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

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Abstract

Background: Asthma is a heterogeneous complex disease with high prevalence and natural course chronicity. The disease presented as cough variant asthma or shortness of breath which mainly due to inflammation and immunological responses that are induced in respiratory system.

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 heterogeneous complex disease with high prevalence and natural course chronicity [1]. The disease presented as cough variant asthma or shortness of breath which mainly due to inflammation and immunological responses that are induced in respiratory system [2-4]. Asthma is with symptomatic or asymptomatic phenotypes and both are associated with inflammation that contribute to release of cytokines that lead to subsequent airway htperresponsiveness and remodeling [4-6].

Asthma initiated as a response to an external allergic substance and followed by a subsequent cascade events of immunological, metabolic and inflammatory responses which involve many structural and inflammatory cells activation [7,8]. Many cytokines are interacted and induce the changes that contribute to appearance of asthma symptoms and signs and subsequent disease episodes and chronicity. The role of some of these cytokines in modulation the development and disease course are established [9,10]. However, there are many unresolved and unknown roles of cytokines in the pathophysiological processes of the disease still need more research

to uncover the underlying etiology. Although asthma phenotypes are documented, however, the disease endotypes need to be distinguished.

Previous studies indicated that cytokines of type 2 T- helper cell play a potential role in asthma pathogenesis and disease progression. These cytokines include interleukin-13, interleukin-5 and interleukin-4. Cells involved in inflammatory and immunologic responses in patients with asthma are controlled by type 2 T- helper cell [11,12]. Platelets, eosinophils, neutrophils, mast cells, basophils, macrophages, and dendritic cells are accumulated in airway of patients with asthma and followed by local activation. However, asthma pathogenesis inflammatory responses are mainly induced and influenced by eosinophils [7-10]. Eosinophil produced, differentiated, maturated and activated as an effect of interleukin-5 [13,14]. Glucocorticoids ant-inflammatory effects in asthmatic patients included vascular permeability reduction, eicosanoid synthesis inhibition, eosinophils, basophils and other leukocyte recruitment in tissue of the lung inhibition, the most effective therapeutic agent as asthma treatment [15,16]. Eosinophils production, differentiation, maturation, activation and survival are under the influence of interleukin-5 [17-19]. Previous studies reported asthma control in some patients are achieved by using anti-interleukin-5 drugs as treatment approach [20-27]. In patients with severe asthma systemic corticosteroids reduced airway mucous membrane interleukin-5 mRNA expression and induce an improve in asthma symptoms and exacerbation [28,29].

Although systemic corticosteroids are effective treatment approach in asthma, however, their side effects and partially effectiveness encourages for research to develop and produce effective drugs with limited side effects. Interleukin-5 play an important role in the pathogenesis of many asthma phenotypes and with selectivity to eosinophils [30]. Additionally, interleukin-5 produced by a wide range of cells [31,32], thus attention was shifted to develop anti-interleukin-5 drugs.

Asthma is a heterogeneous disease with different inflammatory and immunologic phenotypes, and these phenotypic diversity affected the therapeutic outcomes of the different treatment approaches.[33] Additionally, gene and environment gene interaction may ameliorate therapeutic response [34] Responses to current asthma therapies varies greatly, which is probably related to the inter-patients differences in pathogenesis. [35] In the present anti-IL-5 antibodies and interleukin-5 receptor antagonists produced and evaluated in many clinical trials which indicated its effectiveness in control asthma [30]. However, these drugs clinical effectiveness influenced by patients proper section as treatment target. In addition, these drugs are not widely used in our community and patient selection for their usage depends on the eosinophils count. 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 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 Ethical Committee of Tikrit University College of Medicine approved the research proposal. Participant informed consent was taken 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 Immunosorbant Assay according to the manufacturer instructions.

Statistical analysis:

The results were analysed using Statistical Package for the Social Sciences (SPSS, version 16). ANOVA test was used to determine the significance of differences between groups mean, while Chi- square was used to determine the significance of frequency differences between groups. Significance considered when $P < 0.05$.

