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## Serum Levels of Total IgE and Interleukin-13 in a Sample of Allergic Asthma Patients in Baghdad

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### Abstract

Allergic asthma is a type of asthma that provokes symptoms when an individual is exposed to certain triggers, such as pollen, animal sources of allergens, and other various types of allergens. These allergens cause an immune response that influences lungs and leads to difficulties in breathing. The current study is performed to estimate the concentrations of immunoglobulin E (IgE) and interleukin-13 (IL-13), tested by using the enzyme-linked immunosorbent assay (ELISA) and the numbers of eosinophils, calculated by using hematological analyzers, in the blood of patients with allergic asthma. A total of 150 patients and 50 healthy individuals were randomly selected for the study. The results revealed that IgE and IL-13 levels as well as eosinophil percentage were significantly increased ( $p < 0.001$ ) in the patients in comparison to the healthy individuals. These parameters deem to be a key element in allergic asthma pathogenesis. They also help in the diagnosis and management of the disease.

**Keywords:** IL-13, Allergic asthma, Immunoglobulin E, Eosinophils counts

## المستويات المصلية لمجموع الغلوبولين المناعي IgE و البين ابيضاض-13 في عينة من مرضى الربو التحسسي في بغداد

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### الخلاصة

يعتبر الربو التحسسي نوع من انواع الربو، وتظهر الأعراض عند وجود بعض المحفزات حول الفرد، مثل حبوب اللقاح، وبر الحيوانات، وأنواع مختلفة أخرى من مسببات الحساسية. تسبب هذه المواد استجابة مناعية تؤثر على الرئتين وتجعل التنفس صعباً. اجريت الدراسة الحالية لتقدير تركيز الغلوبولين المناعي E و البين ابيضاضين-13 بواسطة تقنية الإيلايزا (مقايسة امتصاصية مناعية للإنزيم المرتبط) في مرضى الربو التحسسي بالإضافة الى ذلك، هذه الدراسة أيضاً تهدف الى حساب عدد الحمضات بواسطة أجهزة تحليل أمراض الدم لدى مرضى الربو التحسسي. اختيرت مجموعة عشوائية من إجمالي 150 مريضاً و 50 من الأفراد الأصحاء. كشفت الدراسة أن مستويات الغلوبولين المناعي و البين ابيضاضي-13 و أيضاً عدد الحمضات ارتفعت بشكل ملحوظ ( $p < 0.0001$ ) في المرضى مقارنة بالأشخاص الاصحاء. يمكن اعتبار هذه المعلومات كعنصر رئيسي في مرض الربو التحسسي. كما أنها تساعد في تشخيص الربو التحسسي وتساعد في السيطرة عليه.

## Introduction

Asthma is a heterogeneous disorder, commonly described as chronic airway hypersensitivity. It is characterized by several symptoms such as cough, wheeze, shortness of breath, and chest tightness. These symptoms vary in terms of their intensity and occurrence and these two factors are subject to expiratory airflow limitation [1, 2]. IgE is an immunoglobulin related to allergic and hypersensitivity reactions. This immunoglobulin fundamentally binds on high-affinity with IgE receptors on basophils and mast cells [3-5]. It is responsible for type I hypersensitivity to reactions. It binds to the mast cells after the first exposure to different types of an allergen in a process called sensitization. Later, with re-exposure to the same type of allergen, mast cells release various inflammatory mediators such as cytokines, leukotrienes, histamine and pro-inflammatory cytokines. These immunological markers promote allergic symptoms [6, 7]. Effective *in vivo* analysis of the functions of cytokines from animal samples proved that Th2 lymphocytes are considered among the major participants of airway hypersensitivity that underlies asthma. In addition, they might cause bronchial hypersensitivity [6]. Allergic asthma is related with Th2 immune response. Th2 cytokines are known to produce many features of allergic asthma disease [8]. Type2-immunity is promoted by allergens and characterized by the differentiation of naive T CD4 cells toward Th2 effector cells. Th2 cytokines are typically related with eosinophilia, IgE production, and mast cell activation. The foundational cytokines in type2 immune response include interleukin IL-4, IL-5, and IL-13 [9]. Interleukin-13 belongs to T-helper 2 cytokines and plays critical role in the inflammatory process in asthmatic [10]. IL-13 contributes in many of the distinguishing features of pathophysiology of asthma, including, immunoglobulin E class switching, mucus production, airway hyper-responsiveness and subepithelial fibrosis [11]. Allergic asthma is related with eosinophilic hypersensitivity in the airways [12]. Eosinophils are granulocytes that are regenerating and circulating in the bone marrow with other types of white blood cells. Eosinophils migrate at relatively low levels in the bloodstream and constitute up to 1–3% of white blood cells. These cells are considered as a main cell type and can be recruited to sites where inflammatory or immunological responses occur [13, 14].

