INTERLEUKIN 23 AND INTERLEUKIN17 IN PSORIASIS, ATOPIC DERMATITIS AND LICHEN PLANUS: A SEROLOGICAL STUDY

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ABSTRACT

Introduction: The classical Th1/Th2 paradigm previously defining atopic dermatitis, psoriasis and lichen planus has recently been challenged with the discovery of Th17 that is now recognized to produce a group of distinctive cytokines such as interleukin 17 under the control of interleukin 23. **Aim:** to evaluate the role of Interleukin 23 and interleukin17 in the pathogenesis of psoriasis, atopic dermatitis and lichen planus. **Methods:** The study included three groups of patients, psoriasis, atopic dermatitis and cutaneous lichen planus each group containing 20 patients, in addition to 20 age and sex matched healthy controls. The levels of IL23 and IL17A were determined in serum samples by ELISA and correlated with the disease severity which was evaluated using specific severity index for each disease. **Results:** The serum levels of IL23 and IL17 were found to be significantly higher in the diseased groups when compared to healthy controls. The highest levels of IL-23 and IL-17 were reported in psoriasis vulgaris followed by lichen planus and the lowest levels of IL23 and IL17 were reported in atopic dermatitis. The correlation of IL23 and IL17 levels with disease severity was not statistically significant in the three groups. **Conclusion:** IL23 and IL17 may play a role in the pathogenesis of the three diseases but cannot be used as a marker of disease severity. On the other hand, the significant positive correlation between IL-23 and IL-17 in the three diseases suggests that those factors affect IL-23 will be reflected on IL-17 levels.

Key words: IL23 , IL17, psoriasis, atopic dermatitis and lichen planus

1- INTRODUCTION

Inflammatory skin diseases involve a very wide range of disorders such as psoriasis, atopic dermatitis and lichen planus. These diseases are multifactorial with complex interactions of immune responses based on a strong genetic predisposition and triggered by environmental factors **(1).**

The classical Th1/Th2 paradigm previously defining atopic dermatitis, psoriasis and lichen planus has recently been challenged with the discovery of T-helper 17 (Th17). Interleukin (IL) 23 is required for an optimal development of human Th17 cells, it is a key cytokine in bridging the innate and adaptive arms of the immune response. It is produced by sentinel dendritic cells and macrophages within a few hours after exposure to lipopolysaccharide and other microbial products**.** This in turn triggers rapid IL17 responses from tissue-resident T cells and natural killer (NK) T lymphocytes **(2).**

Most parenchymal cells express interleukin-17 receptors and signaling through these receptors induces target cells to produce pro inflammatory factors such as interleukin-6, interleukin-1, tumor necrosis factor (TNF), interleukin-8 and matrix metalloproteinases. If the IL23/IL17 immune pathway becomes dysregulated, there is a danger of severe autoimmune pathologies and chronic inflammation **(3).**

2-PATIENTS AND METHODS

2.1. Patients

Sixty patients were collected from dermatology and venereology department at Zagazig university hospitals after being consented to participate in the study. They were divided into three groups; psoriasis vulgaris, atopic dermatitis and lichen planus, twenty patients in each. Different demographic and clinical data were recorded for all patients. The disease severity was evaluated by Psoriasis Area and Severity Index (PASI) for psoriasis, SCORING AD (SCORAD) index for atopic dermatitis and visual analogue scales (VAS) for lichen planus.

Twenty age and sex matched healthy controls were recruited from health care personnel, medical students and patients present at the outpatient clinic.

Three ml venous blood samples were collected on sterile plane tube and were allowed to stand for 30 minutes at room temperature then centrifuged at 300 g for 5 minutes. Sera were immediately separated and stored at -20 C until the time of analysis.

2.2. Interleukins detection

A- IL-23 assay kit (e Bioscience INC, 1030 Vienna, Austria): Employs the quantitative sandwich

enzyme immunoassay technique. A mono-clonal antibody specific for IL-23 has been pre coated onto a micro plate. Standards and samples are pipetted into the wells and any IL-23 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for IL-23 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-23 bound in the initial step. The color development is stopped, the intensity of the color is measured be monitored by the ELISA reader at 620 nm e Bioscience.

B- IL-17A assay kit: The Koma biotech INC. (Human IL-17 Enzyme-linked Immunosorbent Assay). This assay employs an antibody specific for human IL-17 coated on a 96-well plate. Standards and samples are pipetted into the wells and IL-17 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IL-17 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IL-17 bound. The stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm Koma biotech inc.

2.3 Statistical Analysis

Data were checked, entered and analyzed by using SPSS (version 19). Data were represented as mean ± SD for quantitative variables. Number and percentage were used for categorical variables. Chi–square (X2) or Fisher exact test were used when appropriate. $P<0.05$ was considered statistically significant. T-test was used to compare means. Kruskal Wallis test was used for the nonparametric variables.

3- RESULTS

3.1. Characteristics of the studied cases

This study included sixty patients, 20 in each disease group in addition to 20 healthy controls. Descriptive and comparative statistics of the demographic data of the studied patients and controls are demonstrated in **(Table 1)**. The disease severity in all diseased groups was estimated at the time of examination using specific severity index for each disease. The Descriptive statistics of the severity index of the studied groups are shown in **(Table 2)**.

