

## Serum Levels of IL-4 and IgE in bronchial asthma in syrian children

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### ABSTRACT

**Objective:** Asthma is the most prevalent chronic respiratory disease among children causing considerable morbidity and mortality.

Interleukin-4 (IL-4) is an important mediator of inflammatory cytokines derived from T helper and mast cells. It mediates essential pro-inflammatory functions in asthma such as induction of the IgE isotype switch, enhancing of eosinophil migration across endothelium, expression of vascular cell adhesion molecule-1 (VCAM-1), mucus secretion, and differentiation of T helper type 2 lymphocytes leading to cytokine release. The aim of our study was measuring the serum IL-4 and IgE levels in bronchial Asthma in Syrian children at different stages of the Asthma disease and in exacerbations, as biomarkers in clinical evaluation of Asthma patients and studying the correlation between IL-4 and IgE levels in order to determine their effects in asthma.

**Methods:** This study included 88 individuals: 38 children with acute Asthma exacerbations (E Asthma), 30 children with stable Asthma (S Asthma), and 20 healthy controls. The serum IgE and IL-4 levels were assessed with ELLSA technique.

**Results:** The results showed that IL-4 and IgE levels were significantly increased ( $p < 0.05$ ) in serum of Asthma children ( $7.16 \pm 5.22$  pg/ml,  $280.5127 \pm 297.65711$  IU/ml) respectively as compared with healthy controls ( $4.67 \pm 0.50$  pg/ml,  $86.1818 \pm 65.40959$  IU/ml). Asthma exacerbations showed also significant increased serum levels of IL-4 and IgE ( $8.19 \pm 6.68$  pg/ml,  $363.51 \pm 353.48$  IU/ml) compared to stable Asthma ( $5.87 \pm 1.75$  pg/ml,  $175.8 \pm 157.55$  IU/ml) and healthy controls ( $4.67 \pm 0.50$  pg/ml,  $86.1818 \pm 65.40959$  IU/ml) respectively. The relationship between IgE and IL-4 was found to be correlated positively ( $r = 0.61$ ). This suggests the possibility to use serum IL-4 as a diagnostic biomarker and to evaluate the inflammatory response in Asthma exacerbations, beside the distinctive role of IgE, indicating the Atopy associated with Asthma.

**KEY WORDS:** Asthma, serum IgE, serum IL-4, Acute Exacerbation.

### 1. INTRODUCTION

Bronchial asthma is a fundamental concern of public health that affects over 300 million people Worldwide (GINA, 2012), and projected that this number witness an outgrowth to over 400 million by 2025, as countries will have become more urbanized (To, 2012), and It is considered the most prevalent chronic respiratory disease among children causing considerable morbidity and mortality (AL- eryani, 2016).

Asthma illness is an inflammatory condition of the lung airways (Liu, 2011), and the chronic inflammation is accompanied with airway hyper-responsiveness ( an overstated airway-narrowing response to triggers like exercise and allergens) (Kim, 2011), which leads to frequent episodes of shortness of breath (breathlessness), wheezing, chest tightness and coughing, especially at night or during early morning. These episodes are generally related with diffuse, but variable, airflow obstruction during the lung that is often reversible either by treatment or spontaneously (GINA, 2012).

