Original Article / Çalışma - Araştırma

Advanced oxidation protein products as an oxidative stress marker in allergic rhinitis

Alerjik rinitte oksidatif stres göstergesi olarak ileri oksidasyon protein ürünleri

Fadlullah Aksoy, M.D.,[1] Hasan Demirhan, M.D.,[1] Bayram Veyseller, M.D.,[1] Yavuz Selim Yıldırım, M.D.,[1] Orhan Özturan, M.D.,[1] Filiz Basınoğlu, M.D.[2]

Department of [1]Otolaryngology, [2]Biochemistry, Haseki Research and Training Hospital, İstanbul, Turkey

Objectives: This study was designed to compare the levels of dityrosine-containing protein cross-link products, also known as advanced oxidation protein products (AOPP); in patients with allergic rhinitis with those in healthy individuals considering the fact that AOPP has the potential to be a marker of oxidative stress specific to proteins in mammalian systems.

Patients and Methods: This study was performed on 40 patients (18 males, 22 females; mean age 29 years; range 10 to 53 years) with allergic rhinitis admitted to our clinic between December 2008 and January 2009 and on 40 healthy volunteers (16 males, 24 females; mean age 31 years; range 13 to 48 years). Skin prick test was performed to establish a diagnosis of allergic rhinitis in patients with major symptoms and allergic sensitization was also supported with serum IgE levels. Blood samples were obtained and examined in all patients to determine AOPP.

Results: Serum AOPP levels were significantly higher in patients with allergic rhinitis (169.0±14.2 µmol/L) compared to controls (43.9±3.5 µmol/L; p<0.001). In addition, mean serum IgE levels of patients with allergic rhinitis (308.2±38.5 IU/ml) were found to support the presence of allergic sensitization.

Conclusion: Determining the levels of serum AOPP, a well-known marker of protein oxidation, appears to be a useful method in determining the role of oxidative stress in the etiopathogenesis of allergic rhinitis.

Key Words: Advanced oxidation protein products; allergic rhinitis; oxidative stress; protein oxidation.

Amaç: Bu çalışma memeli sistemlerde proteinlere özgü oksidatif stres varlığının bir göstergesi olma potansiyeline dayanarak, alerjik rinitli hastaların plazmalarında ileri oksidasyon protein ürünleri (İOPÜ) olarak da bilinen ditirozin içerikli protein çapraz bağ ürünleri düzeyinin sağlıklı bireylerle karşılaştırılması amacıyla tasarlandı.

Hastalar ve Yöntemler: Bu çalışma Aralık 2008 ile Ocak 2009 tarihleri arasında kliniğimize başvuran ve alerjik rinit tanısı konan 40 hasta (18 erkek, 22 kadın; ort. yaş 29 yıl; dağılım 10-53 yıl) ve sağlıklı 40 gönüllü (16 erkek, 24 kadın; ort. yaş 31 yıl; dağılım 13-48 yıl) ile gerçekleştirildi. Majör semptomları olan hastalara deri prik testi yapılarak alerjik rinit tanısı kondu ve alerjik sensitizasyon serum IgE düzeyleri ile de desteklendi. İleri oksidasyon protein ürünlerinin tespiti için tüm hastalardan kan örnekleri alındı ve incelendi.

Bulgular: Serum İOPÜ düzeyleri alerjik rinitli hastalarda (169.0±14.2 µmol/L) kontrol grubuna göre anlamlı şekilde daha yüksek bulundu (43.9±3.5 µmol/L; p<0.001). Buna ek olarak, alerjik rinitli hastalarda saptanan ortalama serum IgE düzeyleri (308.2±38.5 IU/mL) alerjik sensitizasyon varlığını destekler düzeyde bulundu.

Sonuç: Protein oksidasyonunun bilinen bir göstergesi olan serum İOPÜ düzeyinin tespiti, alerjik rinit etyopatogenezinde oksidatif stresin rolünü belirlemek açısından faydalı bir yöntem olarak gözükmektedir.

Anahtar Sözcükler: İleri oksidasyon protein ürünleri; alerjik rinit; oksidatif stres; protein oksidasyonu.

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Correspondence / İletişim adresi: Yavuz Selim Yıldırım, M.D. Haseki Eğitim ve Araştırma Hastanesi Kulak Burun Boğaz Hastalıkları Kliniği, 34089 Fatih, İstanbul, Turkey.
Tel: +90 212 - 529 44 00 / 1286 Fax *(Faks):* +90 212 - 510 37 01 e-mail *(e-posta):* dryavuzselim@yahoo.com

Allergic rhinitis and allergic asthma are IgE dependent and these are the most commonly seen type 1 hypersensitivity reaction forms with the prevalence figures ranging between 5 to 30% in developing countries.[1] These are inflammatory nasal mucosal diseases characterized with sneezing, runny nose and nasal congestion and itching.

