# Trace Elements (Zn and Cu) and Oxidative Stress in Pediatric Patients with Persistent Allergic Rhinitis

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## ABSTRACT

**Objective:** To investigate the relationship between complaints and serum trace elements and oxidative stress levels in pediatric patients with allergic rhinitis (AR).

**Study Design:** Descriptive study.

**Place and Duration of Study:** Department of Otolaryngorhinology, Medicine Faculty, Alanya Alaaddin Keykubat University, between May and September 2020.

**Methodology:** Thirty-five patients diagnosed with AR in the pediatric age group and 35 healthy control groups were examined. The patients were evaluated according to the ARIA (Allergic rhinitis and its ımpact on asthma) guidelines. Blood samples were taken during the attack to measure serum Zn and Cu levels and oxidative stress levels from patients diagnosed with AR. Measurements of serum Zinc and Copper levels, and recently developed new generation oxidant-antioxidant balance markers were performed spectrophotometrically using a commercial kit.]

**Results:** The mean ages of the study patients and healthy controls were 10.9 ± 4.7 and 10.5 ± 4.9 years, respectively. Female/-Male ratio was 20 (57.1%)/15 (42.9%) in both groups. Zn level was lower in the patient group ($p < 0.05$). Total thiol and native thiol values were higher in the control group ($p < 0.05$). Statistically significant high disulfide values were found in allergic patients ($p < 0.05$).

**Conclusion:** Oxidative stress plays a role in the pathophysiology of allergic rhinitis, and thiol-disulfide hemostasis may be an indicator in allergic rhinitis.

**Key Words:** *Oxidative stress, Allergic rhinitis, Thiol, Disulfide, Cu, Zn.*

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## INTRODUCTION

Allergic rhinitis is a common complaint among the reasons for applying to the otorhinolaryngology clinic. It is a common disease affecting approximately 10-40% of the general population.[1] In the industrial world and in modern life, where chemical exposure is increasing, environmental allergen exposure significantly increases the incidence of AR. AR is an inflammatory process. İn this process, histamine, interleukin, and prostaglandins play a role after contact with an allergen substance with the nasal mucosa.[2] Nasal itching, nasal congestion, and runny nose are the classic symptoms of AR.[3] Allergy can be considered as a single disease affecting the respiratory tract, if we consider it as a whole with the upper and lower respiratory tract.[4]

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AR can decrease the quality of life by daily activity loss, labour loss, and causing sleep disorders.[5] If the symptoms of allergic rhinitis last more than four weeks in a year and there are allergic rhinitis complaints more than four days a week, it is called persistent allergic rhinitis. Guidelines for diagnosis of allergic rhinitis suggest that diagnosis should be made when history and physical findings are positive as skin prick tests, specific IgE levels, and nasal smear for eosinophil.[6]

Free radicals formed as a result of the metabolism of foods with oxygen cause oxidative cell damage. The resulting free radicals cause oxidative cell damage, cellular death, and tissue damage.[2] Hydroxyl radicals, superoxide anions, and hydrogen peroxide are reactive oxygen derivatives (ROS).[7] Reactive oxygen species (ROS), which are byproducts of the metabolic pathway, react with cellular proteins and cause cell damage. The concentrations of substances such as SOD, native tyhol, disulfide, which are serum oxidative stress markers, were measured to detect this process.[7] Thiol groups known as mercaptans are important components. Thiols are organic compounds consisting of a hydrogen atom attached to a carbon atom containing a sulfur atom and a sulfhydryl group (-SH).[8] In oxidative stress, thiol oxidises and converts to a disulfide. Thiol/di-

sulfide homeostasis was first measured in 1979, using methods such as high-performance liquid chromatography and bioluminescence.[5] Thiol-Disulfide hemostasis (TDH) is an important and dual indicator of oxidant-antioxidant balance.[9]

Zinc is an enzymatic activator and catalyst, which plays an important role in hormonal processes and the amino acid cycle. İt is used to treat functional dyspepsia, Wilson's disease, diabetes mellitus, rheumatoid arthritis, *helicobacter pylori* infection, and various malignancies.[10,11] Copper is a trace element necessary for the controlled functioning of enzymes in oxidation. It is found in the structure of superoxide dismutase, monoamine oxidase, tyrosinase, dopamine beta-hydroxylase, and ceruloplasmin.[10] Therefore, the role of Zn and Cu in the antioxidant system is very important.

