

# Comparison of Preoperative and Postoperative Antioxidant Levels in Patients with Adenotonsillar Hypertrophy

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## Abstract

**Objective:** This study aimed to compare the serum oxidative stress levels of patients with adenotonsillar hypertrophy (ATH) with those of controls and to investigate the effects of adenotonsillectomy on the oxidative stress levels.

**Material and Methods:** Thirty healthy children (mean age, 6 years) and 30 patients with ATH (mean age, 7 years) aged 2-12 years were included in the study. Serum total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), and paraoxonase (PON) levels were compared between the patient and control groups. The preoperative and postoperative levels were also compared within the patient group.

**Results:** The TOS and OSI levels were significantly higher in the patient group than in the control group ( $p=0.007$  and  $p=0.027$ , respectively). There was no significant difference between the patient and control groups in terms of the TAS and PON levels ( $p=0.399$  and  $p=0.237$ , respectively). The TOS and OSI levels in the patient group were significantly higher in the preoperative than in the postoperative period ( $p=0.005$  and  $p=0.023$ , respectively), whereas there were no significant differences in the TAS and PON levels between the preoperative and postoperative period ( $p=0.192$  and  $p=0.262$ , respectively).

**Conclusion:** These findings demonstrate that patients with ATH are exposed to high levels of oxidative stress and that adenotonsillectomy normalizes the oxidative stress.

**Keywords:** Adenotonsillar hypertrophy, total oxidant status, total antioxidant status, oxidative stress index, paraoxonase

## INTRODUCTION

Adenotonsillar hypertrophy (ATH) is caused by mucosal lymphoid tissue hyperplasia (Waldeyer ring) after recurrent inflammation and infections in children (1). The palatine tonsils are the first reactive lymphoid organs that encounter the antigenic and microbial agents present in the air and food. Tonsils are found at the entrance of the respiratory and digestive systems, and they undergo changes in cellular and humoral immunity after recurrent upper respiratory tract infections (2). Adenotonsillar disease should be examined in two groups, including the main infection and hypertrophy (2). The pathogenesis of ATH has not yet been fully elucidated, but it has been reported that there is an increase in oxygen free radicals (OFR) and lipid peroxidation due to inflammation and infection (3). Adenotonsillectomy is one of the most common surgical procedures performed by otolaryngologists (3-5).

There is an equilibrium between oxidant and antioxidant molecules in the body. If oxidants increase or antioxidants decrease, oxidative stress increases and cellular damage occurs (6). OFR play a role in the pathogenesis of diabetes mellitus, Behçet's disease, rheumatoid arthritis, atherosclerosis, and various cancers (7).

There are recent interesting investigations regarding the effects of oxidant and antioxidant molecules in many diseases. Although it is possible to individually measure the plasma concentrations of oxidant molecules, it is not practical because these molecules may affect each other's blood levels. Recently, total oxidant status (TOS) measurement is used as an indicator of the sum of all oxidants in the body (8-10). Instead of the individual measurement of antiox-

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idants, measuring the level of all antioxidants provides more useful information. Hence, total antioxidant status (TAS) measurement is used more widely as an indicator of the antioxidant status of blood (8). Paraoxonase (PON) is an ester hydrolase having a glycoprotein structure, related with calcium-dependent high-density protein (HDL), acts as an antioxidant, and can inhibit paraoxon, which is a strong inhibitor of cholinesterase (11, 12). This study aimed to determine the oxidative stress index (OSI) levels in children with ATH and to investigate the effects of adenotonsillectomy on oxidative stress levels.

## MATERIAL AND METHODS

This study was conducted from June 1, 2016 to August 31, 2018 in the Ear, Nose, and Throat Clinic at Hitit University School of Medicine. Approval for the study protocol was obtained from the Clinical Studies Ethics Committee of Hitit University with the project no. 2017-195 (Approval Date: December 19, 2017). Thirty patients diagnosed with ATH between the ages of 2 and 12 and 30 healthy individuals forming the control group were included into the study.

