Evaluation of Interleukin-5 in Mild and Moderate Asthmatic Patients Following Corticosteroid Therapy
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Abstract

Background: Asthma is a very common chronic disease in Iraqi community. Involving the respiratory system in which the airways occasionally constrict, become inflamed, and are lined with excessive amounts of mucus often in response to one or more triggers.

Aim: This study was conducted to determine the role of interleukin 5 as asthmatic biomarker in Iraqi community in order to target the trait.

Patients and Methods: Serum level of interleukin-5 [SIL-5] which is one of the inflammatory markers in asthma was estimated in 90 persons including those with asthma on steroid therapy (35 patients) and those without steroid (35 patients) therapy and the healthy control group (20 persons).

Results: The mean level of SIL-5 was high in moderate asthmatic patients without steroid in their treatment protocol (16.56 pg/ml), while it was less in mild asthmatic patients without steroid therapy. SIL-5 mean values were lower in mild and moderate asthmatic patients who receive steroid therapy (3.94 pg/ml, 4.10 pg/ml respectively). While it was least in the control healthy group (3.32 pg/ml).

Conclusion: Serum IL-5 is predictive biomarkers for asthma severity and treatment response monitoring in Iraqi subjects with asthma.

Key Words: Asthma, IL-5, Steroid therapy, Inflammatory marker.

Introduction

Asthma is a very common chronic disease in Iraqi community [1], involving the respiratory system in which the airways occasionally constrict, become inflamed, and are lined with excessive amounts of mucus often in response to one or more triggers.[2-4] Even in asymptomatic periods asthmatic lungs show evidence of inflammation compared to controls and there is much interest in how chronic or repeated episodes of inflammation which associated by induction of different mediators that may cause remodeling of the airways and supporting vasculature leading to disease progression.[4-6]

Asthma is a complex chronic inflammatory and immunologically mediated disease of the airways that involves the activation of many inflammatory and structural cells all of which release inflammatory mediators that result in the typical pathophysiological changes of asthma.[7,8] The airway inflammation underlying asthma is regulated by a network of mutually interacting cytokines. The exact functional role of each individual cytokine in the pathogenesis of the disease remains to be fully established.[9,10]

The potential role of type 2 T-helper cell- cytokines in asthma is increasingly being recognized in particular interleukin -4, interleukin -5 and interleukin -13. Th2 cells are thought to control the growth and effectors function of those cell types that
are involved in allergic inflammatory responses in asthmatic patients.[11,12] Many inflammatory cells are recruited to asthmatic airways or are activated in situ. These include eosinophils, mast cells, macrophages, T lymphocytes, dendritic cells, basophiles, neutrophils, and platelets. From these inflammatory cells the eosinophil is considered a key effector cell in the pathogenesis of allergic inflammation in asthma. [7-10] IL-5 seems to be the primary cytokine involved in the production, differentiation, maturation and activation of eosinophils.[13,14]

The anti-inflammatory effects of glucocorticoids in asthma include modulation of cytokine and chemokine production, inhibition of eicosanoid synthesis, marked inhibition of accumulation of basophils, eosinophils and other leukocyte in lung tissue and decreased vascular permeability. The profound and generalized anti-inflammatory action of this class of drugs explains why they are currently the most effective drugs used in the treatment of asthma.[15,16]

IL-5 can influence the production, maturation and activation of eosinophils, it acts predominantly at the later stages of eosinophil maturation and activation. IL-5 can also prolong the survival of eosinophils.[17-19] Studies of the use of anti-IL-5 antibodies in the treatment of human asthma are indicating the effectiveness of this approach in the control of asthma in some patients [20-27]

The effects of systemic corticosteroid treatment in patients with worsening asthma indicate that there is a reduction in the expression of IL-5 mRNA in the airway mucosa that is associated with an improvement in asthma.[28,29] The anti-inflammatory effects of corticosteroids in asthma include modulation of cytokine and chemokine production, inhibition of eicosanoid synthesis, marked inhibition of accumulation of basophils, eosinophils and other leukocytes in lung tissue and decreased vascular permeability. The profound and generalized anti-inflammatory action of this class of drugs explains why they are currently the most effective drugs used in treatment of asthma. [15,16]

Asthma is a heterogeneous disease with different inflammatory and immunologic phenotypes, and these phenotypic diversity affected the therapeutic outcomes of the different treatment approaches. [30] Additionally, gene and environment gene interaction may ameliorate therapeutic response [31] Responses to current asthma therapies varies greatly, which is probably related to the inter-patients differences in pathogenesis. [32] Thus due to several phenotypes, subtypes, and asthma endotypes, this study was conducted to determine the role of interleukin 5 as asthmatic biomarker in Iraqi community in order to target the trait.