The aims of this study are to estimate the total serum levels of IgE and interleukins 13 and calculate eosinophils count in the blood of allergic asthma patients from Al-Rusafa side of Baghdad governorate. Changes in these parameters in the patients are evaluated as compared to those in the blood of healthy subjects.

## Materials and Methods

After obtaining the approval of the Ethics Committee in Biotechnology Department, College of Science, University of Baghdad and written consent was taken from all the participants, the study was conducted on allergic asthma patients at the in Baghdad-Iraq. The study included 150 patients who were diagnosed with allergic asthma. The participants, with an age range between 10 to 65 years, attended the Allergy Specialized Center in Al- Rusafa from September 2019 to February 2020. Furthermore, there were 50 apparently healthy individuals as controls, with an age range between 10 to 65 years. Diagnosis for patients was based on pre-diagnosis performed by a physician and a diagnosis of allergic asthma disease depending on the guidelines described by Global Initiative for Asthma (GINA) [1]. The patients and controls were tested for total serum levels of IgE as well as IL-13. Blood (3 ml) was withdrawn from each subject (patients and controls), divided in sterile gel tubes, and left for about two hours to clot. The sample was then centrifuged at 3000 rpm for 15 minutes to separate the serum which was stored at -20°C until assayed. ELISA kits were employed to assess levels of total IgE (Euroimmun Company, Germany) and IL-13 (BT LAB Company, China). Two milliliters of venous blood was withdrawn from each subject by vein-puncture under aseptic technique by multi-sample syringe. For estimating eosinophils count, whole blood was placed in EDTA tubes and tested by a Hematology analyzer according to the manual procedure of the manufacturing company (Beckman Coulter, United States). The Body Mass Indexes (BMI) was calculated by considering both weight and height of the body, using the following formula [15]:  
$$\text{BMI} = \frac{\text{weight (in Kg)}}{\text{height}^2 \text{ (in meter)}}$$

## Statistical Analysis

Data of the subjects were analyzed using SPSS software (Statistical Package for the Social Sciences) version-13. For the quantitative variables (level of total IgE, IL-13 and eosinophil count) data were tested using the normality (Shapiro-Wilk and Kolmogorov-Smirnov) tests. Significant

differences between medians were estimated by the nonparametric (Mann-Whitney-U-test and Kruskal-Wallis test, using probability value (p-value) of < 0.05 [16].

### Results

The number of the allergic asthma patients selected for the current study was 150 in total. This number involved 84 females and 66 males. The healthy individuals (N = 50) represented the control group, involving 22 females and 28 males. The age range of allergic asthma patients was between 10 to 65 years with mean and standard deviation values equal to  $34.9 \pm 15.8$  years. The age range of the controls was identical to the age range of patients ( $37.3 \pm 12.4$  years). The participants were divided into three age groups; 10-25, 26-41 and > 42 years. The frequencies of patients of these age groups were 54, 46 and 50, respectively. In contrast, the frequencies of healthy subjects for the same age groups were 10, 22 and 18, respectively. In addition, the percentage of allergic asthma patients in the 10-25 years age group was 36 %, which is higher than that in the age groups of 26-41 years (30.6%) and > 42 years (33.3 %). BMI value in the patients was  $29.4 \pm 6.4$  kg/m<sup>2</sup>, which was significantly (P = 0.001) higher than that of the controls ( $23.7 \pm 2.6$  kg/m<sup>2</sup>), as shown in Table-1.

