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RESEARCH ARTICLE

Oxidative stress parameters and serum magnesium levels in patients with seasonal allergic conjunctivitisZeynep Dadaci¹, Mufide Oncel², Nursen Oncel Acir¹, Emel Sahin², and Mehmet Borazan¹¹Department of Ophthalmology, Mevlana (Rumi) University School of Medicine, Konya, Turkey and ²Department of Clinical Biochemistry, Mevlana (Rumi) University School of Medicine, Konya, Turkey**Abstract**

Objective: To evaluate oxidative stress parameters and serum magnesium (Mg) levels in patients with seasonal allergic conjunctivitis (SAC) during the pollen season.

Methods: This observational cross-sectional study involved 35 patients with SAC without any other ocular and systemic diseases, and 38 consecutive, age- and sex-matched healthy subjects. Serum malondialdehyde (MDA), adjusted ischemia modified albumin (IMA), and Mg levels were quantified, and the results were compared between the groups.

Results: No significant differences were found between the groups with respect to age ($p=0.416$) and sex ($p=0.362$). Serum MDA and adjusted IMA levels of the subjects with SAC ($69.54 \pm 7.71 \mu\text{M}$ and $0.74 \pm 0.39 \text{ ABSU}$) were significantly higher than the control group ($64.61 \pm 5.89 \mu\text{M}$ and $0.57 \pm 0.19 \text{ ABSU}$) ($p=0.002$ and $p=0.025$, respectively). There was no significant difference for serum Mg levels between the groups ($p=0.177$).

Conclusion: We demonstrated higher levels of oxidative stress parameters in patients with SAC compared to the control group, which imply a possible role of oxidative stress in the pathogenesis of SAC.

Keywords

Adjusted ischemia modified albumin, allergy, magnesium, malondialdehyde, oxidative stress, seasonal allergic conjunctivitis

History

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Introduction

The prevalence of allergic diseases is increasing worldwide¹. Seasonal allergic conjunctivitis (SAC), besides its being the most common ocular allergy, is also among the most common allergic diseases encountered worldwide². The main signs and symptoms are itching, conjunctival hyperemia, tearing, chemosis, and lid edema which cause discomfort and lead to a decline in quality of life. SAC usually has a chronic course with relapsing-remitting intervals and is associated with other allergies in most of the cases^{2,3}.

Ocular surface is among the organs that are frequently exposed to environmental insults. In SAC, environmental allergens (usually pollens) induce an allergic inflammation which is a type 1 hypersensitivity reaction mediated by immunoglobulin E (IgE)³. Mast cells play a pivotal role in type 1 hypersensitivity reactions. In susceptible individuals, airborne environmental allergens trigger a series of reactions resulting in degranulation of sensitized mast cells and release of a considerable number of mediators, particularly histamine³. Also, mast cells are known to play role in a variety of diseases other than allergies, such as atherosclerosis, cardiac and cerebral ischemia, and autoimmune diseases⁴. Mast cells

release mediators depending on local tissue conditions⁵. Ischemia and products related with oxidative stress, such as oxidized low-density lipoprotein⁶, are supposed to trigger degranulation of mast cells in most of these diseases.

Oxidative substances (reactive oxygen species, free radicals) are continually produced and destroyed in living organisms. Shifting this balance toward oxidants is called oxidative stress⁷. In recent years, there are several reports demonstrating the role of oxidative stress in the pathophysiology of various diseases including allergies⁸. Generally, the end products generated by modification of lipids, proteins, and DNA by oxidants are used as markers of oxidative stress, because oxidants have much shorter half-lives (only seconds)⁷. Malondialdehyde (MDA), a lipid peroxidation product, and ischemia modified albumin (IMA), a marker of protein modification related to oxidative stress, are both sensitive and well-known biomarkers for assessing oxidative stress^{7,9}.

Several factors such as industrialization, dietary intake, and exposure to environmental factors are blamed for the rise in the incidence of allergic diseases¹⁰, which might be through induction of oxidative stress. In studies investigating the relationship between dietary intake and allergic diseases, high serum magnesium (Mg) levels were found protective^{11,12}. Mg, which has a role as a cofactor in more than 300 enzymatic reactions, is one of the most abundant cations in the intracellular environment and its deficiency is associated

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with a wide variety of ocular diseases through several mechanisms including oxidative stress¹³.

