J MEDICINE 2023; 24: 89-95

# Association of Interleukin-4 in Immunoglobulin-E Mediated Asthma: A Cross Sectional Study

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**Abstract**

***Backgrounds:*** *Interleukin 4 (IL 4) is a cytokine associated with the cause of several allergic diseases such as asthma due to their function in the differentiation of T helper type 2 lymphocytes and induction of the IgE isotype switch.*

***Object:*** *The study aims to determine the association IL-4 level with total serum IgE levels and asthma severity.*

***Methods:*** *A cross-section study was performed. Eighty-seven subjects were recruited from Karbala Teaching Hospital for Children in the period extending from January 25 to May 24, 2022, including children with asthma, were subjected to measure IL 4 level using Elabscience ELISA kit and measured total IgE level using AccuBind IgE ELISA kit.*

***Results:*** *Eighty-seven subjects of asthmatic children in which the mean age was 7.833 ± 3.652. There are 65.52% and 34.48% of asthmatic children (male and female, respectively). Total serum IgE was 398.889 ± 227.156 IU/ml, while the IL-4 level was 5.18 ± 8.224. There was a significant difference in IL 4 levels depending on asthma severity (P= 0.037). IL-4 levels in severe persistent asthma were higher than IL-4 levels in mild and moderate persistent asthma. Further, there was a highly significant difference in IL 4 levels depending on asthma controlled (P= 0.004). IL-4 levels in not well-controlled asthma were higher than IL-4 levels in well and partial controlled asthma. In addition, there was no significant correlation between IL-4 levels and total serum IgE levels (P=0.436).*

***Conclusion:*** *IL 4 has an important role in predicting asthma severity and asthma control in children. These findings have important implications for the treatment of IgE-mediated asthma. Despite this, IL 4 levels have no significant correlation with total serum IgE levels.*

***Keywords:*** *Asthma, IgE, IL-4, Severity*

*DOI: https://doi.org/10.3329/jom.v24i2.67270*



***Received:*** *27.04.23;* ***Accepted:*** *11.06.23*

## Introduction

Asthma is a common chronic inflammatory disorder of the airways arising from not fully understood heterogenic gene-environment interactions. It's characterized by variable airway obstruction and bronchial hyperresponsiveness. It is an obstructive pulmonary disease with exacerbations characterized by symptoms of shortness of breath, chest tightness, cough, and wheezing[1-3].

The asthma phenotype involves a variable degree of bronchoconstriction, airway remodeling, and increased mucus production. The allergic diseases are characterized by skewing the immune system towards a T-helper 2 (Th2) phenotype. From multiple interactions of infiltrating and structural cells in the context of chronic airway inflammation orchestrated by Th2 cells, airway inflammation arises. Th2 cells produce cytokines that lead to mast cell stimulation, eosinophilia, and leukocytosis. Further, Th2 cytokines can enhance B-cell IgE production[2, 4, 5]. Th2-type cytokines, such as interleukin-4 (IL-4), IL-5, and IL-13, are thought to drive the disease pathology in patients with asthma and play a role in driving many of the hallmarks of allergic inflammation[6, 7].

IL-4 is essential in regulating antibody production, hematopoiesis and inflammation, and the development of effector T-cell responses[8]. IL-4 plays a critical role in activating mature B cells as a cofactor for Lipopoly-saccharide (LPS), CD40L, and Ag stimulation to induce B cell differentiation, proliferation, and Ab secretion, mainly of IgG1 and IgE isotypes[9]. IgE production by B cells requires IL-4 and physical interaction between T and B cells, involving several surfaces and adhesion molecules such as CD40-CD40L and CD28/CD80[10].

