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Measurement of Total Immunoglobulin E and Vitamin D3 Levels in the Sample to Asthma Patients of Iraq-Baghdad

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Abstract

Asthma is the hypersensitivity-reaction. Numerous elements were mentioned as being crucial in the pathogenicity of asthma. By using the ELISA technique on the sera of 100 asthmatics and 50 supposedly healthy controls, the amount of total IgE was measured, and the level of vitamin D3 was estimated using the same procedure on the subject sera (60 asthma-patient and 30 controls). According to this study, asthmatic patients' sera had significantly higher total IgE levels than control groups (P=0.001). Also, the study found that asthmatic patients' serum vitamin D levels declined significantly (P≤0.05) compared to the healthy control group. In addition, demonstrates decrease in vitamin D levels in severe asthma compared to moderate, mild asthma, and control at high significance (p= 0.001), helps to explain the relationship between vitamin d and asthma severity. Furthermore, Total IgE and Vit. D3 test found to be the ideal test for those with asthma.

Keywords: Asthma, IgE, ELISA, Vitamin D3

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قياس مستويات الغلوبيولين المناعي الكلي E و فيتامين د في عينة لمرضى الربو في العراق - بغداد

محمد صالح جبر [1]

الخلاصة

الربو هو تفاعل من تفاعلات فرط الحساسية. هنالك العديد من العناصر تم ذكرها لما لها من دور في امراضية الربو. باستخدام تقنية *الاليزا* على مصل 100 مصاب بالربو و 50 شخص يفترض أنه يتمتعون بصحة جيدة، تم قياس كمية إجمالي الغلوبيولين المناعي E ، وتم قياس مستوى فيتامين د باستخدام نفس التقنية على مصل (60 مريض ربو و 30 شخص من المجموعة الضابطة). ووفقًا لهذه الدراسة، فان مستويات اجمالي الغلوبيولين المناعي E لدى مرضى الربو أعلى بكثير من المجموعة الضابطة (P= 0.001). كما وجدت الدراسة أن مستويات فيتامين د في مصل مرضى الربو انخفضت بشكل ملحوظ (P≤0.05) مقارنة بالمجموعة الضابطة. بالإضافة الى ذالك يظهر في هذه الدراسة انخفاضًا في مستويات فيتامين (د) في حالات الربو الحاد مقارنةً بالربو المعتدل والخفيف، السيطرة بدلالة احصائية عالية (P = 0.001) ،وهذه يساعد في تفسير العلاقة بين فيتامين د وشدة الربو. علاوة على ذلك، وجد ان اختبار إجمالي الغلوبيولين المناعيE و فيتامين د هو الاختبار المثالي لمن يعانون من الربو.

الكلمات المفتاحية: الربو، اجمالي الغلوبيولين المناعي E تقنية الاليزا، فيتامين د

انتساب الباحث

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معلومات البحث

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Introduction

An allergic reaction to inhaled allergens causes chronic inflammatory airway disease known as asthma [1]. An antibody called immunoglobulin E (IgE) is connected to hypersensitivity and allergic responses [2]. A production of IgE-antibodies in response to exposure to an allergen, binding of IgE to Fc-receptors of mast cells, cross-linking of the bound IgE by an allergen upon reexposure, and product of mediators from mast-cells such as histamine, cytokines and lipid mediators are the initial steps in an allergic reaction. Many of the symptoms are brought on by certain mast cell-mediators, which rapidly increase vascular permeability and promote smooth muscle contraction [3]. We would like to concentrate on three findings about chronic asthma, keeping in mind the importance of IgE antibodies and the involvement of allergens in asthmatic patients: The relevant allergens, such as dust mites, cats, dogs, or cockroaches, have been shown to become air-borne on particles of 5 to 35 m in diameter, and many or most acute episodes of wheezing cannot be related to increased or even high-level exposure to allergens. A strongest-association is with sensitization to common perennial inhalant-allergens, which is true in particular for high-titer IgE antibodies to allergens that are prevalent in the patient's community [4]. Influences of vitamin D on inflammatory pathways brought on by allergens are immunomodulatory [5] by acting on VDR expressed on several immune cells, including T and B cells, dendritic-cells, and macrophages [6]. Many of these cells, such as activated macrophages and dendritic cells can synthesize biologically-active vitamin-D from circulating 25-OHD [7]. Additionally, D vitamin has demonstrated the capacity to block both T-helper (Th-1 and Th-2type) responses by inhibiting the generation of IFN- produced by IL-12 as well as IL-4 and Interlukine-4 induced expression of IL-13 [8]. This study is a trail to estimate vitamin D distribution of patients according to asthma severs as well estimates the level of total IgE and vitamin D3 asthma in comparison with apparent healthy-controls.

