

Total Antioxidant Status and Oxidative Stress and Their Relationship to Total IgE Levels and Eosinophil Counts in Children With Allergic Rhinitis

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■ Abstract

Background: Oxidative stress may play a role in the pathophysiology of several diseases including allergic rhinitis.

Objectives: To evaluate whether plasma total oxidant status (TOS) in the form of plasma reactive oxidants differs between children with allergic rhinitis and healthy controls and to investigate associations between plasma antioxidants and other allergic rhinitis-related immunological markers.

Materials and Methods: Plasma total antioxidant status (TAS), TOS, total eosinophil count, and total serum immunoglobulin (Ig) E levels were measured in 106 children newly diagnosed with allergic rhinitis and 70 nonallergic children (7-12 years of age). Plasma TAS and TOS were measured using novel automated measurement methods. Blood eosinophils (absolute counts and percentage of total white blood cells) and total IgE were elevated in children with allergic rhinitis after adjusting for age and sex.

Results: TAS and TOS were higher in the patient group than in the control group ($P < .001$ and $P < .002$, respectively). The association between plasma TAS and TOS and allergic rhinitis status was similar when eosinophils, total IgE, and allergic sensitization were included as possible confounders in logistic regression models. Multivariate logistic regression identified allergic rhinitis as the only independent factor contributing to TOS.

Conclusion: Plasma TAS and TOS levels are elevated in children with allergic rhinitis. Moreover, the high level of TOS indicates that these patients are exposed to severe oxidative stress. This stress factor may have a role in the pathogenesis of allergic rhinitis.

Key words: Total oxidant stress. Allergic rhinitis. Children.

■ Resumen

Antecedentes: El estrés oxidativo puede desempeñar un papel importante en la fisiopatología de varias enfermedades, incluida la rinitis alérgica.

Objetivos: Evaluar si el estado oxidativo total del plasma en forma de oxidantes reactivos al plasma difiere entre los niños con rinitis alérgica y los controles sanos e investigar las asociaciones entre los antioxidantes plasmáticos y otros marcadores inmunológicos relacionados con la rinitis alérgica.

Materiales y métodos: Se determinaron el estado antioxidante total (EAT) y el estado oxidante total (EOT) del plasma, el recuento total de eosinófilos y los niveles de inmunoglobulina (Ig) E sérica total en 106 niños recién diagnosticados de rinitis alérgica y 70 niños no alérgicos (de entre 7 y 12 años de edad). El EAT y el EOT plasmáticos se determinaron mediante nuevos métodos de medición automáticos. Los niveles de eosinófilos en sangre (recuentos absolutos y porcentaje de leucocitos totales) y de Ig E total fueron elevados en niños con rinitis alérgica después del ajuste por edad y sexo.

Resultados: El EAT y el EOT fueron más elevados en el grupo de pacientes que en el grupo de control ($p < 0,001$ y $p < 0,002$, respectivamente). La asociación entre el EAT y el EOT plasmáticos y el estado de la rinitis alérgica fueron similares cuando los eosinófilos, la IgE total y la sensibilización alérgica se incluyeron como factores de confusión posibles en los modelos de regresión logística. En la regresión logística multifactorial se identificó la rinitis alérgica como el único factor independiente que contribuye al EOT.

Conclusión: Los niveles de EAT y EOT plasmáticos son elevados en niños con rinitis alérgica. Asimismo, el nivel elevado de EOT indica que estos pacientes están expuestos a un estrés oxidativo considerable. Este factor de estrés puede desempeñar un papel importante en la patogenia de la rinitis alérgica.

Palabras clave: Estrés oxidativo total. Rinitis alérgica. Niños.

Introduction

Allergic rhinitis (AR), with a prevalence ranging from 5% to 40%, is the most common allergic disease, and one of the main chronic diseases in children [1-3]. As with other atopic disorders such as asthma, eczema, and food allergy, AR is part of a systemic disease complex [4]. There is a very close relationship between AR and allergic asthma. In fact, the 2 conditions are manifestations of what is known as the chronic allergic respiratory syndrome, which affects 2 regions of the respiratory tract. As stressed recently, AR and asthma can be considered within the concept of "one airway, one disease" [5]. It is well recognized that over 80% of people with allergic asthma have AR and that AR is a risk factor for the development of asthma. The 2 conditions also share common immunopathology and pathophysiology [6,7].

