

RESEARCH ARTICLE

# Systemic levels of ceruloplasmin oxidase activity in allergic asthma and allergic rhinitis

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## Abstract

**Context:** The role of ceruloplasmin oxidase activity (COA) involving the interaction of oxidant and antioxidant balance in allergic diseases is still unknown.

**Objective:** Our study was designed to examine the changes in COAs in severe persistent asthma-allergic rhinitis, new diagnosed allergic asthma-allergic rhinitis and allergic rhinitis patients.

**Methods:** The study included 20 age- and sex-matched healthy individuals as control (Group I); Group II included 15 newly diagnosed allergic asthma-allergic rhinitis; Group III included 15 patients with severe persistent asthma-allergic rhinitis and in the fourth group there were 20 patients with allergic rhinitis. Group III was divided in two groups, severe persistent asthma-allergic rhinitis who were pre-(III-A) and post-treated (III-B) with omalizumab. Group IV was divided to two groups, pretreatment (IV-A) and posttreatment (IV-B) with specific subcutaneous immunotherapy modalities. All the posttreatment measurements were 12 months after the therapy. All the patients were assessed by the skin prick test, high sensitive C-reactive protein (hs-CRP) and COA.

**Results:** There were significant differences between Group I and Groups III-A, III-B, IV-A and IV-B; Group II and Groups III-A, III-B, IV-A and IV-B; Group III-A and Groups III-B, IV-A and IV-B; Group III-A and Groups IV-A and IV-B; and Group IV-A and IV-B. Interestingly, there was a correlation between the hs-CRP and COA levels in Group III-A.

**Conclusions:** Our data suggest that hs-CRP and COA levels might be an indicator of an inflammation and important in revelation of patients with allergy related diseases, especially of asthma patients.

**Keywords:** Omalizumab, allergic asthma, ceruloplasmin oxidase activity, allergic rhinitis, high-sensitive CRP, anti IgE, skin prick test

## Introduction

Allergen specific subcutaneous immunotherapy has been used in the management of allergic diseases for nearly 100 years. Allergen means a biologic agent, including allergenic extracts and others, that is administered to man for diagnosis, prevention and treatment of allergy and allergic diseases.<sup>(1)</sup> The quality of allergen products is a key issue for both diagnosis and specific subcutaneous immunotherapy. The heterogeneity of allergen extracts

and the unknown properties of many of the components of each preparation have made it necessary to develop methodologies to assess their potency and ensure their consistency. In addition, the variable response of each patient to the different allergen components (mainly proteins) may influence the potential clinical outcome and led to different approaches to the allergen standardization.<sup>(1)</sup> Our previous study suggests that 51.8% of the allergens determined by prick test were mite

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and 42.3% were pollens. In the cases that had immunotherapy, the pollen positivity rate was 61.8%, and the mite positivity rate was 60.4%. In most of the cases the rhinitis symptoms were due to pollens and mite. Patients generally complained about their symptoms got worse with exposure to dust, smoke, heavy odors, perfumes, detergents. Air pollution was the most important factor that exacerbated the symptoms of allergic rhinitis and allergic asthma (77.0%). Allergens are known to exhibit regional variation in expression, which helps explain why allergen profiles and skin prick tests should be designed with reference to individual locales.<sup>(2)</sup>

An increase in serum C-reactive protein levels measured by high-sensitivity assays may be associated with airflow obstruction and airway inflammation, and may serve as a surrogate marker of airway inflammation in asthma.<sup>(3)</sup> Asthma is recognized as a complex condition with differences in severity, natural history, comorbidities, and treatment response. It has been defined as "a common chronic disorder of the airways that is complex and characterized by variable and recurring symptoms, airflow obstruction, bronchial hyperresponsiveness, and an underlying inflammation".<sup>(4)</sup>

