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RESEARCH ARTICLE

EVALUATION OF INTERFERON- γ LEVEL IN ASTHMA PATIENTS.

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Abstract

Background: Bronchial asthma is a chronic inflammatory disease of the airways. It manifests with recurrent episodes of coughing, breathlessness, wheezing, and chest tightness. There is a noticeable increase in health care burden from asthma in several areas of the world. In asthmatic patients the reduced production of IFN-γ has been reported.

Methodology: A total of 53 individuals were included in a comparative study. Full history was taken especially regarding the family history, disease presentation, in addition to careful physical examination. Serum IFN- γ assessment, WBC count, and Eosinophil count were measured.

Results: The study covered 37 patients with bronchial asthma, and 16 apparently healthy individual as a control group. The highest rate 27% was in the age group of (20-29) year. There was no statistical significant difference between severity of asthma and residence of the patients (p=0.893). IFN- γ was significantly higher in those with bronchial asthma in comparison with control group (p=0.00001).

Conclusion: All asthmatics have low serum IFN-γ level but high eosinophil count. Further detailed study of IFN-γ effectiveness in asthmatic patients, also carrying out a clinical trial about the use of IFN-γ in the treatment of asthma. Considering its role in treatment.

Introduction:-

Bronchial asthma is a chronic inflammatory disease of the airways [(1)]. It manifests with recurrent episodes of coughing, breathlessness, wheezing, and chest tightness. These episodes are associated with airflow obstruction [(2)], which is often reversible either spontaneously or with treatment [(1)].

Asthma is an allergic multifactorial disease, including environment and heredity, and closely related to the immunity [(3)]. The immune imbalance of Th1/Th2 cytokines is one of the key mechanisms of this disease. IL-4 and IFN-γ are the main cytokines secreted by the Th2 and Th1 cells. Nowadays, it's considered that IL-4 and IFN-γ-caused imbalance of Thl and Th2 is a key factor in the pathogenesis of asthma [(4)].

IFN-γ was originally identified as a product of mitogen-stimulated T lymphocytes that inhibited viral replication in fibroblasts. The only known sources of (Interferon gamma) IFN-γ are CD4+ and CD8+ T cells and natural killer cells [(5)]. In asthmatic patients the reduced production of IFN-γ has been reported. This suggests that defective IFN-γ

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production may be important in asthma [6], although no polymorphisms of the IFN-γ gene have been associated with asthma [7].

There is a noticeable increase in health care burden from asthma in several areas of the world [8]. Asthma is a problem worldwide, with an estimated 300 million affected individuals. the global prevalence of asthma ranges from 1% to 18% in different countries, this is due to absence of definite definition of the disease [8].

Over 100 different mediators are now recognized to be involved in asthma and mediate the complex inflammatory response in the airways [9]. Eosinophils and T-lymphocytes present in increased numbers in the airways, release specific cytokines, including Interleukin-4 (IL-4) that orchestrate eosinophilic inflammation and IgE production by B-lymphocytes [10].

The aim of the present work was to assess serum level of IFN-γ in asthmatic patients and to compare it with control group who are apparently normal.

Patients and Methods:-

Study design:
This is a comparative study.

Patients and control:
The study was conducted in Tikrit Teaching Hospital, during the period from April to the end of October 2008. A total of 53 individuals were included in this study, 37 patients and 16 control. Patients composed of (17 males and 20 females), control group consist of (11 males and 5 females). The same examinations and investigations were done for apparently healthy control group as for the diseased groups.

Clinical Examination:
Full history was taken especially regarding the family history, disease presentation, in addition to careful physical examination.

Blood Sampling:
Alcohol 70% is used for sterilization of the area of blood aspiration from cubital vein. A blood sample (5 ml) was collected from each patient and control group by using disposable needle and syringes. The collected sample was divided into two parts, (3 ml) transferred immediately into a plain tube. The blood in tube was allowed to clot at room temperature, centrifuged at 3000 revolutions per minute (rpm) for 15 minutes and the serum was then transferred to another plain tube, and stored at -20°C until the time of analysis. Samples showing hemolysis were discarded. The remaining (2 ml) of the blood sample was used to determine the total WBC and eosinophil count.

Procedures:
Kit for determination Interferon Gamma 5×96 wells: A plate of 5×96 wells, two lyophilized vials for calibration, biotinylated monoclonal antibodies (five ml vials), diluents (two 25 ml vials), streptavidin-HRP conjugate (five, 12 ml vials), wash solution (20x) (three, 50 ml vials), substrate (five, 12 ml vials), stop solution (five, 5 ml vials).

Measurement of serum IFN- γ:
Specimen processing:
Sample processing is critical for cytokines assay. Any stimulation of the cells while performing the procedure should be avoided. All sampling material must be pyrogen-free.

Collection and storage:
Most cytokines are labile molecule in biological fluids. The samples were immediately assayed or kept in plastic tubes and stored at temperature below -20°C (maximum for two months).

