Interferon-γ Level in Patients with Atopic Dermatitis

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Abstract
Atopic Dermatitis is an itchy, inflammatory skin condition with a predilection for the skin flexures. Studies have found the expression of IL-4 and decreased IFN-γ expression was more pronounced in allergen-specific T cells stimulated by various allergens. A comparative descriptive study cover 21 case of AD and 16 control individuals. The mean level of INF-γ was higher among the control than the cases of AD but there was no significant difference between the mean INF-γ level (P = 0.261). There was no significant difference in age between cases and control (P = 0.053).

1. Introduction:

Atopic Dermatitis (AD) is an itchy, inflammatory skin condition with a predilection for the skin flexures [1]. It is characterized by poorly defined erythema with edema, vesicles, and weeping in the acute stage and skin thickening (lichenification) in the chronic stage. Approximately 70% of cases of AD start in children under five years of age [2], although 10% of cases seen in hospital settings start in adults [3].

The prevalence of symptoms of AD in children six or seven years of age during a one-year period varied from less than 2% in Iran and China to approximately 20% in Australia, England, and Scandinavia [4]. A high prevalence has also been found in the United States [5]. Approximately 60% of patients with childhood AD are free of symptoms in early adolescence, up to 50% may have recurrences in adulthood [6].

1.1. Pathogenesis:

Genetic and environmental factors induce a complex series of cellular interactions leading to the symptoms and signs of AD (7). A possible mechanism is that Langerhans cells, which are surface IgE positive, present antigen to T cells leading to their activation and release of cytokines, IL-1, IL-6, IFN-γ and Tumor Necrosis Factor-a (TNF-a) (8).

Consistent with this result, studies have found the expression of IL-4 and decreased IFN-γ expression was more pronounced in allergen-specific T cells stimulated by various allergens including group I grass pollen allergen [9].

Recent studies, however, have revealed that in the chronic eczematous skin lesions, the expression of the TH1 cytokine, IFN-γ predominates compared to TH2 phenotype in the acute lesions. This has also been confirming by patch testing, when the majority of T cells were found to express IFN-γ and mRNA, also to secrete IFN-γ protein, either alone or in combination with IL-4 in late lesions [10, 11]. These results suggest that the chronic lesions are not completely TH2, but more likely a combination of TH2 and TH1 phenotypes [12].

2. Patients and Methods:

A descriptive study conducted in Tikrit Teaching Hospital, from April to the end of October 2008. A total number of (21) patients with AD were included in the study (14 males and 7 females). A control group of (16) apparently healthy individuals (11 males and 5 females) were also included. Both patients and the control group were submitted to same examination and laboratory tests.

2.1. Blood Sampling:

A (5ml) blood sample was drawn from cubital vein of each patient and control group by using disposable syringes after sterilization of the area with 70% alcohol. 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. The serum was then isolated, and stored at -20°C until the time of analysis. Samples showing hemolysis were discarded. While the (2 ml) of the blood sample was used to determine the total W.B.C. and eosinophil count. Kit for determination of Interferon Gamma 5×96 wells.

2.2. Measurement of serum IFN- γ:

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. The samples were immediately assayed or kept in plastic tubes and stored at temperature below -20°C (maximum of two month), because most cytokines are

labile molecule in biological fluids.

2.3. Protocol:
- Step l: 50 micro liters of calibrator or sample was added per well, incubate 2 hrs. at 18-25°C while shaking, wash the wells.
- Step 2: 50 micro liter of biotinylated antibody and 100 micro liters of streptavidin-HRP conjugate was added per well, incubate 30 minutes at 18-25°C while shaking, Wash the wells.
- Step 3: 100 micro liters of substrate was added then incubate 20 minutes at 18-25°C. After that 50 micro liter of stop solution then absorbance was read at 450 nm.

2.4. 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.

2.5. WBC count:
A sample of whole blood is mixed with White-count diluting fluid that lyses nonnucleated red blood cells. 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 sq. mm multiple by 20 (the resulting dilution is 1:20)/4.

2.6. Blood Eosinophil Count:
Routine blood film was stained with Lishman's stain, 100 leukocytes 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.

3. Statistical Analysis:
All results were given as the mean ± standard deviation value and data analysis was performed by SPSS statistical program (version 11.5). Differences between cases (atopic eczema) and controls were tested by using t-test and chi-square. Analysis of variance (ANOVA) was used to calculate the relation within the groups. Any P value less than 0.05 was considered as a significant value.

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

5. Results:
Age distribution of the cases (21) patients and the control group (16) apparently healthy individuals are showed in table 1. Children below nine years forming more than half of the cases 12(57.1%), among the control group 7 individual constitute (43.75%) were in the age group (10-29). There was no significant difference in age between cases and control.