Understanding the influence of oxidative stress and environmental factors, such as Mg deficiency, in the pathogenesis of allergic diseases could modify our dealing of these diseases. Therefore, we aimed to investigate oxidative stress parameters and serum Mg levels of patients with SAC and compare with healthy controls without any allergies. We are unaware of any previous reports evaluating these parameters in patients with SAC.

Methods

Study population

This observational, case–control study comprised 73 subjects (35 patients with SAC and 38 control subjects). All patients were recruited in the study between April and June 2014, the peak spring allergy season in Turkey¹⁴ and evaluated at the outpatient ophthalmology clinic of Mevlana University. Patients with a history of SAC for ≥ 2 years' duration, with signs and symptoms of clinically active allergic conjunctivitis (conjunctival hyperemia and/or chemosis, itching, tearing or lid edema) were included in the SAC group. We graded the signs and symptoms as mild, moderate, and severe, and included the subjects in the moderate and severe group. All subjects in the SAC group had a positive skin prick test to at least one antigen common in the geographical area (mixed grass pollens were the most common allergen). SAC patients with allergic rhinitis (allergic rhinoconjunctivitis) were also included. Control group was composed of 38 consecutive subjects of similar age (aged 14–40 years) and gender with normal ocular examination except refractive disorders within ± 3.0 diopters, without any history of allergic disorders, systemic diseases, or drug consumption (both systemic and topical), and with negative skin prick test results. Ethics committee approval (2014/016) was obtained before the initiation of the study. The study was performed in compliance with good clinical practice guidelines and in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from each patient prior to enrollment in the study.

Pregnant or breast-feeding women were not included in the study. Subjects with an active ocular infection or with ocular pathology other than allergy, such as dry eye syndrome, blepharitis, uveitis, and glaucoma were excluded. Subjects who are smokers (both current and previous) and who have a known systemic disease or history of consumption of any drugs, including antibiotics, antihistamines, and corticosteroids, vitamin supplements, or alcohol during the 4 weeks prior to the study were excluded. We also excluded obese subjects, as defined by body mass index and weight under the 95th percentile of age- and gender-specific centile curves, because obesity is related with an oxidative stress status¹⁵.

Biochemical analysis

Venous blood samples were driven into serum separator tubes without any anticoagulants. After centrifugation at $1500 \times g$ for 10-min serum samples were stored at -80°C until analysis. Serum MDA levels were determined by the

method of Draper and Hadley¹⁶ based on the reaction of MDA with thiobarbituric acid. In this reaction, MDA and TBA react and form a colored pigment and results are obtained by analyzing the color of pigment at 532 nm using UV1601 spectrophotometer (Shimadzu, Sydney, Australia) and the results are reported as μM MDA. Mg concentrations were determined using Cobas Integra 400 analyzer (Roche, Mannheim, Germany) with colorimetric method and results were expressed as milligrams per deciliter. IMA levels were determined by using colorimetric method described by Bar-Or et al.¹⁷. The colorimetric test is based on decreased binding of exogenous cobalt to albumin and spectrophotometric measurement at 470 nm. Results were reported in absorbance units (ABSU). Serum albumin concentrations were measured with BCG method by using Cobas Integra 400 analyzer (Roche, Mannheim, Germany). Results were expressed as milligrams per deciliter. Adjusted IMA levels were calculated with the formula defined by Lippi et al: albumin adjusted IMA = [(individual serum albumin concentration/median albumin concentration of the group) $\times$ individual IMA value]¹⁸.

Statistical analysis

Statistical analyses were performed using SPSS V.15.0 (SPSS Sciences, Chicago, IL). The distribution of normality was tested with Kolmogorov–Smirnov. Mann–Whitney *U* test was used for the data that were not normally distributed and independent-samples *t*-test was used for normally distributed data. Mann–Whitney *U* test was used for testing the difference between MDA levels for patient and control groups. Mean age, adjusted IMA, and Mg levels between groups were compared by independent-samples *t*-test and distribution of sex was compared by Pearson chi-square test. *p* Values < 0.05 were considered as statistically significant. Data are reported as mean $\pm$ standard deviation.