Material and Method

Asthma patient and healthy control:

One hundred patients with allergic asthma were enrolled in this study. They attended the Allergy Specialized Center in Baghdad, Iraq from September 2019 to February 2020, with ages ranging from 10 to 65, and included 66 ladies and 44 males. Additionally, 50 seemingly healthy individual subjects control (22 females and 28 males). The pre-diagnostic performed by the doctor using the WHO and GINA guidelines served as the foundation for the patient's diagnosis.

Sample collection and Immunological test:

Each individual had a vein punctured to remove five samples of venous blood using an aseptic procedure and a syringe. The blood sample was drawn into sterile gel tubes, allowed to clot for roughly one hour, and then centrifuged for 15 minutes at 3000 rpm to separate the serum. The serum was then refrigerated at -20 °C until it was tested. According to the manual approach of the Euroimmun Company, immunoglobulin E (IgE) has been quantified in the serum of patients and control groups using the ELISA method (Germany). According to the Eagle Biosciences, Inc., (United States) manual, the serum 25-hydroxyvitamin D levels were determined using

the ELISA method with a sensitivity of up to 1.6 ng/mL.

Statistical Analysis:

Data were analyzed by using statistical-system SPSS (Statistical-Package for the Social-Sciences) version-13. For quantitative-variables, the original data were tested for normality, (Shapiro-Wilk and Kolmogorov-Smirnov-test), significant (sig.) differences-between medians were assessed by the nonparametric-tests (Mann-Whitney and Kruskal-Wallis) p= ≤0.05 was considered-significant as well probability-value >0.05 was considered non-significant.

Result

Demographic characteristic:

The distribution of asthma patients by age and sex can be shown in this table. Most patients are between the ages of 26 and 41 and 42, with a percentage of 44% and 36%, respectively. The frequency of patients between the ages of 10 and 25 is also high, at 20%, but it is not as high as in the other three groups. As opposed to the controls. The distribution of patients by gender was also shown in the table. 66 asthmatic women were more likely to develop the condition than 44 asthmatic men, as shown in Table 1.

Table (1): Frequency of Subject Groups according to Gender and Age groups

Age group:	**Asthma (N=100)**		**Healthy (N=50)**		**Studying Groups**
	No	**%**	**No**	**%**	
10-25	20	20	10	20	Chi square =200
26-41	44	44	22	44	p-value =0.001
<42	36	36	18	36	
Gender:	**No**	**%**	**No**	**%**	Chi square =20
Female	66	66	22	44	p-value =0.001
Male	44	44	28	66	

Reference: Results of Researcher analysis by SPSS Programs.

Estimation of Total IgE:

Total immunoglobulin E is one of the crucial variables in the diagnosis of asthma. The compares between a level of T-IgE in serum to asthma patients and control groups was significantly significant-difference (330.0 vs.42.5; P=0.001), as show in Table (2) and Figures (1).

Table (2):Total-IgE Concentration in Sera of Asthma-Patients in comparison with Controls.

Groups	**No. of subject**	**Total IgE (IU/ml) Median, (min-max)**	**p-value**
Patients	100	330.0, (100-500) IU/ml	<0.001
Healthy	50	42.5, (10-75.2) IU/ml	

Reference: Results of Researcher analysis by SPSS Programs.

