

# Investigating the Level of Vitamin E, C and Superoxide Dismutase in Patients with Allergic Asthma and Allergic Asthma with Helminths Infection

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## Abstract

The aim of this study was to investigate the relationship of antioxidant activity, vitamin E, vitamin C and superoxide dismutase with the severity of allergic asthma by estimating their level in the patients' sera, as well as evaluating their association with the level of total and specific serum IgE in allergic asthmatics and allergic asthmatics with helminths infection. The study applied on 180 subjects divided into three groups, 100 patients with allergic asthma and 50 patients having allergic asthma with helminths infection. The group of patients included 50 males and 100 females age 15-60 years. The control group comprised of 30 healthy individuals, included 14 males and 16 females age range 16-60 years. For each participant, the medical history was considered; the total and specific IgE levels, vitamin E, vitamin C and superoxide dismutase levels were determined in the patient's sera using ELISA technique. The present study has demonstrated an increased level in serum total IgE in the patients' groups (allergic asthma group and allergic asthma with helminths) compare to the control group, mean 374.54 IU/mL; 472.24 IU/mL; 29.21 IU/mL respectively. The level differences among the three groups are highly significant when compared statistically.

**Keywords:** Allergic asthma, vitamin E, vitamin C, superoxide dismutase, antioxidant, asthma with helminths infection, total IgE, specific IgE.

## Introduction

Asthma is a major public health problem affecting many people around the world; men and women, young and old all over the world <sup>1</sup>. Asthma is a chronic disease of airways with acute lung inflammation and bronchial hyperresponsiveness that characterized by different respiratory symptoms including wheezing, dyspnea, chest discomfort, cough and variable airflow limitation (2,3). These symptoms are often caused by several factors such as allergen exposure, exercise, irritant exposure, viral respiratory infections or change in the weather <sup>4</sup>. Asthma is believed to be trigger by a combination

of complex genetic and environmental interactions. It is divided into two types, both of them have the same signs. These types are allergic (or extrinsic) asthma and non-allergic (or intrinsic) asthma <sup>5</sup>. Allergens can activate the inflammatory cells in the airways and lungs. This activation leads to generate oxidants (free radicals) (6,7). The antioxidant such as vitamin E, vitamin C and superoxide dismutase (SOD) terminate and inhibits oxidant reaction. As a result of this reaction, antioxidant reducing external attacks in the lung <sup>8</sup>. However, asthma's patients have been reported to have reduced antioxidant defences in their peripheral blood <sup>9</sup>. Vitamin E is an important antioxidant vitamin in the body, playing a protective role by which damage free radical and scavenge reactive oxygen species (ROS) <sup>10</sup>. Vitamin E becomes oxidized itself while preventing the oxidation of other factors more important metabolically <sup>11</sup>. In allergic asthma and allergic lung inflammation, vitamin E acts as an anti-inflammatory factor and blocks the airway hyperreactivity by regulating leukocytes'

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signals of trans-endothelial migration from the blood to the lung<sup>12</sup>. Vitamin E intake is associated with low serum IgE concentrations and low frequency of allergen sensitization<sup>13</sup>. Vitamin C is a critical water soluble vitamin that presents into two active forms, ascorbic acid which is oxidized derivative and dehydroascorbic acid. Vitamin C can act as a hydrogen donor to converse oxidation; therefore, it functions as a non-enzymatic antioxidant which deactivates and reacts with the free radicals<sup>(14, 15)</sup>.

## Materials and Method

### Subjects

The current study included 150 allergic asthmatic patients, 100 with allergic asthma and 50 having allergic asthma with helminths infection confirmed by general stool examination (GSE). The group of patients comprised of 50 males and 100 females age 15-60 years. These cases were followed up in the Specialized Center of Allergy in Baghdad/ Al-Rusafah and Al-Zahraa Center of Allergy in Baghdad/ Al- Karkh for the period from July 2018 to January 2019.

