequent upper respiratory tract infections by adversely affecting their immune system. The discovery of antioxidants and oxidation products in the tonsil tissues of patients with chronic tonsillitis shows that these substances are associated with this disease².

Chronic tonsillitis is a chronic inflammatory illness, and the role of FORs in the pathogenesis of this disease has been reported in diverse studies^{2,14}. Kiroglu et al.¹⁵ reported that the preoperative blood erythrocyte malondialdehyde (MDA), serum MDA, erythrocyte catalase and serum catalase levels and adenoid and tonsil tissue levels of MDA and catalase were higher in chronic adenotonsillitis patients than in children with adenotonsillary hypertrophy. Kaygusuz et al.¹⁶ investigated chronic tonsillitis patients, and found that oxidative stress increased and SOD activity decreased in parallel with an increase in the generation of MDA by lipid peroxidation that resulted in tissue damage. The authors also reported that in the same patient group, oxidative stress declined during the postoperative period together with an increment in SOD activity and a decline in MDA level. Yılmaz et al.² com-

pared the pre- and postoperative (1 month) blood levels of antioxidants (carotene, retinol, lycopene, tocopherol, ascorbic acid, SOD, glutathione peroxidase) and MDA in patients with adenotonsillary disease. They reported that the blood levels of antioxidants increased, while those of the oxidant decreased significantly after operation in patients with adenotonsillary disease. Garca et al.¹⁷ investigated the effects of adenosine deaminase, an enzyme that plays significant roles in the differentiation of lymphoid cells, and oxidative stress in patients with chronic tonsillitis. The authors found that tissue and serum adenosine deaminase activity was elevated in patients with chronic tonsillitis.

AOPP levels have been mentioned as a marker of oxidative stress in the literature concerning the ear, nose and throat region. Balikci et al.¹⁸ investigated AOPP levels in children with chronic otitis media with effusion; they found that AOPP levels were increased in the effusion fluid, but not in the plasma, of these patients. The authors, therefore, concluded that chronic otitis media with effusion was associated with protein oxidation abnormalities. Veyseller et al.¹⁹ reported that AOPPs could be used as markers of oxidative stress during the development of nasal polyposis. Aksoy et al.²⁰ investigated the levels of AOPP as a marker of oxidative stress in patients with allergic rhinitis. They concluded that as a known indicator of protein oxidation, the serum AOPP level could be used as a marker of increased oxidative stress in response to allergen exposure in allergic rhinitis. In the present study, which is the first to investigate the relationship between AOPP levels and chronic tonsillitis, we found that AOPP levels were higher in the tonsil tissue and serum samples obtained from patients with chronic tonsillitis than in serum samples obtained from the control group.

A limitation of our study was not comparing the postoperative serum levels of AOPPs of patients with the control group. Ethical rules limited us to take blood samples from patients postoperatively. This is because we do not routinely take blood samples in every patient in the postoperative period if patients recover well. In addition, because it was not ethically acceptable to generate a control group for tonsillar tissue measurements to statistically compare these values with any corresponding value, and only blood samples were attained from the control group. We hope that this preliminary study will encourage larger studies to be undertaken.

Conclusions

AOPPs may represent a novel class of pro-inflammatory molecules that are involved in oxidative stress in patients with chronic tonsillitis. Examination of the pathophysiological processes associated with AOPPs could deepen our knowledge concerning the relationship between oxidative stress and the course of chronic tonsillitis. AOPPs

may be used as a marker of oxidative stress in paediatric patients with chronic tonsillitis. In addition, AOPPs blood levels as biochemical parameter may have possible role for tonsillectomy indication in paediatric patients with chronic tonsillitis. Further studies are required to elaborate on this relationship.

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Received: October 26, 2015 - Accepted: March 26, 2016