Results

Thirty-five patients with SAC (12 male, 23 female) and 38 control subjects (17 male, 21 female) were enrolled in our study. The mean ages of patients with SAC and control subjects were 23.80 ± 9.55 years (range 13–42 years) and 25.45 ± 7.61 years (range 14–40 years), respectively. No significant differences were found between the groups with respect to age ($p = 0.416$) and sex ($p = 0.362$). Serum MDA and adjusted IMA levels of the subjects with SAC were significantly higher than the control group ($p = 0.002$ and $p = 0.025$, respectively), but there was no significant difference for serum Mg levels between the groups ($p = 0.177$) (Table 1). Serum MDA and adjusted IMA levels of the SAC and control groups are shown in Figures 1 and 2, respectively.

Discussion

This is a novel study evaluating the relationship between SAC and oxidative stress. MDA and adjusted IMA levels were significantly higher in patients with SAC compared to the control group. This finding implicates for the first time that oxidative stress may play a role in the pathogenesis of SAC.

Oxidants are produced as a by-product of normal aerobic cellular metabolism¹⁹. Although oxidants are used as signaling molecules and a basal level of oxidative stress is necessary for cell functions such as controlling cell division, migration, and mediator production²⁰, increased levels of oxidative stress is linked with aging²¹ and a considerable number of diseases including atherosclerosis, diabetes²², neurodegenerative diseases, cancer²³, and also allergies^{8,24}.

SAC is an antibody-mediated immune reaction against antigenic stimuli³. Helper T (Th) cells regulate the antigen-specific immune responses. Differentiation of naïve helper T cells into effector T cells (Th₁ or Th₂) or regulatory T cells is dependent on the microenvironment to which they are exposed²⁵. The differentiated Th₁ and Th₂ cells regulate cell-mediated and humoral immune responses, respectively. There should be a balance between these two subtypes of helper T cells for a normal immune response. The deviation of balance in helper T cell responses toward Th₂ phenotype is associated with allergic diseases²⁵. Oxidative stress may provide a microenvironment that enhance deviation to Th₂ cells^{25,26}. In a previous study in which the effects of oxidative stress on human T cell differentiation was investigated, the presence of oxidative stress was shown to promote phenotypic deviation of purified naïve helper T cells to Th₂ cells²⁶. In an animal

study, the antioxidant desferrioxamine was shown to inhibit Th₂ cell responses. The authors propose that this inhibition is either due to a direct effect of antioxidants on Th₂ cells, or an indirect effect via the inhibition of mast cell driven activation²⁷. The higher levels of oxidative stress markers in patients with SAC in the present study may imply the role of oxidative stress in the pathogenesis of allergy which might be through inducing deviation of immune response or activation of mast cells.

Mast cells play role in various diseases most known of which are allergies. A series of reactions triggered by antigen exposure causes sensitization of mast cells, and allergen linkage to membrane-bound IgE induces degranulation of pre-sensitized mast cells and the release of various mediators results in the signs and symptoms of allergy³. Besides the immunologic stimuli, non-immunologic stimuli may also lead to mediator release from mast cells⁵. In a rat model of ischemia-reperfusion injury, reactive oxygen species are reported to contribute to mast cell degranulation in lungs²⁸. Oxidative stress may also contribute to the development of allergic reactions by facilitating degranulation of mast cells in response to allergens. In fact, there are some reports in the literature demonstrating a link between oxidative stress and various types of allergic diseases^{8,24}. Patella et al. reported that in patients with hymenoptera venom allergy levels of advanced oxidation protein products were significantly higher than the healthy controls which did not change during immunotherapy. They suggested that, although hymenoptera venom allergy is not included among chronic inflammatory diseases, an oxidative stress status occurs in patients with this kind of allergy²⁴. Supportively, we observed higher levels of oxidative stress markers in patients with SAC, which has a course of exacerbations and remissions.