Oxidant generation is part of the normal metabolism of many types of cells and it is critical for cell homeostasis. To protect itself against exposure to noxious oxidants, the airway mucosa has developed an antioxidant system [8]. Cells infiltrating the nasal mucosa in patients with AR produce a variety of mediators, including reactive oxygen species (ROS). This leads to an imbalance between the oxidative forces and the antioxidant defense systems, which is believed to favor an oxidative injury that has been implicated in the pathogenesis of asthma and AR [7,9]. The association between chronic inflammation and oxidative stress is well documented. Elevated levels of ROS such as hydroxyl radicals, superoxides, and peroxides may induce a variety of pathological changes that are highly relevant in nasal and airway mucosae. These include lipid peroxidation, increased airway reactivity, increased nasal mucosal sensitivity and secretions, production of chemoattractant molecules, and increased vascular permeability [10,11].

The main difficulties associated with the use of oxidative stress markers are the complexity and cost of the methods and equipment required. Since the measurement of individual antioxidant molecules is not feasible and since the antioxidant effects of these molecules are additive, we measured total oxidative status (TOS) and total antioxidant status (TAS) [12]. These measurements, combined with total peroxide (TPX) levels, have shown good correlation with other oxidant stress markers in various diseases [13-15]. Inflammatory disorders such as asthma and AR may be mediated by oxidative stress [15]. There is increasing evidence in the literature that oxidative status and antioxidant capacity play a role in allergic inflammation, but most studies have focused on the relationship with asthma [9,13]. The role of oxidative stress in AR has not been widely studied, but the pathogenesis may be similar to that of asthma. To our knowledge, there are no published reports on serum oxidative stress markers such as TAS and TOS in children with AR. Therefore, in order to determine the oxidant/antioxidant imbalance in a large number of individuals, define the determinants of this imbalance, and investigate associations between plasma antioxidants and other AR-related immunological markers, we measured TOS and TAS in the serum of children with perennial AR (PAR) and healthy controls.

Methods

Study Population

One-hundred-and-six children aged 7 to 12 years newly diagnosed with PAR at the pediatric allergy outpatient unit at Bezmialem Vakif University Hospital in Istanbul, Turkey between May 2010 and May 2011 were included in the study. The diagnosis and severity of PAR were defined according to the Allergic Rhinitis and its Impact on Asthma guidelines [18]. The patients were classified as having PAR if they had had at least 2 rhinitis symptoms (sneezing, rhinorrhea, nasal obstruction, itching) for at least 6 months a year in the previous 2 years and if they had a positive skin prick test response to at least 1 clinically significant perennial allergen (eg, house dust mites, molds, cockroach, cockroach excrement, or animal dander). None of these newly diagnosed children had received oral, inhaled, or nasal corticosteroids, or any other controller medication in the 4 weeks before inclusion in the study. We excluded children with clinical evidence of recurrent or active airway infection, children diagnosed with concomitant asthma, and children with severe comorbidities. We also excluded any patients being treated with antibiotics, nasal vasoconstrictors, antihistamines, or topical or systemic corticosteroids. Total immunoglobulin (Ig) E levels and eosinophil counts were determined, and skin testing was performed with a panel of 16 aeroallergens on the upper back of the children. Reactions with an induration 3 mm larger than that of the negative control were considered positive, and children with at least 1 positive result were considered atopic.

The control group was composed of 70 age-matched children seen at the outpatient department of the same hospital during the study period. They were scheduled for routine preoperative examination (eg, for inguinal hernia, tonsillectomy) or regular follow-up. The controls were evaluated for chronic and severe infections, rheumatological and autoimmune disorders, and personal and family history of atopy. Laboratory tests were also performed. Children were included if they responded negatively to an established, validated AR questionnaire [19], and if they had no personal or family history of atopy, or signs of an atopic disorder. Skin prick testing and measurement of total IgE levels, total eosinophil counts, TAS, and TOS were performed in all cases. As smoking is known to have an effect on oxidative status, we selected both patients and controls from nonsmoking households.

Blood Samples

Total IgE

Serum samples for in vitro total IgE were obtained from all children after 8 hours of fasting. Venous blood samples of 2 mL were obtained and left to clot for 60 minutes at room temperature. They were centrifuged for 10 minutes at 1200 rpm and stored at -20°C . Serum total IgE (IU/mL) was measured on an Immulite 2000 automated analyzer (DPC) using the chemiluminescent enzyme immunoassay method.

Eosinophil and Neutrophil Counts

Eosinophil counts were determined from venous blood samples using a Coulter Counter for leukocyte measurements (Beckman Coulter, Inc.).