Dilution:
Dilute samples containing IFN- γ concentrations above 25 IU/ml in the same diluents as the one used to dilute the calibrator. From the 250 IU/ml calibrator solution and the appropriate diluents, prepare a fresh dilution series in plastic tubes prior to each assay as indicated below. This dilution series cannot be stored.

Protocol:
Three steps protocol used: in step one 50 micro liters of calibrator of sample was added per well, incubated for 2 hrs at 18-25°C while shaking, wash the wells; step two 50 micro liter of biotinylated antibody and 100 micro liter of streptavidin-HRP conjugate was added per well, incubate 30 minute at 18-25°C while shaking, Wash the wells; step three 100 micro liter of substrate was added then incubated 20 minutes at 18-25°C, after that 50 micro liter of stop solution then absorbance was read at 450 nm.

Measures:
The results are calculated by interpolation from a calibrator curve that is performed in the same assay as that sample. Draw the curve, plotting on the horizontal axis the IFN- γ concentration of the calibrator and on the vertical axis the corresponding absorbance. Locate the absorbance for each sample on the vertical axis and read off the corresponding IFN- γ concentration on the horizontal axis.

WBC count:
A sample of whole blood is mixed with White-count diluting fluid that lyses non-nucleated red blood cells. Either of the following diluting fluids may be used: (Two percent acetic acid plus 2 ml glacial acetic acid) or one percent hydrochloric acid plus one ml hydrochloric acid). Following adequate mixing, the specimen is introduced into a counting chamber where the white blood cells (leukocytes) in a diluted volume are counted. Calculate the number of WBCs per cubic mm= a total of 4 mm^2 multiple by 20 (the resulting dilution is 1:20)/4.

Blood Eosinophil Count:
Routine blood film was stained with Lishman's stain. One hundred leukocyte were counted and the percentage of eosinophils was obtained accordingly, and then multiples this percentage with the total WBC count to gate eosinophil count / ml.

Statistical Analysis:
All results were given as the mean ±standard deviation value and data analysis was performed by SPSS statistical program (version 19). Differences between cases (atopic eczema and allergic asthma) and controls were tested by using T-test and chi-square. Also ANOVA was used to calculate the relation within the groups. Any P value less than 0.05 was considered significant.

Ethical consideration:
Ethical consideration for the study has been obtained from Tikrit Teaching Hospital. A verbal consent was taken from all participants in this study.

Results:
This study included 37 patients with bronchial asthma, and 16 apparently healthy individual as a control group. The age distribution of both groups was showed in (Table-1). The highest rate 27% was in the age group of (20-29) year.

Table 1:-Distribution of study sample according to age group

Age group	Asthma	Control	Total
	No.(%)	No.(%)	No.(%)
10-19	2(5.4)	2(12.5)	4(7.5)
20-29	10(27)	5(31.3)	15(28.3)
30-39	8(21.6)	6(37.5)	14(26.4)
40-49	3(8.1)	2(12.5)	5(9.5)
50-59	8(21.6)	1(6.25)	9(17.0)
≥60	6(16.2)	0(0)	6(11.3)
Total	37(100)	16(100)	53(100)

Table-2 showed the gender distribution of patients with asthma. Seventeen patients were male, 3 (17.6%) had mild intermittent asthma, 2 (11.8%) had mild persistent asthma, 4 (23.5%) had moderate asthma and 8 (47.1%) had severe asthma. Twenty patients were female, 6 (30%) had mild intermittent asthma, 5 (25%) had mild persistent asthma, 2 (10%) had moderate asthma and 7 (35%) had severe asthma. There was no significant difference in gender distribution and severity of asthma ($P > 0.05$).

Table 2:-Cross tabulation of gender and severity of patients

Severity of the disease	Gender			Test of significance
	Male	Female	Total	
	No. (%)	No. (%)	No. (%)	
Mild intermittent	3(17.6)	6(30)	9(24.3)	Chi-sq = 2.7942
Mild persistent	2(11.8)	5(25)	7(18.9)	P-value = 0.42446
Moderate	4(23.5)	2(10)	6(16.2)	
Sever	8(47.1)	7(35)	15(40.5)	
Total	17(100)	20(100)	37(100)	

Seventeen asthmatic patients were from urban areas and twenty patients were from rural areas as shown in (Table-3). There was no statistical significant difference between severity of asthma and residence of the patients (P = 0.893).

Table 3:-Cross tabulation of gender and severity of patients

Severity of the disease	Residence			Test of significance
	Urban	Rural	Total	
	No. (%)	No. (%)	No. (%)	
Mild intermittent	5(29.4)	4(20)	9(24.3)	Chi-sq = 0.6148
Mild persistent	3(17.6)	4(20)	7(18.9)	p-value = 0.8930
Moderate	3(17.6)	3(15)	6(16.2)	
Sever	6(35.3)	9(45)	15(40.5)	
Total	17(100)	20(100)	37(100)	

Regarding the occupation of the patients, asthma was higher in housewives 17(45.9%), chi-square test was not applicable as shown in (Table-4).