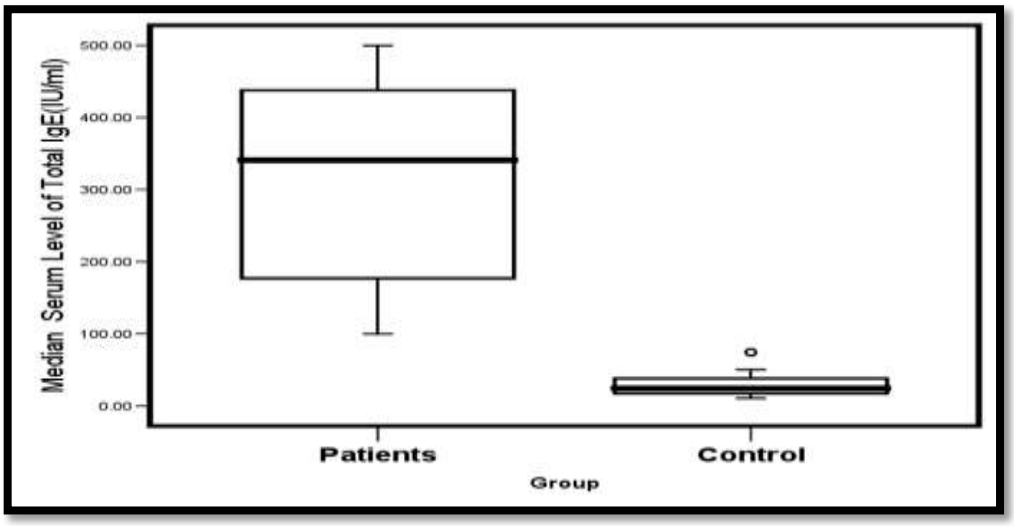

Figure (1): Diagram showing the serum total IgE in the two study groups (asthma patient and control).

ROC test to Total-IgE:

Area under the ROC curve (AUC) =1.00, Cut-off= >75 and Significance level P<0.0001, that T-IgE consider excellent marker to asthma patient according to the percentage of Area-under the ROC curve as show Figure (2).

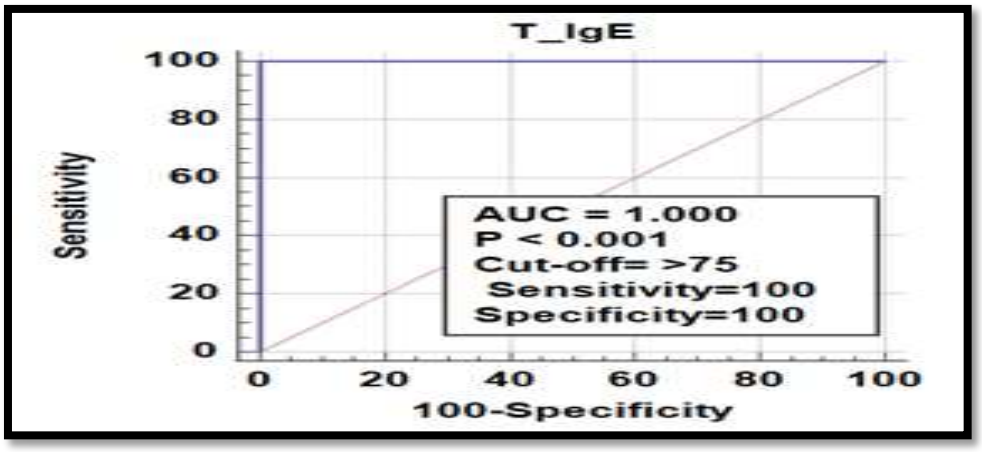

Figure (2): Receiver operating characteristic of T-IgE serum level in asthma patients

Estimation of Vitamin D3:

Vitamin D3 of asthma was significantly, lower than control-group (13.4 ± 6.7 vs 36.01 ± 12.3 ng/ml), (P= ≤0.05), as show in Table (3) and Figure (3).

Table (3): The 25 (OH)D concentrations in asthmatic in comparison with control.

Vit.D3	N	Mean ±S.D.	p –value
Asthma Patients	60	13.4 ±6.7	≤0.05
Controls	30	36.0 ±12.3	

Reference: Results of Researcher analysis by SPSS Programs.

