

Immunological study of patients with asthma

Alaa J.H. and Thanaa A.M

University of Babylon , College of Science , Biology Department

Abstract :

An immunological study were performed to estimation the concentrations of immunoglobulin IgE , IL-4 , IL-13 and PGE2 by ELISA , calculate the total and differential WBCs counts of patients with asthma , as well as the relationship between this disease and gender in Babylon province . The study reveals that the IgE and IL – 4 levels were significantly increased ($p<0.05$) in patients (second and third age categories) compared with healthy subject . The results illustrated that the patients show a significant elevation in IL – 13 levels in first ($p<0.01$) , second ($p<0.05$) and third ($p<0.01$) age categories respectively compared with control group ;furthermore, the study revealed that the concentration of prostaglandin –E2 (PGE2) show highly significant elevation ($p<0.05$) in patients compared with healthy individuals . The results displays that there is no significant differences ($p<0.05$) in total WBCs count between patients and control group , and the same results was in means of neutrophils , lymphocytes and monocytes ; while there were significantly increased ($p<0.05$) in eosinophils (first and second groups) and basophils (all groups) in patients compared with healthy subject ,furthermore, the results reveals increased ratio of disease in females compared with males .

Key words :- patients . asthma . IL-4 . IL-13 . PGE2 . IgE.

الخلاصة :

أعدت دراسة مناعية لتقدير تراكيز الكلوبولين المناعي E- (IgE) ، IL-4 ، IL-13 ، والموتين E2- (PGE2) بطريقة الأمتزاز المناعي المرتبط بالأنزيم (ELISA) ، حساب العد الكلي و التفريقي لخلايا الدم البيض لمرضى الربو بالإضافة الى علاقة المرض بالجنس في محافظة بابل . أظهرت نتائج الدراسة وجود ارتفاع معنوي ($p<0.05$) في مستويات الضد IgE و الأنترليوكين – 4 للمرضى في الفئتين العمريتين الثانية و الثالثة مقارنة مع مجموعة الأفراد الأصحاء . أوضحت نتائج الدراسة في التحري عن الأنترليوكين – 13 وجود ارتفاع معنوي في المرضى للفئات العمرية الأولى ($p<0.01$) و الثانية ($p<0.05$) و الثالثة ($p<0.01$) على التوالي مقارنة مع مجموعة السيطرة ، بالإضافة الى ذلك بينت النتائج وجود ارتفاع معنوي كبير ($p<0.05$) في تركيز الموتين E2 – لمرضى الربو مقارنة مع الأفراد الأصحاء . أوضحت النتائج عدم وجود فروق معنوية ($p<0.05$) في العد الكلي لخلايا الدم البيض بين المرضى ومجموعة السيطرة ، ولوحظت ذات النتائج في معدلات العدلات ، الخلايا اللمفية والخلايا وحيدة النواة الدموية ، في حين كانت هنالك زيادة معنوية ($p<0.05$) في الحمضات (الفئة العمرية الأولى والثالثة) والقعدات (كل المجاميع) في المرضى مقارنة مع الأفراد الأصحاء ، بالإضافة الى ذلك أظهرت النتائج زيادة نسبة المرض في الأناث مقارنة مع الذكور .

Introduction :

Asthma is a clinical syndrome characterized by episodic reversible airway obstruction, increased bronchial reactivity, and airway inflammation. Asthma results from complex interactions among inflammatory cells, their mediators, airway epithelium and smooth muscle, as well as the nervous system(Harvy and Champe,2008). In genetically susceptible individuals, these interactions can lead the patient with asthma to symptoms of breathlessness, wheezing, cough, and chest tightness (Kenneth et al.,2008) Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, in particular, mast cells, eosinophils, T lymphocytes, macrophages, neutrophils, and epithelial cells. In susceptible individuals, this inflammation causes recurrent episodes of wheezing,breathlessness, chest tightness, and coughing, particularly at night or in the early morning and these episodes are usually associated with wide spread but variable airflow obstruction that is often reversible either spontaneously or with treatment.;furthermore, the inflammation causes an associated increase in the existing