An extremely large group of chemokines, cytokines and another pro-inflammatory mediators which are released by airway structural and immune-inflammatory cells, importantly contribute to shape several different asthma phenotypes (Pelaia, 2015). Cytokines play a key role in organizing the chronic inflammation of asthma by recruiting, activating, and induction the survival of multiple inflammatory cells in the respiratory system (Barnes, 2008). IL-4 demonstrates a wide spectrum of biological activities in asthma. In general terms, it can be described as the central cytokine included in the pathophysiology of allergic responses, at the same time downregulating acute inflammatory changes (Kips, 2001). The major cellular sources of IL-4 are thymocytes, mature T-cells, mast cells, basophils and CD4+ Th2 cells (Mahajan, 2006). IL-4 selectively binds to IL-4Ra, which is expressed on eosinophils T-lymphocytes, mononuclear phagocytes, B-lymphocytes endothelial cells, bronchial epithelial cells, lung fibroblasts and smooth muscle cells (Maes, 2012). IL-4 is connected with the induction of the IgE isotype switch and IgE excretion by B lymphocytes. Also, IL-4 promotes the IgE-mediated immune responses by its ability to upregulate the receptors of IgE on the cell surface: the high-affinity IgE receptor (FceRI) on basophils and mast cells and the low-affinity IgE receptor (FceRII; CD23) on mononuclear phagocytic cells and B lymphocytes (Steinke, 2001). IL-4 plays a remarkable role in Th2 differentiation stage. Next to this role in Th2 development (Maes, 2012), IL-4 is one of the responsible factors of airway obstruction in asthma by stimulating of mucin gene expression and the hypersecretion of mucus (Steinke, 2001). IL-4, jointly with IL-13, up-regulates the endothelial expression of vascular cell adhesion molecule-1 (VCAM-1). This simplifies transmigration of T-lymphocytes, eosinophils, monocytes, and basophils that are attracted by mast cell-derived chemokines, therefor sharing in promotion of a local inflammatory response (Maes, 2012). In addition, IL-4 prevents apoptosis of eosinophil and enhances eosinophilic inflammation

via promoting eosinophil chemotaxis and activation by increasing the expression of eotaxin. Also, IL-4 drives the differentiation of naive T helper type 0 (TH0) lymphocytes into TH2 lymphocytes and inhibits apoptosis of T lymphocytes (Steinke, 2001). Inhalation of recombinant human IL-4 motivates airway eosinophilia and causes degrees of bronchial hyper responsiveness in atopic asthmatics. Because of these properties IL-4 has long been considered as a potential target in asthma treatment (Mahajan, 2006).

The aim of our study focused on measuring the serum IL-4 and IgE levels in bronchial Asthma in Syrian children at different stages of asthma disease and in exacerbations, as biomarkers in clinical evaluation of Asthma patients and studying the correlation of interleukin-4 (IL-4) with Immunoglobulin E (IgE) to determine their effects in asthma.

## 2. MATERIALS AND METHODS

**Study subjects:** 88 children (49 males and 39 females) participated to this study and all of them signed an informed consent. Asthmatic children were divided in two groups:

**Stable asthma group (S- Asthma):** 30 children with well-controlled asthma (mean age:  $6.9 \pm 3.4$ , ranges: 1.5- 13 years). All patients had no asthma exacerbation.

Children with asthma exacerbations (E- Asthma): this group consisted of 38 age-matched children (mean age:  $6.5 \pm 3.3$ , range: 1.5–13 years) hospitalized for asthma exacerbation.

The healthy controls group consisted of 20 healthy children (mean age:  $7 \pm 3.4$ , range: 1.6–13 years) with a negative history of allergic diseases.

Exclusion criteria included the use of immune-modulatory drugs like steroids within the past 14 days, autoimmune diseases, allergic, lung diseases, heart diseases and tumors.

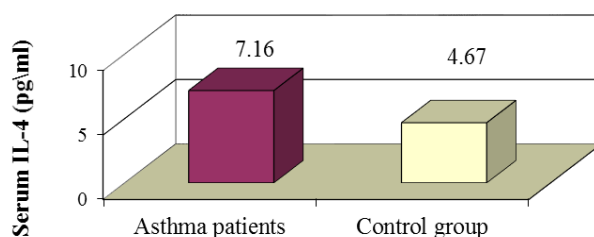
**Sampling:** blood samples from children with asthma and healthy controls were collected at the time of admission to hospital or during the scheduled clinic visit and after centrifugation, serum was acquired and aliquots were stored at  $-40^{\circ}\text{C}$  until assays were done.