The balance between impaired oxidative stress/antioxidant system plays a role in the etiopathogenesis of many diseases. There is no study in the literature investigating the relationship between AR and oxidative stress level and serum Zn and Cu levels in the pediatric age group. This study aimed to investigate the relationship between complaints and serum trace elements, and oxidative stress levels in pediatric patients with AR.

## METHODOLOGY

In this descriptive controlled study, 35 patients attending the outpatient clinic of the Otorhinolaryngology Department of ALKU Medical Faculty Hospital, between May and September 2020, were included, after obtaining ethical approval from the University. These patients diagnosed with AR in the pediatric age group and 35 healthy children in control group were examined. İn the study group, patients who applied to ENT clinics with complaints of itching in the nose and palate, nasal congestion, runny nose, and sneezing; and diagnosed with allergic rhinitis were examined. All patients have these symptoms for more than four weeks, more than four days a week, and they did not receive any medical treatment. The patients (5-18 age) were determined according to the ARIA (Allergic rhinitis and its impact on asthma) guidelines.[1] Patients with a recent diagnosis (less than four weeks ago), a recent history of nasal surgery, nasal polip and an oncological diagnosis, were excluded. Blood samples were taken during the attack to measure serum Zn and Cu levels and oxidative stress levels from patients diagnosed with AR. In the control group, 35 healthy patients of similar age groups and gender with no AR symptoms were included in the study group.

Thiol/disulfide homeostasis tests were performed in the same way as Erel and Neselioglu previously described in their study.[5] Two parallel vessels were used for the samples. For determining total thiol, a 10µL sample was treated with 10µL sodium borohydride in 50% methanol-water solution (v/v; R1). 110µL 6.715mM formaldehyde and 10.0mM ethylenediaminetetraacetic acid (EDTA) in Tris buffer 100mM (pH 8.2) used for Excess reductants eliminating. Ten µL sample was treated with 10µL, 10mM sodium chloride in 50% methanol-water solution (v/v; R1') and 110µL 6.715mM formaldehyde and 10.0mM EDTA in Tris buffer 100mM (pH 8.2) used for determinating native thiol. The first absorbance was taken only after adding R1 and R1' for total and native thiol, respectively. First absorbance was subtracted from the second. All the chemicals were purchased from Merck Chemicals (Darmstadt, Germany) and Sigma-Aldrich Chemie (Milwaukee, Wisconsin, USA).

The amount of dynamic disulfide was calculated as half of the difference between total thiol and native thiol groups and calculated native and total thiols. The serum disulfide levels and the disulfide/native thiol, disulfide/total thiol, and native/total thiol ratios were also calculated.[9]Commercial kits were used to determine the zinc and copper levels by spectrophotometric method (Relassay Diagnostics, Turkey). The absorbance level proportionally alters with the overall zinc and copper content in the samples when at 548 and 572 nm. Absorbance measurement was made in a microplate reader device (Biotek Synergy H1, USA). The units are given as µg/dl.

All analyses were performed using IBM SPSS Statistics Version 20.0 statistical software package (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY). Categorical variables were expressed as numbers and percentages; whereas, continuous variables were summarised as mean and standard deviation. Comparison of laboratory parameters between groups was made by independent t-test. It was accepted as the statistical significance level ($p < 0.05$). ROC analysis was applied for all parameters.

**Table I: Comparison of serum trace elements between study groups.**

| | AR | Control | P |
|---|---|---|---|
| Zn(µg/dL) | 86.3±12.1 | 112.8 ±80.1 | 0.061 |
| Cu(µg/L) | 113.3 ± 38.8 | 104.8± 33.6 | 0.328 |

**Table II: Comparison of study groups thiol, native thiol and disulphide.**

| | AR | Control | P |
|---|---|---|---|
| TT(µmol/L) | 343.0±35.8 | 406.5±25.6 | <0.001 |
| NT(µmol/L) | 283.8±26.2 | 384.7±23.5 | <0.001 |
| SS(µmol/L) | 29.6±16.1 | 10.9±5.1 | <0.001 |
| Reduced Thiol(µmol/L) | 83.2±8.2 | 94.7±2.4 | <0.001 |
| Oxidized Thiol(µmol/L) | 8.4±4.1 | 2.7±1.2 | <0.001 |
| ThiolOx/ThiolRed | 2375.7±4722.7 | 4385.7±2121.1 | 0.025 |