Measurement of TAS in Plasma

Serum samples for the measurement of TAS and TOS were stored at -80°C until needed. Plasma TAS was determined using a novel automated method [12] involving the production of hydroxyl radical, the most potent of biological radicals. The antioxidant effect of the sample against the potent free-radical reactions initiated by the hydroxyl radicals produced is measured. The assay has excellent precision ($<3\%$). Results are expressed as millimoles of Trolox per liter.

Measurement of TOS in Plasma

TOS in plasma was also determined using a novel automated measurement method, developed by Erel [17]. In this method, oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by abundant glycerol molecules in the reaction medium. The ferric ion forms 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 ($\mu\text{mol H}_2\text{O}_2 \text{ Eq/L}$).

Measurement of TPX Concentrations in Plasma

TPX concentrations in plasma were determined by the FOX2 method [18] with minor modifications. The principle of the assay depends on the oxidation of ferrous ion to ferric ion via various oxidants, with measurement of the resulting ferric ion with xylenol orange. TPX is reduced by triphenyl phosphine (TPP), which is a specific reductant for lipids. The difference between results obtained with and without TPP pretreatment gives TPX concentrations.

Ethical approval

The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the local ethics committee of Vakif Gureba Hospital. Informed consent was obtained from the participants' parents or legal guardians.

Statistical Analysis

All the statistical analyses were performed using SPSS version 11.5. Signal intensities are given in arbitrary units with means and SD or SEM. Mean age, serum total IgE, eosinophil and neutrophil counts, TAS, TOS, and TPX concentrations in the 2 groups were compared using the independent-samples *t* test or analysis of variance on ranks. Associations between biological markers (TAS, TOS, eosinophils, neutrophils, total serum IgE, and allergic sensitization) and AR were examined using multivariate logistic regression models. We

also examined whether the relationship between TAS and TOS and AR was independent of other clinical markers of AR (eosinophil count, total serum IgE, and skin test positivity) using multivariate logistic regression models that included TAS, TOS, and other clinical markers as potential predictors of AR status. *P* values of ≤ 0.05 were considered statistically significant.

Results

One-hundred-and-six children with PAR and 70 nonallergic children were included in the study. The characteristics of the study population are summarized in Table 1. As expected, there were significant differences in IgE levels, skin test positivity, and eosinophil counts between the study and the control group. There were no statistically significant differences with regard to age, sex, or body mass index ($P > .05$) (Table 1). There was a strongly significant increase in oxidant stress in plasma (increased TOS) and a similar decrease in antioxidant defense (decreased TAS) in patients with PAR compared with controls ($P \leq .001$) (Table 2). In support of this finding, multivariate logistic regression analysis showed that reduced TAS (odds ratio [OR], 1.25; 95% CI, 1.15-3.35; $P < .05$) and increased TOS (OR, 2.1; 95% CI, 1.9-3.7; $P = .025$) were independently associated with AR status (Table 3).

Table 1. Clinical and Demographic Characteristics of Children With Perennial Allergic Rhinitis and Nonallergic Children^a

Characteristics	Patients	Controls	<i>P</i> Value
No.	106	70	
Male/Female, No.	66/40	45/25	.25
Age, y	10.2 (2.5)	10.7 (2.8)	.14
Serum Total IgE, IU/mL	385 (55.7)	102 (15.3)	.013
Skin Test Positivity, No.	106	7	.005
Eosinophils, mm^3	386 (102.05)	168 (56.7)	.002
Body Mass Index	0.78 (0.30)	0.73 (0.20)	.15

^aResults are shown as mean (SD) unless otherwise indicated.

Table 2. Total Antioxidant Status and Total Oxidative Status in Children With Perennial Allergic Rhinitis and Nonallergic Children^a

Characteristics	Patients (n=106)	Controls (n=70)	<i>P</i> Value ^b
Total antioxidant status, mmol of Trolox/L	5.8 (2.2)	1.45 (0.52)	$<.001$
Total oxidative status, $\mu\text{mol H}_2\text{O}_2/\text{L}$	14.5 (4.4)	7.5 (3.6)	$<.001$
Total peroxide concentration (μmol)	5.5 (1.5)	2.5 (0.2)	$<.001$

^aResults are shown as mean (SD).

^bBy analysis of variance on ranks.