Omalizumab was developed for the treatment of severe allergic asthma. It blocks binding of free IgE on basophils and mast cells, thus inhibiting their activation by allergens. Omalizumab has been evaluated as a treatment for asthma in large, multicenter, double-blind placebo control trials involving patients with moderate to severe asthma who required corticosteroids.<sup>(5)</sup> The pathogenesis of chronic obstructive pulmonary disease involves the recruitment and regulation of neutrophils, macrophages, and lymphocytes to the lung, as well as the induction of oxidative stress, all of which result in lung parenchymal destruction and airway remodeling. Recent research has generated a greater understanding of the mechanisms responsible for chronic obstructive pulmonary disease development, including new concepts in T cell biology and the increasing recognition that the processes governing lung cell apoptosis are up regulated.<sup>(6)</sup> An imbalance between oxidative stress and anti-oxidative capacity may play an important role in the development and progression of bronchial asthma and chronic obstructive pulmonary disease. The systemic oxidant-antioxidant status changes during exacerbation versus stable periods in patients with bronchial asthma and chronic obstructive pulmonary disease.<sup>(7)</sup> Our previous studies on severe persistent allergic asthma suggesting a novel perspective of omalizumab treatment effect, using as markers nitric oxide (NO), malondialdehyde (MDA), hydrogen peroxide ( $H_2O_2$ ) and total antioxidant capacity.<sup>(8)</sup>

Oxidative stress is believed to play a role in the development of human diseases including cancer, cardiovascular disorders and immunologic diseases. Asthma is a chronic inflammatory airway disease, and oxidative stress may be involved in its pathogenesis. In bronchial asthma, oxidative stress aggravates airway inflammation

by inducing diverse proinflammatory mediators which enhance bronchial hyperresponsiveness, stimulate bronchospasm, and increase mucine secretion.<sup>(9,10)</sup> An increase in oxidative stress markers has been previously described in a number of smoke, sulfur dioxide and ammonium inhalation animal models, even in the absence of any evidence of inadequate systemic perfusion.<sup>(11,12,13)</sup>

Ceruloplasmin (CP) is a copper-containing alpha-2-glycoprotein with a molecular weight of approximately 132 kDa CP is essential for iron homeostasis, is involved in angiogenesis and under different conditions can act as either a pro- or anti-oxidant. The known functions of ceruloplasmin oxidase activity (COA) include copper transportation, iron metabolism, antioxidant defense and involvement in angiogenesis and coagulation.<sup>(14,15)</sup> It was previously reported that synthesis of ceruloplasmin was stimulated by interleukin-1 in normal and copper-deficient rat models concluding that ceruloplasmin is dependent for oxidase activity.<sup>(16)</sup> Moreover, the copper ions had been suggested as an explanation for the sensitivity of asthmatic individuals by their biologic effects of inhaled particulate air pollution.<sup>(17)</sup> *In vivo* experiments on finding the cytokines involved in acute-phase protein response showed that there are three major cytokines; interleukin 1-beta, 6 and TNF-alpha.<sup>(18)</sup>

There is no data for alterations in COA in severe persistent asthma, newly diagnosed allergic asthma and allergic rhinoconjunctivitis patients. Given the presumed importance of redox systems in this disease, we aimed to examine changes in COA in severe persistent asthma and newly diagnosed allergic asthma patients. Omalizumab treatment and specific subcutaneous immunotherapy were investigated whether the effect on COA, high-sensitive C-reactive protein (hs-CRP) and albumin levels.

## Patients and methods

### Patients

The study was approved by the local ethics committee. All patients and healthy volunteers gave written, informed content. The study participants ( $n = 70$ ) included 20 healthy individuals and 50 patients. Group I, 20 age- and sex-matched healthy individuals (eight male and 12 female) were used as controls (mean age 45.2 years). Group II, 15 newly diagnosed allergic asthma-allergic rhinitis patients (seven male and eight female) mean had a mean age of 43.6 years. The third group (Group III-A and III-B) included 15 patients (seven male and eight female, mean age 44.8 years) with severe persistent asthma-allergic rhinitis, Group III-A was studied before omalizumab treatment and Group III-B was studied 12 months after omalizumab therapy.

The fourth group (Group IV-A and IV-B) included 20 patients (eight male and 12 female, mean age 46.3 years) with allergic rhinitis, Group IV-A was studied before specific subcutaneous immunotherapy and Group IV-B was studied 12 months after specific subcutaneous immunotherapy.

The demographics of the patients including mean age, sex, duration of disease symptoms, BMI were given in Table 1.

Assessment of clinical changes and adverse effects were made at each bimonthly patient visit, and included vital signs, full physical examination, details of any allergy incidents and asthma control test (ACT) (Quality Metric Incorp.). All patients were followed in the Internal Medicine, Clinical Immunology and Allergy Clinic of Antalya Education and Research Hospital, and were evaluated by clinical status (Tables 1 and 2).

