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Evaluation of total antioxidant and oxidant status in the serum of patients with seasonal allergic conjunctivitis.

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Abstract

Purpose: To investigate the relationship between seasonal allergic conjunctivitis (SAC) and oxidative stress by measuring serum total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI).

Methods: A total of 95 participants, 49 patients with SAC (18 female, 31 male) and age and gender matched 46 controls (23 female, 23 male) were included during allergy season in this observational case-control study. Serum samples were obtained from all participants. The measurements of serum TAS and TOS levels were made by automatic colorimetric method and OSI was calculated.

Results: Serum TAS levels of the patient and control group were measured as 1.45 ± 0.19 mmol Trolox Equ/L and 1.46 ± 0.15 mmol Trolox Equ/L, respectively. The median serum TOS values of the patient and control group were 3.60 (1.34-36.27) µmol H_2O_2 Equ/L and 3.29 (1.00-12.96) µmol H_2O_2 Equ/L respectively. OSI values of the patient and control group were calculated as 37.67 ± 39.32 and 26.78 ± 15.38, respectively. There was no statistically significant difference between serum TAS, TOS and OSI values of two groups (p: 0.974, 0.544 and 0.372, respectively).

Conclusion: We found no significant difference in levels of serum oxidative stress markers in patients with SAC and control group. There may be localized oxidative stress only in the ocular surface, which does not affect serum values in patients with SAC.

Keywords: Oxidative stress index, Seasonal allergic conjunctivitis, Total antioxidant status, Total oxidant status.

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Introduction

Seasonal allergic conjunctivitis (SAC) is the most common ocular allergy and its incidence ranges from 5 to 22% [1]. SAC is characterized by itching, eyelid edema and erythema, conjunctival hyperemia and edema. It has relapsing-remitting intervals causing decrease in the quality of life. In the SAC etiology, environmental factors such as pollens, industrialization, and dietary take are being blamed [2]. SAC occurs via the IgE-mediated type 1 hypersensitivity reaction triggered by environmental allergens [3]. As a result of these reactions, histamine and reactive oxygen species (ROS) such as hydroxyl radicals, superoxides, peroxides released from mast cells cause inflammation [4]. ROS are the mediators necessary for the maintenance of vital functions in physiological concentrations. Therefore, it is constantly produced in certain amounts in the organism and excess part of it is destroyed by antioxidant molecules. Oxidative stress is called as the imbalance in favor of oxidants [5]. Oxidative stress has been associated with many diseases such as inflammation, asthma, rhinitis, obesity, rheumatological diseases, cardiovascular diseases, neurodegenerative diseases [4,6-11].

It is impractical and almost impossible to measure each of the multiple ROS in order to assess oxidative stress. Therefore, it is more practical and reliable to evaluate serum total antioxidant status (TAS) and serum total oxidant status (TOS) levels, which show the total levels of all systemic antioxidants and oxidant molecules [12,13]. Studies in the literature that investigate the relationship between allergy and oxidative stress are generally related to asthma. There are a few studies about the SAC and oxidative stress but we did not find a study examining serum TAS and TOS levels in SAC. We aimed to investigate the relationship between SAC and oxidative stress by measuring serum TAS and TOS levels in our study.

Methods

Subjects

A total of 95 subjects, 49 patients with SAC and 46 controls were included in this observational case-control study. All SAC patients applied to the ophthalmology department of the Abant Izzet Baysal University, Faculty of Medicine between May and August 2017, the allergy season in Bolu. The control group was randomized from age and gender-matched healthy

volunteers who applied to the ophthalmology department for routine control. All patients had an active SAC clinic at moderate or severe grade (itching, eyelid edema and erythema, conjunctival hyperemia and edema) and none had history of the using topical or systemic antihistamines, mast cell stabilizers, or corticosteroids for the past 6 months. Each patient had only SAC and no additional allergic disease such as asthma or rhinitis. Detailed ophthalmologic examination was performed in all cases. Patients with ocular diseases such as active ocular infection, dry eye, blepharitis, uveitis, glaucoma, with topical or systemic drug use history, with obesity, smokers and pregnancies were excluded. This study was done in accordance with the principles of the Helsinki Declaration, with the approval of the Abant Izzet Baysal University Clinical Researches Ethics Committee (Decision no: 2017/39). Written consent was obtained from all participants or their parents for kids before starting study.

Biochemical analysis

Sample collection and preservation: Serum specimens were taken from antecubital vein and placed in gelled tubes without anticoagulant. Samples were centrifuged at 4000 rpm for 10 minutes after coagulation. Then it was stored at -80°C in Eppendorf tubes. Before the procedure, the samples were brought to room temperature and then analyzed with the Abbott Aeroset 2.0 (Abbott Laboratories, Ilinois, USA) analyzer.

Measurement of TAS: The measurement of serum TAS levels was made by automatic colorimetric method developed by Erel [12]. A commercial kit (Rel-Assay-Diagnostics-Total Antioxidant Status, Mega Medicine Ltd, Gaziantep, Turkey) was used for this procedure. In this method, ferrous ion-o-diacinidine complex entering Fenton type reaction with hydrogen peroxide generates hydroxyl radicals. These radicals enter the reaction causing yellow-brown color formation. Antioxidant molecules in the samples suppress this reaction and prevent color formation. The intensity of the color caused by the amount of antioxidant molecules is measured spectrophotometrically in an automatic analyzer. This measurement results indirectly reflect the TAS level. The results are expressed in millimolar Trolox equivalent per liter (mmol Trolox Equ/L)[12] .