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Immunoglobulin E (IgE) is a type of antibody produced by plasma cells located in lymph nodes draining the site of antigen entry or locally, at the sites of allergic reactions, by plasma cells derived from germinal centers developing within the inflamed tissue.[11] Among all antibody classes, IgE is the most potent and can trigger dramatic inflammatory reactions even when present in minute amounts.[12] Despite a half-life of only a few days, there is evidence that the IgE response may last for years without allergen stimulation. This is likely caused by long-lived IgE-producing plasma cells.[13]

The pathogenic role of IgE antibodies in triggering and maintaining allergic inflammation in response to allergens is due to the binding of multivalent allergens to allergen-specific IgE on sensitized effector cells. These interactions trigger effector cell activation, releasing potent inflammatory mediators, recruitment of inflammatory cells, antigen presentation, and production of allergen-specific antibody responses.[14] These events may cause responses at a local level, such as bronchoconstriction, vasodilation, and/or airway mucus secretion, and trigger the allergy-associated symptoms of nasal congestion, wheezing, sneezing, and cough, conjunctivitis, runny nose, dyspnoea, and chest tightness.[15]

This study was conducted to examine the evidence for the effect of Th2 cytokine (IL-4) and total IgE on asthma severity and to investigate the possible ability of asthma control to reduce IL-4 levels and total IgE. Further, this study was done to detect the correlation between IL-4 levels and total IgE antibody production in asthmatic children.

## Material and Methods

### Study Design and Subjects

A cross-section study was performed. Eighty-seven subjects were recruited from Karbala Teaching Hospital for Children in the period extending from January 25 to May 24, 2022, including children with asthma. The mean age of patients was ranging from 1 to 16 years old.

### Recruitment of the patients:

The diagnosis of asthma was obtained based on clinical examination. The patients were divided to 57 male and 30 female children with asthma. All asthmatic children had European Respiratory Society/American Thoracic Society criteria for asthma[16]. All patients underwent a total serum IgE test and excluded asthmatic patients with a total IgE concentration of less than 100 IU/ml from the study. The degree of asthma severity and well controlled patients were identified based on the international standards diagnosed in the NAEPP/EPR 3 Guidelines by the specialist pediatrician. Patients with asthma in this study displayed typical clinical histories of mild, moderate to severe persistent asthma, and most patients had moderate persistent asthma (Table 1).

### Sample collection and processing

After sampling 3 ml peripheral blood, serum was separated, divided into several aliquots in Eppendorf tubes, and immediately frozen at “80 °C. Sera were collected from patients to determine the total serum IgE levels by Combiwash Max-Planck-Ring 21 automated immunoassay analyzer (Human, Germany) using AccuBind total IgE ELISA kit, USA (LOT NO. 25K1D1). IL-4 levels (Human IL-4(Interleukin 4) ELISA Kit, LOT NO. 39CE12C5TF, Elabscience, USA) was measured on all subjects using commercial quantitative ELISA kits in an automated instrument (Combiwash Max-Planck-Ring 21).

### Data collection

The study protocol was approved by the Ethical Committee in the Babylon medical college (1354, 2022) and the relevant ethical committee in the health directorate (20719, 2022). The parents of participants who revealed readiness to participate in this study were supplied with written informed consent and verbal information regarding the aim of the study.

### Statistical Analyses

All the statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) version 21 (San Diego, California, USA). Kruskal Wallis Test is a non-parametric test used to compare the median ± Interquartile Range (IQR) when the p-value of Levene’s test was less than 0.05. Spearman’s test measured the correlation between IL-4 levels and total IgE. P-value < 0.05 was considered to indicate the statistical significance and highly significant if P-value < 0.001.

## Result

The Characteristics of the Subjects

In the present study, 31 confirmed patients with mild asthma symptoms, 42 had moderate asthma, and ten children with severe asthma were enrolled (Figure 1). Of the total of 87 asthmatic children, 33 (37.9%) cases were well controlled, and 33 (37.9%) cases were partially controlled, while 13 (14.9%) cases were not well controlled, and 8 (9.2%) cases were without control (Table 1). Approximately 65.52% of asthmatic children were male, and 34.48% were female. Total serum IgE was 398.889 ± 227.156 IU/ml, while the IL-4 level was 5.18 ± 8.224, as shown in Table 1.



identified based on the international standards diagnosed in

Association between Immunological Parameters and Clinical Characteristics of Asthma

The median ± IQR of total IgE in mild, moderate, and severe asthma was 392.444±337.978, 387.419±405.952, and 414.424±422.571, respectively. The median ± IQR of the total IgE among three groups of asthma severity was shown in Table 2. According to the Kruskal Wallis test, there was no statistically significant difference in total IgE among the three groups (P value=0.955). Paradoxically, there was a statistically significant difference in IL-4 levels among the three groups of asthma severity (p value= 0.037). In which the median ± IQR of IL-4 in mild, moderate, and severe asthma was .384± 4.814, 2.316± 4.937, and 4.577± 9.392, respectively.