Children with snoring, mouth breathing, and breathing arrest during sleep were included in the patient group. This was confirmed by the information obtained from the parents of the patients who had complaints for at least 6 months. Adenoid hypertrophy was detected by lateral skull radiography and nasopharynx endoscopic examination in all the patients. Adenoid size was visualized radiographically. Radiologically, the adenoidal depth/nasopharyngeal diameter ratio was measured on lateral radiography and the adenoidal nasopharyngeal (AN) ratio was determined. Patients with AN ratio greater than 0.67 were selected (13).

Brodsky criteria were used in the classification of tonsil hypertrophy (14) (Class I: tonsils hidden behind the front pillars; II. Grade: The tonsils are easily seen behind the front pillars; III. Degrees: tonsils block three-quarters of the airway; IV. Degrees: tonsils completely obstruct the airway). The control group consisted of children without adenoid and tonsil diseases, upper respiratory tract disease, nasal congestion, allergy, and asthma. Malignancy, serious systemic and neurological diseases, allergies, neck malformation, authenticated syndromes and nasal polyp patients were excluded from the study in both groups.

Adenotonsillectomy was performed using the cold knife and curettage method by the same surgeon under general anesthesia. Participants provided a signed consent form to voluntarily participate in the study. We collected 10 mL of venous blood from the patients into a vacuum chemistry tube between 8:00 and 10:00 a.m. From the patient group with ATH, venous blood was collected two times: one in the preoperative period and one at the 1-month postoperative follow-up. Pre- and postoperative measurements of the patients were individually compared with each other and with the control group. After 30-45 min of waiting, the blood samples were centrifuged at 4000 RPM and maintained in an Eppendorf tube at -80°C until biochemical analysis was performed.

## Biochemical Analysis

TOS and TAS were studied using the methods developed by Erel (8, 10), regarded as the most popular methods in our day. The TOS assay was performed in the Vital Scientific, Selectra/Flexor E (The Netherlands) autoanalyzer by a colorimetric method using the fully automated RelAssay (RelAssay Diagnostic's kit, Mega Tip, Gaziantep, Turkey) kit developed by Erel. The results were expressed as  $\mu\text{mol H}_2\text{O}_2$  equivalent/L (8). The TAS measurement was performed in the Abbott Architect® c16000 autoanalyzer using a fully automated RL0031 RelAssay® (Gaziantep,

Turkey) commercial kit. Results were expressed as micromolar Trolox/liter in this fully automated colorimetric assay method developed by Erel (8). The OSI is the ratio of TOS to TAS levels, and is indicative of the degree of oxidative stress (8). The following formula is used when the OSI is calculated. The mmol value in the unit of the TAS test is converted to  $\mu\text{mol}$  and the results are expressed as arbitrary unit (AU).  $\text{OSI} = \text{TOS (mmol H}_2\text{O}_2 \text{ equiv./L)}/\text{TAS (mmol Trolox equiv./L)} \times 10$ . The PON level was measured in the Abbott Architect® c16000 autoanalyzer using the fully automated RelAssay® (Gaziantep, Turkey) commercial kit. The results were expressed as unit/mol.

## Statistical Analysis

Statistical analyses were performed IBM Statistical Package for the Social Sciences software version 22.0 (IBM SPSS Corp.; Armonk, NY, USA). The normality distribution was analyzed with the Shapiro-Wilk test. Descriptive statistics were expressed as mean  $\pm$  standard deviation, median (min-max) for continuous variables based on their distribution assumptions, and as number and percentage for categorical data. In the analysis of continuous variables, independent-sample t-test was used to compare the means of two independent samples with a normal distribution, while the Mann Whitney U test was used for independent groups without normal distribution, Wilcoxon signed sequence test was used for normal non-distributed dependent groups and while the paired t-test was used for normally distributed dependent groups. For the level of statistical significance, p was accepted to be  $<0.05$ .