**Table 1**-Distribution of subjects according to gender, age, age group, and BMI

| Groups   | Patients ( n=150) | Controls (n=50)  | p-value |
|--|-------------------|------------------|---------|
| Gender (F/M), ( N,% )                                | 84/66, 56.0/44.0  | 22/28, 44.0/56.0 | 0.14    |
| Age (mean $\pm$ S. D.) year                          | $34.9 \pm 15.8$   | $37 \pm 12.4$    | 0.3     |
| Age Group ( N,% ): 10-25 years                       | 54, 36            | 10, 20           | 0.072   |
| 26-41 years  | 46, 30.6          | 22, 44           |         |
| <42 years  | 50, 33.3          | 18, 36           |         |
| Body mass index (mean $\pm$ S. D.) kg/m <sup>2</sup> | $29.4 \pm 6.4$    | $23.7 \pm 2.6$   | 0.001   |

M: Male; F: Female; N: Frequency; %: percentage; S. D.; standard deviation; kilogram; m<sup>2</sup>: Square meter; p: probability.

One of the essential parameters in asthma diagnosis is the serum level of total IgE. Table-2 shows the concentrations of total IgE in the serum of allergic asthma patients in comparison with that of the control. Total IgE median level of allergic asthma group (341.04 IU/ml) was higher than that of the control group (24.5 IU/ml), with a highly significant difference (P<0.001).

**Table 2**-Total IgE concentrations in the serum of patients with allergic asthma and controls

| Groups                     | No. of subject | Total IgE (IU/ml) Median, (min-max) |
|----------------------------|----------------|-------------------------------------|
| Allergic asthma            | 100            | 341.04, (100.03-500) IU/ml          |
| Control                    | 50             | 24.5, (10-75) IU/ml                 |
| <b>P -value &lt; 0.001</b> |                |                                     |

Min: Minimum; mix: Maximum; p-value: probability; No.; Number; IgE: immunoglobulin (Ig) E; International Units; ml: Milliliter.

As shown in Table-3, the concentration of IL-13 was higher in asthmatic patients (Median 5.7, range 2.06 - 128) compared with healthy control (Median 2.9, range 1.1- 6.9), with highly significant differences (P = 0.001).

**Table 3**-IL-13 level of allergic asthma patients in comparison with controls

| Groups          | No. of subjects | IL-13 (ng/ml) Median, (min-max) | p-value |
|-----------------|-----------------|---------------------------------|---------|
| Allergic asthma | 60              | 5.7, (2.06-128) ng/ml           | 0.001   |
| Control         | 25              | 2.9, (1.1-6.9) ng/ml            |         |

Min: Minimum; mix: Maximum; p-value: probability; No.; Number; IL: Interleukin; ng: Nano-gram. The correlations between median levels of Interleukin-13 and gender and age groups are shown in (Table-4). The table shows that allergic asthma patients of both genders had higher levels of IL-13 in comparison with their corresponding controls. In addition, allergic asthma patients of 10-25, 26-41 and >42 age groups had an elevation in the level of IL-13 as compared to their corresponding controls.

**Table 4-**Correlation between level of Interlukin-13 (ng/ml) and gender and age group of patients and controls

|             | Patients |                    |      |       | Control |                    |      |      | p-value |
|-------------|----------|--------------------|------|-------|---------|--------------------|------|------|---------|
|             | No.      | Median IL-13 ng/ml | Min  | Max   | No.     | Median IL-13 ng/ml | Min  | Max  |         |
| Gender:     |          |                    |      |       |         |                    |      |      |         |
| Male        | 26       | 4.4                | 2.08 | 128.0 | 11      | 2.8                | 1.1  | 6.94 | 0.003   |
| Female      | 34       | 8.1                | 2.06 | 27.64 | 9       | 3.2                | 1.10 | 4.53 | 0.001   |
| Age Group:  |          |                    |      |       |         |                    |      |      |         |
| 10-25 years | 20       | 6.8                | 2.66 | 128.7 | 5       | 2.6                | 1.1  | 4.44 | 0.001   |
| 26-41 years | 22       | 5.0                | 2.08 | 56.10 | 13      | 2.8                | 1.10 | 6.94 |         |
| >42 years   | 18       | 8.1                | 2.87 | 128.0 | 7       | 4.4                | 1.35 | 4.80 |         |

Min: Minimum; mix: Maximum; p-value: probability; No.; Number; IL: Interleukin; ng: Nano-gram.

The correlation between the serum levels of interleukin-13 in pre- and post-treatment patients who inhaled corticosteroid/a long-acting  $\beta_2$ -agonist (CSI/LABA) immunotherapy is illustrated in Table-5. The results showed a significant increase of interleukin-13 level in AA (allergic asthma) patients at post-treatment with CSI /LABA immunotherapy in comparison with those at pretreatment (9.1 vs. 3.6 ng/ml, p-value <0.001).