There was a significant difference in the IL 4 levels in different asthma-controlled groups (p-value 0.004) by using Kruskal Wallis Test. The median ± IQR of IL-4 in well, partially, not well, and patients without controlled was 2.624±5.252, 2.566±6.458, 5.625±10.410, and .10±1.136, respectively. On the other hand, there was no significant difference in total IgE in controlled groups of asthma (p-value= 0.900), as shown in Table 3.

Correlation between Total IgE and IL-4 levels in asthmatic patients

In this study, the correlation between total IgE and IL-4 levels in asthmatic patients was investigated by Spearman's test. There was no significant correlation between total IgE and IL-4 levels (P value=0.436, *r =-0.018), as shown in Table 4 and Figure 2.

**Table 1.** *Demographic and Clinical Characteristics of Asthmatic Children*

| Demographic and Clinical Characteristics | | |
|---|---|---|
| Age mean ±SD | | 7.833 ± 3.652 (years) |
| Sex No. (%) | Male | 57 (65.52%) |
| | Female | 30 (34.48%) |
| Total IgE IU/ml mean ±SD | | 398.889 ± 227.156 |
| IL-4 level pg/mL mean ±SD | | 5.18 ± 8.224 |
| Severity [a] No. (%) | Mild | 31 (35.6%) |
| | Moderate | 42 (48.3%) |
| | Severe | 10 (11.5 %) |
| Control No. (%) | Well controlled | 33 (37.9%) |
| | Partial well controlled | 33 (37.9%) |
| | Not well controlled | 13 (14.9%) |
| | Without [b] controlled | 8 (9.2%) |

[a] 4-samples are missed, [b] Some of them are new diagnoses, and others are not committed to treatment.

***Table 2.*** *The Association between Immunological Parameters and Asthma Severity in Asthmatic Patients*

| Parameter | Severity | Number [b] | Median ± Interquartile Range (IQR) | Kruskal Wallis Test | P-value |
|---|---|---|---|---|---|
| Total IgE | Mild | 31 | 392.444±337.978 | .091 | .955 |
| | Moderate | 42 | 387.419±405.952 | | |
| | Severe | 10 | 414.424±422.571 | | |
| IL-4 level | Mild | 27 | .384± 4.814 | 6.589[a] | .037* |
| | Moderate | 40 | 2.316± 4.937 | | |
| | Severe | 10 | 4.577± 9.392 | | |

The significant level was intended as *p<0.05, [a] 6-sample excluded because its outliers, [b]4-sample missed

**Table 3** *The Association between Immunological Parameters and Controlled Asthma*

| Parameter | Type of Controlled groups | Number | Median ± Interquartile Range (IQR) | Kruskal Wallis Test | P-value |
|---|---|---|---|---|---|
| Total IgE | Well controlled | 33 | 403.6±387.218 | .585 | .900 |
| | Partial controlled | 33 | 372.55±409.921 | | |
| | Not well controlled | 13 | 356.362±394.985 | | |
| | Without controlled [a] | 8 | 350.935±379.143 | | |
| IL-4 level | Well controlled | 31 | 2.624±5.252 | 13.256 | .004** |
| | Partial controlled | 33 | 2.566±6.458 | | |
| | Not well controlled | 12 | 5.625±10.410 | | |
| | Without controlled [a] | 8 | .10±1.136 | | |

The significant level was intended as **p<0.01, [a] some of them new diagnosis and other not committed to treatment.