bronchial hyper response to a variety of stimuli (NHLBI, 1995 ;Woessner et al.,1999). production of immunoglobulin E (IgE). Allergens that enter the airway are presented by antigen presenting cells (APC) to the naïve CD4 T cell which differentiates into effector T helper type 2 cells (Th2) ; Th2 cells themselves secrete a number of cytokines including IL-4 , IL-9 and IL-13 acting on different target cells such as mast cells, basophils , eosinophils, epithelial cells, smooth muscle cells and B-lymphocytes (NAEPP,1997). The IL-4 stimulated B cells to synthesizing IgE (Elias et al.,1999; Busse and Lemanske,2001). IgE is tightly bound to mast cells and basophils surfaces through the FcεR1 ; furthermore , repeated exposure to the same allergen and its binding to IgE cross – links these FcεR1 stimulating these cells to release chemical mediators including ; cytokines (IL- 4 and IL-13) , enzymes ,histamine , heparin , leukotrienes and prostaglandins (D2 and E2) , the later cause smooth muscle contraction , increase vascular permeability and stimulate mucus secretions , therefore all these changes in the respiratory airways can lead to the airflow obstruction and asthmatic symptoms(Janeway et al.,2005; Kenneth et al.,2008) .The aim of this study to evaluate the changes in some Immunological parameters of patients with asthma and the relationship between this disease and gender at different age categories in Babylon governorate .

Materials and Methods :

1- Patients and Control :

The work was applied on 60 asthma patients (27 males and 33 females) admitted to the center of asthma and allergy , maternity and pediatrics hospital as well as murjan hospital in Babylon province , and 30 apparently subject(14 males and 16 females) with no symptoms of asthma were selected as control group . The cases of this study were divided into three age categories for comparison : (1-20) years , (21-40) years and (41-60) years . The number of cases was 15 , 18 and 27 in each groups respectively . The diagnosis for patients was based on the pre- diagnosis by physician.

2- Blood Samples :

The blood samples were drawn from each patients and controls (5 ml) by vein puncture using disposable syringes . The blood was divided into two parts , one of them placed in disposable tube , kept to clot at room temperature , and then centrifuged at 3000 r.p.m (Bishop et al , 1985) for 10 minutes , after that sera samples were carefully transferred to appendrof tubes and stored in a liquotes at deep freezing until used . The other part of blood sample was placed in EDTA tube in order to account the total and differential WBCs (Haen,1995).

3- Immunological Tests :

A. The concentrations of IL-4 , IL-13 and PGE2 were estimated by ELISA according to the manual procedure of Cusabio company (China) .

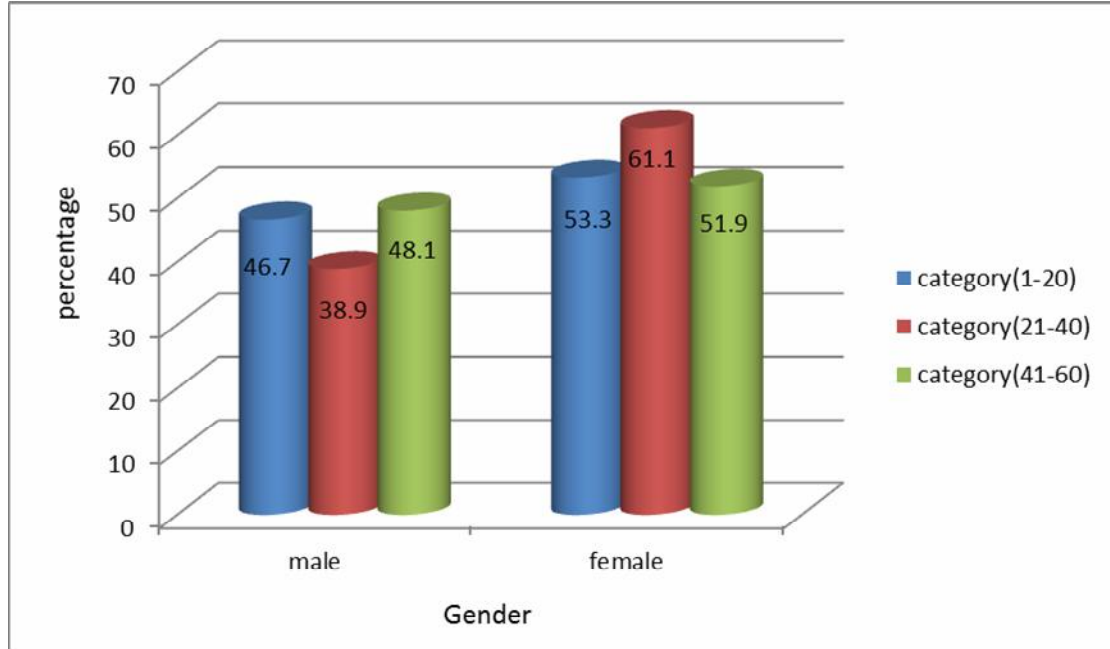
B. The concentration of IgE was estimated by ELISA according to the manual procedure of SRG company (Germany) .