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

Recently asthma has considered not a single disease and thus defined as an heterogeneous disease with multiple endotypes and phenotypes. The disease pathogenesis not rely on inflammatory responses as previously thought, but included a multiple immunological, inflammatory and metabolic changes [4-11]. Local and systemic corticosteroids which is used extensively in asthma treatment especially in severe cases exert their therapeutic effect by reduction of cytokines induced intracellular signaling and cytokines production by the effector cells [15,16]. In asthma, many cytokines mRNA expression were reduced by corticosteroids therapy as shown in biopsy studies [36,37]. 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) [38]

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. 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.[39] 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,[40] who found that the serum level of IL-5 in asthmatic patients (mild and moderate) were significantly lower in controls and interleukin-5 serum level was lower in mild asthmatic as compared to moderate asthmatic. On the other hand, corticosteroids not show a significant effect on serum interleukin-5 between patients not using and those using the drug [41]. To date, the most effective anti-inflammatory agents in asthmatic patients are corticosteroids [15,16]. Corticosteroids modulate the transcription of cytokine interleukin-5 genes through inhibitory effects on transcription factor such as activator protein-1 (AP-1)[41]

The serum levels of interleukin-5 as this study indicated was higher in patients with asthma than in controls confirm the role of interleukin-5 in pathogenesis of non-atopic and atopic asthma phenotypes. This study also indicated for the first time that serum interleukin-5 increased in mild and moderate asthma phenotypes, who form the large number in asthmatic cases. Previous studies reported that moderate asthma show elevated interleukin-5 serum levels than in those with mild asthma [17-19]. Corticosteroids treatment significantly reduced serum interleukin-5 in patients with moderate and mild asthma, however, the mean serum values still higher than that in controls.[42].

The serum interleukin-5 sequential measurement not performed in the present study and thus sequential reduction of serum IL-5 during the corticosteroids treatment course cannot be excluded. A large scale longitudinal study is warranted to confirm or exclude such hypothesis. In the present study cohort, serum interleukin-5 elevation in mild and moderate cases may be attributed to bronchus smooth muscle cholinergic hyperresponsiveness. Interleukin-5 receptors antibodies may be used for blocking such cholinergic response and improve asthmatic patients clinically and prevent exacerbation and repeated episodes. T-helper 2 cytokines suppression by drugs may be of value on long term asthma control [43,44]

In conclusion, it is evident from this study that steroid therapies in asthmatic patients suppress the serum level of IL-5. Interleukin-5 level seems to be proportional to the severity of asthma and is a proper candidates as target of therapy in asthma.

Recommendations:

1. Perform studies that evaluate the role of another inflammatory markers like/ IL-2, IL-3, IL-4, IL-13 and TNF in the pathogenesis of asthma.
2. Further study by using leukotriene receptor antagonist drugs (Montelukast & Zafirlukast) in asthmatic patients to evaluate their effect on different inflammatory markers are warranted.

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

Group	Number	Mean pg/ml	SD
Mild asthma without steroid	**16**	**10.78**	**10.19**
Moderate asthma without steroid	**19**	**16.65**	**11.99**
Mild asthma with steroid	**13**	**3.94**	**0.82**
Moderate asthma with steroid	**22**	**4.10**	**1.08**
Control	**20**	**3.32**	**0.36**
		t value	**P**
Mild asthma without steroid versus moderate asthma without steroid		**1.51**	**>0.05**
Mild asthma with steroid versus moderate asthma with steroid		**0.46**	**>0.05**
Mild asthma without steroid versus mild asthma with steroid		**2.41**	**0.0233**
Moderate asthma without steroid versus moderate asthma with steroid		**4.89**	**0.0001**
Mild asthma without steroid versus control		**3.28**	**0.0024**
Mild asthma with steroid versus control		**2.98**	**0.0055**
Moderate asthma without steroid control		**4.97**	**0.0001**
Moderate asthma with steroid versus control		**3.08**	**0.0038**