Subjects with kidney disease, heart disease, liver disease, diabetes mellitus, cancer status, smoker, BMI >30 kg/m<sup>2</sup> and autoimmune disease were excluded clinically and serologically. Subjects whose serum hs-CRP levels by standard assay were >10 mg/L were excluded. None of the subjects were passive smoker or had a respiratory tract infection or exacerbation of asthma during the month before enrolment.

### Skin prick test

Skin prick tests (SPT) on the forearm were performed in all patients using standardized latex extract containing high ammonia natural rubber latex, and a full set of 35 common allergens. In addition, venom SPT was performed on one patient based on the subject's clinical history. SPTs were performed by skilled nursing personnel. Positive tests were counted as wheals of 3 mm in diameter after 20 min. Tests were compared with positive histamine controls and negative saline controls. Commercial extracts used were manufactured by Alyostal ST-IR (Starallergenes S.A., France). No intradermal tests were performed.

### Laboratory investigations

Blood samples were obtained in the morning after 12 h fast. Blood samples were collected into 5 mL plain vacutainer tubes and centrifuged at 3000×g for 10 min. Serum samples were stored at -70°C until the assays for the determination of ceruloplasmin oxidase activity, high-sensitive C-reactive protein and albumin levels were done. All assays were carried out in duplicate.

Total and specific IgE levels were enumerated by fluoro-enzyme immunoassay (ImmunoCAP-FEIA) using an ImmunoCAP (Pharmacia, Uppsala, Sweden) kit. Values above 100 and 0.35 kU/L for total and specific IgE levels were considered abnormal.

### hs-CRP assay

Serum hs-CRP levels were measured using a hs-CRP assay kit (Behring Latex-Enhanced using the Behring Nephelometer BN-100; Behring Diagnostics, Westwood, MA, USA). The sensitivity of the assay ranged 0.04–5.0 mg/L.

### Albumin assay

Serum albumin levels were measured using the bromocresol green dye binding method.<sup>(19)</sup> Stock bromocresol green reagent solution was prepared by dissolving 0.419 g bromocresol green in 10 mL sodium hydroxide (0.1 N) and diluting it to 1 L with distilled water. Serum (20 µL) was added to the bromocresol working solution (5 mL), which was prepared by mixing the stock bromocresol green solution (250 mL) with a citrate buffer (pH 4.2, 750 mL) and polyoxyethylene lauryl ether (30%, 4 mL) solution. The absorbance of this mixture

Table 1. Demographics for healthy individuals (Group I), newly diagnosed allergic asthma-allergic rhinitis patients (Group II), severe persistent asthma patients (Group III) and allergic rhinitis patients (Group IV).

|                                                                       | N  | Mean Age (years) | BMI (kg·m <sup>-2</sup> ) | Sex (M/F) | Duration of disease symptoms (years) |
|-----------------------------------------------------------------------|----|------------------|---------------------------|-----------|--------------------------------------|
| Group I (healthy control)                                             | 20 | 45.2             | 22 ± 4.6                  | 8/12      | -                                    |
| Group II (newly diagnosed allergic asthma-allergic rhinitis patients) | 15 | 43.6             | 24 ± 3.3                  | 7/8       | 2.4                                  |
| Group III (severe persistent asthma-allergic rhinitis patients)       | 15 | 44.8             | 23 ± 1.8                  | 7/8       | 18.1                                 |
| Group IV (allergic rhinitis patients)                                 | 20 | 46.3             | 24 ± 3.1                  | 8/12      | 5.2                                  |

Values represent mean ± standard deviation (SD).

M, male; F, female; BMI, body mass index.

Table 2. Baseline characteristics of controls and patients.

|                                                        | N  | Skin prick test | Mean Total serum IgE (IU/mL) | Specific IgE (kU/L) | Eosinophils mm <sup>3</sup> | Treatment modality                                                                    |
|--------------------------------------------------------|----|-----------------|------------------------------|---------------------|-----------------------------|---------------------------------------------------------------------------------------|
| Group I (healthy control)                              | 20 | Negative        | 43.21                        | Negative            | 245 ± 52                    | None                                                                                  |
| Group II (newly diagnosed allergic asthma patients)    | 15 | Mite and grass  | 156.41                       | Mite and grass      | 635 ± 68                    | Salmeterol xinafoate-fluticasone propionate<br>Desloratadine                          |
| Group III (severe persistent allergic asthma patients) | 15 | Mite and grass  | 451.73                       | Mite and grass      | 871 ± 96                    | Anti-IgE (Omalizumab)<br>Salmeterol xinafoate-fluticasone propionate<br>Desloratadine |
| Group IV (allergic rhinoconjunctivitis patients)       | 20 | Mite and grass  | 149.60                       | Mite and grass      | 639 ± 41                    | Desloratadine<br>Specific subcutaneous immunotherapy                                  |

Values represent mean ± standard deviation (SD).

was measured at 628 nm. Albumin concentrations were expressed as g/L using albumin as a standard.

