Possible Role for Interleukin-5 in Asthma

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Summary:

Background: *Asthma is an allergic disease characterized by airway obstruction as a result of cellular accumulation due to the liberation of certain mediators. Among those mediators are the cytokines such as IL-5.*

Patients & Methods: *Interleukin-5 concentration has been estimated in 94 sera samples of Asthmatic patients in comparison with 41 non-asthmatic bronchitis as patient controls in addition to 30 apparent healthy control group using ELISA method.*

Results & Conclusions: *There is highly significant elevation of IL-5 in the asthmatic cases in comparison with healthy controls (P< 0.001). We conclude that this cytokine may play the major role in asthmatic attack & it may be a good marker for the disease.*

Key words: *Asthma, IL-5, bronchitis, Eosinophilia, IgE*

Introduction:

Recent Studies have shown that initial Sensitization to airborne environmental allergens occurs typically in early childhood. Subsequent progression to persistent atopic asthma **[1]**.

The cells which participate in asthma & atopy include, Eosinophils,

Basophiles & mast cells besides others with secondary role in **[2].**

lt has been observed that Eosinophils are important for their contribution to allergic disease particularly asthma **[3].**. Correlation exists between the numbers of airway Eosinophils and the severity of asthma **[3]**

Cytokines are defined as hormone like, low molecular weight, proteins that enable immune cells to communicate, and play an integral role in the initiation, perpetuation and subsequent down regulation of the immune response **[4-5].** They are soluble factors released by cells (cyto-) to communicate with, and influence the function (-kines) of other cells through specific surface receptors **[6].** Abundant evidence now exist that asthmatic primary inflammatory lesion consists of accumulation of CD^{+4} Th_2 lymphocytes and Eosinophils in the airway mucosa. Th_2 orchestrate the asthmatic inflammation through the secretion of a series of cytokines particularly lL-4, lL-1, lL-5 and lL-9, in contrast, Th_1 associated cytokines such as 1FN-γ induce protective effect **[7].**

Interleukin-5 acts predominantly as an Eosinophilo-poietic factor and contributes greatly to Eosinophils production and activation in vivo **[8].**. Considerable evidence from experimental animals and human asthmatic indicates IL-5 can also modulate the survival and inflammatory activity of Eosinophils and may play a crucial role in the pathogenesis of asthma **[9].**

It was reported that IL-5 binds its receptors. These receptors are expressed on small number of inflammatory cells, including Eosinophils and basophiles. This step triggers a series of intercellular protein phosphorylation events that promote the accumulation of IL-5 responding cells in the target tissue and the release of inflammatory mediators that contribute to tissue injury **[8, 10].**

Patients & Methods:

Ninety-four asthmatic sera samples for asthma patients volunteers [i.e. there is an oral permission of these patients to perform these tests] have been estimated for the Interleukin-5 (IL-5) level in comparison with 41 samples for non-asthmatic bronchitis patients beside 30 samples for apparent healthy controls, using

ELISA method {according to Diaclone Fleming Co. Kit instruction , France}. All the patients have been diagnosed according to the patient's medical history, physical examination and laboratory test's results under supervision of consultant committee in the Al-Zahra'a Center of Asthma & Allergy. The main criteria for asthma are nocturnal coughing [often in children], wheezing and shortness of breath besides IgE elevation & Eosinophilia **[6].**

All the patients and control groups were matched in age and sex. Asthmatic patients ' ages ranged between (6-59) years. Thirty apparent healthy individuals with age range from (11 -52)

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years. Besides this group, there was another group comprised of patients suffering from bronchitis, which is considered as a patient control group, with age ranges from (7 - 51) years.

All the data has been statistically analyzed using Semirnov-Kolmogorov test and ANOVA (Kruskal-Wallis test). While the statistical significance of difference in median between 2 groups was assessed by Mann-Whitney test **[11].**

Results:

Frequency of Asthmatic patients according to Age & Gender:

The frequency of patients in comparison with control groups was listed in Table 1.

