Adenoid Hipertrofisi Bulunan Çocuklarda Plazma Selenyum, Çinko ve Bakır Seviyeleri

Plasma Selenium, Zinc, and Copper Levels in Children with Adenoid Hypertrophy

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ÖZET

Amaç: Oksidatif strese bağlı doku hasarının birçok otolaringolojik hastalığın patogenezinde rol oynadığı gösterilmiştir. Bu çalışmanın amacı adenoid hipertrofisi (AH) bulunan çocukların plazmalarında selenyum (Se), çinko (Zn) ve bakır (Cu) seviyelerini değerlendirmektir.

Gereç ve Yöntem: Çalışma popülasyonu 60 çocuktan oluşmaktaydı; 30 sağlıklı çocuk (Grup 1) ve AH'si bulunan 30 çocuk (Grup 2). Se, Zn ve Cu ölçümleri atomik absorpsiyon spektrofotometre cihazı kullanılarak yapıldı.

Bulgular: Grup 1'e göre Grup 2'de plazma C-reaktif protein (CRP) ve Cu düzeyleri anlamlı olarak yüksek ve plazma Se ve Zn düzeyleri anlamlı olarak düşük bulundu ($p < 0,05$). Tüm olgular birlikte değerlendirildiğinde, Zn ile CRP ($r = -0,322$, $p = 0,012$) ve WBC ($r = -0,262$, $p = 0,043$) arasında istatistiksel olarak negatif korelasyon saptandı. Se ile Zn arasında istatistiksel olarak pozitif korelasyon saptandı ($r=0,276$, $p=0,033$).

Sonuç: Sağlıklı kontrollerle karşılaştırıldığında AH'li çocuklarda saptanan azalmış Se ve Zn gibi serum antioksidan eser element seviyeleri ile artmış Cu seviyeleri, AH'nin azalmış antioksidan yanıt ve artmış inflamasyon ile ilişkili olduğu hipotezini desteklemektedir.

Anahtar Sözcükler: Adenoid hipertrofisi; selenyum; çinko; bakır

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ABSTRACT

Objective: It has been demonstrated that oxidative stress induced tissue damage plays a role in the pathogenesis of several otolaryngological diseases. The aim of the present study was to evaluate the selenium (Se), zinc (Zn) and copper (Cu) levels in the plasma of children with adenoid hypertrophy (AH).

Material and Methods: The study population consisted of 60 children, 30 healthy children (Group 1) and 30 children with AH (Group 2). Se, Zn, and Cu measurements were taken using an atomic absorption spectrophotometer.

Results: The plasma C-reactive protein (CRP) and Cu levels were significantly higher, and the plasma Se and Zn levels significantly lower, in Group 2 than in Group 1 ($p < 0.05$). When all the cases were evaluated together, a statistically significant negative correlation was determined between Zn and CRP ($r=-0.322$, $p=0.012$) and WBC ($r=-0.262$, $p=0.043$), and a positive correlation was found between Se and Zn ($r=0.276$, $p=0.033$).

Conclusion: Decreased serum antioxidant trace element Se and Zn and increased Cu in children with AH when compared with healthy controls supported the hypothesis that AH is associated with decreased antioxidant response and increased inflammation.

Key words: Adenoid hypertrophy; selenium; zinc; copper

INTRODUCTION

As a part of Waldeyer's Ring, the adenoid is the accumulation of lymphoid tissue known as a pharyngeal tonsil that is located at the back of the nose or on the posterosuperior wall of the nasopharynx. Adenoid hypertrophy (AH) develops as a result of parenchymal hyperplasia or fibrinoid degeneration, and it is the most common cause of nasal obstruction during childhood (1). Adenoids are parts of the immune system that are the first barrier for preventing bacteria, viruses, and toxins from entering the body. Immunologic reactions within adenoids may lead to hypertrophy and chronic infection. Although the pathophysiology of AH is still unclear, impaired immune system parameters and oxidative stress play a significant role in the etiology of AH (2).