Table 4:-Distribution of the study sample according to occupational status

Occupation	Asthma	Control	Total	Test of significance
	No. (%)	No. (%)	No. (%)	
Child	2(5.4)	3(18.8)	5(9.4)	Not applicable
Farmer	5(13.5)	0(0)	5(9.4)	
House wife	17(45.9)	1(6.25)	18(34.0)	
Professional	9(24.3)	12(75)	21(39.6)	
Businessman	4(10.8)	0(0)	4(7.6)	
Total	37(100)	16(100)	53(100)	

Table-5 showed mean WBC count was higher among asthmatic patients than control group this difference was found to be significant (p=0.00001). Mean eosinophil count was significantly higher in those with asthma in comparison with control group (p=0.025). IFN- γ was significantly higher in those with bronchial asthma in comparison with control group (p=0.00001).

Table 5:- Mean difference of some variables between asthmatic and control group

Variable	Asthma N=37	Control N=16	p-value*
Mean WBC (cell*10^9/l)	6619	6300	0.00001
Mean Eosinophil count (cell/ml)	3.3	2.7	0.025
Mean INF- γ (IU/ml)	1.09	0.21	0.00001
* t-test, WBC: White blood cell count, INF- γ: Gamma-Inferon			

Discussion:-

Asthmatic patients in the age groups 20-29 years and 30-39 years were higher than other age groups. This result may agree with a study conducted by Drazen, who showed that Asthma. approximately affects 5% of adult population, most cases begin before the age of 25 years but it may develop at any time throughout life [(11)].

The data demonstrated that severe asthma was significantly higher in those with positive family history than those with negative family history of atopy. These observations are in agreement with the findings of Allen et al [12], who stated that several potential gene linkages (e.g. chromosome 11 q l3) to asthma and atopy have been suggested; however, the genetic contribution to asthma remains poorly defined. It possibly involves polygenic inheritance with several genes contributing to the asthmatic tendency in any one individual, and genetic heterogeneity where different combinations of genes lead to asthma in different individuals [13].

The severity of asthma was similar in men and women in young patients and with age become more in women than men.

In this study there was no statistically significant difference between severity of diseases and residence of the patients ($P > 0.05$). This finding is in agreement with several studies of asthma and atopy in rural and urban populations that have primarily been cross-sectional surveys. The prevalence rates from these studies are difficult to compare because varying methods were used to define asthma and atopy. These studies primarily rely on self-reported questionnaire responses about wheeze, allergy symptoms, and diagnosed asthma. Even the more "clinical" measures of atopic sensitization (skin prick testing and serum IgE) and bronchial hyper-reactivity (methacholine challenge testing) are difficult to compare across studies because of varying methodologies [14].

It is also difficult to establish the timing of exposure and disease using cross-sectional studies. Several authors have suggested that the decreased prevalence of asthma and atopy observed in some studies may reflect a "healthy farmer effect" [15]. Over time, persons with asthma or atopy symptoms may self-select out of farming into other occupations with less respiratory exposures. Thus, when a cross-sectional survey is performed, asthma and atopy appear less common in the farming population because only the "healthy farmers" have remained in agriculture [16].

Also these studies of farm children have attempted to adjust for this "healthy farmer effect" by collecting information about parental history of asthma and atopy. Parental history of atopic conditions was more common among urban children in some studies, while in others it was not [17][18].
So that the result of this study appear non-significant or less sever in rural area than urban area.

According to our study, eosinophil cell count was significantly higher in those with bronchial asthma in comparison with control group. IFN- γ was significantly higher in those with bronchial asthma in comparison with control group, this mean IFN- γ increase in asthmatic patients in comparison to control group this is because use of corticosteroids therapies by asthmatic patients or may be due to the small size sample of the study. These findings were in agreement with the results of Parish and Luckhurst, who reported that T cells from airways, but not from peripheral blood, that were obtained from asthmatic subjects released mediators that promoted eosinophil chemokinesis and chemotaxis, but not neutrophil chemokinesis and chemotaxis [19].

Although IFN- γ injections are considered safe and effective for many people who have severe asthma, they aren't widely used because of their inconvenience and expense. However, if we have severe symptoms that have not responded to other treatments, injections are worth considering [20].

Conclusions and Recommendations:-

Conclusion:

Asthma is common in age group 20-29 years old. Most of patients have positive family history of atopic diseases. All asthmatics have low serum IFN- γ level but high eosinophil count.

Recommendations:

Further detailed study of IFN-γ effectiveness in asthmatic patients, also carrying out a clinical trial about the use of IFN-γ in the treatment of asthma. Considering its role in treatment.

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