Table 1 Frequency of Asthmatic patients & Control groups according to Gender & Age groups:

Age groups in years:	*Study Groups*					
	Asthmatic Patients		*Healthy Control*		*Patients Control*	
	No	**%**	**No**	**%**	**No**	**%**
< 16	**22**	**23.4**	**4**	**12.9**	**9**	**22**
16-29	**33**	**35.1**	**18**	**58.1**	**15**	**36.6**
30-49	**34**	**36.2**	**6**	**19.4**	**13**	**31.7**
> 50	**5**	**5.3**	**3**	**9.7**	**4**	**9.8**
Gender:	**No**	**%**	**No**	**%**	**No**	**%**
Female	**59**	**62.8**	**16**	**51**	**23**	**56.1**
Male	**35**	**37.2**	**14**	**49**	**18**	**43.9**
Total	**94**	**100**	**30**	**100**	**41**	**100**

This table shows that the majority of patients are between (30-49) years with a percentage of 36.2 % also the frequency of patients at age (16-29) is high 35.1%, though it is not high as the second group. More over it is clear that the asthmatic patients above 50 years are few (only 5.3 %). In comparison with the patient controls, the young patients and teenagers are the major number. The table revealed also the distribution of patients according to gender. It was obviously that the women are at a great risk for developing disease either asthma or bronchitis [59 asthmatic- and 24 bronchitis women] with no significant differences between their female to male ratios (1.7: 1 and 1.3: 1 respectively).

Interleukin-5 Level:

Table 2. shows that there was a highly significant difference between the IL-5 concentration in the sera of patients [mean concentration was 49 pg. / ml with a median of 45 pg. / ml], and apparent healthy control group[mean concentration was 4.6 with a median of 4.5 pg. / ml] (P value < 0.001). The same significant difference was noticed between patient controls and apparent healthy controls since the mean of IL-5 concentration in the patients controls was 64.3 pg. / ml. While no significant difference observed between asthma cases and patient or case controls levels (P < 0.98).

Table 2 Cytokines sera levels of asthmatic patients in comparison with patients and healthy controls groups:

Cytokines Concentration pg /ml	**Asthma Cases**	**Healthy Controls**	**Patient Controls**	**P value****
IL-5 Level *	45 ± 24.7	4.5 ± 1.5	33 ± 56.3	**< 0.001**
Number	35	30	9	
****P value for difference in median between:** **Asthma cases Vs Healthy Controls < 0. 001.** **Patient Controls Vs Healthy Controls < 0.001.** **Asthma cases Vs Patient Controls = 0.98 NS * = Median ± SD**				

- ***= Meadian ± SD***

Figure 2. reveals the frequency of IL-5 among the patients' sera in addition to controls. It is clear that the majority of healthy control's values are within low level in comparison with the asthma cases. While the patient controls values accumulate at middle region between the pervious other groups

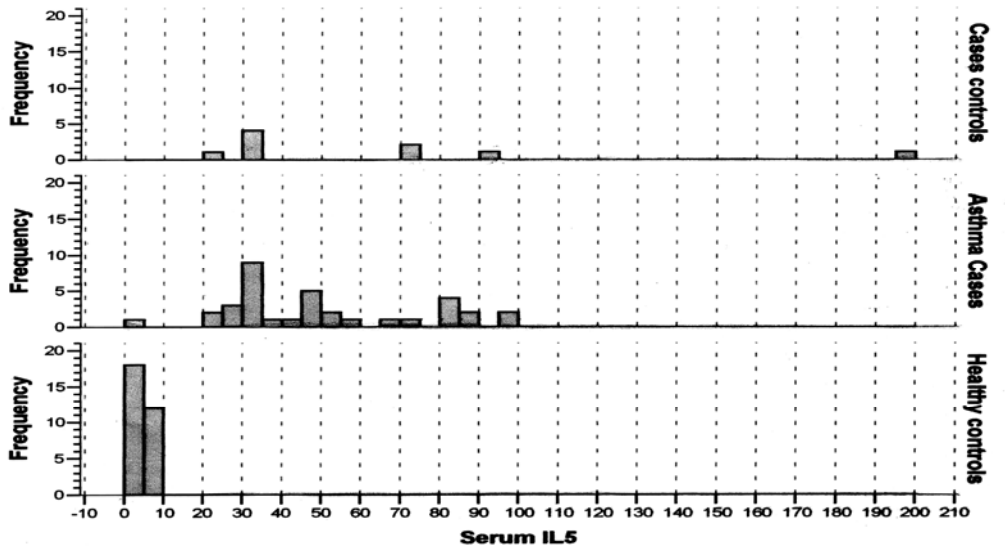

Figure 2. The frequency of IL-5 levels in the sera of Asthma patients in comparison with control groups.