**Patients & Methods**

**Design of the Study:**

An observational cohort study which was conducted in Tikrit Teaching Hospital during the period from April 2016 to the end of November 2016. Ninety subjects were included in this study from both genders. Their age are ranging from 18-78 years.

**Patient Selection**

A total of 70 adult asthmatic patients from both genders attending outpatient department of medicine in Tikrit Teaching Hospital included in this study. Their age are ranging from 18 -78 years (37.6±16.9 years). All patients presented with signs and symptoms' of asthma, and they had been checked for their age and severity of the disease (mild and moderate). The patients were divided into two main groups: First group consist of 35 asthmatic patients without steroid therapy (16 males and 19 females), the second group consist of 35 asthmatic patients, and they are on steroid therapy (15 males and 20 females). Exclusion criteria were; Patients with severe
asthma, Patients with chronic obstructive pulmonary diseases, Patients with hyperthyroidism, Patients with diabetes mellitus, Pregnancy, Those who are smoker, Uncooperative patients and Patients with cardiovascular disease. The study protocol was approved by Tikrit University College of Medicine Ethical Committee and informed consent was taken from each participant before enrolment in the study.

**Control Group:**

Twenty apparently healthy subjects of both genders matched with patient age and ethnicity were selected as a control group. A detailed history of asthma and atopic disease in their families were questioned. The entire control group had normal pulmonary function test. The control subjects with positive findings of any disease were excluded.

**Clinical Evaluation:**

Full history was taken according to paper sheet questionnaire prepared previously given to all patients include name, age, gender, address, occupation, detail history of asthma: including asthma symptoms and signs, asthma treatment and control, family history of asthma and other atopic disease, drug allergy, food allergy, relieving factors, aggravating factors and history of associated allergic disease.

**Clinical Examination and Measurements:**

Careful physical examination was performed for every patient to identify the disease which includes general examination and chest examination. Measurements of height, weight and peak expiratory flow rate (PEFR) by using portable peak flow meter.

**Estimation of Serum Interleukin 5 by ELISA test:**

The concentration of IL-5 in the sera of patients and control group was measured by sandwich Enzyme Linked Immunosorban Assay according to the manufacturer instructions.

**Statistical analysis:**

The results were arranged in figures and tables and given as mean ± SD. Values and data were analyzed using (SPSS) version (16). The differences between the study's groups were tested by using ANOVA and Chi-square. P < 0.05 was considered as statistically significant.

**Results:**

From the 90 persons included in the study (35 patients without steroid therapy and 35 with steroid therapy), and (20 control healthy subject), the mean level of serum interleukin -5 was (10.78 pg/ml) in mild asthma without steroid therapy, (16.65 pg/ml) in moderate asthma without steroid therapy, (3.94 pg/ml) in mild asthma on steroid therapy, (4.10 pg/ml) in moderate asthma on steroid therapy and (3.32 pg/ml) in control group, table (1).

Figure (1) shows that there was statistically significant (P<0.05) difference in SIL-5 between mild asthma without steroid therapy (10.78 pg/ml) and mild asthma on steroid therapy (3.94 pg/ml) in comparison with control group (3.32 pg/ml). Figure (2) shows that there was significant (P<0.05) difference in SIL-5 between moderate asthma without steroid therapy (16.65 pg/ml) and moderate asthma on steroid therapy (4.1 pg/ml) in comparison with control group (3.32 pg/ml).

**Discussion**

Asthma is defined as a chronic inflammatory disorder of the airway mucosa. The inflammatory process is orchestrated and regulated by a complex network of mutually interacting cytokines and growth factors. The therapeutic effect of corticosteroid, the current mainstay of asthma treatment, has been largely attributed to an anti-cytokine effect, inhibiting the production of cytokines as well as the
cytokine-induced intracellular signaling.[15,16] Biopsy studies confirm that in asthma, steroids reduce messenger ribonucleic acid (mRNA) expression of several cytokines. [33,34] Corticosteroid inhibition of cytokine IL-5 will diminish the effects of this cytokine on eosinophil (which include hematopoiesis, potentiating of mediator release, prolongation of survival, increases in cytotoxicity, migratory properties, and other functions) [35]

The result of this study revealed that the mean level of serum interleukine-5 was significantly higher in those asthmatic patients without steroid therapy in comparison with asthmatic patients on steroid therapy and control groups in both mild and moderate asthma.(p value <0.05),

Additionally, the mean of serum IL-5 levels roughly correlated with the disease severity i.e in moderate asthma was higher than that of mild asthma. These finding was in agreement with the results of El-Radhi et al.[36] who reported that corticosteroid treatment in asthma was associated with clinical improvement and also with significant reduction in serum concentrations of IL-5 levels.