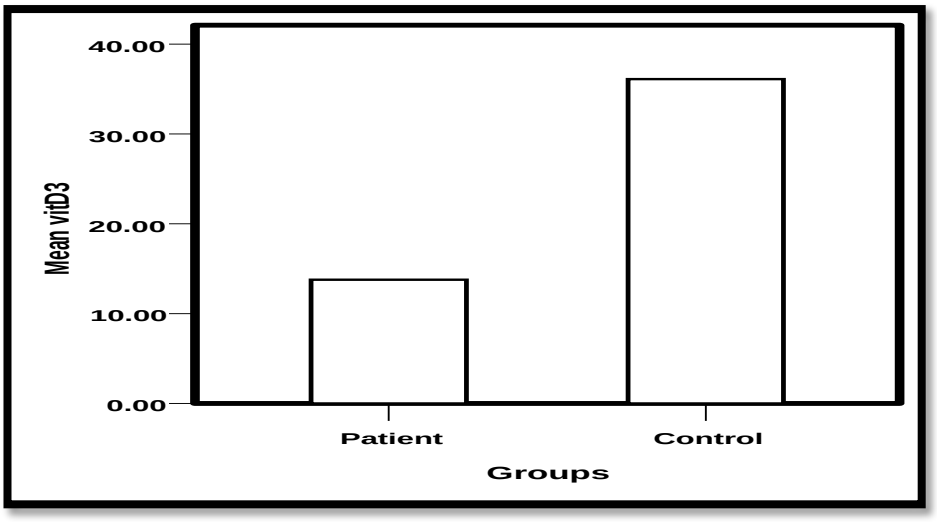

Figure (3): Diagram showing the mean levels of vitamin D3 in subjects.

The reference value of Vit.D3 as show in the table (4).

Table (4): A rang of Vitamin D and Classified into Five Levels.

Vitamin D 25	Range
Sever deficiency	Less 10ng/ml
Deficient	10to20ng/ml
In-sufficient	20to30ng/ml
Normal	30to100ng/ml
Toxic	150ng/ml>

References: [9].

Vitamin D3 and Severity of Asthma:

The reduces level of vit. D in severe asthma compare with moderate, mild asthma and control at high significant (p= 0.001) as show in Table (5)

Table (5): The Mean Serum Level of vit. D according Asthma Severs.

Vitamin D3	%	N	Mean ±S.D.	Level of Vit. D3 (ng/ml)	P-value
Severe Asthma	38.4%	23	7.0±1.7	**Sever deficiency**	0.001
Moderate Asthma	53.3%	32	15.7±2.5	**Deficient**	
Mild Asthma	8.3%	5	28.0±12.3	**Insufficient**	
Control	100%	30	36.0±13.9	**Normal**	

Reference: Results of Researcher analysis by SPSS Programs.

ROC test to 25OH-D3:

The Area under the ROC curve (AUC) =0.966, Cut-off= ≤19 and Significance level (P<0.0001), that vitamin D consider excellent-marker to asthma patients according to the percentage of Area under the ROC curve as show in Figure (4).

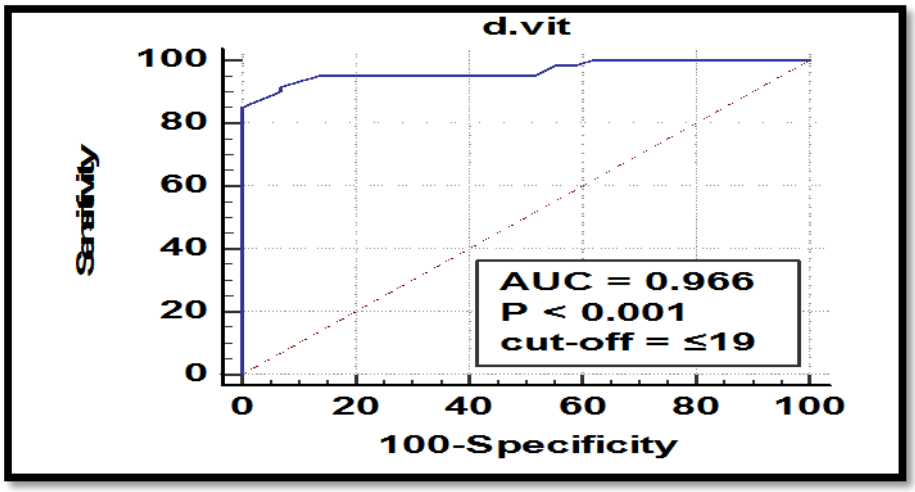

Figure (4): Receiver operating characteristic of vitamin d serum level in asthma.