Studies from the literature have demonstrated that oxidative stress induced tissue damage plays a role in the pathogenesis of many pathological otolaryngological conditions such as otitis media, rhinosinusitis, chronic tonsillitis, nasal polyps, laryngeal cancer, and hearing loss (3). Since AH is a chronic inflammatory disease of the nasopharynx, there is a significant possibility that the balance between oxidation products and antioxidants is involved in the appearance and chronicity of this disease in the pharynx. Several studies have demonstrated high levels of oxidative stress in children with AH (3-5). During the chronic inflammation in adenotonsillar pathology, excessive production of reactive oxygen species (ROS) occurs, which leads to damage of nuclear and mitochondrial DNA, cell membrane lipids, and intracellular proteins, resulting in tissue damage (6). Studies have also shown that AH can develop as a result of hypofunction in local and systemic immunity, which could be caused by free radical damage to the membrane lipids of leukocytes (7). Additionally, because AH develops secondary to lymphoid hyperplasia, as Onal et al. (7) reported, apoptosis may be playing an important role in the pathogenesis of AH.

Trace elements are essential to biochemical processes in the body and are involved in immunological and inflammatory reactions. Microminerals such as selenium (Se), zinc (Zn), and copper (Cu) as the essential components of the antioxidant defense system utilized to synthesize antioxidant enzymes against free radical induced damage to tissues (8). Selenium, from the Greek Selene (meaning moon), is a chemical element with the atomic number 34 that was discovered by Berzelius as early as 1817 and is a well-known essential trace element important for human health (9). Studies of Se typically begin with the characterization of the first mammalian enzyme containing the

unusual amino acid selenocysteine (SeCys) in its catalytic center as well as cellular glutathione peroxidase (GPx). The main selenoprotein family consists of seven GPx genes that protect cells against the harmful effects of free radicals (10). In addition, Se functions as a redox center for GPx synthesis, which plays a key role in antioxidative defenses (10). It has been reported that Se deficiency could cause many inflammatory diseases by inhibiting the growth of immune organs and the resultant decreased immune function (11).

Superoxide dismutase (SOD) is one of the most important enzymatic antioxidants, and it protects aerobic organisms from the damage caused by free radicals. The cytoplasmic Cu/Zn-SOD, one of three forms of SOD, has antioxidant and anti-inflammatory activity and contains Cu and Zn as cofactors. It is believed to play a predominant role in the first step of antioxidant defense (2). Cu, one of the oldest known metals, is the third most abundant transition metal in the human body, and its excess is harmful by promoting excessive oxidative stress responses, which causes a reduction in the permeability of biological membranes. An improved oxidative stress response, permanent inflammatory status, and Cu overload have been documented in pathological conditions in both animal models and humans (12).

Zn is one of the most common trace elements in the human body and is required for activating enzymes that contribute to antioxidant defense mechanisms, protein synthesis, and nucleic acid replication. It reduces inflammatory cytokines, and so plays a role in the regulation of chronic inflammation. Zn deficiency could alter immune functions and increase the production of pro-inflammatory cytokines that influence the onset of numerous inflammatory diseases (2).

A number of studies have demonstrated that patients with AH have altered immune function and increased oxidative stress, so in this study, we aimed to evaluate plasma concentrations of Se, Zn, and Cu in children with AH.

MATERIALS AND METHODS

This cross-sectional study included participants recruited from among patients treated at the outpatient clinic of the Department of Otolaryngology of the Medical Faculty of Kahramanmaras Sutcu Imam University. The 60 participants were split into two groups, 30 healthy children in Group 1 (18 males, 12 females; mean age 8.30 ± 2.37 years; range 4 to 12 years) and 30 children with AH in Group 2 (16 males, 14 females; mean age 9.33 ± 1.95 years; range 4 to 12 years). Research ethics approval was obtained from the Ethics Committee of Kahramanmaras Sutcu Imam University before the initiation of the study. Signed informed consent was obtained from the parents of all the patients and healthy controls before enrollment in the study.

All the subjects had detailed otolaryngological and head and neck examinations. AH was diagnosed by history, ENT, and radiological examination of children who suffered from open mouth breathing, snoring, sleep apnea, difficulty in swallowing, and a lack of appetite.

The control group consisted of healthy children who were admitted to the otorhinolaryngology clinic with no symptoms related to the head and neck region and proven to be normal by otoscopic examination.

Excluded from the study were children with nasal septal deviation, major craniofacial abnormalities, nasal polyposis, hypertrophic concha, or acute or chronic inflammatory diseases as well as those taking nasal steroids or antihistaminic medication within the four weeks prior to examination.