Allergic rhinitis is a disease characterized with nasal secretion and increased eosinophils, basophiles and mast cells in the nasal mucosa. Immunological or nonimmunological stimulation of these cells results in the production of oxygen-derived free radicals (OFR) including superoxide anion, hydrogen peroxide and hydroxyl radicals. Oxygen free radicals are reactive molecules that are produced physiologically in the body and contain an unbound electron.[2] The levels of these molecules are balanced by the antioxidant defence systems of the body via neutralization. Disruption of this balance in favour of OFR might initiate destructive reactions in some important molecules including the proteins, lipids and nucleic acids. Reactive oxygen species are used in the regulation of cellular functions including intracellular communication, activation of transcription, cellular proliferation, inflammation and apoptosis. While low dosage and physiological levels of these substances participate in cellular communication and defence mechanisms, higher dosages and/or inadequate elimination of active oxygen results in oxidative stress.[3] Release of these toxic molecules into the extracellular space is directly responsible for the inflammation. Oxidative stress plays an important role in the pathogenesis of all diseases.

Although there are several methods that might be utilized in the evaluation of oxidative stress, most of these methods are not used in clinical laboratories due to the lack of automation and/or complicated methodologies. Protein oxidations may consist of several groups. These include the formation of protein carbonyls, cross linked molecules via sulphydryl group oxidation or advanced oxidation products. Oxidative modifications of proteins are good markers of oxidative stress since proteins are much more stable than lipids. Among several forms of protein oxidation, the dityrosine-containing cross linked protein products,[4,5] also known as advanced oxidation protein products (AOPP), are markers that are considered to indicate oxidative stress.[5]

The balance between oxygen free radicals and antioxidants is termed oxidative stress. Oxidative stress might occur in several allergic and immunological disorders. Endogenous antioxidants with enzymatic and non-enzymatic subgroups are the primary defence mechanism against the oxygen free radicals. The enzymatic antioxidants include the superoxide dismutase (SOD) family, catalase, glutathione peroxidase, glutathione S-transferase and thioredoxin. The non-enzymatic category of antioxidant defence includes low molecular weight compounds such as glutathione, ascorbic acid, urate, alfa-tocopherol, bilirubin and lipoic acid. The concentration of these antioxidants depends on the subcellular and anatomical location.[2]

Similar to all the cells in an organism, the respiratory system cells are constantly in contact with reactive oxygen and nitrogen species via both endogenous and exogenous sources. One way of exposure to reactive oxygen metabolites specific to airways is the direct contact with oxidants present in cigarette smoke, ozone and muffler smoke and it has been reported that this type of exposure might facilitate response to allergens and trigger the exacerbation of allergic respiratory diseases.[6] Although the role of oxidative stress in the pathogenesis of allergic rhinitis has not been investigated as detailed as in asthma, it is most likely that similar mechanisms are valid for both.

Studies have shown varying results for antioxidant enzyme activities in patients with allergic rhinitis. These varying results indicate that the role of antioxidant defence mechanisms has not been clarified yet and that clarification of this issue should lead to new advances in the treatment of allergic rhinitis. In the light of these data, this study was designed to compare levels of dityrosine containing protein cross-link products, also known as AOPP, in newly diagnosed patients with allergic rhinitis who have not received any medical treatments with healthy individuals.

PATIENTS AND METHODS

This study was performed on 40 patients (18 males, 22 females; mean age 29 years; range 10 to 53 years) with allergic rhinitis presented to our clinic between December 2008 and January 2009 and 40 healthy individuals (16 males, 24 females; mean age 31 years; range 13 to 48 years). The diagnosis of allergic rhinitis was established by looking at a combination of physical examination (anterior rhinoscopy, nasal endoscopy, autoscopy, examination of oropharyngeal and entire mucosal structures) and laboratory tests (Prick test and

total IgE). The control group was chosen from individuals who did not have any nasal complaints or any nasal pathologies (nasal septal deviation, hypertrophic concha and nasal polyp) which might cause nasal congestion. Allergic sensitization was evaluated with skin prick test and the results were supported with serum IgE levels.

Age at the onset of symptoms, factors initiating and aggravating the symptoms, medication and food allergies, history of nasal and paranasal surgeries, family history, pets at home, the environment and occupation were also questioned in patients diagnosed with allergic rhinitis. Anterior rhinoscopic examinations were performed including a careful examination of septum, concha and nasal mucosa. All data and examination results were recorded. Afterwards, eight standard aeroallergens (grasses, cereals, trees, mites, foods, mushrooms, wools, insects, histamine as positive control, serum physiologic as negative control) were used in the Prick test and the diagnosis was confirmed with total IgE tests. Detailed otolaryngology and head-neck examinations were performed prior to the test in all subjects. Patients with deviation, nasal polyposis and hypertrophic concha as well as those with signs of infection, history of use of nasal steroids and antihistaminic medication in the last four weeks and pregnant women were excluded from the study. It was ensured that the control group did not include any patients with systemic diseases. The group consisted of individuals with no systemic or local signs or complaints of atopy or allergy. Blood samples were obtained from all patients to determine the AOPP. The trial protocol, prepared in accordance with the Declaration of Helsinki and approved by our institutional ethics committee, was extensively explained to all subjects prior to obtaining their informed consent.