## RESULTS

The age and sex of the patients and controls were similar ( $p>0.05$  for both). The mean TOS levels in the patient and control groups were  $3.35 \pm 2.58$  and  $1.98 \pm 1.57$  mmol/L, respectively. The serum TOS levels in the patient group were significantly higher than those in the control. ( $p=0.007$ ) (Table 1). The mean OSI level in the patient and control groups was  $0.29 \pm 0.21$  and  $0.17 \pm 0.14$  AU, respectively. The OSI level was significantly higher in the patient group ( $p=0.027$ ) (Table 1). There was no significant difference in terms of the TAS and PON levels ( $p=0.399$  and  $p=0.237$ ) (Table 1, Figure 1). The mean TAS level in the patient and control groups was  $1.15 \pm 0.20$  and  $1.11 \pm 0.7$  mmol/L, respectively. The mean PON level in the patient and control groups was  $325.53 \pm 267.67$  and  $368.86 \pm 209.72$  U/L, respectively. When pre- and postoperative ATH groups were compared, no significant difference was found between the TAS and PON levels ( $p=0.192$  and  $p=0.262$ , respectively) (Table 2). The mean TAS level in the pre- and postoperative periods was  $1.15 \pm 0.20$  and  $1.21 \pm 0.14$  mmol/L, respectively.

The mean PON level in the pre- and postoperative group was  $325.53 \pm 267.67$  U/L and  $255.60 \pm 152.58$ , respectively. The serum TOS and OSI levels in the preoperative group were significantly higher than those in the postoperative group ( $p=0.005$ ), ( $p=0.023$ ) (Table 2). The mean TOS in the preoperative and postoperative groups was  $3.35 \pm 2.58$  mmol/L, and  $1.88 \pm 2.68$  mmol/L, respectively. The mean OSI level in the preoperative and postoperative groups was  $0.28 \pm 0.21$  AU and  $0.17 \pm 0.24$  AU, respectively.

## DISCUSSION

Although tonsillectomy and adenotonsillectomy are among the most frequently performed operations in children, the pathogenesis of ATH is not yet clearly understood. Hypoxia/reoxygenation periods are observed because of ATH and respiratory arrest and resumption (3). Therefore, we assume that the increase in oxidative stress plays an important role in the

**Table 1.** Comparison of the patient and control groups in terms of TOS, TAS, OSI, and PON

	Group	N	Mean±SD	Median (min-max)	p
TOS	Patient	30	3.35±2.58	2.61 (0.49-11.44)	0.007*
	Control	30	1.98±1.57	1.87 (0.14-8.55)	
TAS	Patient	30	1.15±0.20	1.14 (0.79-1.70)	0.399
	Control	30	1.11±0.7	1.12 (0.71-1.52)	
OSI	Patient	30	0.29±0.21	0.20 (0.04-1.04)	0.027*
	Control	30	0.17±0.14	0.17 (0.01-0.76)	
PON	Patient	30	325.53±267.67	189 (88-1193)	0.237
	Control	30	368.86±209.72	354 (94-814)	

\*Statistically significant (p<0.01).

TOS: total oxidant status; TAS: total antioxidant status; OSI: oxidative stress index; PON: paraoxonase; N: number; SD: standard deviation; min: minimum, max: maximum.

**Table 2.** Comparison of the patient and control groups in terms of the TOS, TAS, OSI, and PON levels

	Group	N	Mean±SD	p
TOS	Preoperative	30	3.35±2.58	0.005*
	Postoperative	30	1.88±2.68	
TAS	Preoperative	30	1.15±0.20	0.192
	Postoperative	30	1.21±0.14	
OSI	Preoperative	30	0.28±0.21	0.023*
	Postoperative	30	0.17±0.24	
PON	Preoperative	30	325.53±267.67	0.262
	Postoperative	30	255.60±152.58	

\*Statistically significant (p<0.05).

TOS: total oxidant status; TAS: total antioxidant status; OSI: oxidative stress index; PON: paraoxonase; N: number; SD: standard deviation.

pathogenesis of the disease. We believe that tonsillectomy has a positive effect on patients by decreasing the oxidative stress levels. The most important result found in the present study is that the TOS and OSI levels in the patient group were significantly higher than those in the control and postoperative groups. We found that there was no difference between the groups in terms of the TAS and PON levels. Generally, when oxidative stress increases, antioxidant levels increase as a response of the body. However, the antioxidant levels may decrease if the oxidative stress is severe and consumes excess antioxidants. Therefore, we suggest that TAS and PON activities change in patients with ATH.

