Serum level of interleukin-1beta in asthma patient and its relation to the severity of the diseases.

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Abstract Asthma is a heterogeneous disease, in which asthmatic patients present with different clinical phenotypes, variable endotypes, and different response to asthma medicines. Asthma is characterized by the involvement of multiple inflammatory pathways. During airway inflammation, many cytokines and chemokines are released and some are detectable in the serum.

Interleukin-1 is a pro-inflammatory cytokine found in two forms(α and β). The α form is mainly cell-bound, whereas IL-1β is primarily secreted by macrophages in response to immune system stimulation.

Objective:to study serum level of IL-1βin bronchial athma and its relation to the severity of the disease.

Methods: 45 cases were included in this study: 30 athma patients and 15 healthy subjects as a control. According to GINA guidelines 2018 we classified the patients into three groups mild ,moderate and severe.serum IL-1β was measured by (ELISA) kits

Results: serum level of interleukin-1β was higher among BA patients as compared with healthy control subjects where the mean levels were (4.52 vs. 4.18) in BA patients and healthy controls respectively but without a statistically significant difference; p-value= 0.549.

Key words: asthma ,mediator, 1β.

Introduction

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation**.(1)**

Asthma is a common, chronic respiratory disease affecting 1–18% of the population in different countries(2).

The World Health Organization Global Burden of Disease Study estimates that 13.8 million disability- adjusted life years (DALYs) are lost annually due to asthma, representing 1.8% of the total global disease burden.(3)

Asthma is a heterogeneous disease, with different underlying disease processes. Recognizable clusters of demographic, clinical and/or pathophysiological characteristics are often called‘asthma phenotypes. (2,3)

IL-1 was first described as a protein that induced fever and was called human leukocytic pyrogen, which comprises 2 major proteins: IL-1a and IL-1b**(4).**

IL-1 affects cells of the innate and adaptive immune system and exerts a wide range of biological activities including promoting fever, vasodilation, hematopoiesis, lymphocyte activation, leukocyte attraction and extravasation, angiogenesis,and antibody synthesis **(5).**

IL-1β is the mainly secreted isoform of interleukin 1 that mediates the development of allergic diseases(6) and asthma(7) via differentiating and activating Th2 cells(8,9)and Th17 cells.(10)

This interleukin also plays a role in host defense system in response to pathogens (bacteria, fungi, viruses) as a part of PAMP pathway (pathogen-associated molecularpatterns) as well as to biomolecules produced by host upon tissue injury, that is, DAMP (danger-associated molecular patterns) that activate non-infectious inflammatory response.(11) (In asthma, it was reported that bothPAMP andDAMPfactors may contribute to asthma exacerbation and increased inflammation in the airways via activation

of inflammasome NLRP3 that produces mature IL-1β.(12)

Recently, IL-1β was shown to mediate functional effects of inflammasome by activating resident epithelial cells and controlling epithelial permeability.(13)

From the previous finding,we studied serum level of IL-1β in athma patients and its relation to the severity of the disease.

Subjects and methods

A case control study was performed on thirty Patients with bronchial athma attending to the chest outpatient clinic of Beni-Suef University hospital in the period between January 2018 to july 2018 . fifteen healthy volunteers were included as a control group. A written consent was taken from all participants.

The study was approved by the local ethical committee at the faculty of medicine ,Beni- Suef university .

Inclusion and exclusion criteria:-

The criteria for inclusion in the study are:

Patients with bronchial athma according to GINA guidelines 2018.

Exclusion criteria are:COPD ,Bronchiectasis,Tuberculosis ,Any inflammatory condition .

Each patient was subjected to the following:

1- Full history taking:- Age, occupation and history of smoking.
- Presenting symptoms (onset, course and duration of the symptoms) especially: cough, symptoms of breathlessness).

-family history of athma.

-History of other allergy: food allergy,eczema or allergic rhinitis.

-General examination:

Eczema,allergic rhinitis ,conjunctivitis.

Local examination:

Physical examination in people with asthma is often normal. The most frequent abnormality is expiratory wheezing (rhonchi) on auscultation.

Plain chest x ray:

Usually normal in athmatic patients.