**Assays:** Serum IL-4 and IgE were assessed by ELISA (Enzyme-Linked Immunosorbent Assay) kits. The IL-4 Human ELISA Kit (DRG, Germany) utilizes an antibody specific for human IL-4 coated on a 96-well plate. The Immunoglobulin E (IgE) ELISA Kit (DRG, Germany) utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the IgE molecule. Absorbance is determined at 450 nm

**Statistical analyses:** were completed by using SPSS version 22 and Excel (2013). Results were presented as a means  $\pm$  standard deviation (mean  $\pm$  SD). Comparisons between the means of two independent groups were performed using student's t-test. The area under ROC curve was concluded to definition the diagnostic value for IL-4 and IgE. The P values less than 0.05 were accepted to indicate statistically significant difference.

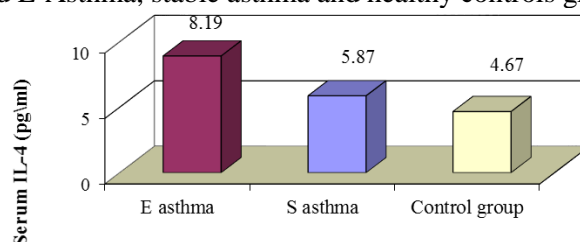
## 3. RESULTS AND DISCUSSION

**Levels of serum IL-4 in patients with Asthma and healthy group:** Serum IL-4 levels were obviously higher in children with asthma ( $7.16 \pm 5.22$  pg/ml) than in healthy controls ( $4.67 \pm 0.50$  pg/ml), ( $P= 0.0002$ ), figure (1).



**Figure.1. Serum levels of IL-4 in Asthma patients and healthy controls**

Serum levels of IL-4 were  $8.19 \pm 6.68$  pg/ml in E Asthma,  $5.87 \pm 1.75$  pg/ml in S Asthma,  $4.67 \pm 0.50$  pg/ml in healthy controls, figure (2). There were statistically significant differences between Asthma exacerbations group and S Asthma, control group and E-Asthma, stable asthma and healthy controls group in our study, table.2.



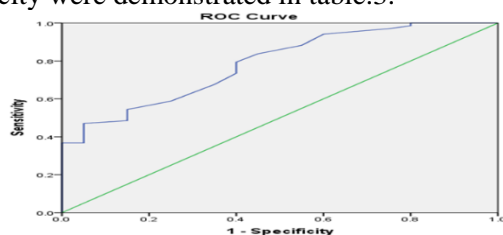
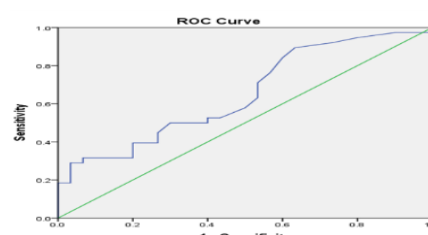
**Figure.2. Serum levels of IL-4 in study groups**

**Table.2. Significance of differences of means of IL-4 levels between the 3 studied groups using the student's t-test**

The Studied parameter	Group (I)	Group (J)	Difference Between two means (I-J)	Standard Error	P-value	Statistically significant differences
		S Asthma	2.3173	1.1290	0.046	Yes
concentration of IL-4	E Asthma	Healthy control	3.51971	1.08888	0.003	Yes
	S Asthma	Healthy control	1.20238	0.33786	0.001	Yes

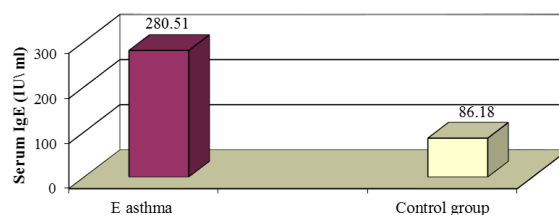
**Serum IL-4 sensitivity and specificity for differentiation between Asthma patients and controls group:** The best proportionally between sensitivity which was 47.1% and specificity which was 9.67% was given at the cut-off value 5.3125 pg/ml which showed a moderate differentiation between Asthmatic children and controls group. In our study, the best value which appears the threshold (diagnostic value) was between patients with asthma and controls group. Table.3, shows some cut-offs value of levels of serum IL-4 sensitivity and specificity. The area under roc curve was equals 0.786, figure.3.