Measurement of stable by-products generated by the effect of oxidants is a widely used method of determining oxidative stress status⁷. Lipids contain multiple double bonds which make them susceptible targets of oxidants and lipid

Table 1. Serum malondialdehyde (MDA), adjusted ischemia modified albumin (IMA), and magnesium (mg) levels in patients with seasonal allergic conjunctivitis (SAC) and controls.

	SAC group (n = 35)	Control group (n = 38)	p Values
MDA (μM)	69.54 ± 7.71	64.61 ± 5.89	0.002*
Adjusted IMA (ABSU)	0.74 ± 0.39	0.57 ± 0.19	0.025†
Mg (mg/dl)	1.82 ± 0.12	1.78 ± 0.13	0.177†

Data presented as mean ± standard deviation.

*Mann-Whitney U test.

†Independent-samples t-test.

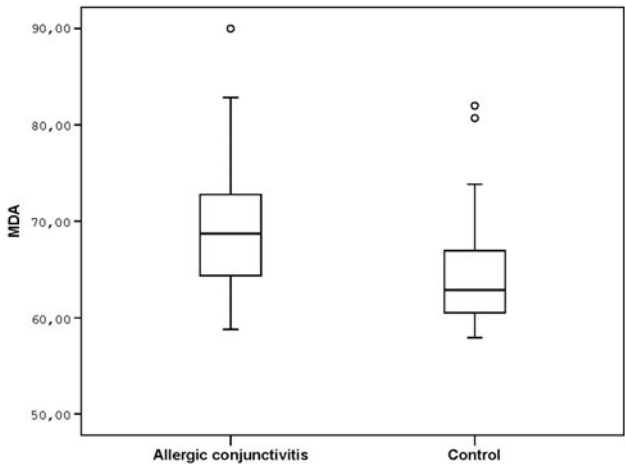


Figure 1. Comparison of serum malondialdehyde (MDA) (μM) levels of patients with seasonal allergic conjunctivitis and control subjects. Serum MDA levels of patients with SAC were significantly higher when compared to the control group ($p = 0.002$). The central box covers the interquartile range, and the horizontal line indicates the median. The end of whiskers corresponds to the most extreme observations and O indicates outliers.

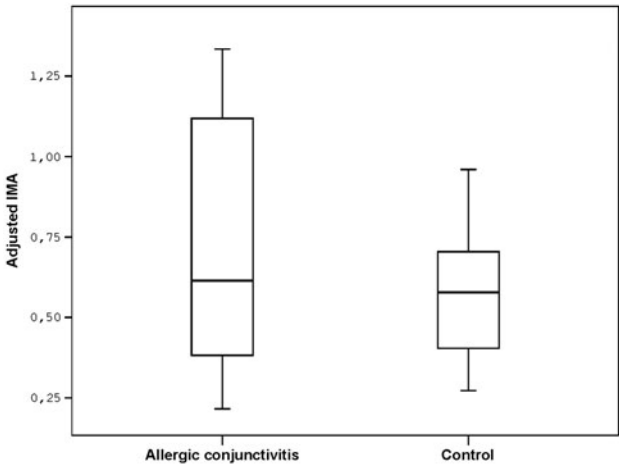


Figure 2. Comparison of serum-adjusted ischemia modified albumin (IMA) (ABSU) levels of patients with seasonal allergic conjunctivitis and control subjects. Serum-adjusted IMA levels of patients with SAC were significantly higher when compared to the control group ($p = 0.025$). The central box covers the interquartile range, and the horizontal line indicates the median. The end of whiskers corresponds to the most extreme observations.

peroxidation products, especially MDA, are among the most frequently used markers of oxidation⁷. Oxidants can also modify proteins such as albumin. IMA was suggested as a novel, rapid, and simple marker for detection of myocardial ischemia in patients with acute symptoms such as chest pain¹⁷. In intact human albumin, N-terminal region with the amino acid sequence N-Asp-Ala-His-Lys has been shown to be a strong binding site for transition metals such as Co, Cu, and Ni. Histidine at position 3 in human albumin is essential for copper binding¹⁷. The colorimetric test performed on serum is based on decreased binding of exogenous cobalt to albumin which occurs in hypoxia, acidosis, sodium, and calcium pump malfunctions, and tissue damage caused by free radicals²⁹. This issue was supported by the reports suggesting that IMA is strongly related with oxidative stress rather than being a myocardial ischemia marker^{9,30,31}. Albumin adjusted IMA index has been reported to be more sensitive than IMA because albumin level interferes with the IMA estimation³¹. So, we used adjusted IMA levels for assessing oxidative stress in our study.