Pulmonary function tests:

Resting spirometry:

This was performed by Master Screen Jaeger-Hochberg,

Germany PFT. No.781040.

The degree of reversibility in FEV1, is generally accepted as 12% and 200 mL from the pre-bronchodilator value.

According to GINA guidelines 2018 we classified the patients into three groups Mild asthma is asthma that is well controlled with Step 1 or Step 2 treatment i.e. with as-needed reliever medication alone, or with low-intensity controller treatment such as low dose ICS, leukotriene receptor antagonists or chromones.

Moderate asthma is asthma that is well controlled with Step 3 treatment e.g. low dose ICS/LABA.

Severe asthma is asthma that requires Step 4 or 5 treatment e.g. high-dose ICS/LABA.

Measurement of interleukin-1 beta:

3cc blood sample was taken from each person, poured into a clot tube and then coagulated. Serum sample was separated by centrifugation and stored at -20 °C in 0.5 cc vials. After collecting of samples, serum il-1 beta determination using commercial sandwich enzyme- linked immunosorbent assay (ELISA) kits(**Human interleukin1β(IL-1β) ELISA Kit, SinoGeneClon Biotech)** was performed.

Statistical methods:

Data were analyzed using the software, Statistical Package for Social Science (SPSS Inc. Released 2009, PASW Statistics for Windows, version 18.0: SPSS Inc., Chicago, Illinois, USA).Frequency distribution as percentage and descriptive statistics in

the form of mean and standard deviation were calculated. Chisquare,t-test and correlations were done whenever needed. P values of less than 0.05 were considered significant. P value > 0.05 (NS) Not significant.

Results: The results are presented in Tables 1:9.

Table (1): Demographic Data of the Studied Population; (N= 45):

	Healthy Control n= 15	Patients with BA n= 30	TOTAL	*p-value**
Age (years);				
Mean ±SD	38.73 ±15.7	44.27 ±15.9	50.98 ±8.9	0.631[a]
Minimum	14	14	35	
Maximum	68	72	72	
Sex; N (%)				
Male	7 (46.7%)	6 (20.0%)	13 (28.9%)	0.086[b]
Female	8 (53.3%)	24 (80.0%)	32 (71.1%)	
Smoking Habit; N (%)				
Smoker	2 (13.3%)	3 (10.0%)	5 (11.1%)	0.547[b]
Non-Smoker	13 (86.7%)	27 (90.0%)	40 (88.9%)	
Working Status; N (%)				
Working	6 (40.0%)	7 (23.3%)	13 (28.9%)	0.423[b]
Not Working	9 (60.0%)	23 (76.7%)	32 (71.1%)	