## RESULTS

Thirty-five patients diagnosed with AR in the pediatric age group and 35 healthy control groups, were examined prospectively. The mean ages of the study patients and healthy controls were 10.9 ± 4.7 and 10.5 ± 4.9, respectively. The female/male ratio (20/15) was the same in both groups. Serum Cu level was higher in the AR group than in the control group. Zn levels in patients with AR were lower than the control group, But both of these levels were statistically insignificant ($p > 0.05$, Table I). Serum total thiol (406.5 ± 25.6 µmol/L /343.0 ± 35.8 µmol/L) and native thiol (384.7 ± 23.5 µmol/L /283.8 ± 26.2 µmol/L) values were higher in the control group ($p < 0.05$). High disulfide (29.6 ± 16 µmol/L) values were found in allergic patients, and they were statistically significant ($p < 0.05$). Reduced thiol (94.6 ± 2.4 µmol/L) was higher in the control group, and oxidised thiol

(8.4 ± 4.1 µmol/L) was higher in the AR group. Thiol oxidised / Thiol reduced ratios were higher (4385.7 ± 2121.1 µmol/L) in the control group (Table II). According to ROC analysis, disulfide and oxidised thiol were found to be strong independent predictive factor in patients with allergic rhinitis (Table III, Figure 1).

**Table III: ROC analysis of Zn, Cu, TT, NT, SS, reduced thiol, oxidised thiol, thiolOx/thiolRed ratio.**

| | Area | p-values | 95%C.I |
|---|---|---|---|
| Zn | 0.535 | 0.614 | 0.391-0.679 |
| Cu | 0.579 | 0.257 | 0.443-0.714 |
| TotalThiol | 0.091 | 0.000 | 0.013-0.170 |
| NT | 0.012 | 0.000 | 0.000-0.033 |
| SS | 0.833 | 0.000 | 0.727-0.938 |
| Reduced Thiol | 0.133 | 0.000 | 0.037-0.228 |
| Oxidized Thiol | 0.867 | 0.000 | 0.771-0.962 |
| ThiolOx/ThiolRed | 0.133 | 0.000 | 0.038-0.228 |

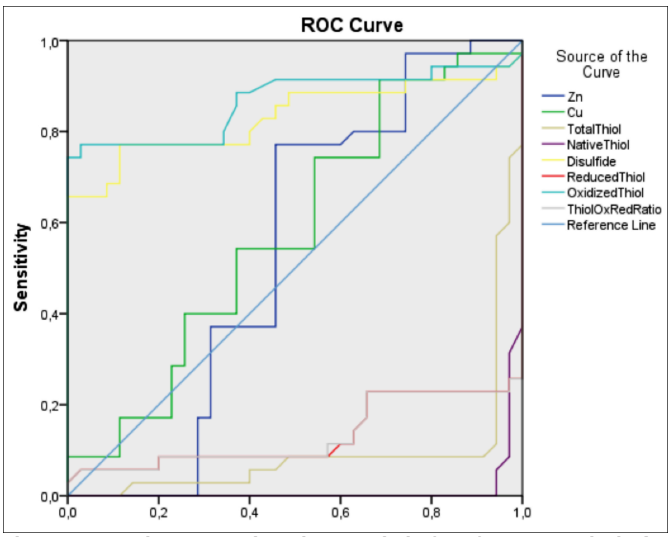


**Figure 1: Receiver operating characteristic (ROC) curve analysis for serum Zn, Cu, TT, NT, SS, reduced T, oxidized T, and thioloxred ratio.**

## DISCUSSION

Various studies in the literature investigate the role of oxidative stress in the etiology of ENT diseases.[11-13] It has been reported to play a role in the etiology of hearing loss, rhinosinusitis, otitis media, chronic tonsillitis, and laryngeal cancer.[2,13] The concentrations of substances such as SOD, native tyhol, disulfide, which are serum oxidative stress markers, were measured to detect this process.[8] Thiol groups known as mercaptans are important components. Thiols are organic compounds consisting of a hydrogen atom attached to a carbon atom containing a sulfur atom and a sulfhydryl group (-SH).[8] In oxidative stress, thiol oxidises and turns into disulfide. Thiol balance is an important and dual indicator of oxidant-antioxidant balance. TDH impairment is thought to play a role in the pathogenesis of diseases by causing increased oxidative stress and tissue inflammation. High-performance liquid chromatography, capillary fluorescence electrophoresis, and bioluminescent systems were used for serum SS and SH measurement. These techniques are time-consuming and costly. The new spectrophotometric analysis technique, described by Neselioglu and Erel, is frequently used in recent studies due to its easy accessibility and cheapness.[9]