**Table 5-**Median serum levels of IL-13 in response to treatment with CSI /LABA immunotherapy in patients with allergic asthma

| Immunotherapy                        | N  | Median IL-13 ng/ml | Minimum | Maximum |
|--------------------------------------|----|--------------------|---------|---------|
| At pre-treatment with immunotherapy  | 42 | 9.1                | 2.77    | 128.00  |
| At post-treatment with immunotherapy | 18 | 3.6                | 2.08    | 13.05   |
| <b>P -value &lt;0.001</b>            |    |                    |         |         |

P-value: probability; IL: Interleukin; ng: Nano-gram; CSI /LABA: inhaled corticosteroid / a long-acting  $\beta_2$ -agonist; ml: Milliliter.

In general, the eosinophils count was above the normal range in all groups of allergic asthma patients. Eosinophils count of allergic asthma patients was significantly higher (P< 0.001) than the controls (6.5 ± 3.1 vs. 2.1 ± 1.0%).

**Table 6-**Eosinophils count in asthma patients in comparison with controls

| Groups                     | No. of subject | Eosinophils count (Mean ± S.D.) % |
|----------------------------|----------------|-----------------------------------|
| Allergic asthma            | 60             | 6.5 ± 3.1                         |
| Control                    | 30             | 2.1 ± 1.0                         |
| <b>P -value &lt; 0.001</b> |                |                                   |

No.: Number; %: percentage; S. D.: standard deviation; EO: eosinophils; p-value: probability.

Correlations between IL-13 levels, total IgE and eosinophils count were measured in allergic asthma patients using Pearson correlation, as shown in Table-7. Statistically, there was a positive correlation between IL-13 and the total IgE levels in allergic asthma (p-value= 0.1 and r = 0.157). A positive correlation between IL-13 levels and eosinophils count was also found (p-value= 0.001 and r = 0.521). In addition, there was a positive correlation between eosinophils count and total IgE which is positive correlation (p-value= 0.001 and r = 0.453).

**Table 7-**Correlation Coefficient between IL-13 (ng/ml), eosinophils count (%) and total IgE (IU/ml).

| Pearson Correlation | IL-13 | EO%   | Total IgE |
|---------------------|-------|-------|-----------|
| IL-13               | 1     | 0.521 | 0.157     |
| p value             |       | 0.001 | 0.154     |
| EO%                 | 0.521 | 1     | 0.453     |
| p value             | 0.001 |       | 0.001     |
| Total IgE           | 0.157 | 0.453 | 1         |
| p value             | 0.154 | 0.001 |           |

EO: Eosinophils; %: percentage; Sig.: Significant; IgE: Immunoglobulin (Ig) E; ng: Nan-gram; ml: Milliliter; IL: Interleukin.

### Discussion

According to gender, it is obvious that females are usually at higher risk for developing many diseases, especially, the immunological diseases. This is attributed to different hormonal secretions between males and female. Some of these hormones promote the Th2 cells. These cells encourage auto-antibodies production and polyclonal B cell activation. Additionally, they promote the production of many pro-inflammatory mediators which play a critical role in the inflammatory reactions, and eventually increase the disorder development [17]. When it comes to age of patients in this study, the results suggest that asthma occurrence in patients of the 10-25 years age group is higher than that in patients of the 26-41 and >42 years age groups. The explanation for this variation of asthma occurrence in different age groups is attributed to several factors. For example, in the age group of 10-25 years, the allergic asthma was the highest among the other two age groups; the reason behind this is that the allergic asthma has cumulative effects because of continuous exposure to different types of allergens. In the age group of 10-25 years, the subjects are at higher risk of exposure to infectious agents and their immune system is immature to some extent [18]. Obesity is a significant global health issue for both adults and children. Obesity increases the spread and incidence of asthma and also augments the risk of severe asthma. Epidemiological data elucidates that asthma is more widespread in obese than lean individuals [19]. It was clear that the mean level of BMI of the allergic asthma group was significantly higher than that of the control group (29.4 vs. 23.7 kg/m<sup>2</sup>). The results of this study are supported by another previously published report [20]. The test results in the present study showed a significant increase ( $P = 0.02$ ) in BMI value in patients with allergic asthma as compared to healthy subjects ( $28.89 \pm 3.29$  vs. 23.4 kg/m<sup>2</sup>).