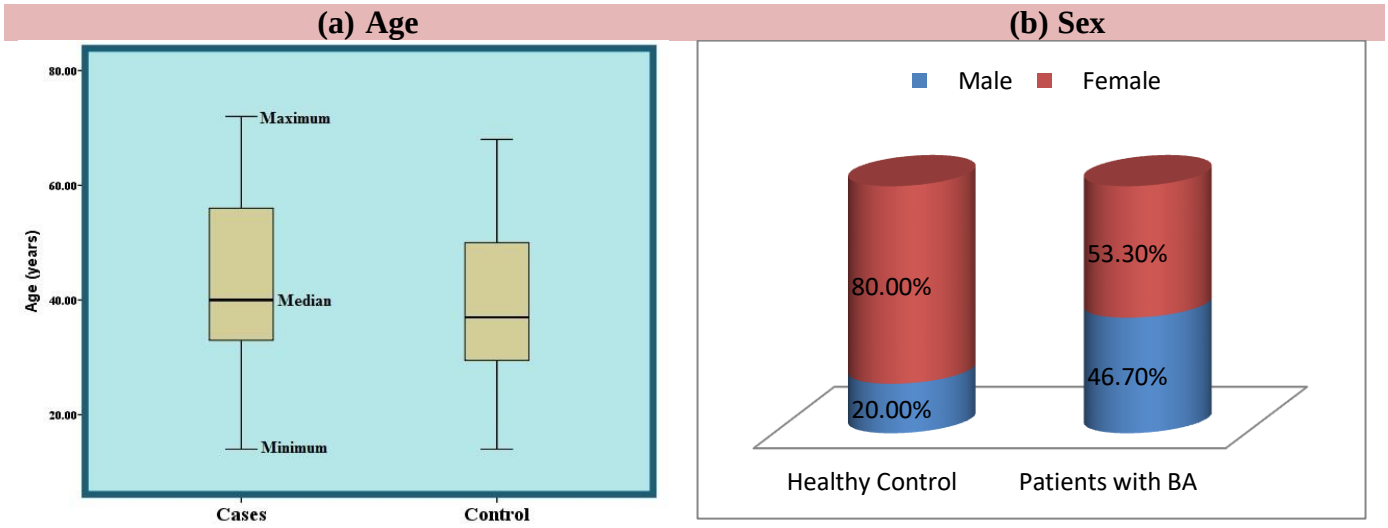

Figure (1): Age and Sex Distribution of the Studied Population
(matched age and sex between cases and controls)

Table (2): Family History and Other Allergies in the Studied Population; (N= 45):

	Patients with BA n= 30	Healthy Control n= 15	TOTAL	*p-value*
Family History; N (%)				
Negative	26 (86.7%)	14 (93.3%)	40 (88.9%)	0.651
Positive	4 (13.3%)	1 (6.7%)	5 (11.1%)	
Allergic History; N (%)				
Negative	17 (56.7%)	15 (100.0%)	32 (71.1%)	0.002*
Allergic Rhinitis	13 (43.3%)	0 (0.00)	13 (28.9%)	

Table (3): Pulmonary Function Tests in the Studied Population before Bronchodilator; (N= 45):

PFT	Studied Population: Healthy Control n= 15	Studied Population: Patients with BA n= 30	*p-value*
FEV1			
Mean ±SD	3.52 ±0.7	1.76 ±0.6	**0.001***
Minimum	2.20	0.60	
Maximum	4.10	2.60	
FEV1/FVC			
Mean ±SD	0.79 ±0.01	0.67 ±0.1	**0.001***
Minimum	0.77	0.42	
Maximum	0.83	0.85	

Table (4): Pulmonary Function Tests in the Studied Patients before and after Bronchodilator; (N= 30):

PFT	Studied Patients		*p-value*
	Before	After	
FEV1			
Mean ±SD	1.76 ±0.6	2.18 ±0.7	**0.001***
Minimum	0.60	1.02	
Maximum	2.60	3.90	
FEV1/FVC			
Mean ±SD	0.67 ±0.1	0.74 ±0.1	**0.001***
Minimum	0.42	0.47	
Maximum	0.85	0.93	

Table (5): Severity of Bronchial asthma and medication used in its treatment among the BA Patients Group; (N= 30):

	BA Patients Group N= 30
Severity of BA	
Mild	11 (36.7%)
Moderate	11 (36.7%)
Severe	8 (26.7%)
Medications used in treatment of BA	
Low dose of ICS/LABA	11 (36.7%)
Medium dose of ICS/LABA	8 (26.7%)
Low dose ICS	11 (36.7%)

Table (6): Serum level of interleukin-1β in the Studied Population :

interleukin-1β	Studied Population		*p-value*
	Healthy Control n= 15	Patients with BA n= 30	
Mean ±SD	4.18 ±0.9	4.52 ±2.8	0.549
Minimum	3.00	0.00	
Maximum	6.70	14.60	