**Serum IL-4 sensitivity and specificity for differentiation between E Asthma and stable Asthma:** The best proportionally between sensitivity which was 28.9% and specificity which was 96.7% was given at the cut-off value 9.4325pg/ml which showed a moderate differentiation between Asthma exacerbation and stable Asthma. In our study, this was the best value which represents the threshold (diagnostic value) between E- Asthma and stable Asthma. The area under roc curve was equals 0.651, figure.4. Some cut-offs value of serum IL-4 sensitivity and specificity were demonstrated in table.3.

**Figure.3. Roc curve for IL-4 between Asthma patients and healthy controls****Figure.4. Roc curve for IL-4 between E Asthma and S Asthma****Table.3. IL-4 sensitivity and specificity of differentiation among study groups at some cut-offs values**

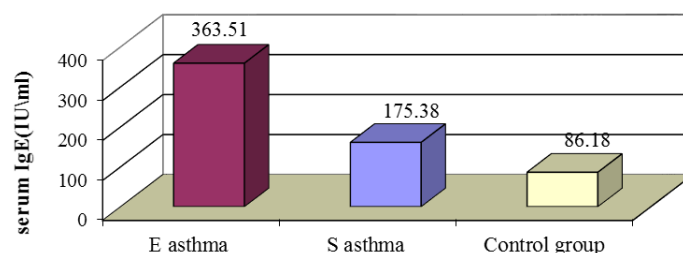
Asthma patients Vs Healthy controls			E asthma Vs S asthma		
Cut- off	Specificity	Sensitivity	Cut-off	Specificity	Sensitivity
4.874	0.65	0.676	8.0915	0.8	0.342
4.948	0.7	0.632	8.283	0.8	0.316
5.023	0.75	0.588	8.3795	0.867	0.316
5.136	0.85	0.544	8.5755	0.933	0.316
5.251	0.85	0.485	8.9745	0.933	0.289
<b>5.3125</b>	<b>0.95</b>	<b>0.471</b>	<b>9.4325</b>	<b>0.967</b>	<b>0.289</b>
5.405	0.95	0.426	9.7435	0.967	0.263
5.563	0.95	0.412	9.954	0.967	0.211
5.721	0.95	0.368	10.2215	0.967	0.184
5.802	1	0.368	10.6015	1	0.184
5.1	1	0.309	10.8755	1	0.158

**Levels of serum IgE in children with Asthma and control group:** The mean of IgE serum levels was significantly higher in Asthma children ( $280.5127 \pm 297.65711$  IU/ml) than control group ( $86.1818 \pm 65.40959$  IU/ml), ( $P < 0.05$ ), figure.5.

**Figure.5. Serum levels of IgE in Asthma patients and healthy controls**

Levels of serum IgE were  $363.51 \pm 353.48$  IU/ml in E Asthma,  $175.8 \pm 157.55$  IU/ml in S Asthma, 86.1818

$\pm 65.40959$  IU/ml in healthy controls (HC, figure.6). Statistically significant differences were found between E Asthma group and S Asthma group, E Asthma group and Healthy controls, S asthma and healthy group in our study, table.4.