### COA assay

Serum COA was measured according to the methods of Schosinky et al.,<sup>(20)</sup> Gumuslu et al.,<sup>(13)</sup> and Gocmen et al.,<sup>(21)</sup> using *o*-dianisidine dihydrochloride as a substrate. COA was estimated in IU/L, using molar extinction coefficient ( $\epsilon = 96 \times 104 \text{ L}/(\text{mol} \times \text{cm})$ ), in terms of substrate consumed.

### Statistical analysis

Data were expressed as mean  $\pm$  standard error of the mean (SEM). The data were analyzed using the SPSS for Windows (Version 13.0; SPSS, Chicago, IL, USA). Comparison of parameters between two groups was performed using Student's *t*-test. The correlations between the variables were evaluated by linear regression analysis. *p* values less than 0.05 were considered statistically significant.

## Results

In this clinical follow-up study, participants' baseline characteristics are shown in Table 2. The mean total IgE levels were 43.21 IU/mL in Group I, 156.41 IU/mL in Group II, 451.73 IU/mL in Group III, 149.60 IU/mL in Group IV. IgE levels in patient groups were higher than in controls ( $p < 0.01$ ). IgE level in Group III was higher than in Group II ( $p < 0.01$ ). IgE level in Group IV was higher than in Group II ( $p < 0.01$ ). Prick test in all patients were detected in mite and grass allergy. These results correlated with specific IgE. Eosinophils levels ( $\text{mm}^3$ ) in patient groups were higher than in controls ( $p < 0.01$ ). The different treatment modalities of the patients were in Table 2. Table 3 reports data of the albumin, hs-CRP and COA of patients and control subjects.

### ACT

Values represent mean  $\pm$  standard deviation (SD).

Pre-omalizumab (Group III-A):  $9.42 \pm 4.26$ .

Post-omalizumab (Group III-B):  $22.48 \pm 2.10$  ( $p < 0.001$ ).

### SPT

There was a statistically significant difference between the skin values before and after specific subcutaneous

immunotherapy (Grasses mixture, *Dermatophagoides pteronyssinus*, *D. farinae*) ( $p < 0.005$ ).

### Albumin levels

The difference of the albumin between control group and the other patient groups except the non-treated allergic rhinitis patients were found non-significant (Table 3).

### COA

COA of control and patients are shown in Table 3. COA in Group II was higher than in Group I ( $p < 0.001$ ). COA in Group III-A was higher than in Group I and Group II ( $p < 0.001$ ). COA in Group III-B was higher than in group I ( $p < 0.001$ ). COA in Group III-B was lower than in Group III-A ( $p < 0.001$ ). COA in Group IV-A was higher than in Group I ( $p < 0.001$ ). COA in Group IV-A was lower than in Group II, Group III-A and Group III-B ( $p < 0.001$ ). COA in Group IV-B was lower than in Group II, Group III-A and Group III-B ( $p < 0.001$ ). COA in Group IV-B was lower than in Group IV-A ( $p < 0.001$ ).

In addition there is a correlation between hs-CRP levels and COA in Group III-A ( $r = 0.837$ ;  $p < 0.005$ ).

### hs-CRP

hs-CRP levels of controls and patients are shown in Table 3. hs-CRP levels in Group III-A were higher than in Group I and Group II ( $p < 0.001$ ). hs-CRP levels in Group III-B were higher than in Group I and Group II ( $p < 0.001$ ). hs-CRP levels in Group III-B were lower than in Group III-A ( $p < 0.001$ ). hs-CRP levels in Group IV-A were lower than in Group III-A and III-B ( $p < 0.001$ ). hs-CRP levels in Group IV-B were lower than in Group III-A and Group III-B ( $p < 0.001$ ).