Temel M. *et al.* found low serum NT and disulfide levels in the child patient with community-acquired pneumonia in their study.[14] In the literature, the effect of TDH in many diseases such as sudden sensorineural hearing loss, vitiligo, Hashimoto thyroiditis, and polycystic ovary syndrome has been investigated.[15,16] In a study conducted with patients with the diagnosis of nasal polyposis, NT levels were found to be significantly lower.[17] In another study, it was reported that allergens increased hydroxyl radicals, peroxide, and superoxide levels. This process initiates the inflammatory process in the respiratory mucosa. There is a protective mechanism against oxidants in the respiratory system mucosa.[13] Choo *et al.* reported that low antioxidant activity increased atopy in children, and clinically, antioxidant supplements may serve as a complementary treatment for a more effective therapeutic approach towards atopic diseases.[2] There is no study about TDH in the pediatric age group, such as this study in the literature. The authors believe that their study will make a significant contribution to the literature on this subject. This study found a significantly higher disulfide level in the AR group compared to the control group during an attack ($p < 0.05$). These findings were consistent with the literature. Besides, low NT and TT levels were found in the patient group. The findings of this study were statistically significant. These findings support the hypothesis that oxidative stress increases and the detoxification pathway slows down in allergic rhinitis patients.

Zn and Cu, which are cofactors in many metalloenzymes, are also very powerful antioxidants, and these are essential elements for antioxidation. Superoxide dismutase (SOD), an antioxidant enzyme, contains Zn and Cu in their structure. Superoxide, the substrate of SOD, reacts with fatty acids in the membranes to form products such as malondialdehyde (MDA) and 4 hydroxy nonenal. These are mutagenic and cellular destructive toxins. SOD gene mutation plays a role in the pathophysiology of Down syndrome and ALS.[18] Studies are reporting that low Zn level is associated with neurological disorders and growth retardation.[19,20] In a study, it was found that low Cu level should be an indicator in pediatric functional dyspepsia.[11] Serum Zn and Cu values can give information about antioxidant capacity. However, there are limited studies in the literature to explain the relationship between Cu and Zn and AR. There is no study in the pediatric AR group. Liu *et al.* in their studies on adult ARs, the patient group reported that they found serum Zn levels significantly lower than the control group.[21] The present authors found the Zn level low in the patient group. However, this variable between groups was not statistically significant ($p > 0.05$).

It can be concluded that a low serum level of Zn triggers AR as a result of increasing free radical formation by decreasing the activity of the SOD enzyme. In this study, similar to other researchers, the authors found higher serum Cu levels in the patient group. This is not a suitable result for this hypothesis. However, studies with large patient groups are needed to make

clearer interpretations.

The deficiency in this study was that the control blood was not checked after the attack. However, the large patient and control groups make this study valuable. As a result, it can be concluded that low Zn levels decrease the antioxidant activity and increase AR formation. The results for thiol showed a statistically significant difference. An increase in disulfide value indicates a decrease in detoxification in oxidative stress metabolism in patients.

## CONCLUSION

Oxidative stress plays a role in the pathophysiology of allergic rhinitis. The authors believe that serum Zn values can be used in the treatment of allergic rhinitis and prevention of new attacks. Thiol-disulfide hemostasis may be a possible laboratory indicator in allergic rhinitis.

**ETHICAL APPROVAL:**
Ethical approval was obtained from the Institutional Review Board of Alaadin Keykubat University.

**PATIENTS' CONSENT:**
Consents were obtained from parents.

**CONFLICT OF INTEREST:**
The authors declared no conflict of interest.

**AUTHORS' CONTRIBUTION:**
HG: Design, acquisition, analysis, interpretation, drafting, revision, and final approval of the version to be published.
HBS: Design, analysis, and interpretation of data.
SG: Acquisition and interpretation of data.

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