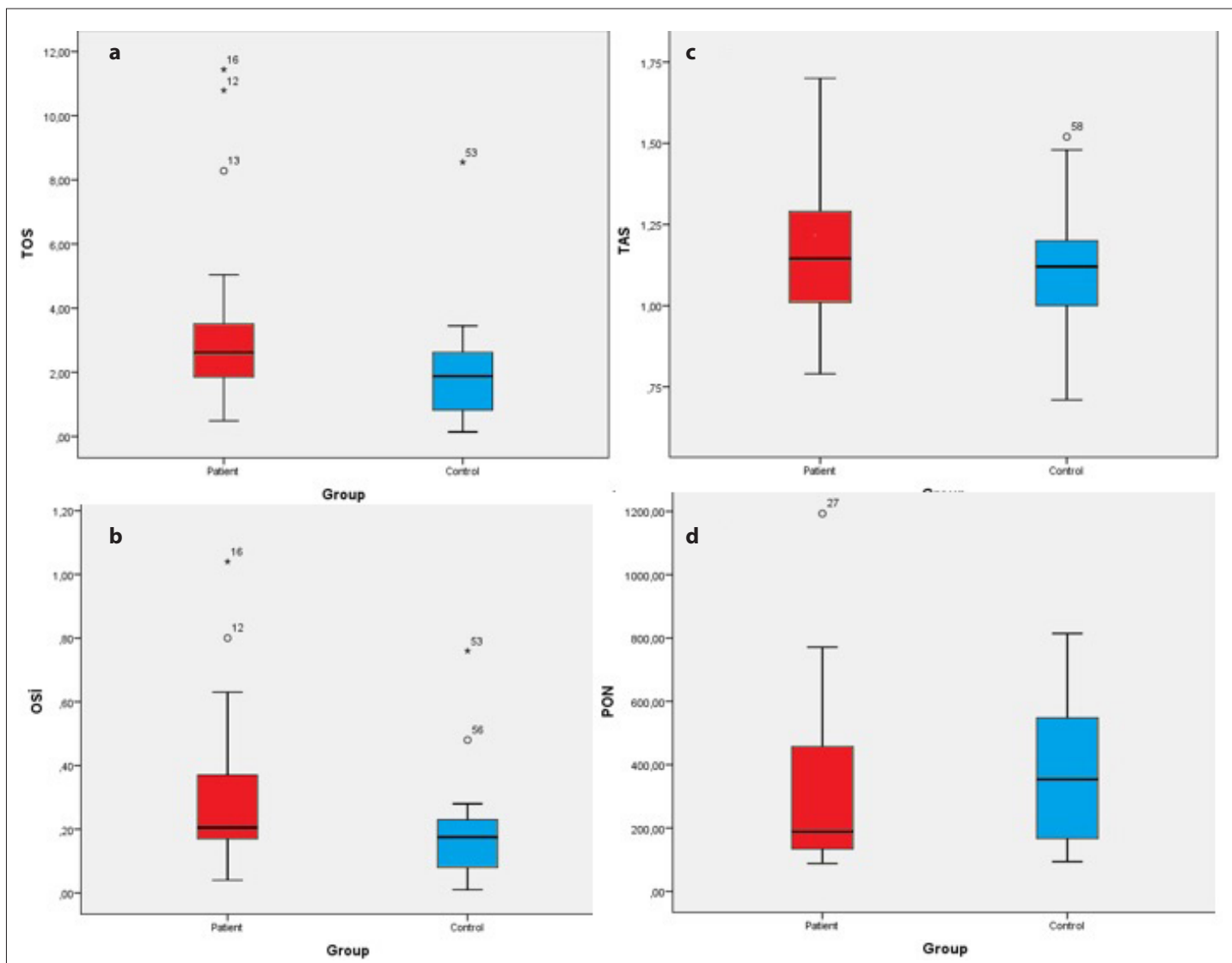
All aerobic organisms produce oxidation products for vital cellular reactions at physiological concentrations. The presence of these products in extreme amounts is called oxidative stress, which is highly detrimental to the body. These detrimental effects of the oxidation products are equilibrated by antioxidants. If this equilibration does not occur properly, oxidants and cellular proteins may cause cellular injury and changes in carbohydrates, lipids, and nucleic acids and may even lead to death. In their enzymatic or nonenzymatic forms, antioxidants play an important role in the defense mechanism of organisms against oxidative stresses (8-10).