4- Statistical Analysis :

The results were analyzed using statistical system Spss version -17 (T-testing) .

Results :

The results showed that the ratio of asthmatic patients were higher in females compared with males in all age categories , which reached 53% , 61.1% and 51.9% in first , second and third age groups respectively , while this ratio in males were 46.7% , 38.9% and 48.1% consuctively (figure 1).



Figure(1) :The relationship between asthmatic patients and gender.

The results illustrated that there was no significant difference in mean of immunoglobulin IgE between patients and control groups in first age category , while the concentrations of IgE were increased significantly($p < 0.05$) in the second and third age groups , which reached 74.276 and 68.106 UI/ml respectively , whereas it was 31.541 and 22.807UI/ml in healthy individuals consuctively . The levels of IL-4 was increased in first age category of patients which reached 19.285 pg/ml , while it was 18.685 pg/ml in healthy subjects , furthermore , the means of IL-4 was increased significantly($p < 0.05$) in second and third age categories of patients which reached 16.381 and 17.626 pg/ml respectively , whereas in control group it was 11.681 and 14.237 pg/ml respectively .

The concentrations of IL-13 and PGE2 were highly significantly increased in all age categories of patients in comparison with control groups as showed in table (1) .

Table(1) : The concentrations of IgE, IL-4, IL-13 and PGE2 of patients with asthma in different age categories .

parameters category	Group	IgE	IL-4	IL-13	PGE2
		UI/ml M±SE	Pg/ml M±SE	Pg/ml M±SE	Pg/ml M±SE
(1-20) Year	Patient	38.840 ± 9.278	19.285 ±3.298	(**) 1488.335 ± 258.778	(*) 92.534 ± 18.868
	Control	38.296 ± 9.138	18.685 ± 2.544	687.343 ± 161.367	43.608 ±9.035
(21-40) Year	Patient	(*) 74.276 ± 15.726	(*) 16.381± 1.391	(*) 2324.835±283.537	(*) 76.150± 13.199
	Control	31.541± 4.856	11.681±1.343	1396.031± 220.547	36.808±5.768
(41-60) Year	Patient	(*) 68.106 ± 14.026	(*) 17.626 ± 0.980	(**) 2206.028± 171.456	(*) 77.711 ±13.098
	Control	22.807± 2.076	14.237 ± 0.907	1371.476± 115.609	26.657± 7.389

Significant level P<0.05*

Significant level P<0.01**

table (2) explained that there is no significant differences in the total WBCs count between asthmatic patients and control groups , as well as in differential WBCs counts of neutrophils , lymphocytes and monocytes patient , while there were significantly increased(p<0.05) in mean of basophils for patients , which reached 0.9 , 0.972 and 0.856 × 10³ (cell\mm³) in first , second and third age groups respectively , whereas it was reached 0.485 , 0.682 and 0.548 × 10³ in healthy subjects consuctively , furthermore , eosinophlis were significantly increased(p<0.05) in first and third age groups in patients, that reached 2.826 and 3.203 × 10³(cell\mm³) respectively , while it was 1.905 and 2.414 × 10³ (cell\mm³) in healthy subjects .