Exclusion criteria were included: smoker, oral corticosteroids, respiratory tract infection, cancer, any chronic disease other than asthma, parasitic infections other than helminths and pregnant females.

The control group involved 30 healthy individuals 14 males and 16 females with age ranged between 16-60 years. Exclusion criteria were chronic respiratory disorders, current or past history of allergy, helminths infection and all chronic diseases mentioned in reference to the study group above.

### Study design

All patients underwent a detailed clinical examination included total immunoglobulin E test (T.IgE), specific immunoglobulin E test (S.IgE), general stool examination (GSE) and serum level of vitamin E, vitamin C and superoxide dismutase (SOD).

Five milliliters of peripheral blood sample were taken from each subject using disposal 10ml syringes under sterile conditions. The blood collected in a gel tube to get the required serum for serological tests (T.IgE and S.IgE). Samples were centrifuged for 10 minutes at 2500 rpm, then serum divided onto five Eppendorf tubes, one for each test. All serum samples kept frozen at -20°C until they used.

### Determination of T-IgE, vit. E, vit. C and SOD

The level of serum T.IgE, vit. E, vit. C and SOD were determined using enzyme linked immunosorbent assay (ELISA) based on the manufacturer's instructions, where microtiter plates have pre-coated with antibodies. The standard and samples were applied on the wells, followed by adding the conjugate, then the TMB substrate to produce a blue color which will be changed to yellow when adding the stop solution. The concentration is proportional to the density of the produced yellow color, which is read using the microplate reader at 450nm to find the concentration of T.IgE, vit. E, vit. C and SOD.

### Determination of S-IgE

The determination of S-IgE was performed using ELISA (polycheck kit, Germany). The polycheck strips were firstly coated with the allergens and calibrators, followed by adding the patients' and control sera which incubated on the rocker shaker. In the next step, the conjugate was applied followed by the substrate to quantify the IgE by the scanner and the computer. The inhalation profile kit that used to diagnose the types of allergens contains 20 parameters from the common allergens including: *Dermatophagoides pteronyssinus* (D1), *Dermatophagoides farina* (D2), Timothy Grass pollen (G6), Rye pollen (G12), Plantain pollen,(W9), Mugwort pollen (W6), *Cladosporium herbarum* (M2), *Aspergillus fumigatus* (M3), *Penicillium notatum* (M1), *Alternaria alternata* (M6), Dog epithelia (E2 / E5), Cats epithelia (E1), Horse epithelia (E3), Guinea epithelia (E6), Hamster epithelia (E84), Rabbit epithelia (E82), White Oak pollen (T7), Birch pollen (T3), Hazel pollen (T4) and Alder pollen (T2).

### Investigation of helminth infections

The association of helminths infection in the group of allergic asthmatic patients were investigated using the GSE. Fecal samples were collected from the patients and control groups. The specimens were directly examined under 10X and 40X objectives.

### Statistical Analysis

Statistical analysis was conducted with Statistical Program for Social Science software (IBM SPSS 23.0). Parametric quantitative data was presented as the mean ± standard error. Least significant difference test (L.S.D) was used to compare among the means of over than three (compare the concentration of T.IgE and vitamin

E, vitamin C and SOD in asthmatics, asthmatic with helminths infection and control groups). Correlation analyses between data were performed using Spearman’s tests, with the correlation coefficients presented as “R”. Level of significance was defined by using ANOVA test with probability  $P \leq 0.05$  (significant),  $P > 0.05$  (none significant) or  $P < 0.01$  (highly significant).

### Results and Discussion

Studying the relationship between the infection with allergic asthma and serum level of antioxidants vit. E, vit. C, SOD as well as its relationship with the level of total and specific serum IgE has been addressed in this study. In addition, the effect of the association of helminths infection with allergic asthma and the effect of this association on the immune response of allergic asthmatic patients was investigated for the first time in

this study, particularly in regard with the biomarkers mentioned above.