Also the results of this study was in agreement with the finding of Joseph et al,[37] who found that the serum level of IL-5 in asthmatic patients (mild and moderate) were significantly higher compared to controls (normal people) and the serum IL-5 level was higher in moderate asthmatics compared to mild asthmatics and there was a trend for the median serum IL-5 to be higher in moderate asthma than in mild persistent type of asthma . On the other hand, there was no significant difference in the serum IL-5 levels between asthmatics using and not using corticosteroids. [38] Corticosteroids are the most effective anti-inflammatory drugs available for asthma [15,16]. Corticosteroids modulate the transcription of cytokine interleukin-5 genes through inhibitory effects on transcription factor such as activator protein-1 (AP-1).[38]

IL-5 has been clearly implicated as an important cytokine mediating airway inflammation in both atopic and non-atopic types of asthma. Elevated serum IL-5 levels in asthmatics as compared with normal controls in the present study support this hypothesis. Whereas interleukin-5 levels are shown to be elevated in severe asthma this is the first demonstration that levels are also elevated in less-severe asthmatics (mild-moderate) that represent the vast majority of patients. There was a tendency for serum IL-5 levels to be higher in moderate asthmatics compared to mild asthmatics,[17-19] Several studies reported that systemic IL-5 levels elevated in mild-moderate asthmatics but significantly reduced following treatment with glucocorticoids. However, the serum IL-5 levels remained above levels observed in normal controls. [39]

We cannot entirely discount the possibility that corticosteroids do reduce serum IL-5 level, as sequential IL-5 level measurement was not possible in this cross sectional study. This issue can be best addressed by a longitudinal study. IL-5 has been shown to elicit a cholinergic type of hyper responsiveness in normal human bronchus and smooth muscle which could be blocked by IL-5 receptor antibodies. Thus, the raised serum IL-5 levels observed in our patients with mild and moderate asthma could have contributed to airway smooth muscle hyper responsiveness and airway inflammation. Although administration of corticosteroids decreases airway inflammation, it does not suppress IL-5 production by peripheral blood mononuclear cells. Administration of agents that could suppress systemic T-helper 2 cytokines may have a beneficial role in the long-term control of asthma. [40,41]
Conclusion:
1. It is evident from this study that steroid therapies in asthmatic patients suppress the serum level of IL-5.
2. Interleukin-5 level seems to be proportional to the severity of asthma.

Recommendations:
1. Studies that concern with another inflammatory markers like IL-2, IL-3, IL-4, IL-13 and TNF in asthmatic patients.
2. Further study by using leukotriene receptor antagonist drugs (Montelukast & Zafirlukast) in asthmatic patients to evaluate their effect on different inflammatory markers.

References:
10. Allobaidi AH, Alsamarai AM, Jawad AK, Janabi J. Association between C reactive protein and asthma. Tur Torak Der 2010;11:98-104.


Table (1): Mean Serum IL-5 Level among Different Study Groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Mean pg/ml</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild asthma without steroid</td>
<td>16</td>
<td>10.78</td>
<td>10.19</td>
</tr>
<tr>
<td>Moderate asthma without steroid</td>
<td>19</td>
<td>16.65</td>
<td>11.99</td>
</tr>
<tr>
<td>Mild asthma with steroid</td>
<td>13</td>
<td>3.94</td>
<td>0.82</td>
</tr>
<tr>
<td>Moderate asthma with steroid</td>
<td>22</td>
<td>4.10</td>
<td>1.08</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>3.32</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Mild asthma without steroid versus moderate asthma without steroid: t value 1.51, P >0.05

Mild asthma with steroid versus moderate asthma with steroid: t value 0.46, P >0.05

Mild asthma without steroid versus mild asthma with steroid: t value 2.41, P 0.0233

Moderate asthma without steroid versus moderate asthma with steroid: t value 4.89, P 0.0001

Mild asthma without steroid versus control: t value 3.28, P 0.0024

Mild asthma with steroid versus control: t value 2.98, P 0.0055

Moderate asthma without steroid control: t value 4.97, P 0.0001

Moderate asthma with steroid versus control: t value 3.08, P 0.0038
Figure (1): Mean Serum Interleukin-5 Level (pg/ml) in Mild Asthma Without Steroid, Mild Asthma on Steroid and Control. ANOVA test, P value < 0.05 (significant).

Figure (2): Mean Serum Interleukin-5 Levels in Moderate Asthma without Steroid, Moderate Asthma on Steroid and control. ANOVA test, P value < 0.05 (significant).