IgE is an immunoglobulin that plays a significant role in chronic inflammatory allergic diseases and acute allergic reactions [21]. Our study showed a significant increase in median serum level of total IgE in patients with allergic asthma in comparison to the control (341.04 vs. 24.5 IU/ml). This is due to the stimulated Th2 cells that are known to produce higher levels of s IL-4 and IL-13 which mediate development of eosinophils and stimulate B-cells to secrete the specific immunoglobulin E. The results of our study are supported by earlier published data [20], which showed that total IgE level of the allergic asthma group ( $470.3 \pm 2.97$  IU/ml) was significantly higher ( $P = 0.009$ ) than that in the control group ( $46.9 \pm 0.3$  IU/ml).

The function of specific cytokines in asthma became obvious; specific immunological biomarkers (e.g. IL-13) of T helper type 2 airway inflammations were already identified. Biomarkers could aid to stratify asthma into different subtypes, reflecting the prevalent pathophysiological mechanism [22]. This study showed that the median level of IL-13 in the allergic asthma group was 5.7 ng/ml which is higher than the control group (2.9 ng/ml). This is probably due to the different stimuli (allergens, super allergens and pollutants etc) promoting epithelial cells to release several types of cytokines such as IL-25, IL-33, and thymic stromal lymphopoietin. These mediators promote a variety of immune cells (T helper 2 cells, Group 2 innate lymphoid cells, macrophages, mast cells, eosinophils, basophils, and B cells) which release several cytokines, including IL-13 [23]. In addition, the IL-13 is produced by a variety of cell types, including both Th1 and Th2, as well as CD4 T cells and CD8 T cells [11]. Elevations of IL-13 levels have been detected in the airways of human asthmatic patients. However, this increment is only observed in a subset of these patients [24]. IL-13 is a pleiotropic type 2 cytokine that has been considered to be essential in the pathogenesis of allergic asthma and other eosinophilic disease [25]. The results of this study are supported by data published in the United Arab Emirates [26], which indicated that IL-13 level in asthma patients (6.67 pg/ml) was higher than that in controls (3.09 pg/ml), with highly significant differences ( $p = 0.001$ ).

Allergic asthma patients in the three age groups considered in this study showed higher IL-13 levels than the controls. However, serum levels of IL-13 in the age group of > 42 years showed a higher increase than the other two age groups of patients as compared to corresponding controls. Allergic asthma patients of both genders appeared to have a significant increase of IL-13 in comparison to their corresponding controls ( $p$ -value = 0.001). Allergic asthma in female patients showed a remarkable increase in IL-13 levels as compared to that in males, as shown in (Table-4). This is attributed to the hormonal differences among males and females, some of which is known to stimulate T helper type 2 cells [17].

The aim of asthma treatment is to control of symptoms of asthma. Combination therapy with inhaled corticosteroid/a long-acting  $\beta_2$ -agonist (ICS/LABA) acts as a gold standard in curing asthma, being an effective and safe treatment as approved by the GINA. ICS-LABA treatment is now approved only in Step 5 (High dosage of ICS-LABA) of the steps of asthma control. This treatment is used in high ICS doses and should be prescribed for only few months. The possibility for any adverse effects should be taken into account [1]. In our study, interleukin-13 level was elevated in all patients before the treatment with ICS/LABA and decreased in all patients after the treatment (9.1 vs. 3.6 ng/ml), ( $p=0.001$ ), (Table-5). The decreased values of IL-13 after treatment with ICS/LABA led to achieving a good control and clinical improvement of the disease. This indicates that IL-13 is a significant marker for monitoring the development of disease and the success of therapy in patients with asthma. The results of this study are consistent with previously reported data [27]. In that study, the results of serum levels of IL-13 were higher before the treatment with the combination of ICS/LABA in all patients with uncontrolled severe persistent asthma, as compared to post-treatment levels (787.3 vs. 350.3 pg/ml), ( $p=0.0014$ ).