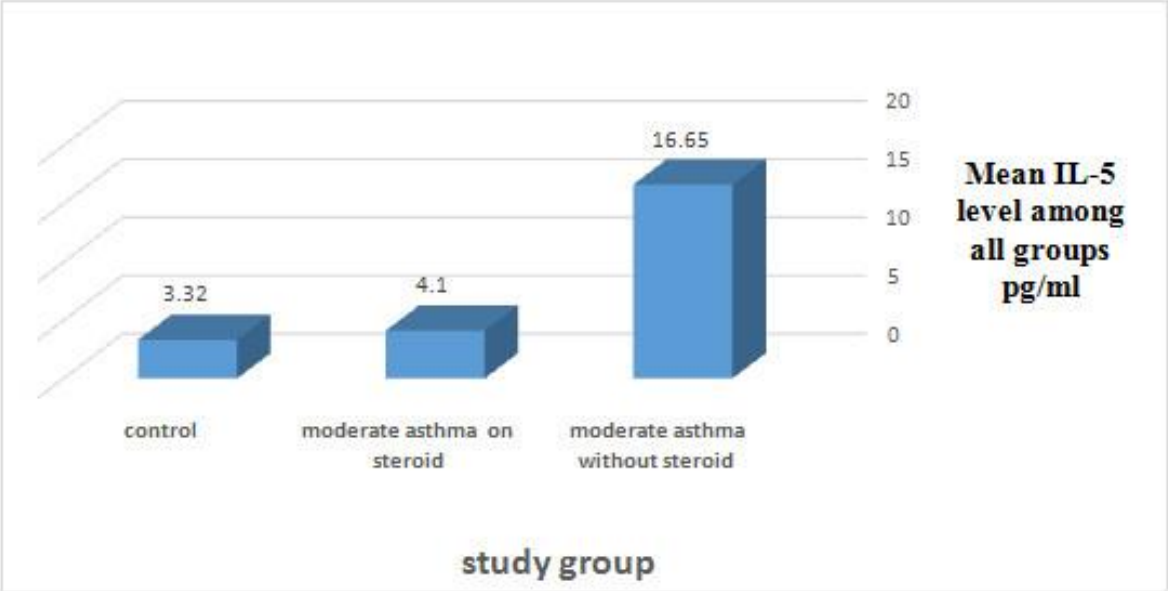

Figure (1): Mean Serum Interleukin-5 Level (pg/ml) in Mild Asthma Without Steroid, Mild Asthma on Steroid and Control. ANOVA test, P value < 0.05 (significant)

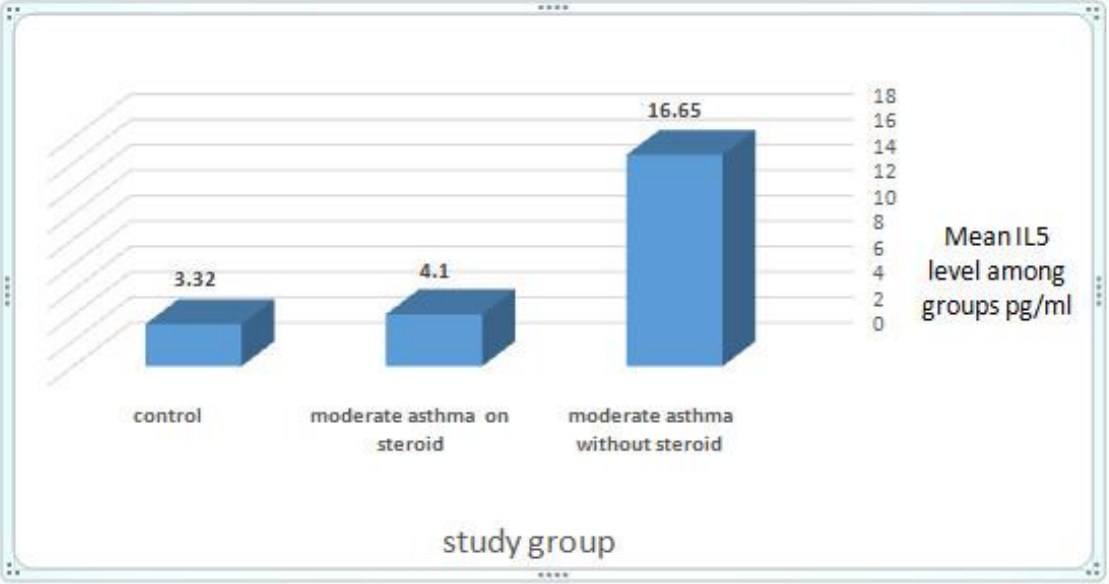

Figure (2): Mean Serum Interleukin-5 Levels in Moderate Asthma without Steroid, Moderate Asthma on Steroid and control. ANOVA test, P value < 0.05 (significant).