The current study has observed a significant elevation in the levels of the total serum IgE parallel with declined serum level of antioxidants in allergic asthmatic subjects and allergic asthmatics with helminth infection compared with healthy individuals. The mean serum level of total IgE of asthmatics was  $374.540 \pm 10.394$  IU/mL, which is highly significant than its level in healthy control, mean value  $29.216 \pm 1.786$  IU/mL,  $p < 0.01$ . In addition, the mean T.IgE level in allergic asthmatic subjects with helminth infection is  $472.242 \pm 6.273$  IU/mL which is highly significant than that of the control. However, the T-IgE level in the patients with allergic asthma and helminth infection was higher than its level in the patients having only allergic asthma, the full comparison is presented in Figure 1.

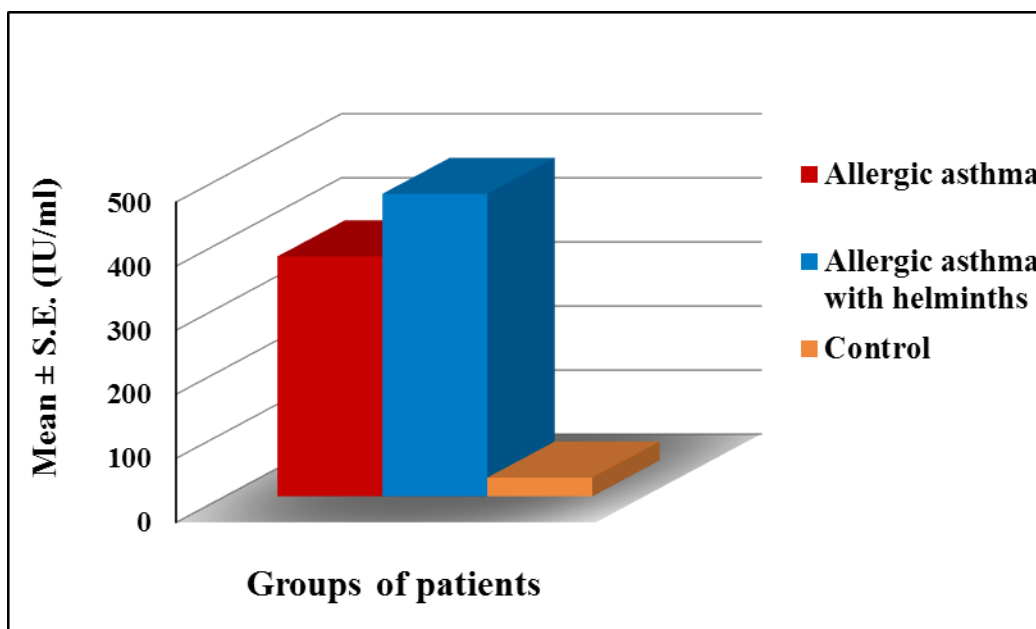
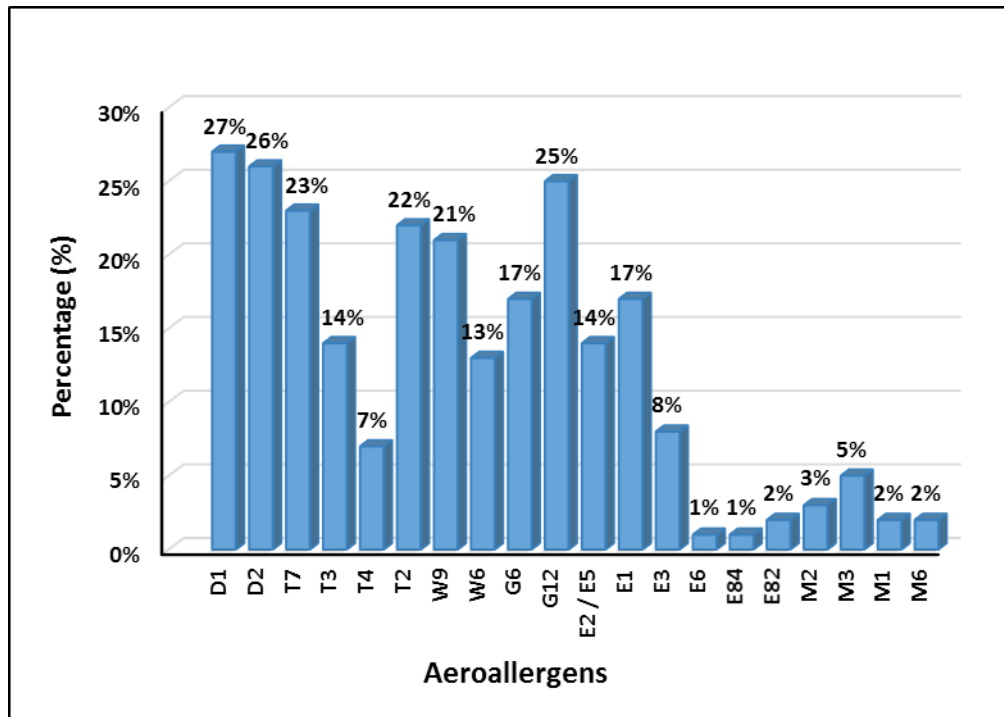