Oxidative stress may be related with either overproduction of oxidant agents or reduced plasma antioxidant capacity. The demonstration of the protective effect of antioxidant intake, including Mg, on allergic diseases^{11,12} supports the hypothesis that oxidative stress is related with allergies. Mg is also reported to exert antioxidant activity and influence the glutathione redox system in asthmatic children³². Increased oxidative stress may be related with decreased antioxidant intake, such as Mg which is reported to be related with allergic diseases, so we decided to investigate Mg levels in our study. But, we were unable to demonstrate a significant difference between the serum Mg levels of the patients with SAC and control subjects. Still, intracellular Mg deficiency may be present in patients with allergy and further studies with larger number of participants are necessary to conclude a relationship between SAC and Mg deficiency.

In conclusion, we demonstrated higher serum oxidative stress markers in patients with SAC compared to the control group. Our demonstration of increased oxidative stress markers in patients with SAC may indicate that oxidative stress leads enhancement of allergic reactions, which might be through the deviation of immune system toward Th₂ cells and/or the activation of mast cells. Another explanation of our results may be that the increase in oxidative stress markers detected in our study may result from a generalized allergy-related inflammation, as most patients with SAC also have other allergies. Although further research is necessary to define the role of oxidative stress in the pathogenesis of SAC, our preliminary study still generates valuable hypotheses and provides a basis for future studies.

Declaration of interest

The authors have no conflict to disclose about this paper.