Table 3. Multivariate Logistic Regression Analysis of Total Oxidative Stress Status and Total IgE, Skin Test Positivity, and Eosinophil Count (n=106)

	Odds Ratio	95% CI
Total IgE	1.74 ^a	1.17-3.54
Skin test positivity	2.67 ^a	1.26-4.89
Eosinophils	1.90 ^a	1.33-3.76

Abbreviation: IgE, immunoglobulin E.

^aP<.05.

Discussion

AR has become an increasing public health concern, especially in industrialized countries. Prevalence of AR has increased significantly, particularly among children, and it is now the most frequent chronic medical condition in the pediatric age group [2]. AR is a complex disease characterized by inflammation of the nasal mucosa, and inflammation is a common finding, even in patients with mild asthma and AR [7,19]. 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 [20]. A linkage between oxidative stress and inflammation is believed to contribute to the pathogenesis of some allergic diseases [21]. Increased oxidative stress has been reported in numerous adult and childhood studies of patients with asthma [13,22,23], but there have been no reports on the antioxidant system in children with AR. The majority of studies on oxidant/antioxidant balance have been performed in patients with asthma [13, 22,25]. AR is considered to be pre-asthma as most patients with AR eventually develop asthma [7]. It therefore seems reasonable to suggest that oxidative stress plays a role in the pathogenesis of AR. To the best of our knowledge, our study is the first to examine plasma levels of oxidant status markers, which are established clinical indicators of immune function, in children with AR. Our study evaluated oxidant stress activity independently of treatment in children with PAR. Our results are in accordance with those of previous studies reporting increased oxidative stress in children with asthma [22,24,25]. We hypothesized that plasma TOS would be increased in children with AR owing to chronic inflammatory airway disease.

It is known that eosinophils are the most dominant inflammatory cells in allergic disease [26]. Our results are consistent with reports in the literature in that we found elevated eosinophil counts in children with PAR. The late phase of AR involves infiltration of nasal mucosa by eosinophils, neutrophils, and basophils, leading to congestion. When activated, eosinophils have an even greater ability of free oxygen radical synthesis than higher neutrophils [27]. We were unable to evaluate the extent to which eosinophils/neutrophils and their derived products act in concert to induce the formation or release of antioxidant or oxidant species.

Total IgE levels and skin prick sensitization were significantly higher in the patient group than in the control group. Allergen-sensitized patients are thought to be more susceptible to oxidant stress-induced inflammation. This

susceptibility appears to be linked to polymorphisms in glutathione-s-transferase that reduce the protein's ability to protect against ROS [28]. It is known that there is reduced antioxidant activity in dust mite-positive asthmatic patients compared to dust mite-negative patients [29]. All of the patients in our study were sensitized to dust mites but as we did not have a comparable group with negative sensitization, we were unable to evaluate the possible effect of this sensitization. Additionally, no significant relationship between plasma TOS/TAS and total IgE or skin sensitization was observed in the present study.

Many observations suggest that oxidative stress levels are increased in children with asthma, not only in the airway but also in the circulation. Excessive spontaneous and stimulated production of oxidants has been observed in the blood leukocytes of patients with stable asthma compared with healthy individuals [30]. The measurement of TAS and TOS [12] using novel automated systems is likely to show the systemic production of free radicals. Plasma TAS and TOS were not evaluated in relation to other potential indicators of allergic or systemic inflammation (eg, eosinophilic cationic protein, ferritin, or other cytokines), or in relation to nasal symptom scores. We were also unable to evaluate AR severity or symptom with respect to TAS, TOS, or immune function indicators. This noninvasive test offers some advantages over invasive techniques such as nasal provocation tests, nasal biopsy, and nasal washing. We are intrigued by the detection of TAS/TOS in frozen plasma as this suggests that the specific TAS and TOS measured in this assay are stable, meaning repeated measurements could be made. Additionally, the present study was not designed to analyze the possible discriminatory capacity of TAS/TOS for AR in the general population. The clinical value of the associations observed in our study will be assessed in another study.

In conclusion, our data indicate that children with PAR have reduced serum antioxidant capacity and increased TOS, which may be markers of allergic inflammation. Plasma TAS and TOS in children with PAR were independent of eosinophil counts, total IgE levels, and allergic sensitization to dust mites, and the association between TOS and AR appeared to be independent of these clinical indicators. For this reason, plasma TOS and TAS may be markers of general inflammation rather than of specific allergic inflammation. In our opinion, this finding also suggests an imbalance between oxidative stress and antioxidant capacity as a consequence of inflammation in AR rather than a cause of AR. This observation should be taken into account in all attempts to explain the pathogenesis of AR.

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