Blood sampling

In addition to routine blood tests, a blood sample was taken from each patient and control subject before administration of any

medication and before any medical or surgical intervention. Blood specimens were drawn from the antecubital vein and collected in no additive vacutainer (Becton-Dickinson, Franklin Lakes, NJ) blood-collecting tubes according to standard hospital guidelines for venipuncture and sample collection. The serum separator tube specimens were allowed to clot and then centrifuged for 10 minutes at 3000 g to separate the serum. Serum hemoglobin (Hb) and white blood cell (WBC) levels were measured the same day using a calibrated automatic blood count analyser (Sysmex XN 3000, Japan). Serum C-reactive protein (CRP) was measured by the nefelometric method (Dade Behring Marburg GmbH, Marburg, Germany). Plasma was separated and stored at −70°C until the analysis of Se, Cu and Zn levels.

Measurement of serum Se levels

Selenium measurement was performed in a graphite furnace atomic absorption spectrophotometer (Perkin Elmer Analyst 800) using Zeeman background correction. Matrix modifiers were palladium (4 mg in a 20-mL sample) and magnesium sulfate (3 mg in a 20-mL sample). Samples and calibration standards were diluted 1:3 with 0.05% Triton X-100 to improve the sample viscosity and the reproducibility of the results. Selenium levels in all groups were evaluated according to a standard curve as μg/L, and Se calibration standards were prepared from the commercial Se standard (1000 mg/L) by serial dilutions (13).

Measurement of serum Cu levels

Serum Cu levels were analyzed in flame photometerof atomic absorption spectrophotometer (Perkin Elmer Analyst 800). Samples and calibration standards for Cu measurement were 1:2 dilutions with 10% glycerol. Commercial Cu calibrators were used as standards (1.000 mg/L) by serial dilutions and samples were evaluated according to a Standard curve (14).

Measurement of serum Zn levels

Serum Zn levels were analyzed inflame photometerof atomic absorption spectrophotometer (Perkin Elmer Analyst 800). Samples and calibration standards for Zn measurement were prepared in 1:4 dilutions with 5% glycerol. Commercial Zn standards (1.000 mg/L) were used by serial dilutions and samples were evaluated according to standard curve (15).

Cu and Zn results were given as ug/dl.

Statistical analyses

All data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows version 25.0 (IBM Corporation, Armonk, New York, U.S.). The normal distribution of data in both groups was tested by a Shapiro-Wilk test, and the variance homogeneity was tested using Levene's test. To determine the statistical significance of differences in age and BMI between two independent groups, the Independent-Samples t Test was used with bootstrap results. The Mann-Whitney U test was used with the Monte Carlo simulation technique for comparison with the Hb, WBC, CRP, Se, Zn, and Cu quantitative variables. Correlations between variables were evaluated using Spearman's rho correlation tests. Data are presented as mean ± SD, minimum-maximum, and median. Variables were examined at the 95% confidence level, and statistical significance was defined as $p < 0.05$.

RESULTS

The clinical characteristics and laboratory results of the groups are given in Table 1. The children were between 4 and 12 years old and were similar in both groups ($p > 0.05$). WBC, CRP and Cu levels were significantly higher in Group 2 (the AH group) than in Group 1 (the healthy control group) ($p < 0.05$) (Table 1). Se and Zn levels were significantly lower in Group 2 than in Group 1 ($p < 0.05$) (Table 1).

When we carried out the correlation analysis between CRP and WBC with Se, Zn, and Cu and between Se, Zn, and Cu, we did not find a correlation between these markers in either the control group or the AH group ($p > 0.05$). When we combined all participants from the two groups and regarded them as a single group (combined group, $n = 60$), a positive correlation between Se and Zn ($r = 0.276$, $p = 0.033$) and a negative correlation between Zn and CRP ($r = -0.322$, $p = 0.012$) and WBC ($r = -0.262$, $p = 0.043$) was revealed (Table 2).

Table 1. The clinical/demographical characteristics and laboratory results of groups.
Tablo 1. Grupların klinik / demografik özellikleri ve laboratuvar sonuçları.

