

**Research Article****Evaluation of Plasma Total Antioxidant Activity in Allergic Rhinitis****Dr. Chandresh Agarwal<sup>1</sup>, Dr. Ijen Bhattacharya<sup>2</sup>, Dr. Rahul Saxena<sup>3</sup>**<sup>1</sup>Associate Professor, Department of Ear Nose and Throat, Saraswati Institute of Medical Sciences, Hapur, U.P., India<sup>2</sup>Professor, Department of Biochemistry, Rama Medical College, Hospital & Research, Hapur, U.P, India Assistant<sup>3</sup>Professor, Department of Biochemistry, SAHS, Sharda University, Greater Noida, U.P, India**\*Corresponding author**

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**Abstract:** The prevalence of allergic rhinitis (AR) increases continuously worldwide. Among various factors, excessive production of free radicals has been implicated in the development of allergic rhinitis. The objectives of present study were to ascertain the plasma total antioxidant activity (TAA) and erythrocyte malondialdehyde (MDA) levels in allergic rhinitis, and to determine their cumulative effect in the disease development. In the present study, aforesaid parameters were estimated in 60 subjects (30-60 years), categorized into two groups: 30 healthy subjects served as healthy control and 30 chronic AR patients; and compared it statistically by using student's t- test. Plasma TAA levels were significantly low ( $p < 0.001$ ) in patient group as compared to healthy controls where as erythrocyte MDA levels were significantly high ( $p < 0.05$ ) in allergic rhinitis with respect to controls. Our findings indicate that alteration in plasma total antioxidant status along with oxidative stress (via MDA production) may be responsible for biomolecular deterioration and induces a variety of pathological changes that are highly relevant in nasal and airway mucosae leading to the etiopathogenesis of AR. Therefore, preventive approach against allergen along with incorporation of antioxidant rich diet to overcome the oxidant overload could be effective in reducing the incidence of allergic rhinitis.**Keywords:** Free radical, inflammation, Lipid peroxidation, Total antioxidant activity.

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**INTRODUCTION**

Allergic rhinitis (AR) is a complex disease characterized by inflammation of the nasal mucosa, along with paroxysms of sneezing, itching of the eyes, nose and palate, rhinorrhoea and nasal obstruction [1]. Although scientific data and treatment facilities increase, this disease continues to cause significant morbidity and mortality. Several risk factors for developing AR have been documented. However, the exact underlying mechanism to explore the hidden facts about AR still needs further investigation. It is well accepted that numerous inflammatory cells, including mast cells, CD4-positive T cells, B cells, macrophages, and eosinophils, infiltrate the nasal lining upon exposure to an inciting allergen (most commonly air borne dust mite fecal particles, cockroach residues, animal dander, moulds, and pollens), triggers the release of histamine, inflammatory cytokines and leukotrienes, and thereby leading to the development of AR [2].

Association of inflammation and oxidative stress with the etiopathogenesis of various diseases is well documented [3, 4, 5]. Oxidative stress ensues when large amount of reactive oxygen species are produced from inflammatory cells during asthma that can evade or overwhelm the antioxidant protective mechanism of

cells and tissues, and produce major interrelated impaired cell metabolism including DNA strand breakage, rises in intracellular free  $Ca^{2+}$ , damage to membrane ion transporters and other specific proteins leading to cell death, increased nasal mucosal sensitivity and vascular permeability [6,7]. Prime target to free radicals attack are the polyunsaturated fatty acids in the membrane lipids, causing lipid peroxidation. Malondialdehyde is the most abundant among the reactive aldehydes derived from lipid peroxidation. It has been suggested that these aldehydes released from cell membrane and increase the risk of AR not only by disturbing endothelial cells, nasal and airway mucosa's via oxidative modification of cellular components but also by adversely affecting membrane bound enzyme activities and cellular functions. [8].