Figure 1: Serum levels of total IgE in the groups of allergic asthma, allergic asthma with helminths and control.

Allergens sensitization test was also performed in the current study by evaluating the level of serum S.IgE. The results have disclosed that the frequency of the most common allergens in 113 allergic asthmatic patients with positive specific IgE was as in the following: D1

(27%), D2 (26%), G12 (25%), T7 (23%), T2 (22%), W9 (21%), G6 (17%), E1 (17%), T3 (14%), E2 / E5 (14%), W6 (14%), E3 (12%), T4 (11%), M3 (7%), M2 (4%), E82 (3%), M1 (2%), M6 (2%), E6 (1%), E84 (1%), as shown in Figure 2.



**Figure 2:** The percentage of aeroallergens that sensitize asthmatic patients’ groups.

As shown in Table 1, the mean level of vitamin E in allergic asthma patients was  $11.545 \pm 0.320$  IU/ml and it was  $9.413 \pm 0.180$  IU/ml in allergic asthma with helminths as compared to its mean level in healthy control group that was  $23.253 \pm 0.726$  IU/ml. Statistical analysis shown that there are a highly significant differences when compared between patients’ groups and control group and also between allergic asthma and allergic asthma with helminths.

**Table 1:** Show the variation in vitamin E concentration among patients’ groups and control.

Groups	Vitamin E ( $\mu\text{g/mL}$ ) Mean $\pm$ S.E.	L.S.D. P-value
Allergic asthma	$11.545 \pm 0.320$	$P1= 0.00^{**}$
Allergic asthma with helminths	$9.413 \pm 0.180$	$P2= 0.00^{**}$
control	$23.253 \pm 0.726$	$P3= 0.00^{**}$

**\*\*Highly significant ( $p < 0.01$ )**

*P*: probability,  $P1$ =Allergic asthma Vs Allergic asthma with helminths,  $P2$ =Allergic asthma Vs control,  $P3$ = Allergic asthma with helminths Vs control.

The serum level of vitamin C in allergic asthmatics was  $36.040 \pm 1.367$   $\mu\text{g/ml}$ , while the recorded mean level in allergic asthmatics with helminths infections was  $21.163 \pm 0.793$   $\mu\text{g/ml}$ . The statistical analysis reached that vit. C level in both patients’ groups are significantly different from the level of vit. C in the sera of the control group which was  $76.288 \pm 2.708$   $\mu\text{g/ml}$ . On the other hand, there was a highly significant difference in the level of vitamin C between the two patients’ groups as shown in Table 2.

