

Oxidative Stress in Children with Noninfectious Acute Urticaria

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Plasma oxidative stress parameters such as total oxidant status (TOS) and total antioxidant status (TAS) were well studied in some chronic allergies, but their role is not well known in acute allergic disease such as acute urticaria (AU). The aim of this study is to investigate the oxidative and antioxidative status in children with AU. Eighty-seven children with noninfectious AU and 70 healthy controls were enrolled in the study. Blood samples were taken for complete blood count, serum immunoglobulin E (IgE), and C-reactive protein (CRP) levels routinely and for TOS and TAS. The skin prick test was performed and/or specific IgE assays were performed as indicated. Blood samples were also taken for TAS and TOS in the control group. Forty-eight (55%) of the children with AU were male, 39 (45%) of them were female. Mean ages were 6 ± 2.02 and 7 ± 1.38 years in the study and control groups, respectively. TOS levels were significantly high and TAS levels were significantly lower in the acute urticarial group ($P < 0.001$) than controls. There was no significant correlation between age, total IgE levels, or eosinophil count and TAS and TOS levels in children with AU. Plasma TOS levels are elevated in children with AU. The high level of TOS indicates that children with AU were exposed to severe oxidative stress.

Introduction

ACUTE URTICARIA (AU) is a common allergic disease characterized by wheals and flares on the skin. It is a type I hypersensitivity reaction to a variety of allergic stimuli.¹ Mast cells are the principle cells in this process, in which the interaction of the antigen with immunoglobulin E (IgE) binds to the high-affinity IgE receptor (FcεRI) on mast cells and leads to release of allergic mediators such as histamine and leukotriens.²

Oxidant generation is part of the normal metabolism of many types of cells and is critical for cell homeostasis. To protect itself against exposure to noxious oxidant, the human body has a developed antioxidant system.³ The association between chronic inflammation and oxidative stress is well documented. Elevated levels of reactive oxygen species (ROS) such as hydroxyl radicals, superoxides, and peroxides are capable of producing a variety of pathological changes that are highly relevant in nasal and airway mucosa. These include lipid peroxidation, increased airway reactivity, increased nasal mucosal sensitivity and secretions, production of chemoattractant molecules, and increased vascular permeability.^{4,5} Plasma oxidative stress parameters such as total oxidant status (TOS) and total antioxidant status (TAS) were well studied in some chronic allergic diseases (ie, asthma, allergic rhinitis),^{6,7} but their role is not well known in acute allergic diseases such as AU.

The main problem for choosing oxidative stress markers arises from the complexity of the methods and highly expensive instruments. Measurement of different antioxidant molecules separately is not practicable, and antioxidant effects of these molecules are additive, so in this study, we measured only TOS and TAS levels.⁸

There were no clinical data in the literature associated with AU and oxidative parameters in children. To determine the oxidant/antioxidant imbalance in a high number of children, define the determinants of this imbalance, and elucidate relationships between plasma antioxidants, we measured a marker of oxidative stress, TOS, and a marker of antioxidant capacity, TAS, in the serum obtained from children with AU and healthy controls.

Materials and Methods

Study design and patients

A total of 135 children who were diagnosed with having AU attended to by Pediatric Allergy and Immunology or Pediatric Emergency Clinics between November 2013 and October 2014 were admitted during the period. The diagnosis of AU is defined as sudden appearance of wheals and/or angioedema, lasting maximum 24 h and, then resolving spontaneously. According to the duration of symptoms, AU is also defined as lasting shorter than 6 weeks. Complete blood

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count, serum IgE, total eosinophil counts, CRP levels, urine analysis, and for TOS and TAS were performed for every patients. The skin prick test was performed and/or specific IgE assays were performed as indicated. However, 45 patients were excluded from the study due to having infectious findings according to their clinical examination (fever higher than 38°C with/or associated physical findings) and laboratory (complete blood analyses, urine analyses) tests. All patients with noninfectious AU were followed at least 2 months. Three of the patients regarded as having chronic urticaria during follow-up were also excluded. Remaining 87 children newly diagnosed with having AU were enrolled into this study. None of the newly diagnosed patients used antihistamines or any drugs that may alter the serum oxidant status before performing laboratory tests.