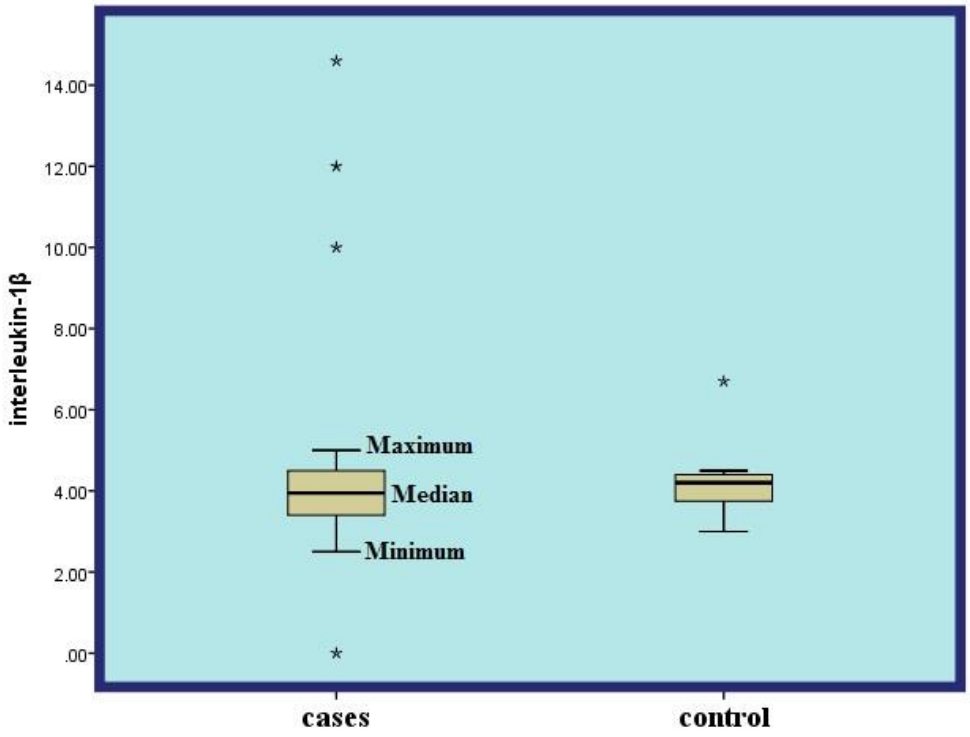

Figure (1): Serum level of interleukin-1β in the Studied Population .

Table (7): Correlation between Serum levels of interleukin-1β and Age of the studied population:

	Serum levels of interleukin-1β	
Patients Age	*r* = - 0.117	*p-value* = 0.445

Table (8): Relation of Serum levels of interleukin-1β to severity of BA in Bronchial Asthma patients:

	Severity of BA			
	Mild n= 11	**Moderate n= 11**	**Severe n= 8**	**p-value***
Mean ±SD	3.35 ±1.3	4.59 ±3.4	3.97 ±0.6	0.440
Minimum	0.00	2.70	3.20	
Maximum	4.50	14.60	5.00	

Table (9): Relation of Serum level of interleukin-1β and Other Allergies in the Studied Population; (N= 45):

Serum level of interleukin-1B	Other Allergies		*p-value*
	Negative n= 32	Allergic Rhinitis n= 13	
Mean ±SD	5.03 ±4.1	4.68 ±3.0	0.783
Minimum	0.00	3.00	
Maximum	21.20	14.60	
Range	21.20	11.60	

Discussion

Asthma is a heterogeneous and a genetically determined disease with different presentations, disease progression, and responses to therapy. Chronic airway inflammation that occurs in asthma is characterized by episodic reversible airway obstruction that variably presents with cough, wheezing, shortness of breath, or chest tightness(**WN Zedan MM, Laimon,et al, 2015).(14)**

Asthma pathogenesis is complex and varies across clinical endotypes. Multifaceted interactions between genetic, epigenetic, and environmental factors predispose patients to develop a number of dysfunctional immunologic regulatory patterns, which in turn dictate the presentation of clinical endotypes **.(olin JT, Wechsler ME.2014(15)**

Asthma has a high prevalence worldwide with increasing mortality and high health care cost burden .(**Zedan M, Settin A,et al,2009).(16)**

About 5% 59 of the patients with asthma require individualized strategies for the optimum control of their disease. Thus, the success of the individualized strategies depends on accurate phenotyping with the help of biomarkers (**Ricciardolo FL. 2014)(17)**Thus, quantifiable noninvasive biomarkers that are informative for assessing asthma pathogenesis and control in a specific patient will be of clinical utility in designing successful personalized treatment plans **(18)(Erzurum SC, Gaston BM.2012).**

Interleukin (IL)-1 is one of the major cytokines involved in the initiation and persistence of inflammation **(Dinarello CA.2000)(19).**

Table (1)shows that the mean age of athma patients was 38.7±15.7 years. The mean age of control group was 44.2±15.9 There was a non-significant difference between athma patients and control group as regards age. These groups were mostly females 24 (80.%) cases (53)% controls,in patient group 27(90%) were non smoker while 3 (10%) were smokers.