**Table 2: Show the difference in the level of serum vitamin C in the groups of allergic asthma, allergic asthma with helminths and control.**

Groups	Vitamin C ( $\mu\text{g/mL}$ ) Mean $\pm$ S.E.	L.S.D. P-value
Allergic asthma	36.040 $\pm$ 1.367	P1= 0.00**
Allergic asthma with helminths	21.163 $\pm$ 0.793	P2= 0.00**
control	76.288 $\pm$ 2.708	P3= 0.00**

\*\*Highly significant ( $p < 0.01$ )

P: probability, P1=Allergic asthma Vs Allergic asthma with helminths, P2=Allergic asthma Vs control, P3=Allergic asthma with helminths Vs control.

Moreover, it has been found that superoxide dismutase is also decreased in both patients' groups. Statistically, there were highly significant differences between the both patients' groups (Table 3) and between the patients in the two groups and the control too. The level of SOD in allergic asthmatic group was 149.048  $\pm$  6.447pg/mL, whereas, it was 78.271  $\pm$  3.635 pg/mL in the group of allergic asthma with helminths. Nevertheless, the SOD level in the serum of the control group was 1034.157  $\pm$  32.659.

**Table 3: Demonstrate the difference in the level of superoxide dismutase (SOD) in the groups of allergic asthma, allergic asthma with helminths and control.**

Groups	SOD (pg/mL) Mean $\pm$ S.E.	L.S.D P-value
Allergic asthma	149.048 $\pm$ 6.447	P1= 0.00**
Allergic asthma with helminths	78.271 $\pm$ 3.635	P2= 0.00**
control	1034.157 $\pm$ 32.659	P3= 0.00**

\*\* Highly significant ( $p < 0.01$ )

P: probability, P1=Allergic asthma Vs Allergic asthma with helminths, P2=Allergic asthma Vs control, P3=Allergic asthma with helminths Vs control.

**Table 4: Show the variations in the level of antioxidant according to different groups of T.IgE level.**

Antioxidants		T.IgE lower than (300) group	T.IgE between (300-500) group	T.IgE upper than (500) group	L.S.D. ANOVA (p-value)
Vitamin C ( $\mu\text{g}/\text{mL}$ )	Mean $\pm$ S.E.	37.162 $\pm$ 2.110	27.380 $\pm$ 2.021	25.257 $\pm$ 1.632	P=0.00**
Vitamin E ( $\mu\text{g}/\text{mL}$ )		11.179 $\pm$ 0.348	11.101 $\pm$ 0.602	9.821 $\pm$ 0.240	P=0.01**
Superoxide dismutase (pg/mL)		138.149 $\pm$ 8.281	116.647 $\pm$ 12.328	100.637 $\pm$ 8.320	P=0.03*

\*Significant ( $p < 0.05$ ) \*\*Highly significant ( $p < 0.01$ )

S.E.= standard error

Allergic asthma is the most common phenotype of asthma among patients. The IgE is a well-known component of allergic reactions where it is produced during the process of sensitization which is triggered on the first exposure to the allergen. However, the level of T.IgE is increased with helminths infections too<sup>22</sup>. The results observed in current study indicating that the T.IgE playing a central role in the initiation and propagation of the inflammatory cascade of the allergic response and also in the immune response to the helminths infection, as shown in Figure 1.

### Conclusion

The level of antioxidants, vit. E, vit. C and SOD are correlated with the severity of allergic asthma, where asthmatic patients having antioxidant insufficiency due to the increased oxidative stress. In addition, antioxidant activity has a negative correlation with pathogenesis parameters of allergic asthma such as total and specific IgE as well as with helminth infections.

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**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Science, Mustansiriyah University, Baghdad-Iraq and all experiments were carried out in accordance with approved guidelines.

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