The control group comprised 70 age-matched children who presented in the same period to the outpatient department of the same hospital. They presented for preoperative routine examination (eg, inguinal hernia, circumcision) or for their regular follow-up. Control patients were evaluated with regard to chronic and/or severe infections, rheumatologic and autoimmune disorders, and familial and personal history of atopy, and also by laboratory tests. None of the study and control group participants had chronic disability (eg, Down's syndrome, Autism), failure to thrive, malnutrition, or obesity. Children were included in the control group if they had no personal and familial history of atopy and no signs of acute infection according to the clinical and laboratory findings. All children in the control group underwent skin prick testing and had their total IgE, total eosinophil count, TAS, and TOS measured in blood samples. As it is known to have an effect on oxidative status, the patients came from nonsmoking households, and the control group was also selected from nonsmoking households.⁹

Blood samples

Total IgE. Serum samples for *in vitro* total IgE were obtained from all children after 8 h of fasting. Venous blood samples of 2 mL were obtained and left to clot for 60 min at room temperature. They were centrifuged for 10 min at 1,200 rpm. Serum samples were stored at -20°C. Serum total IgE (IU/mL) was measured through the chemiluminescent enzyme immunoassay method using the Immulite 2000 automated analyzer (Immulite 2000; DPC, Los Angeles, CA).

Eosinophil and neutrophil counts were determined from venous blood samples through Coulter Counter (Beckman Coulter, Inc., Fullerton, CA) leukocyte measurement.

Measurement of the TAS of plasma

Serum samples for measurement of TAS and TOS levels were stored at -80°C until they were used. TAS of serum was determined with using an automated method.⁸ In this method, the hydroxyl radical, the most potent biological radical, is produced. The antioxidant effect of the sample against the potent free-radical reactions, which are initiated by the hydroxyl radicals produced, is measured. The assay has excellent precision values of lower than 3%. The results are expressed as milimoles of Trolox per liter.

Measurement of TOS of plasma

TOS of plasma was determined using an automated measurement method, developed by Erel.¹⁰ Oxidants present in

the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules in the sample. The assay is calibrated with hydrogen peroxide and results are expressed in terms of micromolar hydrogen peroxide equivalent per liter ($\mu\text{mol H}_2\text{O}_2 \text{ Eqv./L}$).

Determination of the oxidative stress index

The ratio of TOS to TAS is compared using the oxidative stress index (OSI). The OSI value was calculated according to the following formula: $\text{OSI (arbitrary unit)} = [\text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ Eqv./L}) / \text{TAS (mmol Trolox Eqv./L)}] \times 100$.¹⁰

Ethical approval. The study is approved by the local ethics committee of Bezmialem Vakıf University (No: 71306642/050-01-04/39) and performed in accordance with the Declaration of Helsinki Good Clinical Practice Guidelines. The study is performed on children whose parents gave informed consent.

Statistical analyses. SPSS program (v11.5; SPSS Inc., Chicago, IL) was used for all statistical analyses. Signal intensities are given in arbitrary units with mean, standard deviation (SD), or standard error of the mean (SEM). Age means, serum total IgE, eosinophils and neutrophil counts, TAS, and TOS in the two groups were compared by independent sample *t*-test or ANOVA rank. We also examined whether the relationship between TAS and TOS in AU was independent of other clinical markers of AU (eosinophil counts, total serum IgE, and skin test positivity) using multivariate logistic regression models that included TAS/TOS and other clinical markers as potential predictors of AU status. $P \leq 0.05$ was considered statistically significant.