Table(2) : The total and differential WBCs count of patients with asthma at different age categories .

parameters category	Group	WBCs $\times 10^3$	NE $\times 10^3$	Lym $\times 10^3$	Mon $\times 10^3$	Eos $\times 10^3$	Bas $\times 10^3$
		Cell/mm ³ M \pm SE	Cell/mm ³ M \pm SE	Cell/mm ³ M \pm SE	Cell/mm ³ M \pm SE	Cell/mm ³ M \pm SE	Cell/mm ³ M \pm SE
(1-20) Year	Patient	0.7809.842 \pm	59.894 \pm 2.265	29.731 \pm 2.111	6.489 \pm 0.817	(*) 2.826 \pm 0.391	(*) 0.900 \pm 0.168
	control	8.804 \pm 1.019	56.657 \pm 1.900	32.795 \pm 1.530	7.523 \pm 0.813	1.905 \pm 0.207	0.485 \pm 0.139
(21-40) Year	Patient	9.011 \pm 0.809	56.511 \pm 1.690	33.088 \pm 1.660	7.177 \pm 0.779	2.405 \pm 0.259	(*) \pm 0.250 0.972
	Control	8.856 \pm 0.539	58.812 \pm 2.036	28.812 \pm 1.723	8.544 \pm 0.545	2.752 \pm 0.171	0.682 \pm 0.089
(41-60) Year	Patient	9.860 \pm 0.916	57.596 \pm 1.527	31.526 \pm 1.229	6.806 \pm 0.665	(*) 3.203 \pm 0.251	(*) 0.856 \pm 0.151
	Control	9.108 \pm 0.645	55.965 \pm 1.833	32.140 \pm 1.579	8.408 \pm 0.564	2.414 \pm 0.277	0.548 \pm 0.076

Significant level P<0.05*

Discussion :

The patients with asthma showed higher values of serum immunoglobulin IgE compared with control group , and this is due to the stimulated Th2 cells can produce the cytokines IL-4 and IL-13 which mediate development eosinophils and stimulate B-cells to secrete specific IgE . This results consistent with the study of Tavakkol et al., (2007) where found a high significant differences in concentrations of IgE in patients compared with healthy individuals , while there was no significant difference in level of IgE in first age category between asthmatic patients and healthy subjects , this may be due to the differences in racial/ethnic groups , therefore many studies illustrated that there were a correlation between IgE and racial / ethnic groups since ; allergy genes were specific in different racial/ethnic groups (Mathias et al., 2001 ; Xu et al. ,2001; Lester et al .,2001) . Our results revealed a significant difference in level of IL-4 between patients and control group exempt a first age category . The

cytokine IL-4 was mediated the normal and inflammatory immune responses(Rakhmankulova,2010) , meantime , Benjamini et al.,(1996) reported that mast cells may be the source of this cytokine in the early phases of immune response , before Th2 cells are activated .Since, a common human diseases such as allergic diseases (ex. asthma) , autoimmune diseases and chronic infections are characterized by dysregulation of the pro and anti-inflammatory cytokines balance produce by Th1 and Th2 cells (Elenkov et al.,2005). The study showed a highly significant increased in IL-13 of patients in comparison with healthy subjects . Deo et al., (2010) revealed that serum levels of IL-13 were higher in patients with asthma , rhinitis and dermatitis, furthermore , Saha et al., (2008) and Ingram et al., (2011) illustrated that the concentration of IL-13 was increases in respiratory airways in patients with asthma and this cytokine believed mediate many forms of interactions including ; hyper responsiveness ,mucus metaplasia , active proliferate of inflammatory fibroblasts in bronchial passages which contribute in remodeling of respiratory airways , therefore , IL-13 was multi functions (Collins et al.,1995; Mould et al. ,1997) . The sera of patients with asthma showed higher values of PGE2 in all groups in comparison with healthy subjects . Kostikas et al., (2003) cleared that the concentrations of PGE2 were higher in patients with mild asthma as compared with normal individuals ,this is mainly attributed to smoking , also their production was increased by active ϵ cells , especially macrophages .The higher levels of endogenous PGE2 do not have protective role on the bronchospasm , furthermore , increased levels of PGE2 secrete by dendritic cells due to alteration in the expression of both cyclooxygenase -1 and cyclooxygenase -2 in patients (Long et al.,2004). The study showed that there were no significant differences in total WBCs count between patients and control group , this is augmented by Lewis et al.,(2003) in which the total WBCs count remain within normal range, while the differential WBCs counts revealed increases in basophils as well as eosinophils count . Eosinophils and basophils play a major role in allergic reactions ,since ; it contains a high affinity receptor , Fc ϵ R1 to the immunoglobulin IgE on their surfaces and attracted to the anatomic site of inflammation(Metzger et al.,1987;Wenzel et al., 1991; Rubira et al.,1997 ; Weller , 1997 ; Wong et al. ,2004) .

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