Eosinophils are implicated in the pathogenesis of asthma disease. Eosinophils contribute to the induction of airway hyperresponsiveness, the modulation of the immune response, and remodeling characteristic features of asthma [28]. In this study, there is a significant increase ( $P<0.001$ ) in eosinophil percentage in the patients of allergic asthma compared with controls ( $6.5\pm 3.1$  vs.  $2.1\pm 1.0\%$ ). Several studies reported that higher count of eosinophils was present in patients with allergic asthma. The results of this study are confirmed by earlier published data [29]. The results of that study showed that the percentage of eosinophil in allergic asthma patients was higher in comparison to the control ( $4.37 \pm 0.52$  vs.  $2.57 \pm 0.86\%$ ).

### Conclusions

The present study suggests that the increase in the levels of interleukin-13 and total IgE and the proportion of eosinophils in the blood of allergic asthma patients could be considered as a key element in pathogenesis.

### References

1. Global Initiative for Asthma **2019**. Global strategy for asthma management and prevention. . Available from: <http://www.ginasthma.org>.
2. Sura F.Alsaffar, Jabbar H. Yenzeel, Haider F.Ghazi **2019**. "Immunocytochemical assessment of FKBP51 and Glucocorticoid receptor localization in asthmatic patients." *Iraqi Journal of Science*: 2609-2617. <http://doi:10.24996/ij.s.2019.60.12.9>
3. Gould HJ, Sutton BJ, Beavil AJ, Beavil RL, McCloskey N, Coker HA, Fear D, Smurthwaite L **2003**. The biology of IGE and the basis of allergic disease. *Annu Rev Immunol*. **21**: 579–628. <http://doi:10.1146/annurev.immunol.21.120601.141103>
4. Noor. S. Hallab, Fariel. A. Mnaf, Hashm. M. Hashm **2014**. "The Effect of Hyperthyroidism on Leptin and Immunoglobuline E Levels in Asthma Patients." *Iraqi Journal of Science*, **55**(3B): 1213-1217.
5. Louis, R., Pilette, C., Michel, O., Michils, A., Brusselle, G., Poskin, A. ... & Gurdain, S. **2019**. Variability in total serum IgE over 1 year in severe asthmatics. *Allergy, Asthma & Clinical Immunology*, 15.1, 20. <https://doi.org/10.1186/s13223-019-0331-8>
6. Abbas A, Lichtman A, Pallai S **2014**. *Basic Immunology: hypersensitivity*. 4th ed. 209.
7. Tayeb, M. M., & Qutub, M. M **2016**. Predictive Accuracy of Total IgE in Detection of Inhalant Allergens Sensitization. *Journal of Family Medicine and Health Care*, **2**(1): 6-9. <https://doi:10.11648/j.jfmhc.20160201.12>
8. Davoodi, P., Mahesh, P. A., Holla, A. D., Vijayakumar, G. S., Jayaraj, B. S., Chandrashekara, S., & Ramachandra, N. B **2012**. Serum levels of interleukin-13 and interferon-gamma from adult patients with asthma in Mysore. *Cytokine*, **60**(2): 431-437. <https://doi.org/10.1016/j.cyto.2012.05.012>
9. Greenfeder S, Umland SP, Cuss FM, Chapman RW, Egan RW **2001**. Th2 cytokines and asthma. The role of interleukin-5 in allergic eosinophilic disease. *Respir Res*, **2**: 71-9. <https://doi.org/10.1186/rr41>
10. Wynn TA **2015**. Type 2 cytokines: mechanisms and therapeutic strategies. *Nat Rev Immunol*. **15**(5): 271–82. <https://doi.org/10.1038/nri3831>