These free radicals are efficiently removed by antioxidant defense system of airway mucosa, which includes antioxidant enzymes and antioxidants. Total antioxidant activity (TAA) is a complex trait reflecting homeostasis of redox metabolism, affected by the relative contribution of each antioxidant and the stress of oxidative free radicals [9]. TAA may have a significant role in the physiochemical alterations in allergic diseases and received much attention in preventing allergen mediated complications such as

AR. Interestingly, there is no far conclusive evidence on alteration in plasma total antioxidant status in relation with lipid per oxidation in allergic rhinitis patients. Therefore, the overall objectives of present study were to ascertain the plasma levels of TAA and erythrocyte MDA levels in AR and to determine their cumulative effect in the disease etiopathogenesis.

## MATERIAL AND METHODS

In the present study, 30 healthy subjects belonged to age group 30-60 years were included of which 18 volunteers were male and 12 were female (served as controls) whereas in the AR patient group (30-60 years), 20 patients were male and 10 patients were female. Nasal symptoms of AR patients were scored and the results were re- corded. Allergic rhinitis patients with at least 2 rhinitis symptoms (sneezing, rhinorrhea, nasal obstruction, itching) for at least 6 months a year in the previous 2 years and had a positive skin prick test response to at least 1 clinically significant perennial allergen (e.g., house dust mites, molds, cockroach, cockroach excrement grass and tree pollen, cat and dog epithelia and molds, or animal dander) were included in the study.

All subjects were included after taking their informed consent and approval of protocol by the ethics committee of the college. Fasting blood samples were collected in in EDTA vial from anticubital veins avoiding venostasis from each subject after collecting the information of age, sex, family history and drug treatment. Patients with other allergic diseases like food allergy, eczema, pregnant women and those who had taken antioxidants, antidepressants or antihistamines were excluded from the study. Whole blood was separated into plasma and erythrocyte fractions by centrifugation (4000×g, 10 minutes) at 4 °C. As soon as possible after separation, the erythrocyte fractions were washed three times with saline. Then, erythrocytes were lysed with cold distilled water (1:4), stored in refrigerator at 4 °C for 15 minutes and then their membranes were removed by centrifugating them at 4

°C for 30 minutes with 20000×g. Plasma samples and erythrocyte lysate were stored at - 70 °C until assay.

Plasma total antioxidant activity was estimated spectrophotometrically by the method involving reaction of standardized solution of iron EDTA complex with hydrogen peroxide, i.e. Fenton type reaction, leading to the formation of hydroxyl radicals. This reactive oxygen species degrades benzoate, resulting in the release of TBARS. Antioxidants from the added plasma cause the suppression of production of TBARS. The reaction was measured spectrophotometrically at 532 nm [10].

Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, after preparation of hemolysate. In this method, the heat induced reaction of malondialdehyde (MDA) with thio barbituric acid (TBA) in the acid solution forms a trimethine colored substance, which was measured spectrophotometrically at 532 nm (11). The data from both the study group subjects and controls were expressed as Mean  $\pm$  SD and compared by using Student's t-test and distribution of probability (P).

## RESULTS

The observation made reveal significant changes in the levels of plasma TAA and erythrocyte malondialdehyde (Figure 1 and 2 respectively) in patient group subjects with respect to control group. Erythrocyte MDA levels were  $2.74 \pm 0.13$  ( $\mu\text{mol MDA/ml}$ ) in allergic rhinitis patients. On statistical analysis, erythrocyte MDA levels were found to be increased significantly ( $p < 0.001$ ) in AR patient group i.e. 37.2 % high as compared to controls (Figure 1). On the other hand, plasma total antioxidant activity was  $0.988 \pm 0.12$  (m mol/L) in allergic rhinitis patients, whereas it was  $1.382 \pm 0.17$  (m mol/L) in healthy controls. Statistically, plasma TAA levels were found to be significantly low ( $p < 0.05$ ) in AR patient group i.e. 28.5 % low as compared to controls (Figure 2).