	Control (Group 1) (n=30)		**Patient (Group 2) (n=30)**		
	Mean ± SD	**Median (Range)**	**Mean ± SD**	**Median (Range)**	**p**
Age (year)	8.30 ± 2.37	8.00 (4.00-12.00)	9.33 ± 1.95	9.50 (4.00-12.00)	0.498
Hb (gr/dl)	12.79 ± 1.06	12.75 (10.50-14.50)	12.06 ± 1.00	11.90 (10.20-14.40)	0.574
WBC	8847.00 ± 1746.24	8955.00 (4930.00-12120.00)	10165.67 ± 4262.45	9235.00 (5900.00-27490.00)	0.017*
CRP	3.49 ± 1.49	3.11 (3.03-11.00)	15.04 ± 27.98	3.32 (3.03-94.00)	0.002*
Zinc (ug/dl)	111.55 ± 27.21	109.30 (78.06-212.50)	92.05 ± 19.26	91.79 (42.92-147.00)	0.005*
Copper (ug/dl)	170.84 ± 35.74	168.60 (113.60-250.70)	209.49 ± 56.72	214.50 (110.30-301.60)	0.004*
Selenium (μg/L)	43.50 ± 19.63	41.79 (14.78-98.60)	29.08 ± 12.51	28.89 (10.57-65.17)	0.001*

All parameters are given mean±standart deviation, median and range values in table 1.
n: subject number
Hb: hemoglobin
WBC: white blood cell
CRP: C-reactive protein
p values statistically evaluated as $p>0.05$ not significant, $p<0.05$ significant.

Table 2. Correlation between Se, Zn and Cu, and their correlations with the WBC and CRP of participants in the combined group (Control + AH group).
Tablo 2. Birleşik grupta (Kontrol + AH grubu) Se, Zn ve Cu'nun birbirleriyle ve bunların WBC ve CRP ile olan korelasyonları.

Parameters	**Combined group (Group 1+ Group 2) (n=60)**		**Combined group (Group 1+ Group 2) (n=60)**		**Combined group (Group 1+ Group 2) (n=60)**	
	Se		Zn		Cu	
	r	p	r	p	r	p
WBC	-0.052	0.694	-0.262	0.043*	-0.050	0.702
CRP	-0.208	0.111	-0.322	0.012*	0.078	0.554
Cu	-0.141	0.283	-0.067	0.613		
Zn	0.276	0.033*				

n: subject number.
WBC: white blood cell
CRP: C-reactive protein
Se: selenium
Cu: copper
Zn: zinc
r, Spearman's rho correlation coefficient.
p values statistically evaluated as $p>0.05$ not significant, $p<0.05$ significant

DISCUSSION

The present study found for the first time that serum Se and Zn levels were significantly lower and Cu levels were significantly higher in children with AH, which is an important health problem in preschool- and school-aged children. Se, Zn, and Cu are involved in the destruction of free radicals through cascading enzyme systems. In the literature, many investigators have studied oxidative stress markers in patients with adenotonsillar pathology both in blood and tissue. Yılmaz et al. (6) found antioxidants and oxidation products in tonsil and adenoid tissues of patients with chronic adenotonsillitis and AH, and they indicated that these products are associated with these diseases. Michalska-Mosiej et al. (2) investigated the Se, Zn, and Cu levels in patients with chronic tonsillitis and found results similar to those in our study, meaning low Se and Zn levels and high Cu levels. Doğruer et al. (3) studied antioxidant enzymes in children with obstructive adenotonsillar hypertrophy (chronic tonsillitis vs. chronic hypertrophy). They hypothesized that imbalances between ROS and antioxidant defense mechanisms in obstructive adenotonsillar hypertrophy are caused by repeated hypoxia/reoxygenation episodes. Supporting this idea, Cho et al. (16) found elevated levels of oxidative stress markers in children with sleep disordered breathing, and they also reported significantly decreased levels of this marker after tonsillectomy and adenoidectomy. Another cause of oxidative stress in AH is inflammation, and several recent studies have demonstrated that it is closely related to free radical generation. Further, some researchers believe that as a result of increased oxidative stress in the inflammatory process, and by the production of ROS, antioxidant enzymes are concomitantly reduced (2). Further, inflammatory cells are the basal resource of increased oxygen radicals that cause cellular death, tissue damage, and necrosis in AH (17).

The antioxidants that have a significant role in defending the organism from oxidative stress and in the management of diseases caused by the oxidation products can be in enzymatic or nonenzymatic form. The most important antioxidant enzymes are catalase, SOD, and GPx. Se has antioxidant and anti-inflammatory actions, and studies have shown that plasma Se and GPx levels were inversely correlated with disease severity, morbidity, and mortality (18). A Se deficit may impair immune functions and enhance the risk of infection by increasing nuclear factor-κB (NF-κB) protein activation, which plays a crucial role in inflammatory and immune responses in AH (19). As reported in studies, Se supplementation might enhance immunity, including both humoral and cell-mediated responses (20). In addition, high-dose Se supplementation may reduce plasma CRP levels in patients with systemic inflammatory response syndrome/sepsis (21). By modulating selenoprotein gene expression, Se supplements inhibit NF-κB, which takes a role in the production of inflammatory markers and the synthesis of adhesion molecules and so may decrease serum hs-CRP levels (22). Thus, the low levels of Se in AH patients in our study could be the result of excessive Se consumption by the body to eliminate oxidative production as a protective action against damage caused by oxygen radicals. However, the causes of this depletion are not well known, whether a real deficiency of Se or a redistribution of Se from plasma to other organs as a defense mechanism of an organism, which is regulated by some immunoregulatory cytokines.