The serum levels of most oxidant and antioxidant parameters can be measured individually. As oxidant and antioxidant parameters exert an additive effect, the individual levels may not be able to completely reflect the TOS or TAS. OSI is calculated using the TOS/TAS ratio (8-10). ATH is a chronic inflammatory disease, and OFR is thought to play a role in its

pathogenesis (15, 16). Infections are one of the causes of an increase in the level of OFR. Neutrophils, monocytes, eosinophils, and macrophages release free radicals to kill bacteria (17).

Abuhandan et al. (18) performed a clinical study on patients with chronic ATH and healthy children. They found that both the TOS and OSI levels were significantly higher in both the preoperative and postoperative patient groups than in the control group. They also found that the TOS levels in the preoperative patient group were significantly higher than those in the postoperative patient group, but they did not report any difference between the TAS and OSI levels (18). In our study, we found that TOS and OSI levels were higher in the preoperative group than in the control group. In addition, TOS and OSI levels were significantly higher in the preoperative group than in the postoperative group.

Kaygusuz et al. (19) found that malondialdehyde (MDA) increased and superoxide dismutase decreased in patients with chronic tonsillitis and reported that these patients were more exposed to oxidative stress. Kiroglu et al. (4) reported that oxidative parameters were high in patients with chronic adenotonsillitis, but normal in patients with ATH. They reported that ATH and chronic adenotonsillitis may be different diseases occurring on the same tissues (4). Yilmaz et al. (16) investigated the oxidative parameters in patients with ATH and reported an increase in antioxidants and a decrease in oxidants after adenotonsillectomy. Similarly, in patients with tonsillar hypertrophy, we found that TOS and OSI were



**Figure 1. a-d.** Box blots of the mean changes of TOS (a), OSI (b), TAS (c), and PON (d) in the patient and control groups

higher than in the control group. We also found that oxidative stress parameters decreased with tonsillectomy.

PON is a protein consisting of 354 amino acids and was detected for the first time in human serum in 1961 (20). This enzyme hydrolyzes lipid peroxide products and has an important role in the defense of LDL and HDL against oxidation (21). PON activity has been reported to have a possibility of change in inflammatory diseases (10). PON enzyme plays a role in the pathogenesis of many diseases including chronic renal failure, helicobacter pylori infection, HIV, chronic hepatitis B, and acute infection due to EBV (22). Koc et al. (3) found that the PON, TOS, and TAS levels were higher in children with ATH than in the control group. However, they reported no difference in the OSI levels (3). Comparing the preoperative and postoperative levels, only the TOS levels were found to be significantly higher in the preoperative group and they did not find any difference between the other parameters. In contrast to the study of Koc et al. (3), we found that there was no difference in the PON and TAS levels in ATH patients compared to the control and postoperative groups. Similarly, in our study, the TOS and OSI levels were significantly higher in the patient group than in the control group.

The limitation of this study was the small number of patients. In addition, we think that it may be beneficial to re-evaluate the oxidative parameters by scoring the obstructive complaints of the patients.

## CONCLUSION

In our study, we found that the preoperative TOS and OSI levels were higher in the patient than in the control groups. We also found that the TOS and OSI levels decreased significantly after adenotonsillectomy in the patient group. According to the results of our study, patients with ATH are exposed to high oxidative stress and adenotonsillectomy decreased the levels of oxidative stress.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Clinical Trials Ethics Committee of Hitit University (Approval Date: December 19, 2017; Approval Number: 2017/195).

**Informed Consent:** Written informed consent was obtained from the patients who participated in this study

**Author Contributions:** Concept - A.E., H.D., A.A., H.A.; Design - A.E., H.D., A.A., H.A.; Supervision - A.E., H.D., A.A., H.A.; Materials - A.E., H.D., A.A.; Data Collection and/or Processing - A.E., H.D., H.A.; Analysis and/or Interpretation - A.E., H.D., A.A., H.A.; Literature Search - A.E., H.D., A.A., H.A.; Writing Manuscript - A.E.; Critical Review - A.E., H.D., A.A., H.A.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

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