Correlations Spearman between Vitamin D3 and Total IgE:

The relationship between the parameters studied (Vitamin D3 and Total IgE) in this study is shown in this table, (6).

Table (6): Correlation Coefficient between Vitamin D3 and Total IgE

Correlations Spearman		**Vit. D3**	**T-IgE**
Vit. D3	Correlation Coefficient	1	0.066
	P-value (2-tailed)	-	0.60
T-IgE	Correlation Coefficient	0.066	1
	P-value (2-tailed)	0.60	-

Reference: Results of Researcher analysis by SPSS Programs.

Discussion

Immunoglobulin, known as IgE is essential for both acute allergic reactions and long-term inflammatory allergic disorder [10] .Demonstrates in this study that there is a substantial rise in the median serum level of total IgE in asthma patients as compared to the control (330.0 vs. 42.5 IU/ml) as show in Table(2) and Figure(1), and this is because activated Thelper-2 cells can release IL-4 and IL-13, which drive B-cells to secrete particular IgE and mediate the formation of eosinophils. T-IgE is biomarker to asthma according to the ROC-curve as show in Figures (2). The results of this-study are supported by data that have been published by [11-13]. Asthma patients' mean 25(OH) D concentrations were 13.4±6.7 ng/mL, compared to 36.0±12.3 ng/mL in the healthy. Compared to asthmatics and controls, the mean D3

levels in the asthma were lower than those in the control group, and level of probability-valu was significant P = ≤ 0.05 as show in Table-(3) and Figure-(3). Several studies have reported that lower vitamin (d3) levels were found in patients with asthma. The findings of this research are supported by evidence released by Mohammed *et al.*, who found that 25% of asthmatic-patients had serum vit. D level below the 25 ng/ml [14]. Moreover, Boonpiyathad *et al.*, who found that, at the period of asthma-exacerbation, the deficiency and insufficiency of vitamin D compared with levels of serum 25(OH) D in patients (without-asthma-exacerbations) and healthy individuals that have been higher levels of the vitamin. In asthma, the likelihood of developing a vitamin D deficit or insufficiency is significant and could rise [15]. Therefore, it is preferable to measure vitamin D as part of a routine examination to track vitamin D concentrations and to follow up on eventual supplementation regimens. In this regard, the rang to classified into five level of vitamin D as show in (Table 4) [9]. Vit. D3 are excellent-marker to asthma patients according to the ROC curve as show in (4). Lower vitamin D levels had an inverse relationship with asthma severity (P-value=0.001) as show, in Table (5). This findings are consistent with those made by Rashid *et al.*, who found that asthmatics had a high prevalence of vitamin D-deficiency and that there was a connection between serum vitamin D levels and asthma severity (p = 0.001) [16]. Also, Shebl *et al.*, we also observed atopy was higher in asthmatic-patients with vitamin D insufficiency than those without vitamin-D insufficient [17]. In addition, to this result dis agree with Esfandiar *et al.*, demonstrate that the mean serum level of vitamin D in the group with moderate-asthma was high than the patients with severe asthma. Additionally, there was no difference between the mean serum levels of vitamin D in children with controlled-asthma and partially-controlled asthma, but, has shown low in d3 level of uncontrolled-asthma compared with the other two groups [18]. And, this study found a relationship between low serum vitamin D levels and a higher incidence of asthma flare-ups. An increased risk of autoimmune disease, cancer, and allergy-related illnesses is linked to hypovitaminosis D [19, 20]. In this study show correlation coefficient was positive and non-significant ranges as, shown in Table (6), this means very weak positive correlation between Vitamins D3 and T-IgE [21].

Conclusions

This study is the trail to estimate a level of total IgE and Vit. D3 of asthmatics in comparison with apparent-healthy and this critical biomarker to allergic diagnosis.

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