

Level of Interleukin–8 in the Sera of Asthmatic Patients in Baghdad

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Abstract:

Asthma is a common, chronic inflammatory, anaphylactic hypersensitivity disease .It is a world-wide distributed with a range extends from mild to severe fatal episodes. Fifty eight asthmatic patients sera have been collected from Al-Zahra'a consultant for asthma and allergy , during the period between July / 2008 – September / 2008 and thirty two sample of healthy peoples as a control group .The patients age ranged from (12-45) years .Enzyme linked immunosorbent assay (ELISA) has been applied estimation of interleukin – 8(IL-8).

The results showed that highly significant differences ($P < 0.0001$) between asthmatic patients – positive IL–8 and asthmatic patients – negative IL-8. There are no significant differences between residency (urban and rural regions) and levels of IL-8. There is a good relation between body mass index (BMI) and asthmatic patients ($P < 0.005$), but no relation between body mass index and levels of IL-8.

Keywords: Asthma, Interleukin– 8, Body Mass Index

Introduction:

Asthma is a highly complex disease that is still poorly understood and whose cause remains unknown. One of the striking advances in the last decade has been the recognition that cytokines play a critical role in orchestrating, perpetuating and amplifying the inflammatory response in asthma. Indeed the increased and abnormal expression of cytokines in airway cells is one of the major targets of corticosteroid therapy, by far the most effective controller treatment for asthma currently available .Many cytokines and chemokines are involved in the pathophysiology of asthma^(1, 2).

Cytokines are active molecules produced by cells of the immune system other than immunoglobulins also are importantly involved in the communication .These substances are one now referred to as cytokines rather than lymphokines .Cytokines produced by lymphocytes are now referred to as lymphokines , whereas products produced by monocytes or macrophages are reckoned as monokines⁽³⁾.

Interleukin – 8 (IL- 8) is a chemokine produced by macrophages and other cell types such as epithelial cells .It is also synthesized by endothelial cells. Primary function of IL-8 is the induction of chemotaxis in its target cells such as (neutrophil granulocytes) . In neutrophil series of cell-physiological responses required for migration and its target function phagocytosis are also induced like increase of intracellular Ca^{++} , exocytosis such as histamine release and respiratory burst⁽⁴⁾.

IL- 8 is a mainly neutrophil chemoattractant and activator which induces shape change, transient rise in Ca^{++} , exocytosis with release of enzyme and proteins from intracellular storage organelles, and respiratory burst through activation of NADPH oxidase⁽⁵⁾.

It also up regulates the expression of two integrins (CD11b / CD18 and CD11 c / CD18) during exocytosis of specific granules^(6, 7).

IL- 8 activates neutrophil 5-lipoxygenase with the formation of leukotriene B₄⁽⁸⁾.

IL- 8 enhanced Co-expression of IL-8 and GM – CSF in bronchial epithelial tissue of subjects with severe a topic asthma but not in normal subjects or those with mild a topic asthma , suggesting

that IL-8 may be a marker of severe asthma. IL-8 was also found to be complexed with IgA levels of which were raised in bronchial tissue in asthma⁽⁹⁾. IL-8 possesses chemotactic activity for primed eosinophils. In addition human IL-8 is able to induce accumulation of eosinophil in guinea pig skin and an anti IL-8 antibody inhibited IL-1 induced eosinophil accumulation in rat skin^(10,11). Thus, this study aimed to estimate IL-8 concentration in the sera of asthmatic patients in comparison with the controls.

Patients and Methods:

Subjects: this study was carried out in AL- Zahra'a consulting center of allergy and asthma in Baghdad. It was conducted on the following main groups during the period between July / 2008 – September / 2008.

Patients study group:

Fifty-eight asthmatic patients attending to the consulting center allergy & asthma. Their age ranged between (5- 45) years.

Control group: Thirty-two apparent healthy individuals were involved in this study as healthy control group. They were well matched in terms of age and sex.

All the sera of the patients & control group have been stored in deep freezer (- 20 c).

Materials:

*CELL COM IL-8 ELISA IM2237, IM2238: is research use only products, intended for quantification of human IL-8 in serum (Immunotech, France).

*Enzyme linked immunosorbent assay (ELISA) system, USA

Methods:

Measurement of human (IL-8) in the sera of asthmatic and control groups:

- 1- Add 50 µL of each standard , sample for each well
- 2- Incubate 120 min. at 37 c with shaking
- 3- Washing the wells by washing solution five times
- 4- Fifty µL of biotinylated antibody was added
- 5- One hundred µL of streptavidin – HRS conjugate was added
- 6- Incubate for 30 min. at 37 c with shaking
- 7- Washing the wells by washing solution five times
- 8- One hundred µL of substrate was added for each well
- 9- Incubate for 20 min. at 37 c with shaking
- 10- Fifty µL of stop solution was added for each well
- 11- Read absorbance at 450 nm by ELISA.

