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Paraoxonase and arylesterase activity and total oxidative/anti-oxidative status in patients with chronic adenotonsillitis

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ABSTRACT

Objectives: The aim of this study was to investigate serum paraoxonase, arylesterase activities along with determination of oxidative status via measurement of total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) in children with recurrent adenotonsillitis during pre- and post-adenotonsillectomy period and to compare results with data from healthy subjects.

Methods: We performed a prospective controlled trial on adenoidectomy and/or tonsillectomy patients. A total of 47 subjects, including 22 patients with recurrent adenotonsillitis and 25 healthy controls were enrolled in this study. Peripheral venous blood samples were taken from patients before adenotonsillectomy and a second sample was obtained in first month postoperatively. In the control group, blood samples from healthy volunteers were collected for one time only. Serum paraoxonase, arylesterase activities, TOS, TAS and OSI levels were measured.

Results: Paraoxonase, arylesterase activity, TAS and TOS levels were significantly higher in preoperative group compared to control group ($P < 0.001$, $P = 0.003$, 0.003 and 0.005, respectively). However, OSI level was similar in preoperative group compared to control group ($P = 0.25$). In the post-operative group, paraoxonase, arylesterase activities, TAS and OSI levels were lower as compared to preoperative group but differences were statistically insignificant ($P = 0.483$, 0.265, 0.149 and 0.090, respectively). TOS level in post-operative group was significantly lower than the preoperative group ($P < 0.001$). In the post-operative group, paraoxonase and arylesterase activities were significantly higher as compared to control group ($P = 0.004$ and 0.02, respectively). TOS and OSI levels were significantly lower in post-operative group compared to control group ($P = 0.001$ and 0.02, respectively). However, TAS was similar between post-operative and control groups ($P = 0.464$).

Conclusions: Based on data obtained from this study, we may state that paraoxonase, arylesterase activities with TAS, TOS and OSI levels of patients with chronic adenotonsillitis shows alterations due to oxidant/antioxidant imbalance induced by frequent infections.

1. Introduction

Tonsillectomy and/or adenoidectomy are the most common surgical operations performed by otolaryngologist in paediatric population. Routine indications for tonsillectomy and/or adenoidectomy are upper airway obstruction, recurrent adenotonsillitis and eustachian tube dysfunction [1]. Pathophysiology of adenotonsillary disease is still unclear. In aerobic organisms, reactive oxygen species (ROS) are produced during normal immune defense and metabolic activity [2]. Rate of production and destruction of ROS is in a state of balance, which is known as oxidative balance. In cases where this oxidative balance is maintained, ROS have no impact on the organism but in cases where this balance is destroyed in favor of free radicals, oxidative stress develops [3]. Oxidative stress is a consequence of relative overproduction of ROS, as seen in inflammation [4]. Defense system protecting free radical damage involves enzymatic and non-enzymatic antioxidant systems. Enzymatic system includes superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase (CAT). On the other hand, non-enzymatic system includes various substances like vitamin E, vitamin C, beta-carotene, glutathione, uric acid, bilirubin and retinol [5]. Paraoxonase is a high-density lipoprotein (HDL)-associated antioxidant enzyme. In various trials, it was shown that paraoxonase prevents oxidative stress by inhibiting oxidation of cell membrane lipids induced by ROS which develop in acute and chronic inflammation [6,7].

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The aim of this study was to investigate serum paraoxonase and arylesterase activities along with determination of oxidative status via measurement of total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) in children with recurrent adenotonsillitis during pre and post-operative period and to compare results with data from healthy subjects.

2. Patients and methods

2.1. Subjects

We performed a prospective controlled trial on adenoidectomy and/or tonsillectomy patients. Study was approved by Ethical Committee of Cumhuriyet University, Medical Faculty.

A total of 47 subjects, including 22 patients with recurrent adenotonsillitis and 25 healthy controls were enrolled in this study. Children with chronic underlying diseases (including cardiovascular disorders, malignancy, asthma, allergic rhinitis, cystic fibrosis, metabolic disease, renal or liver disease or immunodeficiency) were excluded from study. Chronic adenotonsillitis were diagnosed by patient history, routine otolaryngological and endoscopical examinations and lateral radiography. Indications for tonsillectomy were seven or more well-documented, clinically prominent and adequately treated episodes of throat infection during preceding year or recurrent acute tonsillitis for at least 2 years with 5 or more acute attacks per year. Indications for adenoidectomy were obstructive nasal symptoms due to hypertrophic chronic adenoiditis. In all patients, adenoidectomy and/or tonsillectomy was performed by otolaryngologist, using standard surgical techniques under general anesthesia. Tonsillectomy was performed by routine dissection-snare method with general anesthesia under operating room conditions. Adenoid tissue was removed using curettes under indirect mirror visualisation. Patients were discharged from hospital on the morning of first postoperative day uneventfully.