The figure below (Figure 3) reveals the distribution of serum IL-5 values among asthma cases and control groups in addition to the median level for each. It is clearly that asthmatic patients and bronchitis patient control groups have comparable median results although the frequency of IL-5 was higher among asthma cases.

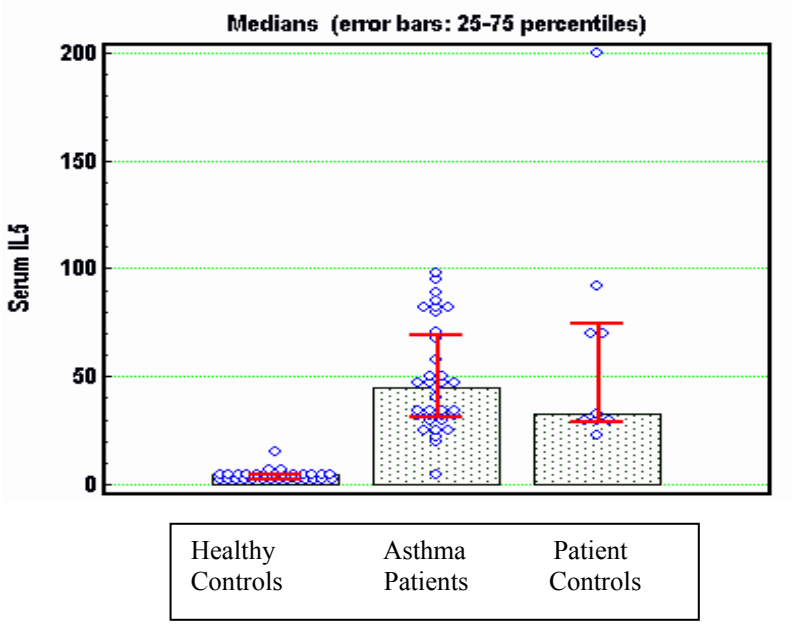

Figure 3. Dot diagram showing the distribution of values, median & interquartile range of serum IL-5 in the three study groups.

The curve below reveals that IL-5 is almost a perfect test in discriminating between asthma cases and healthy control group since ROC table (Table 3.) shows that the sensitivity of the test is 97.1 % with a specificity of 100.0 % while it's accuracy is 98.4 %. The statistical significance of the test is < 0.001 with the highest ROC Curve = 0.99. The optimal cut-off is 17.5.

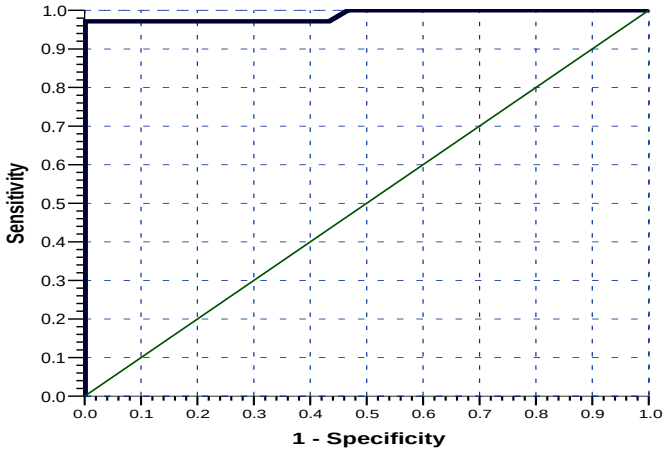

Figure 4: ROC curve showing the trade-off between sensitivity(rate of true positive results) and rate of false negative (1-specificity) of serum IL5 test in differentiating asthma cases from healthy controls. Area under the ROC curve = 0.99 P<0.001