Determining the level of AOPP

The spectrophotometric method of Witko-Sarsat et al.[4] was used to determine the plasma AOPP levels. This method relies on the spectrophotometric measurement of the AOPP levels induced by chlorine oxidants (chloramines and hypochlorous acid) and calibration with chloramine-T solutions with the absorbance at 340 nm in the presence of potassium iodide.[4] Chloramine-T standards were prepared at 750, 500, 250, 100 μmol/L concentrations by diluting the chloramine-T main standard solution (standard 1:1000 μmol/L) with PBS (20 mmol/L, pH 7.4) in order to form the five point calibration curve.

Table 1. Absorbance values for different concentrations of chloramine-T solution

Standards	Concentration (micromol/L)	Absorbance (A)
Standard 1	1000	0,7277
Standard 2	750	0.4646
Standard 3	500	0.3289
Standard 4	250	0.1158
Standard 5	100	0.0458

Chloramine-T standards were subsequently examined at linear cromaline photometer, absorbance (A) values for the concentration of each of the five standards were obtained (Table 1) and a calibration curve was formed for these values by the device (Fig. 1). Samples maintained at room temperature prior to the examination were examined with the linear cromaline photometer device. Therefore, a 160 μl PBS reagent was added to 10 μl of serum and incubated for 25 seconds. Afterwards, 20 μL of acetic acid was also added and incubated for another 25 seconds. Finally, 10 μL of KI solution was added and incubated once more for 25 seconds and the absorbance was measured at 340 nm. Sera above the linearity level were examined after dilution. The AOPP concentration results were given in μmol/L together with the chloramine-T unit. All stages were performed in a single sink at 37 °C and time intervals were arranged as 25 seconds or longer.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) and analyzed with the Mann-Whitney U-test using SPSS (Statistical Package for Social Sciences) version 16.0 (SPSS Inc., Chicago, Illinois, USA). The level of statistical significance was set at $p<0.05$.

RESULTS

Serum AOPP levels were significantly higher in patients with allergic rhinitis (169.0±14.2 μmol/L)

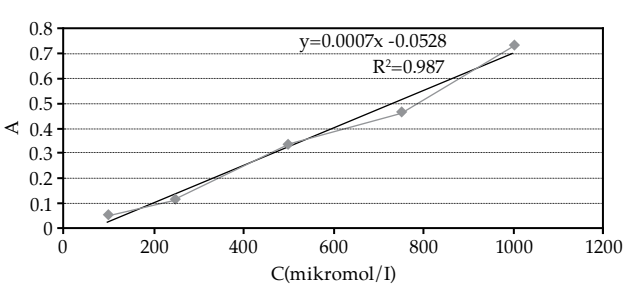

Fig. 1. Advanced oxidation protein products calibration curve.

Table 2. Comparison of the control group and the patients with allergic rhinitis in terms of serum advanced oxidation protein products and IgE levels

	Control group (n=40)	Allergic rhinitis group (n=40)
	Mean±SD	Mean±SD
Serum AOPP levels (μmol/L)	43.9±3.5*	169.0±14.2
Serum IgE levels (IU/mL)	–	308.2±38.5

*: p<0.001. Mean±SD: Mean standard deviation; AOPP: Advanced oxidation protein products

compared to controls (43.9±3.5 μmol/L; p<0.001; Table 2). In addition, the mean serum IgE levels of patients with allergic rhinitis (308.2±38.5 IU/ml) were increased in support of the presence of allergic sensitization (Table 2).

DISCUSSION

Oxidative stress results from the imbalance between the reactive oxygen and nitrogen metabolites and the antioxidant defence systems working against these. Inflammatory reactions, hypersensitivity reaction and autoimmune conditions emerge when antioxidant systems eliminating the free radicals fail.[7] It is already known that oxidative stress plays a role in the pathogenesis of several diseases including neurodegenerative diseases, ischemia/reperfusion damage, arthritis, cataract, asthma, atherosclerosis and cancer.[7,8]

Excessive production of reactive oxygen metabolites is known to cause extensive damage in the lungs including the epithelial and endothelial membranes. Moreover, increased levels of nitrotyrosine[9] and chlorotyrosine[10] in lavage samples obtained from allergic patients indicate protein damage. Inflammatory cells located in the airways and intravascular areas are considered to be responsible from increased hydrogen peroxide and nitric oxide levels in the respiratory air of asthma patients.[2]