Results

Of the 87 patients with AU, 44 (50%) were male, 43 (50%) were female. Mean age of the study group was 5.85 ± 1.02 years. Thirty-four patients of the control group (48.2%) were male, 36 (51.8%) were female. Mean age of the control group was 6.25 ± 1.38 years. There was no statistically significant difference in terms of gender and age between groups. ($P = 0.85$, $P = 0.35$, respectively) (Table 1); 20% ($n = 18$) of the children with AU had at least one atopic disease. Twelve had asthma, 2 had atopic dermatitis, 1 had cephalosporin sensitivity (positive specific IgE and clinical history), 1 had cow's milk sensitization (specific IgE positivity and clinical history), 1 had asthma and atopic dermatitis, and 1 had asthma and allergic rhinitis. Twenty-five patients (28%) had house dust mite sensitivity according to SPT results, and others were negative in the study group (Table 1).

There was a highly significant increase in the oxidant stress parameters in the plasma (increased TOS and OSI) and a similar decrease in the antioxidant defense (decreased reduced TAS) in patients with AU compared with controls ($P \leq 0.001$) (Table 2). Multivariate logistic regression analysis showed that reduced TAS [odds ratio (OR), 1.25; 95% confidence interval (CI), 1.15–3.35; $P < 0.05$] and increased TOS (OR, 2.1; 95% CI, 1.9–3.7; $P = 0.025$) are not associated with total IgE levels and eosinophil counts in patients with AU (Table 3).

TABLE 1. SOME CLINICAL AND DEMOGRAPHIC CHARACTERISTICS OF THE STUDY AND CONTROL GROUPS

	<i>Patients with acute urticaria (N=87) N (%)</i>	<i>Control group (N=70) N (%)</i>	<i>P^a</i>
Gender			
Male	48 (55)	38 (54)	0.85
Female	39 (45)	32 (46)	
Age (years)	6 ± 2.02	7 ± 1.38	0.35
Concomitant atopic disease			
Asthma	12 (13.8)	0	0.0001
Atopic dermatitis	2 (1.14)	0	
Cow's milk protein hypersensitivity	1 (1.14)	0	0.78
Cephalosporin hypersensitivity	1 (1.14)	0	
Asthma + Allergic rhinitis	1 (1.14)	0	
Asthma + Atopic dermatitis	1 (1.14)	0	
IgE (mean, SD)	83 ± 9.70	75 ± 7.30	0.55
CRP (mean, SD)	0.79 ± 0.55	0.65 ± 0.35	0.38
Eosinophil (%)	2.55 ± 0.25	2.00 ± 0.15	0.80
Skin test sensitization positivity (N, %)	25/87 (28)	10/70 (10)	0.032

^aIndependent sample *t*-test.

Bold type indicates that concomitant atopic disease and skin test positivity rates were statistically higher in patients with acute urticaria than controls.

CRP, C-reactive protein; IgE, immunoglobulin E; SD, standard deviation.

Discussion

In this study, serum TAS, TOS, and OSI values that easily performed blood biomarkers were found to be statistically significantly different between children with AU and control group. To the best of our knowledge, our study is the first to examine plasma levels of oxidant status in children with AU, established clinical indicators of acute inflammation and immune function. Our study also showed that there is oxidant stress imbalance by measuring TOS and TAS independently of allergic status in a group of children with AU. Limited studies have explored the importance of oxidative stress in urticaria, especially in chronic idiopathic urticaria (CIU). However, possible interaction between oxidative stress and AU has not been studied in a comprehensive manner as it was performed in our study by measuring these two (TAS, TOS) serum biomarkers.

Many cells and mediators are thought to modulate the formation of ROS (oxidative stress), which in turn may mediate cellular signaling pathways related to inflammation.^{2,11} A linkage between oxidative stress and inflammation is believed to contribute in the pathogenesis of some allergic diseases.^{5,12} However, there is no report on the antioxidant system and ox-

idant stress in children with AU. The majority of studies on oxidant/antioxidant balance have been performed in patients with asthma, allergic rhinitis, and adult urticaria.^{4,6,7,13} Kalkan et al.¹⁴ showed increased levels of enzymes, such as malondialdehyde (MDA), nitric oxide (NO), and copper-zinc superoxide dismutase (Cu-Zn SOD), and decrease in glutathione peroxidase (GSH-Px) activity with decreased levels of protein carbonyl groups in 50 adult patients with AU.