Table 3. ROC Table for the accuracy of IL-5 in term of sensitivity & specificity

Serum IL5 Positive if ≥ Cut-off	Sensitivity	Specificity	Accuracy
1.0	100.0	0.0	53.8
2.1	100.0	3.3	55.4
2.3	100.0	6.7	56.9
2.8	100.0	13.3	60.0
3.1	100.0	16.7	61.6
3.3	100.0	20.0	63.1
3.6	100.0	26.7	66.2
3.8	100.0	30.0	67.7
3.9	100.0	36.7	70.8
4.1	100.0	43.3	73.8
4.2	100.0	46.7	75.4
4.3	100.0	50.0	76.9
4.5	100.0	53.3	78.4
4.7	97.1	56.7	78.5
4.9	97.1	60.0	80.0
5.0	97.1	63.3	81.5
5.1	97.1	66.7	83.1
5.3	97.1	70.0	84.6
5.4	97.1	73.3	86.1
5.7	97.1	76.7	87.7
5.9	97.1	83.3	90.7
6.5	97.1	90.0	93.8
11.0	97.1	96.7	96.9
17.5*	97.1	100.0	98.4
21.0	94.3	100.0	96.9
23.5	91.4	100.0	95.4
25.5	85.7	100.0	92.3
28.0	82.9	100.0	90.8
31.0	77.1	100.0	87.7
32.5	68.6	100.0	83.1
33.5	65.7	100.0	81.5
34.5	57.1	100.0	76.9
37.5	54.3	100.0	75.4
42.5	51.4	100.0	73.8
46.0	48.6	100.0	72.3
48.5	37.1	100.0	66.1
54.0	31.4	100.0	63.1

Serum IL5 Positive if ≥ Cut-off	Sensitivity	Specificity	Accuracy
63.0	**28.6**	**100.0**	**61.6**
69.5	**25.7**	**100.0**	**60.0**
75.5	**22.9**	**100.0**	**58.5**
81.0	**20.0**	**100.0**	**56.9**
82.5	**17.1**	**100.0**	**55.4**
84.0	**11.4**	**100.0**	**52.3**
87.0	**8.6**	**100.0**	**50.8**
92.0	**5.7**	**100.0**	**49.2**
96.5	**2.9**	**100.0**	**47.7**
99.0	**0.0**	**100.0**	**46.2**

Discussion:

Considering the age of disease onset it was shown that the majority of patients are above 50 years while the patient control group represents the teenage. The explanation for this variation is related to the differences between the natures of the two diseases; asthma is a hypersensitivity disease with accumulative effects due to continuous exposure to allergen, which enhance the disease development. While bronchitis is an infectious acute disease usually occurs at the childhood and teenage; the period when either the immune system to some extent is immature or the individuals at great risk to exposure to infectious agents as a results of high activity.

It is well known that the female at maximum risk for developing so many diseases particularly the immunological disorders such as autoimmune diseases. This is attributed to the hormonal differences between male and female which some of them activate T_{H2} cells. These cells enhance polyclonal B cell activation and autoantibodies formation in addition to secretion of many pro-inflammatory mediators that play an important role in inflammatory process and disease pathogenecity **[12].** These facts may explain that the majority of asthmatic patients are females rather than males.

Immunoglobulin E was observed to induce production of a variety of ***Cytokines*** **[13-15]** These cytokines are proved to play a role in asthma pathogenesis **[16-19].** Among those cytokines was ***IL-5***. Particularly IL-5 gene is called nowadays as "Colony-stimulating factor, Eosinophils" **[20].**. This study was planned to estimate IL-5 concentration in sera of asthmatic patients besides bronchitis patients and healthy controls. Interleukin-5 was observed in high level among patients' sera [45 pg /ml] in comparison with 4.3 pg /ml for healthy controls ($P < 0.001$) and [33pg /ml] for patient controls, with one high value. This value is excluded from the statistical analysis and represents abnormal cases and may be due to early allergic case in addition to bronchitis infection. These findings agree with others studies **[21, 14-15, 22]**. While Park *et al*., declared that IL-5 level was higher among normal individuals beside those mild acute and severe chronic asthma cases (35.8, 89.9, 178.7 pg /ml respectively) **[23].**. The interpretation for this variation may be related to race, genetic variation between Iraqi patients and others in addition to the duration of the disease particularly some patients [not all] have been treated with corticosteroid.

The interpretation for total serum IL-5 elevation may be related to the role of IgE in IL-5 liberation by lung mast cells & Eosinophils **[13-14, 17, 20, 22-24].** These facts may explain the tissue Eosinophilia subsequently for cytokine release **[19, 21-22, 25].** There is highly association between IgE-, Il-5- arising levels and Eosinophilia among asthma cases **[13-15, 17, 22].** This correlation with Eosinophilia and IgE has been proved by applying ROC curve for IL-5, which showed highly significance in differentiation between asthma cases from healthy controls ($P < 0.001$ for each marker) with a high sensitivity [97.1%] and perfect specificity [100%] . These results are comparable with those of abroad **[26-27].**

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