## Discussion

Tissue oxidant stress is related to the inflammation in the respiratory system that is common to a wide variety of airborne exposures and infections. Among well-described environmental exposures are the oxidant pollutants, ozone and nitrogen dioxide, ambient particulate matter, and cigarette smoke.<sup>(22–26)</sup> The upper respiratory epithelial mucosa, at the juncture of the

Table 3. Albumin and high-sensitive C-reactive protein (hs-CRP) levels, and ceruloplasmin oxidase activities in control group and patient groups.

|                                                                          | N  | Albumine (g/dL) | hs-CRP (mg/L)   | COA (IU/L)       |
|--------------------------------------------------------------------------|----|-----------------|-----------------|------------------|
| Group I (control) (healthy individuals)                                  | 20 | $3.19 \pm 0.38$ | $1.05 \pm 0.13$ | $38.33 \pm 0.56$ |
| Group II (newly diagnosed asthma-allergic rhinitis patients)             | 15 | $3.20 \pm 0.25$ | $1.26 \pm 0.18$ | $48.97 \pm 0.42$ |
| Group III-A (severe persistent asthma-allergic rhinitis pre-omalizumab)  | 15 | $3.19 \pm 0.42$ | $3.50 \pm 0.21$ | $58.75 \pm 0.91$ |
| Group III-B (severe persistent asthma-allergic rhinitis post-omalizumab) | 15 | $3.20 \pm 0.56$ | $2.66 \pm 0.23$ | $52.81 \pm 0.47$ |
| Group IV-A (allergic rhinitis pre-immunotherapy)                         | 20 | $3.19 \pm 0.67$ | $1.12 \pm 0.17$ | $40.07 \pm 0.58$ |
| Group IV-B (allergic rhinitis post-immunotherapy)                        | 20 | $3.32 \pm 0.85$ | $1.17 \pm 0.15$ | $32.55 \pm 0.36$ |

Values represent mean  $\pm$  standard error of mean (SEM).

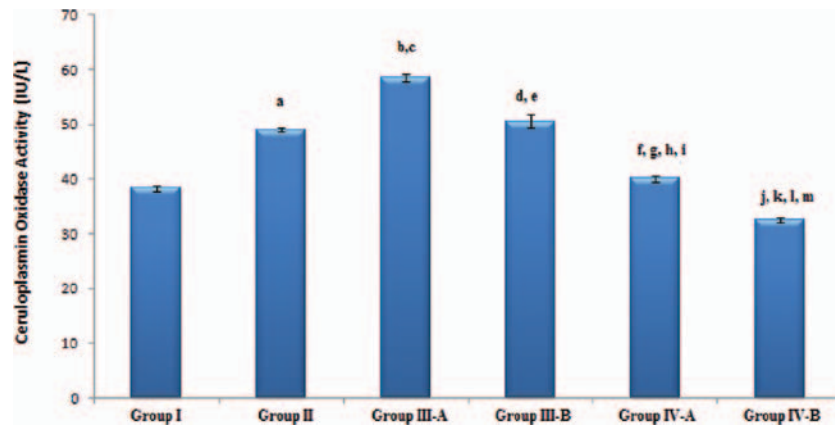


Figure 1. Ceruloplasmin oxidase activity of control and patients groups. The  $p$  values were as follows: a,  $p < 0.001$ , Group II versus Group I; b,  $p < 0.001$ , Group III-A versus Group I; c,  $p < 0.001$ , Group III-A versus Group II; d,  $p < 0.001$ , Group III-B versus Group I; e,  $p < 0.001$ , Group III-B versus Group III-A; f,  $p < 0.001$ , Group IV-A versus Group I; g,  $p < 0.001$ , Group IV-A versus Group II; h,  $p < 0.001$ , Group IV-A versus Group III-A; i,  $p < 0.001$ , Group IV-A versus Group III-B; j,  $p < 0.001$ , Group IV-B versus Group II; k,  $p < 0.001$ , Group IV-B versus Group III-A; l,  $p < 0.001$ , Group IV-B versus Group III-B; m,  $p < 0.001$ , Group IV-B versus Group IV-A. Values represent mean  $\pm$  SEM. Group I: healthy control, Group II: new diagnosed allergic asthma-allergic rhinitis patients, Group III-A: severe persistent allergic asthma-allergic rhinitis pre-omalizumab, Group III-B: severe persistent asthma-allergic rhinitis post-omalizumab, Group IV-A: allergic rhinitis pre-immunotherapy, group IV-B: allergic rhinitis post-immunotherapy.