Table 1: Distribution of the patients with atopic dermatitis and the control group according to age

Age group	Atopic dermatitis n(%)	Control n(%)	Total n(%)	Chi-sq test P-value
<9	12(57.1)	3(18.75)	15(40.5)	5.19 0.053
10-29	6(28.6)	7(43.75)	13(35.2)	
30-60	3(14.3)	6(37.5)	9(24.3)	
Total	21 (100)	16(100)	37(100)	

Table 2 showed that majority of the patients 19(90.5%) had family history of atopy, while all the control group had no family history of atopy (Chi-square test was not applicable). Patients came from urban 14(66.7%) were higher than those came from urban 7(33.3%) residency, the association was not significant (P = 0.851).

Table 2: Distribution of the atopic dermatitis according to the family history and residence

	Atopic dermatitis (n=21)		Control (n=16)		Chi-sq Test
	No.	%	No.	%	P-value
Family history					
Positive	19	90.5	0	0	Not
Negative	2	9.5	16	100	applicable
Residence					
Rural	7	33.3	4	25	0.03*
Urban	14	66.7	12	75	0.851
*Yate's correction					

The rate of atopic dermatitis among male was (61.9%) which was higher than the rate among female patients (38.1%) this was close to distribution among the control group (68.8%, 31.2%) for male and female respectively. The relationship was not significant (P = 0.665). This was represented in table 3 which also revealed that the atopic patients were mostly children 17(81%), this wasn't the case with the control group were the highest group were the workers 12(75%). The relationship was significant (P = 0.0002).

Table 3: Distribution of cases of atopic dermatitis according to gender and occupation

Variable		Atopic dermatitis (n=21)		Control (n=16)		Total (N=37)		Chi-sq test
		n	%	n	%	n	%	P-value
Gender	male	13	61.9	11	68.7	24	64.8	0.19
	female	8	38.1	5	31.3	13	35.2	0.665
Occupation	Child	17	81	3	18.8	31	33.7	16.9
	Housewife	2	9.5	1	6.3	25	27.2	0.0002
	Workers	2	9.5	12	75	36	39.1	

Table 4 showed the mean level of INF-y was higher among the control than the cases of AD but there was no significant difference between the mean Inferon-γ level, WBC, and Eosinophil count (P = 0.261, 0.424, and 0.163 respectively).

Table 4: Mean INF-γ, WBC, and Eosinophil count among the cases and control group in the study sample

	Atopic dermatitis (n=21)	Control (n=16)	t-test P-value
INF- γ	0.18	0.21	0.261
WBC	6414	6300	0.424
Eosinophil	3.4	2.69	0.163

6. Discussion:

6.1. Relationship between AD and Family History of Atopy:

A family history of allergic diseases is common because this is one of the most important factors predisposing a child to the development of AD. Prospective studies suggest that the risk of AD in a child approaches 50% when one parent is atopic and 66% when both parents are atopic [13]. There is tendency for atopic diseases to be more common in some families than others [168]. However, the results of this study show that AD was significantly higher in those with family history of atopy.

6.2. Relationship between Occupation and diseases:

Regarding the occupation of the patients, AD was significantly higher in children (P < 0.001). An increased risk of children developing AD was found when either parent had positive history. This association increase in strength when both parents have positive histories. The result is inconsistent with the conclusions of a large cross-sectional study in Germany, which showed that genetic contribution from each parent had additive effect [14]. Although often taken to be synonymous with genetic risk [15], a shared environment may also explain aggregation of disease in a family [16]. However, shared environmental factors have not been shown to significantly affect familial aggregation in studies which include mathematical models, although their effects cannot be entirely ignored [17, 18].

The indoor environment is a particularly important cause of asthma in housewife since allergen exposure early in life appears to be important in determining sensitization. House dust mites abound in carpets, soft furnishings and bedding, and pet-derived allergens are widespread in houses where dogs or cats are kept. Other allergens of relevance are fungal spores and cockroach allergens [18].

6.3. Comparison of mean level of Eosinophil, mean level of INF-γ, and WBC count among different study groups:

According to our study, eosinophil cell count showed no significant difference between patients with AD and the control group. The eosinophil levels roughly correlated with the disease severity, but the pattern of eosinophilia was not homogeneous. Very high eosinophil counts were common in severe cases of AD who had a personal or family history of respiratory atopy, while normal or moderately elevated counts were obtained in severe cases of pure AD who had neither a personal nor a family history of respiratory atopy. It was suggested that disease severity and personal or family history of respiratory atopy are important factors in determining high blood eosinophil levels in AD [19].

Similarly, the present study found that the absolute eosinophil count showed significant covariance with disease severity. The non-homogeneous distribution of the absolute eosinophil count was reflected in the large range and higher standard deviation. One-way analysis of variance showed a significant association of the absolute eosinophil count with a family history of AD only when both parents were affected. [20].

There was no significant difference in total WBC count among all groups, the pattern of the total WBC, neutrophil, lymphocyte and eosinophil count is predictable during the steroid treatment, active infection secondary to AD and unnecessary use of antibiotics also may be due to included small number of cases in our study give us no significant results.

7. Conclusion:

Measuring the level of IFN-γ is useful in patients with atopic dermatitis, also carrying out a clinical trial about the use of IFN-γ in the treatment of such patients to consider its usage in treatment.

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