Sagdic et al.¹⁵ reported that erythrocyte MDA levels, GSH-Px activities, and Zn levels showed no differences between the patient and control groups, but a statistically significant decrease and increase were observed in erythrocyte Cu-Zn-SOD activities and Cu levels, respectively, in the CIU. Authors concluded that an oxidative stress can be relieved by some preserved antioxidant mechanisms seemed to be involved in patients with CIU even if they are clinically stable and it might have a role in the pathogenesis.

In our study, there was a significant increase in TOS levels and significant decrease in TAS levels in children with AU, which is an acute form of allergic inflammation. This finding correlates with the findings of Tsukahara et al.¹⁶ who stated that oxidative stress and altered antioxidant defenses were involved in the pathophysiology of acute exacerbation of atopic dermatitis. In general, half of the children with atopic dermatitis have no atopic background,¹⁷ and in our study population, there were only 20% with atopic background. Additionally, our results showed that there was no significant correlation between serum TAS/TOS levels and total IgE and

TABLE 2. TOTAL ANTIOXIDANT STATUS AND TOTAL OXIDATIVE STATUS LEVELS IN STUDY GROUP AND CONTROLS

<i>Parameter</i>	<i>Children with acute urticaria (n=87)</i>	<i>Controls (n=70)</i>	<i>P^a</i>
Total antioxidant status (mmol of trolox/L)	1.75 ± 0.20	3.73 ± 0.32	<0.001
Total oxidant status (μmol H ₂ O ₂ /L)	10.46 ± 3.69	4.21 ± 2.51	<0.001
OSI (oxidative stress index, arbitrary units)	5.72 ± 1.22	1.03 ± 0.85	<0.001

^aBy ANOVA on ranks.

TABLE 3. MULTIVARIATE LOGISTIC REGRESSION ANALYSIS OF TOTAL OXIDANT STATUS AND TOTAL IgE, AND EOSINOPHILS, (N=87) IN CHILDREN WITH ACUTE URTICARIA

	<i>OR</i>	<i>95% CI</i>	<i>P</i>
Total IgE	1.54	[1.27–3.84]	0.05
Eosinophils	1.40	[1.84–3.45]	

OR, odds ratio; CI, confidence interval.

eosinophils in children with AU. This finding suggests that the imbalance between oxidative stress and antioxidant capacity is a consequence of inflammation in AU rather than caused by urticaria. Further studies regarding the association of oxidant/antioxidant imbalance and IgE production are necessary to determine the reason for such discrepancies.

There are numerous compounds with diverse antioxidant activities in the plasma, so total antioxidant capacity is more accurate and sensitive to represent the antioxidant status.^{12,14} Because there are many oxidant and antioxidant enzymes and their effects are additive, individual measurements may not reflect the actual oxidative status accurately.^{4,12} Measuring different oxidants and antioxidants in serum requires complicated techniques and is time-consuming. So, it is more cost-effective to measure total oxidative and antioxidative status instead of individual enzymes.¹⁰ For all the above reasons, we measured TAS and TOS instead of different oxidant and antioxidant enzymes in our study.

The primary aim of our study was to evaluate oxidant status in children with AU and a possible relationship between each other rather than other etiologic causes. The causes of AU in the elicited subjects were not further evaluated. Because of ethical approval for these possible causative laboratory studies outside the approved scope of this study and financial constraints, these diagnostic additional studies were not performed.

Conclusion

This is the first study in the literature, which was done to evaluate the role of oxidative stress in children with AU. Our data showed that children with AU had reduced levels of TAS, a biomarker of the serum antioxidant capacity, with increased TOS, two biological markers for evaluation of inflammation. Plasma TAS and TOS levels in children with AU were independent of total IgE levels, eosinophil number, allergic sensitization, and atopic background. For this reason, plasma TAS and TOS levels may be inflammation markers rather than specifically of allergic inflammation. This finding suggests that the imbalance between oxidative stress and antioxidant capacity is a consequence of inflammation in AU rather than caused by urticaria. This observation should be taken into account in all attempts to explain the pathogenesis in children with AU.

Author Disclosure Statement

No competing financial interests exist.

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