Measurement of TOS: The measurements of serum TOS levels was made by automatic colorimetric method developed by Erel [13]. A commercial kit (Rel-Assay-Diagnostics-Total-Oxidant Status, Mega Medicine Ltd, Gaziantep, Turkey) was used for this procedure. In this method, oxidant molecules in the sample oxidize the ferrous ion-o-diacinidine complex to ferric ion. The ferric ions to form a colored complex with xylenol orange contained in the medium. The intensity of the color caused by the amount of oxidant molecules is measured spectrophotometrically in an automatic analyzer. This measurement results indirectly reflect the TOS level. The results are expressed in micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equ/L) [13].

Oxidative stress index (OSI): Oxidative Stress Index (OSI) is the percentage of TOS to TAS ratio [14]. Before the calculation, the TOS unit must be converted to μmol trolox Equ/L from mmol trolox Equ/L. The calculation was done automatically by the computer.

Oxidative Stress Index (OSI)=TOS (μmol H_2O_2 Equ/L)/TAS (μmol Trolox Equ/L) × 100

Statistical analysis

Statistical analysis of all data was performed with SPSS software version 22.0 for Windows (SPSS Inc, Chicago, USA). Data with a normal distribution (age, gender, TAS and OSI) were compared by using independent-sample t-test. Data without a normal distribution (TOS) were compared using Mann-Whitney U test. The results were expressed as mean ± standard deviation or median value (min-max value range). $P<0.05$ value was considered as statistically significant.

Results

A total of 95 participants, 49 patients with SAC (18 female, 31 male) and 46 controls (23 female, 23 male) were included in the study. The mean age of patients with SAC was 25.57 ± 15.49 years and the control group was 19.36 ± 9.80 years. There was no statistically significant difference between groups in terms of gender (p:0.095) and age (p:0.235).

Table 1. *Comparison of patients with SAC and control group.*

	SAC Group (N=49)	Control Group (N=46)	P value
Age (years)	25.57 ± 15.49	19.36 ± 9.80	0.235
Gender (female/male)	18/31	23/23	0.095
TAS (mmol Trolox Equ/L)	1.45 ± 0.19	1.46 ± 0.15	0.974
TOS (μmol H2O2 Equ/L)	3.60 (1.34-36.27)	3.29 (1.00-12.96)	0.544
OSI	37.67 ± 39.32	26.78 ± 15.38	0.372

SAC: Seasonal Allergic Conjunctivitis; TAS: Serum Total Antioxidant Status; TOS: Serum Total Oxidant Status; OSI: Oxidative Stress Index

Serum TAS levels of the patient and control group were measured as 1.45 ± 0.19 mmol Trolox Equ/L and 1.46 ± 0.15 mmol Trolox Equ/L, respectively. No statistically significant difference was found between them (p:0.974). Serum TOS values were expressed as median value and minimum-maximum value. The median serum TOS values of the patient and control group were 3.60 (1.34-36.27) μmol H_2O_2 Equ/L and 3.29 (1.00-12.96) μmol H_2O_2 Equ/L respectively. The difference between them was not statistically significant (p: 0.544). OSI values of the patient and control group were calculated as 37.67 ± 39.32 and 26.78 ± 15.38, respectively. There was no significant difference between OSI values of two groups (p: 0.372). We did not detect any deterioration in the oxidative balance (Table 1).

Discussion

To our knowledge, this study is the first study to investigate the relationship between oxidative stress and SAC using serum TAS, TOS and OSI levels in the literature. In the present study, we did not detect any significant differences between serums TAS, TOS and OSI values of patients with SAC and control group. Dadaci et al. stated that SAC is associated with oxidative stress [15]. However, they only achieved this result with serum malondialdehyde (MDA), adjusted ischemia modified albumin (IMA) levels they did not measured serum TAS and TOS levels. However, some of the SAC patients in the study group also had rhinitis, which reduces the reliability of the results. It has been shown in previous studies that allergic rhinitis is associated with oxidative stress [9,16]. Wakamatsu et al. reported that oxidative stress mediators increased in the conjunctiva of patients with atopic keratoconjunctivitis (AKC) [17]. Wakamatsu measured hexanoyl-lysine (HEL), 4-hydroxy-2-nonenal (4-HNE) and cytokine levels by examining patients' tear and brush cytology specimens. But he did not evaluate blood samples. Besides his working groups were very small and only total of 23 people participated in the study. Basci and his colleagues also histologically examined the conjunctiva of experimental murine models with allergic conjunctivitis and found that the level of ROS was high [18]. But he did not measure serum levels of ROS.

ROS occur as a result of multiple immunological or non-immunological stimulation and it takes a physiological role in many places such as the organism's defense system [19-21]. Excessive production, however, can lead to cell damage causing many diseases such as ischemic cardiovascular diseases, rheumatologic diseases, obesity, keratoconus, glaucoma and asthma [4,6-8,10,11,22,23]. There are hundreds of oxidant and antioxidant molecules and organisms, many of which are destroyed in seconds. For this reason, it is almost impossible to quantify the amount of each of the ROS mediators. Therefore only a few specific mediator levels have been examined in previous studies to investigate the association of various diseases with oxidative stress. This reduces the reliability of the results. Erel and colleagues have developed a new method that automatically measures the TAS and TOS levels to assess the cumulative synergistic effects of all oxidant and antioxidant molecules and their interactions with each other [12,13]. Furthermore, OSI measurement allows the evaluation of redox imbalance and the balance between oxidants and antioxidants [14]. This automatic colorimetric TAS and TOS measurement method developed by Erel is easy, sensitive, reliable, and reproducible [12,13,24].

In conclusion, we did not find any significant difference in serum TAS, TOS and OSI values of SAC patients in our study. The limitation of our study is that TAS, TOS and OSI measurements have not been made in tear samples. There may be localized oxidative stress only in the ocular surface, which does not affect serum values in patients with SAC. Further studies are needed for the investigation of this situation.

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