References

1. Pawankar R. White book on allergy 2011–2012: executive summary. Milwaukee, WI: World Allergy Organization; 2011.

2. Rosario N, Bielory L. Epidemiology of allergic conjunctivitis. *Curr Opin Allergy Clin Immunol* 2011;11:471–476.
3. Ono SJ, Abelson MB. Allergic conjunctivitis: update on pathophysiology and prospects for future treatment. *J Allergy Clin Immunol* 2005;115:118–122.
4. Anand P, Singh B, Jaggi AS, et al. Mast cells: an expanding pathophysiological role from allergy to other disorders. *Naunyn Schmiedeberg Arch Pharmacol* 2012;385:657–670.
5. Lowman MA, Rees PH, Benyon RC, et al. Human mast cell heterogeneity: histamine release from mast cells dispersed from skin, lung, adenoids, tonsils, and colon in response to IgE-dependent and nonimmunologic stimuli. *J Allergy Clin Immunol* 1988;81:590–597.
6. Kelley J, Hemontolor G, Younis W, et al. Mast cell activation by lipoproteins. *Methods Mol Biol Clifton NJ* 2006;315:341–348.
7. Ho E, Karimi Galougahi K, Liu C-C, et al. Biological markers of oxidative stress: applications to cardiovascular research and practice. *Redox Biol* 2013;1:483–491.
8. Ozkaya E, Akduman H, Erenberk U, et al. Plasma paraoxonase activity and oxidative stress and their relationship to disease severity in children with allergic rhinitis. *Am J Rhinol Allergy* 2013;27:13–17.
9. Borderie D, Allanore Y, Meune C, et al. High ischemia-modified albumin concentration reflects oxidative stress but not myocardial involvement in systemic sclerosis. *Clin Chem* 2004;50:2190–2193.
10. Prescott SL. Early-life environmental determinants of allergic diseases and the wider pandemic of inflammatory noncommunicable diseases. *J Allergy Clin Immunol* 2013;131:23–30.
11. Rosenlund H, Magnusson J, Kull I, et al. Antioxidant intake and allergic disease in children. *Clin Exp Allergy* 2012;42:1491–1500.
12. Van Oeffelen AAM, Bekkers MBM, Smit HA, et al. Serum micronutrient concentrations and childhood asthma: the PIAMA birth cohort study. *Pediatr Allergy Immunol* 2011;22:784–793.
13. Agarwal R, Iezhitsa L, Agarwal P. Pathogenetic role of magnesium deficiency in ophthalmic diseases. *Biometals Int J Role Met Ions Biol Biochem Med* 2014;27:5–18.
14. Yaylali V, Demirlen I, Tatlipinar S, et al. Comparative study of 0.1% olopatadine hydrochloride and 0.5% ketorolac tromethamine in the treatment of seasonal allergic conjunctivitis. *Acta Ophthalmol Scand* 2003;81:378–382.
15. Marseglia L, Manti S, D'Angelo G, et al. Oxidative stress in obesity: a critical component in human diseases. *Int J Mol Sci* 2014;16:378–400.
16. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. *Meth Enzymol* 1990;186:421–431.
17. Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia – a preliminary report. *J Emerg Med* 2000;19:311–315.
18. Lippi G, Montagnana M, Salvagno GL, et al. Standardization of ischemia-modified albumin testing: adjustment for serum albumin. *Clin Chem Lab Med* 2007;45:261–262.
19. Groeger G, Quiney C, Cotter TG. Hydrogen peroxide as a cell-survival signaling molecule. *Antioxid Redox Signal* 2009;11:2655–2671.
20. Janssen-Heininger YMW, Mossman BT, Heintz NH, et al. Redox-based regulation of signal transduction: principles, pitfalls, and promises. *Free Radic Biol Med* 2008;45:1–17.
21. Syslová K, Böhmová A, Mikoška M, et al. Multimarker screening of oxidative stress in aging. *Oxid Med Cell Longev* 2014;2014:562860.
22. Jesmin J, Rashid MS, Jamil H, et al. Gene regulatory network reveals oxidative stress as the underlying molecular mechanism of type 2 diabetes and hypertension. *BMC Med Genomics* 2010;3:45.
23. Thanan R, Oikawa S, Hiraku Y, et al. Oxidative stress and its significant roles in neurodegenerative diseases and cancer. *Int J Mol Sci* 2014;16:193–217.
24. Patella V, Incorvaia C, Minciullo PL, et al. Oxidative stress markers in patients with hymenoptera venom allergy. *Allergy Asthma Proc* 2015;36:9–13.
25. Pulendran B, Artis D. New paradigms in type 2 immunity. *Science* 2012;337:431–435.

26. King MR, Ismail AS, Davis LS, et al. Oxidative stress promotes polarization of human T cell differentiation toward a T helper 2 phenotype. *J Immunol* 1950 2006;176:2765–2772.
27. Wu Z, Holwill SDJ, Oliveira DBG. Desferrioxamine modulates chemically induced T helper 2-mediated autoimmunity in the rat. *Clin Exp Immunol* 2004;135:194–199.
28. Zhao W, Gan X, Su G, et al. The interaction between oxidative stress and mast cell activation plays a role in acute lung injuries induced by intestinal ischemia-reperfusion. *J Surg Res* 2014;187: 542–552.
29. Roy D, Quiles J, Gaze DC, et al. Role of reactive oxygen species on the formation of the novel diagnostic marker ischaemia modified albumin. *Heart Br Card Soc* 2006;92:113–114.
30. Kaefer M, Piva SJ, De Carvalho JAM, et al. Association between ischemia modified albumin, inflammation and hyperglycemia in type 2 diabetes mellitus. *Clin Biochem* 2010; 43:450–454.
31. Kiyici A, Mehmetoğlu I, Karaoğlu H, et al. Ischemia-modified albumin levels in patients with end-stage renal disease patients on hemodialysis: does albumin analysis method affect albumin-adjusted ischemia-modified albumin levels? *J Clin Lab Anal* 2010; 24:273–277.
32. Bede O, Nagy D, Surányi A, et al. Effects of magnesium supplementation on the glutathione redox system in atopic asthmatic children. *Inflamm Res off J Eur Histamine Res Soc* 2008;57:279–286.