11. Wills-Karp, M **2004**. "Interleukin-13 in asthma pathogenesis." *Immunological reviews*, **202**(1): 175-190. <https://doi.org/10.1111/j.0105-2896.2004.00215.x>
12. Lu, Y., Sjöstrand, M., Malmhäll, C., Rådinger, M., Jeurink, P., Lötval, J., et al **2010**. New production of eosinophils and the corresponding TH1/TH2 balance in the lungs after allergen exposure in BALB/c and C57BL/6 mice. *Scand. J. Immunol.* **71**: 176–185. <https://doi.org/10.1111/j.1365-3083.2009.02363.x>
13. Isobe, Y., Kato, T., and Arita, M. **2012**. Emerging roles of eosinophils and eosinophil-derived lipid mediators in the resolution of inflammation. *Front. Immunol.* **3**: 270. doi:10.3389/fimmu.2012.00270
14. Uhm, T. G., Kim, B. S., and Chung, I. Y. **2012**. Eosinophil development, regulation of eosinophil-specific genes, and role of eosinophils in the pathogenesis of asthma. *Allergy Asthma Immunol. Res.* **4**: 68–79. <https://doi.org/10.4168/air.2012.4.2.68>
15. Printice, A.M and Jebb, S. A. **2002**. Beyond Body Mass Index, *Obesity Rev.* **2**(3):141-7. <https://doi.org/10.1046/j.1467-789x.2001.00031.x>
16. McLeod, S. A. **2019**. What a p-value tells you about statistical significance. *Simply Psychology*. <https://www.simplypsychology.org/p-value.html>
17. Goldsby RA, Kindt TJ & Osborne BA **2000**. *Kuby Immunology* [4<sup>th</sup> ed] Freeman & Company, New York, PP: 585.
18. Al-rubayei, Yacoob Y **2006**. Interleukin-5 Level in the sera of Iraqi Asthma Patients. *Al-Mustansiriyah Journal of Science*, 2006, **17**(3): 1-10. <https://www.iasj.net/iasj?func=fulltext&aId=68818>
19. Tashiro, H. and S. A. Shore **2019**. "Obesity and severe asthma." *Allergology International*, **168**(2): 135-142. <https://doi.org/10.1016/j.alit.2018.10.004>
20. Qasim, A.J. **2019**. The Role of Vitamin D and Interleukin-25 in Iraqi Patients with Allergic Asthma. MSc. thesis. Mmustansiriyah University, College of Science, 2019.
21. Navinés-Ferrer, A., Serrano-Candelas, E., Molina-Molina, G. J., & Martín, M **2016**. IgE-related chronic diseases and anti-IgE-based treatments. *Journal of immunology research*. <https://doi.org/10.1155/2016/8163803>
22. Bagnasco, Diego, et al **2016**. A critical evaluation of anti-IL-13 and anti-IL-4 strategies in severe asthma. *International archives of allergy and immunology*, **170**(2): 122-131. <https://doi.org/10.1159/000447692>
23. Marone, G., Granata, F., Pucino, V., Pecoraro, A., Heffler, E., Loffredo, S. ... & Varricchi, G **2019**. The Intriguing Role of Interleukin 13 in the Pathophysiology of Asthma. *Frontiers in Pharmacology*, 2019, **10**. <https://doi.org/10.3389/fphar.2019.01387>
24. Jia, G., Erickson, R. W., Choy, D. F., Mosesova, S., Wu, L. C., Solberg, O. D. ... & Bradding, P **2012**. Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *Journal of Allergy and Clinical Immunology*, **130**(3): 647-654. <https://doi.org/10.1016/j.jaci.2012.06.025>
25. Doran, E., Cai, F., Holweg, C. T., Wong, K., Brumm, J., & Arron, J. R **2017**. Interleukin-13 in asthma and other eosinophilic disorders. *Frontiers in medicine*, **4**: 139. <https://doi.org/10.3389/fmed.2017.00139>
26. Joseph J, Benedict S, Al-sowaidi S, Joseph M, Zoubeidi T **2005**. Serum interleukin-13 levels are elevated in mild and moderate persistent asthma. *Internet J Asthma Allergy Immunol*; **4**. <https://doi.org/10.1186/1471-2466-4-2>
27. Janeva, E. J., Goseva, Z., Gyorchev, A., Debreslioska, A., Spiroski, M., Zafirova, B., & Dimitrova, M. G **2015**. The effect of combined therapy ICS/LABA and ICS/LABA plus montelukast in patients with uncontrolled severe persistent asthma based on the serum IL-13 and FEV1. *Open access Macedonian journal of medical sciences*, **3**(2), 268. doi: 10.3889/oamjms.2015.053
28. Possa, S. S., Leick, E. A., Prado, C. M., Martins, M. A., & Tibério, I. F. L. C **2013**. Eosinophilic inflammation in allergic asthma. *Frontiers in pharmacology*, **4**: 46. <https://doi.org/10.3389/fphar.2013.00046>
29. Brakhas, Suaad A.; Hassan, Abrar J.; Jasim, Amna N **2016**. Study of total Immunoglobulin E and Eosinophil count in allergic disease. *Baghdad Science Journal*, **13**(2): 298-304. <https://dx.doi.org/10.21123/bsj.2016.13.2.0298>