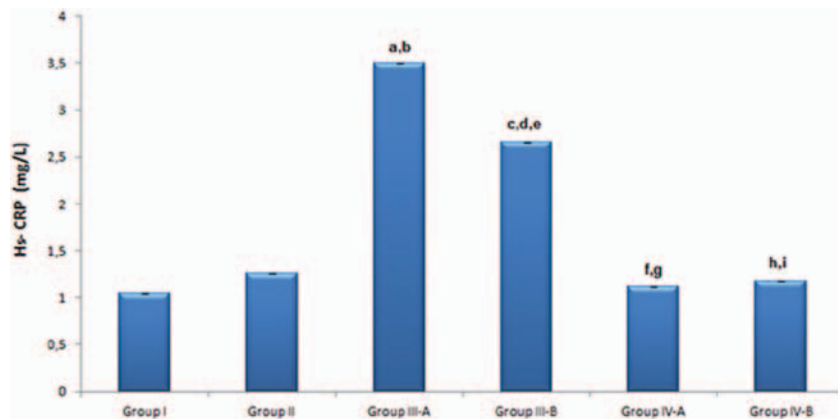


Figure 2. hs-CRP levels of control and patient groups. The  $p$  values were as follows: a,  $p < 0.001$ , Group III-A versus Group I; b,  $p < 0.001$ , Group III-A versus Group II; c,  $p < 0.001$ , Group III-B versus Group I; d,  $p < 0.001$ , Group III-B versus Group II; e,  $p < 0.05$ , Group III-B versus Group III-A; f,  $p < 0.001$ , Group IV-A versus Group III-A; g,  $p < 0.001$ , Group IV-A versus Group III-B; h,  $p < 0.001$ , Group IV-B versus Group III-A; i,  $p < 0.001$ , Group IV-B versus Group III-B. Values represent mean  $\pm$  SEM. Group I: healthy control, Group II: new diagnosed allergic asthma-allergic rhinitis patients, Group III-A: severe persistent allergic asthma-allergic rhinitis pre-omalizumab, Group III-B: severe persistent asthma-allergic rhinitis post-omalizumab, Group IV-A: allergic rhinitis pre-immunotherapy, Group IV-B: allergic rhinitis post-immunotherapy.

external and internal environments, plays a key role in the success of adaptive responses to oxidant-stress related challenges, such as those associated with pollutant exposures, microbial infections and allergic challenge.<sup>(27)</sup>

Serum hs-CRP levels were suggested as a prognostic marker for the development of diabetes mellitus<sup>(25)</sup> or future cardiovascular events in previous studies.<sup>(29,30)</sup> Serum levels of the well-known inflammatory marker CRP is still the simplest and inexpensive way to measure the systemic inflammation in related diseases.<sup>(31)</sup> However, standard assays for CRP, with a lower detection limit of 3–8 mg/L, lack the sensitivity required to determine levels of inflammation within the normal range.<sup>(32)</sup>

In our study, subjects with kidney disease, heart disease, liver disease, diabetes mellitus, cancer status, obesity, smoker and autoimmune diseased patients were excluded clinically and serologically. Subjects whose serum CRP levels by standard assay were  $>10$  mg/L were also excluded.

Asthma is characterized by airway hyperresponsiveness and inflammation, in which various cells (such as basophils, mast cells, macrophages, eosinophils, neutrophils and T lymphocytes), cytokines and mediators play a role. Beside local inflammation, systemic inflammation is also present in asthma, as shown by increased levels of plasma fibrinogen and serum amyloid-A.<sup>(33,34)</sup> In this study, there was a significant difference in hs-CRP levels between Group I and Groups III-A and III-B; Group II and

Groups III-A and III-B; Group III-A and Groups III-B, IV-A and IV-B; and Group III-B and Groups IV-A and IV-B; and the healthy controls and severe persistent asthma-allergic rhinitis and allergic rhinitis patients. Interestingly, there was a correlation between the hs-CRP levels and COA in non-treated severe persistent asthma-allergic rhinitis patients (Group III-A).