The sample results are calculated by interpolation from a standard curve that is performed in the same assay as that of the sample. Drawing the curve, plotting on the horizontal axis the IL-8 concentration of the standards and on the vertical axis the corresponding absorbance. By location of the absorbance for each sample on the vertical axis, we can read off the corresponding IL-8 concentration on the horizontal axis.

Body mass index (BMI) calculation: BMI had been estimated from person's weight & height. It is calculated according to following formula⁽¹²⁾:

$$\text{BMI} = \frac{\text{Weight per Kg}}{\text{Height per m}^2} = \text{Kg/m}^2$$

Height per m²

BMI = 18.5-24.9 = normal weight

BMI = 25-29.9 = overweight

BMI ≥ 30 = obese⁽¹³⁾.

Statistical analysis:

Analysis of data obtained was made by the use of a computer using statistical packages available (SPSS-15).The test of significance used for comparing difference in proportions by using chi-squared (X^2) test and the limit of the p- value accepted was < 0.05 as level of significance.

Result:

The mean concentration of IL-8 in control group =111.801(pg/ml) which has been considered as cut off value of the ELISA kit between positive and negative samples.

Table (1): shows that there is a highly significant difference ($p<0.0001$) between patients with Asthma and control groups in the results of IL-8.

Table (2): shows highly significant differences ($P<0.0001$) between asthmatic patients – positive IL-8 and asthmatic patients – negative IL- 8 .In addition, it was found that there are no significant differences between residency (urban and rural regions) and IL-8 results (Table 3).Table (4) shows no significant difference between body mass index and IL-8 results .However, table (5) shows highly statistically significant differences ($p<0.005$) in body mass index between patients with and without asthma.

Table: (1) Distribution of asthma and control groups according to Interleukin -8 (IL-8).

IL-8	Groups			
	Cases		Controls	
	No.	%	No.	%
Positive	51	87.93	13	40.62
Negative	7	12.07	19	59.38

Chi-square =22.465 df=1 $p<0.0001$

Table: (2) Distribution according to Interleukin -8 with asthmatic patients.

IL-8	Asthma			
	Negative		Positive	
	No.	%	No.	%
Positive	7	29.17	57	86.36
Negative	17	70.83	9	13.64

Chi-square =28.028 df=1 $p<0.0001$

Table: (3) Distribution according to residency and Interleukin-8.

Residency	IL8			
	Positive		Negative	
	No.	%	No.	%
Urban	43	67.19	14	53.85
Rural	21	32.81	12	46.15

Chi-square =1.417 df=1 $p<0.234$

Table: (4) Distribution according to body mass index (BMI) with Interleukin -8.

BMI	IL8			
	Positive		Negative	
	No.	%	No.	%
20-25	15	23.44	6	23.08
25-30	27	42.19	16	61.54
>30	22	34.38	4	15.38

Chi-square =3.758 df=2 $p=0.153$

Table: (5) Distribution according to body mass index (BMI) and asthma.

BMI	Asthma			
	Negative		Positive	
	No.	%	No.	%
20-25	10	41.67	11	16.67
25-30	12	50.00	31	46.97
>30	2	8.33	24	36.36

Chi-square =9.535 df=2 $p<0.009$

Discussion:

IL-8 possesses chemotactic activity for period eosinophils⁽¹⁰⁾, also it includes the release of histamine from human blood basophils with enhanced release with IL-3, IL-5, small of Ca^{++} and respiratory burst^(14, 15).

The present results are consistent with what was found before⁽¹⁶⁾; IL-8 was elevated in patients with cystic fibrosis and asthma. They hypothesized that IL-8 acts directly on airway smooth muscle cells in a way that may contribute to the enhanced airway responsiveness and any way remodeling observed in cystic fibrosis and asthma.

The results of IL -8 in the urban and rural regions showed no significant difference, and the reason may be due to the environmental exposure in both regions to air pollution, pollens, house dust mites and others. Thus this finding was found to be in agreement with the previous observations elsewhere⁽¹⁷⁾.

In view of our findings; there is no significant differences ($p>0.005$) between IL-8 results and BMI. Results in this scope were consistent with the results of previous studies elsewhere^(18, 19); they said that no proof of a direct evidence about the source of the elevated plasma IL-8. However, previous studies showed IL-8 production by the adipocytes were related to the fat mass⁽²⁰⁾. Moreover, others observed for the first time to our knowledge, an increase in plasma IL-8 in obese individuals⁽²¹⁾.

Findings of present study also revealed that there was a highly significant differences ($p<0.005$) in body mass index between patients with and without asthma. Previously it was confirmed that BMI is a good indicator for obesity⁽²²⁾, thereafter, overweight or obesity were proposed to be a risk factor that enhancing the chance for asthma development⁽²³⁾. As already mentioned, this might be one of the factors responsible for the increased cardiovascular risk in obesity⁽²⁴⁾.

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