Studies have shown that oxidative stress plays an important role in the pathogenesis of allergic diseases including the asthma,[11-13] rhinitis[14-16] and atopic dermatitis.[17] The in vivo differences in the defence mechanisms of allergic patients against free radicals are evidences of the important role of antioxidant detoxification mechanisms in the pathogenesis of allergy.[7]

Moreover, it has been reported that contact with house mites trigger the production of hydrogen peroxide by nasal eosinophils.[14] Ozone concentrations, on the other hand, have been reported to significantly affect the symptoms of rhinitis in adults allergic to pollen.

Indeed, it has been reported that the oxidation ability is significantly increased in patients with atopic asthma and allergic rhinitis compared to healthy individuals and that this might be interpreted as an indicator of a link between rapid oxidation phenotype and a predisposition to develop atopic asthma and allergic rhinitis.[18] Oxidative stress has been shown to exhibit marked destructive effects on lipids, proteins and nucleic acids.[2] Currently, proteins are invariably considered as the main targets of free radicals and other oxidant agents, and the principle of accumulation of relatively durable proteins in areas of oxidative stress dictates that these lesions on protein surfaces might become high-sensitivity markers of oxidative stress in mammalian systems.[19,20] Protein damage via reactive oxygen species includes a series of reactions including the formation of dityrosine by in vitro oxidation of amino acid residues such as tyrosine, protein aggregation, cross-linkage and fragmentation. However, such an oxidative damage has not been shown in vivo and its possible clinical significance has not been explained.[4,19]

The reduction observed in hydrogen peroxide, nitrotyrosine and ethane production in response to anti-inflammatory treatment (steroids) is considered in favour of a significant correlation between inflammation and oxidative stress.[2] Nitration occurring in cellular lipids, proteins and nucleotides in the presence of oxidative stress triggers marked inflammatory changes and causes damage that creates predisposition to pulmonary dysfunction observed in the clinical course of allergic diseases.[2] In addition, the role of oxidative stress has been demonstrated in patients allergic to pollens or mites, and it has been reported that inflammatory cells might release oxygen radicals that are responsible for the tissue damage observed in asthma.[21] These might also lead to an exacerbation of inflammation and increased tissue injury by altering the expression of various inflammatory molecules.[22]

Immunological or non-immunological stimulation of increased eosinophils, basophiles and mast cells in the nasal mucosa result in the production of OFR including superoxide anion, hydro-

gen peroxide and hydroxyl radicals. Studies have shown that reduced dietary intake of antioxidants including carotenoids, tocopherols and vitamin-C is involved in the increased incidence of allergic rhinitis and allergic sensitization.[2] Similarly, case reports also indicate that dietary intake of antioxidant foods correlate negatively with the symptoms of asthma patients.[23,24]

One study performed on guinea pigs demonstrated that exposure to ozone is associated with rhinitis, sneezing, nasal secretion, hyperactivity and eosinophil infiltration.[15]

Although the oxidative modification of proteins is one of the best known biochemical effects of free radicals, extensive studies could not be performed on protein oxidation until the recent development of easily applicable methods for determining protein oxidation products.[25] The development of markers for in vivo protein oxidation[20] has allowed the documentation of a predisposition of proteins to oxidant injury.[26] Hence, the oxidative modification of proteins has been reported to be a more stable and better-known marker of oxidative stress compared to the lipids.[27] Our results also emphasize the role of oxidative stress resulting from the imbalance between the oxidation process and the antioxidant mechanisms[28] on the etiopathogenesis of allergic rhinitis via AOPP.

The wide age distribution range of our subjects (10-53 years) appears to be disregardable relying on the literature knowledge that the difference observed in the age dependent analysis in allergic sensitization is statistically insignificant in terms of sensitivity between the elderly and younger individuals.[3] In terms of the distribution of sexes, the incidence of allergic rhinitis has been reported to be higher in females for some unknown reason, despite the fact that the prevalence of allergic sensitization is higher in males.[3] In our study, males and females diagnosed with allergic rhinitis were equal in number, at least for the period they were recruited.

The significantly higher AOPP levels in patients with allergic rhinitis compared to the control group in our study indicates the relationship between oxidative stress and type 1 hypersensitivity reactions.

Consequently, determining the serum AOPP levels, a well-known marker of serum protein oxidation, might be a beneficial tool in establishing the role of oxidative stress in the etiopathogenesis of allergic rhinitis. Therefore, the measurement of AOPP should be considered as a non-invasive, in vivo and valid marker of free radical reactions necessary for explaining the oxidative mechanisms involved in the development of allergic rhinitis.

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