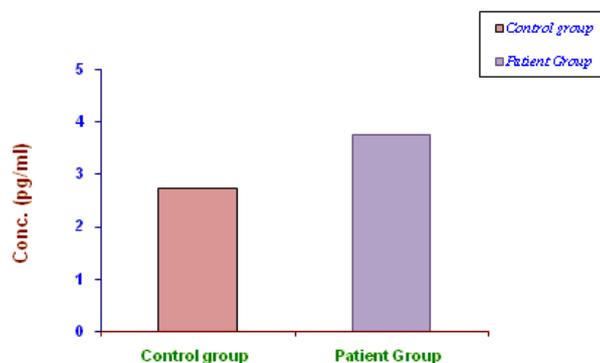
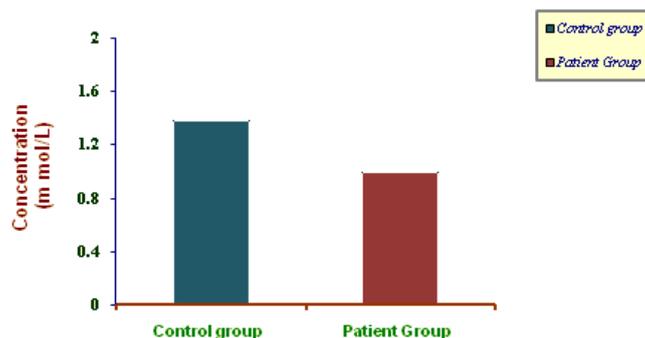


Fig 1: Erythrocyte Malondialdehyde levels in study group subjects



**Fig 2: Plasma Total Antioxidant Activity in study group subjects**

## DISCUSSION

Excessive production of free radicals eventually lead to cellular death, tissue damage and necrosis, have been implicated in the pathogenesis of many inflammatory diseases including allergic rhinitis [1,2]. It is well known that eosinophils are not only the most dominant inflammatory cell but also have a greater ability to produce free radicals in allergic rhinitis [12]. Superoxide radical produced via activation of NADPH oxidase activity in neutrophils and eosinophils that leads not only the depletion of antioxidant enzymes as reported in previous studies but also amplify further deterioration by producing  $H_2O_2$ , highly reactive hydroxyl radical, peroxynitrite anion and hypochlorous acid [13]. The mechanisms whereby these free radicals may exert cytotoxic effect related to AR development, which include damage to cell membrane via lipid peroxidation, increased airway reactivity, increased nasal mucosal sensitivity and secretions, production of chemoattractant molecules, and increased vascular permeability [6, 8].

Lipid peroxidation, characterized by the production of malondialdehyde, ethane and pentane, is a deleterious process leading to structural modification of complex lipid protein assemblies associated with cellular malfunction and death [14]. In the present study, malondialdehyde levels, the most abundant reactive aldehydes derived from lipid peroxidation, were also found to be significantly high in allergic rhinitis patients ( $P < 0.001$ ; Figure 1.0) which authenticate the contention that development of allergic rhinitis is closely associated with oxidative stress mediated lipid peroxidation. Similarly, enhanced incidence of lipid peroxidation in erythrocytes of allergic rhinitis patients after exposure to pollen and house dust mite, have been well documented [15].

A rise in lipid peroxidation also explains oxidant overload in allergic rhinitis which might have reflected by alteration in antioxidant defense system. In this context, reduction in total antioxidant activity (TAA) indicates the disturbance in the antioxidant defense system of the body, which could be due to decrease in individual antioxidants. In the present study, plasma TAA levels decreases significantly in allergic

rhinitis patients ( $p < 0.05$ ,  $p < 0.001$ ) along with rise in erythrocyte MDA levels, which clarify the contributory effect of reduced antioxidant status due to augmented oxidative stress. Recently, low TAA levels have been observed in smokers, which are considered as one of the important risk factor of allergic rhinitis [16]. Moreover, consistent findings have been documented by Akbay *et al.* and suggested that imbalances between oxidative stress and antioxidant defense mechanisms may play a role in the etiopathogenesis of allergic rhinitis [17]. However, TAA levels in the upper airways in patients with allergic rhinitis are still undefined.

## CONCLUSION

On the basis of present study findings, it is apparent that patients with allergic rhinitis have low plasma total antioxidant activity due to inflammation mediated oxidant overload as characterized by enhanced lipid peroxidation. Therefore, supplementation of diet rich in antioxidants along with anti-inflammatory drugs may be an effective approach in the prevention of allergic rhinitis progression. Furthermore, adoption of preventive measures and development of future drugs based on aforementioned targets are obligatory to reduce inflammation and oxidative stress, which collectively constitute the chief contributors to the global burden of allergic rhinitis.

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