**Figure.6. Serum levels of IgE in study groups**

**Table.4. Significance of differences of means of IgE levels between the 3 studied groups using the student's t-test**

The Studied parameter	Group (I)	Group (J)	Difference Between two means (I-J)	Standad Error	P-value	Statistically significant differences
		S Asthma	188.13710	64.15142	0.005	Yes
concentration of IgE	E Asthma	Healthy control	277.33255	59.17730	0.000	Yes
	S Asthma	Healthy control	89.19545	32.26907	0.008	Yes

We have also demonstrated an important correlation between serum IL-4 and IgE levels in both E-Asthma ( $R=0.512$ ,  $P=0.001$ ) and S-Asthma ( $R=0.794$ ,  $P=0.0001$ ). The results of present study showed clear increases in the serum of both IL-4 and IgE levels in asthma exacerbation compared to both stable asthma and healthy controls and significant increase was shown in Asthmatic children compared with controls group ( $P<0.05$ ). Also obvious increases were shown in stable asthma compared with healthy controls group. In this study, the association between the increase of both IgE and IL-4 and the severity of the Asthma disorder especially with exacerbations was clear. In addition, the high specificity of IL-4 serum levels allow us determining the occurrence of Asthma disease and predicting exacerbations in some cases.

These increased levels of serum IL-4 in Asthma patients reflects the airway inflammation and severity of disease in asthma, and indicates to the role of IL-4 in pathophysiology of asthma where it acts on several levels; it is associated with switching of IgE isotype from B cells, induction of contraction of Airway smooth muscles and mucus secretion.

In the present study, it was also observed that there was a considerable correlation between serum total IgE and IL-4 levels in both E-Asthma ( $R=0.512$ ,  $P=0.001$ ) and S-Asthma ( $R=0.794$ ,  $P=0.0001$ ).

Our results were in agreement with (AL-Daghri, 2014), who found significant high level of serum IL-4 in children with asthma when compared to healthy children, and with (Guangyi, 2003), who found significant high level of serum IL-4 in asthmatic children when compared with controls group and proved precense positive correlation between levels of IL 4 and IgE in remission and attack stages of asthma. Also in agreement with (Ai-hua, 2006) who reported statistically significant variations of IL-4 serum levels between remission and acute asthma attack stage ( $p= 0.05$ ) and between remission stage and healthy controls ( $P= 0.05$ ).

The findings from our study were consistent with (Meiyu, 2005) study which demonstrated that IL-4 serum levels were higher in both stable and exacerbation of asthma than in healthy subjects with a statistically important difference. Also (Miao, 2004) reported significant high level of IL-4 in both remission group and acute asthma attack group when compared to normal group and between acute attack groups compared to remission group. Further, (Zong-Zhi, 2003) in china have reported statistically significant differences between stage of attack and healthy controls for both IL-4 ( $p<0.01$ ) and IgE ( $p<0.01$ ) serum levels.

Also we were in accordance with (Yue hai, 2003) in china who found that average levels of both serum IL-4 and IgE in children with acute attack of asthma were higher than those in control group, and proved also existence positive correlation between the levels of serum IL 4 and IgE during the phase of asthmatic attack.

Our results were in accordance with (Xian-di, 2000) who evidenced significant high level of serum IgE in both stable asthma and asthma exacerbation when compared with controls group. Also significant increases were shown in asthma exacerbation when compared to stable asthma with (Yue-sha, 2006) study which showed that levels of IgE were higher in both asthma exacerbation and stable asthma than in healthy subjects with a statistically significant difference.

In 2013, Yan-li et al, found also that levels of serum IgE in asthmatic children were higher than in healthy controls, and the levels of serum IgE in acute exacerbation group were clearly higher compared to remission group (Yan-li, 2013).

**4. CONCLUSION**

We demonstrated increased serum levels of IL-4 and IgE in Asthmatic children including children with exacerbation with a moderate diagnostic value given by IL-4 concentrations in the differentiation between healthy controls and Asthmatic children.

Levels of serum IL-4 also had a moderate differentiation between stable Asthma and Asthma exacerbation. So we can use IL-4 as biomarker for diagnostic purpose and for the evaluation of the inflammatory response in Asthma patients, beside the serious role of IgE, indicating the Atopy associated with Asthma.

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