**Table 4.** *Correlation between IL-4 Levels and Total IgE in Asthmatic Patients*

| IL-4 levels pg/mL | Parameter | Spearman's rho | p-value |
|---|---|---|---|
| | Total IgE IU/ml | -.018 | .436 |

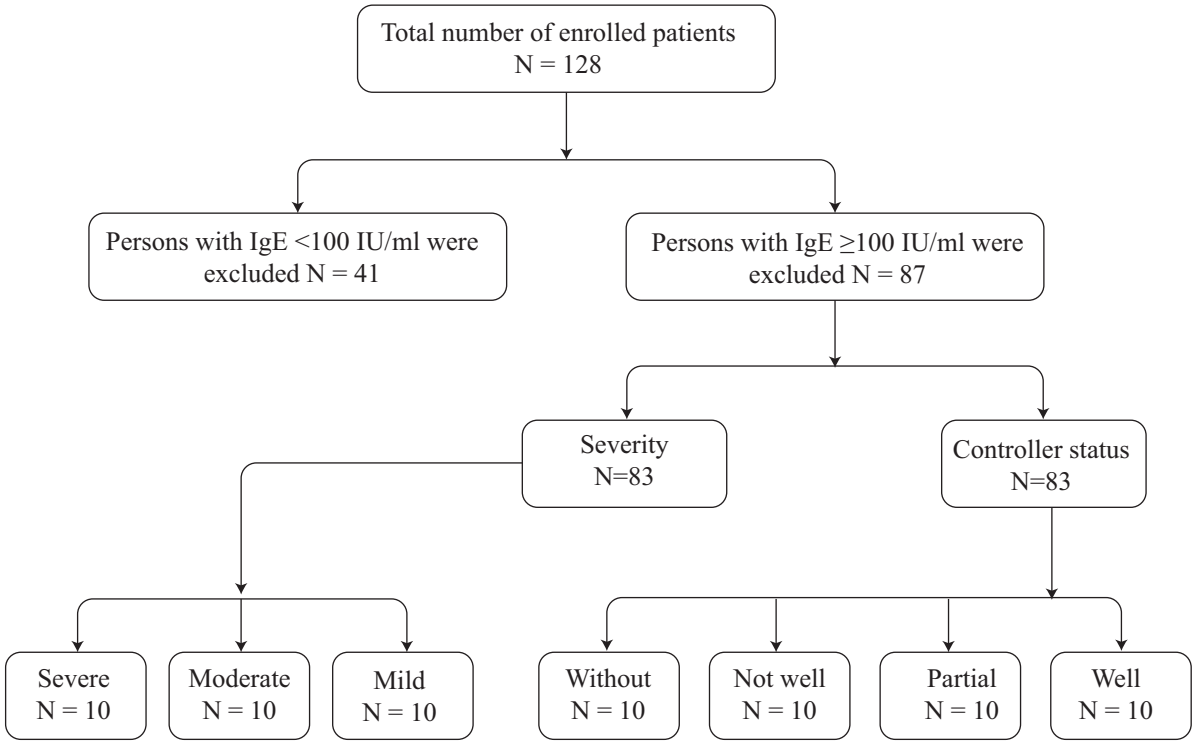


**Figure 1:** *Characteristics of study group*

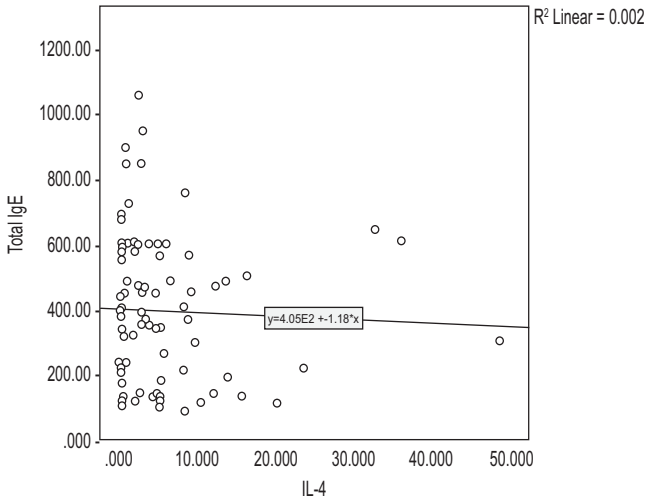


**Figure 2:** *scatter plot of the correlation between IL-4 levels and total serum IgE.*

**Discussion:**

In the present study, IL-4 level was at higher concentration at severe and not welled controlled asthma.