Table (2)4 patients (13.3%) have family history of bronchial athma while one control (6.7%).13 (43.3%) athma patients have positive history and clinical symptoms of allergic rhinitis compared to 0% in control group with significant difference (p <.002).

Regarding spirometry

Pulmonary function tests as demonstrated in **table (3**) were significantly higher among healthy control subjects as compared with patients with BA P- value <.001.

Table (4) demonstrates that FEV1 in the studied patients was significantly increased after bronchodilator. FEV1/FVC was significantly increased after bronchodilator.P-value 001.

Regarding severity of bronchial athma : Table (5) show that 11 (36.7%) patients have mild athma and on low dose ICS, 11(36.7%) patients have moderate athma and on low dose ICS/LABAand 8(26.7%) patients have severe bronchial athma and on medium dose ICS/LABA.

As demonstrated in **table (6);** serum level of interleukin-1β was higher among BA patients as compared with healthy control subjects where the mean levels were (4.52 vs. 4.18) in BA patients and healthy controls respectively but without a statistically significant difference; p-value= 0.549.

This is agreed with **(20) (Aneta Kleniewska,et al,2016)** who compared sputum and serum markers of inflammation in patients with occupational asthma and COPD.The study group included 20 patients with stable COPD, 24 patients with asthma, and 22 healthy subjects. Interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF)-α, matrix metalloproteinase (MMP)-9 levels in serum and induced sputum as well as fibrinogen and CRP in serum were determined in all the subjects.

Higher concentrations of IL-1β, IL-6, TNF-α, and MMP-9 in induced sputum and an increased concentration of acute-phase proteins in serum were observed in COPD patients compared with healthy subjects. Higher concentrations of IL-1β and MMP-9 in induced sputum and a higher concentration of C-reactive protein (CRP) were detected in COPD patients than in asthmatic subjects.serum IL-1β of athmatic pateints and control wasnot significant.

This study is also with agreement with**(21) (Wendy A Neveu1,2010)** who investigated the potential relationship between IL-6 and other proinflammatory cytokines, Th2/Th17 cytokines and lung function in allergic asthma, Cytokine levels in serum and lung function were measured in 16 healthy control and 18 mild-moderate allergic asthmatic subjects. The levels of the proinflammatory biomarkers TNFa and IL-1b were not different between the control and asthmatic group. In contrast, IL-6 levels were specifically elevated in asthmatic subjects compared with healthy controls ($p < 0.01$).

We also agreed with **(**22)**(Pagnoux C, et al,2019)** who analysed a panel of serum cytokines and chemokines as markers of disease activity in patients with eosinophilic asthma, eosinophilic granulomatosis with polyangiitis and primary hypereosinophilic syndrome (HES). The levels of 54 cytokines and chemokines were measured in the sera of 40 patients with active EGPA, 10 of these patients during inactive disease, 6 patients with HES, 8 with asthma, and 10 healthy controls. Serum cytokine/chemokines measured included interleukin (IL)-1α, 1β, 3, 4, 5, 6, 8, 13, 15, 17A, 17E(25), 18, 23 and 33, soluble IL-2 receptor alpha, eotaxin-1 (CCL11), -2 (CCL24) and -3 (CCL26), macrophage-derived chemokine (MDC/CCL22), macrophage inflammatory protein (MIP)-1a and -1b, and tumour necrosis factor (TNF)-α. Results were compared between disease and control

groups. RESULTS Differences regarding interleukin-1β between patients with active disease, inactive disease, eosinophilic asthma, primary hypereosinophilic syndrome and control group did not reach significance.