Zn and Cu, which are cofactors in many metalloenzymes, are also basic nutritional substances known as antioxidants. Both play an important role in ensuring the function and integrity of biomembranes and in protecting against free radical damage (23). In addition, Cu, which presents in the blood as being composed of nearly 90% ceruloplasmin, has been shown to play a role in the development and maintenance of the immune system. Increased oxidative stress, persistent inflammation, and Cu overload have been reported in pathological conditions in both animal experiments and in humans. In fact, Cu is important for immune response, including the production of IL-2 by activated lymphocytic cells, and it supports

the activity and effectiveness of cellular and humoral immunity (24). In parallel to studies that reported high serum levels of Cu in infection and inflammation, we found increased Cu levels in patients with AH. Contrary to our results showing high Cu levels in patients with AH, Aydoğan et al. (25) did not find a significant difference in serum Cu levels among groups. Increased plasma Cu concentrations in our study might be the result of the inflammatory response of the organism against infections.

Zn, one of the most valuable trace elements has catalyzer, structural, and regulatory functions in organisms. Zn has many biological actions that are essential for human health by preventing free radical formation in protecting biological structures from damage, in correcting immune functioning, and in apoptosis. Zn also plays an essential role with SOD, carbonic anhydrase, and matrix metalloproteinases enzyme activities (26). Zn is a cofactor of the antioxidant enzyme CuZnSOD, which is suppressed in an environment of Zn deficiency (27). Park et al. (28) reported a relationship of a postnasal drip, paranasal sinusitis, and middle ear effusion with CuZnSOD expression in the adenoids. Önerci et al. (29) and Somuk et al. (23) found significantly lower levels of Zn in patients with recurrent tonsillitis. They emphasized that because Zn is an element essential to the immune system, lower levels could create a predisposition for increased susceptibility to pathogens. In contrast to our result of low level of Zn in patients with AH, Aydoğan et al. (22) found smilar Zn levels in patients with AH in their study.

In our study we found a negative correlation between Zn and CRP. The high levels of the acute phase reactant, CRP, in our study indicated a concurrent inflammatory process, which may be secondary to AH. Also Zn is a negative acute-phase reactant; hence, its concentration decreases in the presence of inflammation. Similarly to our study Karakochuk et al. (30) reported that Zn concentrations were significantly negatively associated with CRP in population of Congolese children aged 6-59 months. Ebrahimi et al. (31) in women with polycystic ovary syndrome reported that Zn supplementation led to a significant reduction in serum hs-CRP. The possible mechanism causing the anti-inflammatory effect of Zn may be the downregulation of NF-κB, which may lead to a reduction in serum concentrations of inflammatory cytokines including CRP (32).

As seen from the studies Se, Zn, and Cu are essential trace elements for biochemical processes in the body and are involved in immunological and inflammatory reactions. They are also involved in the destruction of free radicals through cascading enzyme systems. The deficiencies of Se and Zn and high levels of Cu found in our study contribute to similar disease formation pathways in patients with AH through increased oxidative stress and reduced immune function.

This study had some limitations. Although we investigated the antioxidant trace elements in our study we could not investigate the total oxidant and antioxidant status. Also in the current study, the sample size was small. Future studies with a larger sample size are needed to confirm the validity of our findings.

CONCLUSION

The present study demonstrated that circulating levels of Se and Zn were significantly decreased, and Cu was significantly increased, in children with AH. Oxidative stress and lipid peroxidation may be key participants in the pathogenesis of AH. However, further investigation with more patients is necessary to determine the exact roles of Se, Cu, and Zn in the etiopathogenesis of AH and to estimate a possible therapeutic role of antioxidants, especially dietary supplementation with Zn and Se, in preventing AH.

Conflict of interest

There is no conflict of interest in this study and publication.

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