IL-4 is a critical cytokine in the development of allergic inflammation. It is associated with differentiation of T helper type 2 lymphocytes leading to cytokine release, induction of the IgE isotype switch, and secretion of IgE by B lymphocytes[17].

The current study showed a high IL-4 levels in severe asthma groups compared with mild and moderate asthma groups (*P*=0.037). The results were consistent with the previous studies [18, 19], Poon *et al.,*[18] mentioned that various lines of evidence demonstrate the importance of IL-4 in allergic asthma in a subset of severe asthmatics with allergen-associated symptoms, eosinophil, and high serum IgE levels. Furthermore, Colley *et al.,*[19] said that severe asthmatic patients are poorly responsive to currently available asthma therapies, and a high level of IL-4 has been observed. This result because that IL-4 is involved in differentiation and stimulation of Th2 cells, synthesis of IgE and activation of macrophages[18].

Serum IL-4 levels indicate a predisposition to atopic status. Measurement of serum IL-4 levels can serve as a low-cost investigative tool to differentiate between allergic and nonallergic asthma, which can be further confirmed by a skin prick test or serum levels of allergen-specific IgE. As there was an association between serum IL-4 levels and the degree of asthma severity, serum IL-4 levels can provide helpful information regarding the severity of asthma and the persistence of bronchial hyperresponsiveness in later adulthood. Quantitative measurement of IL-4, when integrated with other clinical indicators, can be used to predict the development of asthma and risk stratification. It can help choose treatment modalities, including using anti-IL-4 therapy with pascolizumab. Pascolizumab is a humanized anti-IL-4 monoclonal antibody that can inhibit upstream and downstream events associated with asthma, including TH2 cell activation and IgE production[20].

The study showed no significant association between total serum IgE levels and asthma severity (P = 0.955). The results were inconsistent with other previous results[21-23], which mentioned that the total serum IgE levels increased as the severity of asthma increased. The difference in the current results compared with the previous results, was due to the effect of high-dose inhaled corticosteroids (ICS) combined with a long-acting â2 agonist (LABA) on total serum IgE levels in severe asthma. Further, mast cell-bound IgE could be a more sensitive indicator of IgE production in vivo than serum IgE levels since the half-life of IgE in serum is only 8–12 h, but in tissues, it extends to 6 days[24].

The study results revealed that the level of IL 4 in asthmatic children on well and partial controlled therapy was lower than in patients with not well controlled (*P* = 0.004). Not well control asthma is difficult-to-treatment asthma, uncontrolled despite prescribing medium or high ICS with a second controller (usually a LABA) or requires high dose treatment to maintain reasonable symptom control and reduce the risk of exacerbations. The cause of this result is that IL-4 levels are elevated in severe persistent asthma since severe persistent asthma is a subset of difficult-to-treat asthma[25].

The study showed no significant correlation between IL 4 levels and total serum IgE in asthmatic children (*P* = 0.436). This result consistence with the previous research[26], which mentioned that no significant correlation between serum levels of IL-4 and total serum IgE. On the other hand, the recent result inconsistence with another study earlier, which detected a relation between levels of serum IgE and IL-4 in 73% of cases[27]. This could result from many reasons: Firstly, cytokines are transient and non-permanent products. The cause could be because some cytokines might not be detectable while their genes have been expressed. In most asthmatic patients, there was a correlation between the expression of the IL-4 gene and the level of serum IgE while they had normal serum IL-4[28]. Secondly, under some conditions, IgE could be produced independently of IL-4 and IL-13. The previous results suggested that even without IL-4 and IL-13, alternative pathways to IgE may exist, enhanced by immunization, resulting in IgE effector

responses in vivo. Finally, IL-15 can trigger STAT6 phosphorylation in mast cells, raising the possibility that cytokines other than IL-4 or IL-13 could use STAT6 to drive IgE production[24].

**Conclusion**

IL 4 has an important role in predicting asthma severity and asthma control in children. These findings have important implications for the treatment of IgE-mediated asthma. Despite this, IL 4 levels have no significant correlation with total serum IgE levels.

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