A major characteristic of asthma is a persistent airway inflammation that fails to resolve spontaneously. Dysregulated expression patterns of pro- and anti-inflammatory mechanisms are thought to be responsible for the development of chronic inflammation. Detection and quantification of airway inflammation is influenced by many factors, including: the use of biologic samples obtained in a non-invasive way; standardized analytical methods to determine biomarkers that can identify inflammatory processes determining the role of systemic inflammation; correlation of biomarkers of inflammation with clinical parameters and their diagnostic efficacy; available tool(s) to monitor disease and evaluate the adequacy of therapy; and the ability to predict the clinical course of inflammation and prognosis of asthma. Using standardized analytical tools, it is now possible to determine direct markers of local inflammation, i.e., the fraction of nitric oxide in exhaled breath; the pH in breath condensate; as well as indirect markers in blood/serum, i.e., numbers of eosinophils and granulocytes, levels of eosinophil cationic protein and CRP. However, none of these biomarkers are specific for asthma.<sup>(35)</sup> Many drugs in the use of allergic diseases can increase albumin levels. However, our results suggesting that the use of omalizumab therapy in asthma was independent of the effect on albumin concentration.

The effects of omalizumab on IgE receptors have been reviewed previously. In brief, omalizumab decreases the levels of circulating IgE by binding to the constant region of the IgE molecule, preventing IgE from interacting with IgE receptors on inflammatory cells. Omalizumab does not bind to the variable allergen specific region of IgE and thus inhibits allergen-induced responses regardless of allergen specificity. The rapid reduction in free IgE levels resulting from omalizumab administration leads to a down regulation of FcεRI expression on inflammatory cells.<sup>(36)</sup> Anti-IgE therapy is an innovative and promising treatment modality that mediates its effects in part at least through decreased inflammation following improved antioxidant capability.<sup>(8)</sup> The balance between oxidant production and sophisticated enzymatic and non-enzymatic antioxidant defense systems maintains physiological homeostasis and counteracts against the oxidative damage of proteins, lipids and DNA. In a previous study, it was reported that the oxidative effects of COA on serum lipids predominated in coronary artery disease (CAD) patients in association with decreased antioxidant protection.<sup>(18)</sup> The known functions of COA include copper transportation, iron metabolism, anti-oxidant defense, and involvement in angiogenesis and coagulation.<sup>(15)</sup> It has been shown that CP catalyzes the oxidation of iron(II) to iron(III), with a catalytic cycle that

involves four of the six atoms of copper associated with COA, and uses dioxygen as an electron acceptor without the mediation of an incompletely reduced reactive oxygen species, such as a superoxide anion or hydrogen peroxide.<sup>(15)</sup> According to our results, we had a comparison of the COA assays. There were significant differences of ceruloplasmin levels between Group I and Groups III-A, III-B, and IV-B; Group II and Groups III-A, and IV-B; Group III-A and Groups III-B, IV-A and IV-B; Group III-B and Groups IV-A and IV-B; and Group IV-A and Group IV-B.

Desloratadine (DCL) is non-sedating anti-histamine approved for the treatment of allergic rhinitis or chronic idiopathic urticaria. DCL acts through its antioxidant effect as a therapeutic action.<sup>(37,38)</sup> In our study Groups of II, III-A/B and IV-A/B had a DCL usage history during the exacerbation of their symptoms on May and November. The blood samples from the patients were obtained on January, when they experienced minimum allergic symptoms. In our study Group of II and III-A and B, salmeterol xinafoate-fluticasone propionate was indicated for the maintenance treatment of asthma. The lower inhalation doses (250 mcg/day) were used for the newly diagnosed Group II and 500 mcg × 2/day inhalation doses were used for Group III. After about the fourth month of the salmeterol xinafoate-fluticasone propionate therapy, the symptoms of the patients had been under controlled and the need of drug use decreased.

In conclusion, anti-IgE therapy is an innovative and promising asthma treatment modality that affects through the inhibition of the inflammation. As a result of our study, the measures of the latter may prove to be useful surrogate markers to monitor efficacy of treatment in patients suffering from this disease. Our observations rather reflect the need to investigate and clarify the proper antioxidants for their usage in specific subcutaneous immunotherapy and anti-IgE treatment.

## Authors' Contributions

ADY participated in the design and execution of the study. ADY, AK and MY followed-up the patients. ADY drafted the manuscript. SG performed the statistical analysis and participated in its coordination. GEP and AB helped with immunoassays. RMG participated in coordination. All authors read and approved the final manuscript.

## Declaration of interest

The authors declare that they have no competing interests.

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