We disagreed with **(23**) **(Bhawna Mahajan et al,2008)**who used Serum interleukin-1β as a marker for differentiation of asthma and chronic obstructive pulmonary disease. Out of a total of 1023 patients screened, they included in the study ten patients each with atopic asthma, non-atopic asthma and COPD and ten healthy subjects. Skin prick tests with 14 inhalant allergens were performed on each patient. Blood was collected in the symptomatic and asymptomatic phases of the diseases and serum IL-1β and IgE levels were determined. their results showed that in the symptomatic phase in asthmatics, serum IL-1β levels were higher ($P<0.05$) than in patients with COPD and control. Serum IgE levels were higher ($P<0.05$) in atopic asthmatics than in non-atopic asthmatics and in COPD patients. they concluded that serum IL-1β level determination during the symptomatic phase of the diseases may help to differentiate asthmatics from patients with COPD.

We also disagreed with **(24) (Mara De Amici,et al,2001)** who studied eventual variations in the serum levels of IL-1β, IL-2, IL-6, and TNF-α in allergic subjects during SIT.Serum levels of IL-1β, IL-2, IL-6, and TNF-α were determined before and after 3, 6, and 9 months of SIT by an immunoradiometric assay (IRMA) in 11 adults with perennial allergic asthma and/or rhinitis caused by house dust mites and in 6 nonatopic healthy volunteers. Median serum IL-1β and TNF-α levels of the patients were significantly higher at baseline than those of the controls.

Table (7) showed that no detected correlation between Serum levels of interleukin-1β and Age of the studied population as p-value > 0.05.

This is in agreement with **(25) (Di Iorio A, et al, 2003)** who evaluated the factors influencing the serum levels of Interleukin-1 beta, in a large and representative population. Data were from the InCHIANTI project, a study of factors contributing to the decline of mobility in late life, which sampled people living in two sites in the surroundings of Florence. Blood samples were obtained from 1,292 participants. The serum levels of several cytokines were measured by enzyme

linked immunosorbent assay using an ultrasensitive commercial kit. They found no association between serum IL-1beta level and age, sex.

Table (8) demonstrates no detected relation of serum levels of interleukin-1β to severity of BA in Bronchial Asthma patients.

Table (9) demonstrates no detected relation of serum levels of interleukin-1β to association with other allergies in the studied population.
This is in agreement with **(26)(Thomas SS1, Chhabra SK.2003)** who studied inter-relationship between IL-1beta and IgE in the serum of asthmatic patients. The study included 30 patients of asthma and 10 as control. Out of these 30 cases, 20 patients had stable and 10 had acute asthma. Of the 20 stable patients, 9 were allergic and 11 were non-allergic to either of the 12 allergens used for skin prick test. Serum IgE and IL-1beta levels were measured by enzyme linked immunosorbent assay (ELISA). Total serum IgE levels increased significantly ($p < 0.05$) in asthma [200.5 +/- 30.91 IU/ml, mean +/- standard error of mean (SEM)] in comparison with the controls (18.15 +/- 4.35 IU/ml). Serum IL-1beta level was higher in allergic (1.94 +/- 0.63 pg/ml) than in non-allergic patients (0.64 +/- 0.21 pg/ml) but it was not statistically significant ($p > 0.05$).

Limitations of the study

First ,its relatively small size affected the power to detect associations between systemic markers and clinical parameters .Second ,the medications for patients with bronchial athma were not investigated and the effect of inhaled corticosteroids and bronchodilators on the systemic level of interleukin-1β could not be evaluated.

Conclusion

serum level of interleukin-1β was higher among BA patients as compared with healthy control subjects where the mean levels were (4.52 vs. 4.18) in BA patients and healthy controls respectively but without a statistically significant difference; p-value= 0.549.
No detected relation of serum levels of interleukin-1β to severity of Bronchial Asthma in Bronchial Asthma patients.

Serum IL- 1β is a poor indicator of bronchial asthma and disease severity therefore, monitoring by serum IL-1β concentration is of limited value. The clinical value of serum IL-1β as a marker of bronchial athma remains to be established.

Recommendation

Since this study population was limited in size, these conclusionsshould be confirmed by further studies. Further follow-up cohortstudies with bigger samples that measure level interleukin-1βprospectively should help to determine the validity of these findings.Further studies on large number of bronchial athma patients are required to determine the value of interleukin-1β as biomarker in bronchial asthma.

Studies with follow up of the patients with bronchial athma by measuring level of interleukin-1β before and after treatment to determine its role in follow up of the disease.

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