2.2. Blood samples collection

After overnight fasting, peripheral venous blood samples were taken from patients into empty tubes before adenotonsillectomy and a second sample was obtained in first month postoperatively. In the control group, blood samples from healthy volunteers were collected for one time only. Samples were immediately separated from the cells by centrifugation at $3000 \times g$ for 10 min, and then stored at $-80\,^{\circ}C$ until further analysis of paraoxonase and arylesterase activities along with determination of oxidative status via measurement of TOS, TAS and OSI.

2.3. Measurement of paraoxonase and arylesterase activities

Paraoxonase activity was measured in absence (basal activity) and presence of NaCl (salt-stimulated activity) [8]. Briefly, rate of paraoxon hydrolysis was measured by the increase of absorbance at 412 nm at 25 °C. Amount of generated p-nitrophenol was calculated from molar absorptivity coefficient at pH 8, which was $17.100\,M^{-1}\,cm^{-1}$. Paraoxonase activity was expressed as U/L serum. Phenylacetate was used as a substrate to measure arylesterase activity. Reaction was initiated by addition of serum and increase in absorbance was read at 270 nm. Blanks were included to correct spontaneous hydrolysis of phenylacetate. Enzymatic activity was calculated from molar absorptivity coefficient of the produced phenol, $1310\,M^{-1}\,cm^{-1}$. One unit of arylesterase activity was defined as 1 μmol phenol generated/min under the above conditions and expressed as U/L serum. Phenotype distribution of paraoxonase was determined in presence of 1 mol/L NaCl (salt-stimulated paraoxonase). Ratio of salt-stimulated paraoxonase activity to arylesterase activity was used to assign individuals to one of the three possible phenotypes [9].

2.4. Measurement of total oxidant status

Total oxidant status of serum was determined using a novel automated measurement method [10]. Oxidants present in the sample oxidize ferrous ion-o-dianisidine complex to ferric ion. Oxidation reaction is enhanced by glycerol molecules, which are abundantly present in reaction medium. Ferric ion reacts with xylenol orange in an acidic medium to produce a colored complex. Intensity of color, which can be measured spectrophotometrically, is related to total amount of oxidant molecules in the sample. Assay is calibrated with hydrogen peroxide and results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equiv./L). The assay has excellent precision values lower than 2%.

2.5. Measurement of the total antioxidant status

Total antioxidant status of serum was determined using an automated measurement method [11]. Briefly, potent free radical reactions were initiated with the production of a hydroxyl radical via Fenton reaction and rate of reactions was monitored by following the absorbance of colored dianisidyl radicals. Using this method, antioxidative effect of the sample against potent free radical reactions, which were initiated by synthesized hydroxyl radical, was measured. Method was applied to an automated analyzer (Aeroset®, Abbott). Both intra- and interassay coefficients of variations were lower than 3%. Data were expressed as TAS (mmol Trolox equiv./L).

2.6. Oxidative stress index (OSI)

The ratio of TOS to TAS yields the OSI, an indicator of the degree of oxidative stress [10,11]. For calculations, the resulting unit of TAS was changed to mmol/L, and the OSI level was calculated according to the following formula: OSI (arbitrary unit) = TOS (μmol H_2O_2 equiv./L)/TAS (mmol Trolox equiv./L).

2.7. Statistical analysis

Each study group was consisted of 22 subjects when Alpha was accepted as 0.05, Beta was 0.20, and (1 − Beta) was 0.80 (power: 0.82).

Pearson's chi-square test was used to compare the gender between groups. Gender was presented as count and percentage. The Kolmogorov–Smirnov test was used to evaluate whether the distribution of variables was normal. The two independent sample *t* test or Mann–Whitney *U* test was used to compare continuous variables between control and patient groups. Continuous variables were presented as mean (standard deviation [SD]). Paired *t* test was used to detect differences between preoperative and postoperative periods. SPSS software 15.0 for Windows (Chicago, IL, USA) was used for all statistical analysis. Calculated *P*-values were considered statistically significant when they were <0.05.

3. Results

Chronic adenotonsillitis group consisted a total of 22 children, 13 (59%) boys and 9 (41%) girls, aged 8 ± 2 and control group consisted 25 children, 15 (60%) boys and 10 (40%) girls, aged 9 ± 4. There were no significant differences between chronic adenotonsillitis group and control groups in terms of age and gender.