3.2. IL23 and IL17A in psoriasis, LP and AD compared with those in healthy subjects

The serum levels of IL23 and IL17A of the three diseases were significantly higher than the control group. The mean serum level of IL23 of the control group was (19 pg/ml), while it was (41.2 pg/ml) in psoriasis, (27 pg/ml) in AD and (37.5pg/ml) in lichen planus. The mean serum level of IL17A was (7.4 $\pm$ 5.3 pg/ml) in control group, (24.8$\pm$4.1 pg/ml) in psoriasis, (20.5$\pm$3.8 pg/ml) in AD and (23.3$\pm$2.17 pg/ml) in lichen planus **(Table3).**

3.3. Comparison of IL23 and IL17A in psoriasis, LP and AD

The serum levels of IL23 and IL17 in psoriasis were higher than atopic dermatitis and lichen planus but the difference between psoriasis and lichen planus was not statistically significant **(Table 3, 4)**. As regard lichen planus patients, the serum levels of IL23 and IL-17 were significantly higher in patients with both cutaneous and oral LP compared with patients of cutaneous LP only **(Table 8)**.

There was no statistical significant correlation between the serum IL-17 and IL23 levels and gender, age and disease duration. An analysis of the correlations between the serum levels of IL23 and IL17 was conducted, a significant positive correlation between the IL23 and IL17 values was found in the three groups of patients ($P < 0.05$) **(Fig. 1, 2 and 3).**

3.4. Correlation between serum interleukins levels and disease severity of the studied patients

There was no statistical significant correlation between the serum levels of IL23, IL17 and the severity of psoriasis, atopic dermatitis or lichen planus as assessed by PASI, SCORAD and VAS respectively **(Table 5, 6 and 7)**.

4-DISCUSSION

Psoriasis, lichen planus and atopic dermatitis are chronic relapsing inflammatory skin diseases that result in great morbidity for those severely affected**.** These diseases are multifactorial with complex interactions of innate and adaptive immune responses based on a strong genetic predisposition and triggered by environmental factors. The exact pathogenesis of these diseases until now is not fully determined **(1)**.

The interleukin-17 cytokines are emerging as key players in immune responses. Importantly, IL17A deregulation favors chronic inflammation, autoimmune disease and tumour development **(4).** Although it is becoming evident that many Th1 diseases including psoriasis have a strong IL17

signal, the importance of the Th17 cells in AD and lichen planus is still unclear, leading us to investigate its role in these diseases, as compared with psoriasis.

In the current study, we evaluated IL17A and IL23 serum levels in psoriasis, cutaneous lichen planus and atopic dermatitis using ELISA technique. The results revealed that the three diseases had significantly higher serum levels than the control group. Psoriasis showed higher serum levels of both IL23 and IL17 than atopic dermatitis and lichen planus but the difference between psoriasis and lichen planus was not statistically significant.

No significant correlations were found between the serum levels of IL23, IL17 and the sex, age or diseases duration of the studied groups. On the other hand, a significant positive correlation was demonstrated between the serum levels of IL23 and IL17 in the three groups of patients ($P < 0.05$). This came in agreement with **Stoma et al. (5),** who have also reported positive correlation between the serum levels of IL-23 and IL-17 in their psoriatic patients.

As regards psoriasis, several recent studies have identified high levels of IL17 and IL23 expression in skin lesions and in serum, that positively correlated with disease severity measured by PASI score **(6, 7)**. In the present study, the serum levels of IL23 and IL17 were higher than the control group; however, there was no significant correlation between the serum levels of IL23, IL17 and psoriasis severity as measured by PASI score.

Nakajima et al. (8) could not detect IL23 or IL17 in the serum of psoriatic patients, while reported higher IL22serum levels than the controls that positively correlated with PASI score. So **Nakajima et al. (8)** suggested that IL23 and IL17 might be involved in the very early phase of psoriasis development or be present only in the lesional skin.

Similarly, **Stoma et al. (5),** found no significant differences in the serum IL17 and IL23 concentrations between the psoriatic patients and control group, however, a significantly higher increase in IL22 was observed in psoriatic patients in comparison with the healthy controls that positively correlated to psoriasis severity measured by PASI. According to these findings **Stoma et al. (5),** suggested that TH17 might be more important in the very early phase of psoriasis development while Th22 role is more significant in the other phases of psoriasis. This may raise the question about the exact role of IL-17 and IL-23 in the pathogenesis of psoriasis.

Concerning atopic dermatitis, This study revealed significantly higher serum levels of both IL23 and IL17 in atopic patients compared with control healthy group but their levels were significantly lower than psoriasis, in accordance with **Yassky et al. (9)** who revealed increased numbers of TH17 cells in the peripheral blood of patients with AD and in skin lesions, however, TH17 cell production of IL-17 was found to be reduced in patients with AD compared with that seen in patients with psoriasis. These findings can be explained by the fact that the cytokines produced by TH2 cells (IL-4 and IL-13) inhibit IL-17 production from T cells, which further reduces TH17 cell activity **(10).**

Another evidence that supports the possible role of IL-23 in the pathogenesis of psoriasis as compared to AD was shown by **Boguniewicz and Leung (11)** who detected higher levels of IL23 in the skin lesions of psoriasis than those detected in AD lesions.

The results of the present study revealed that the correlation between the serum levels of IL23, IL17 and the disease severity as measured by SCORAD index was insignificant. This was not in agreement with the results reported by **Cuparri et al. (12)** who have found significant positive correlation between the serum IL-17 levels and the disease severity. This may be explained by the different age group in their study (children) versus adults in our study.

As regards lichen planus, there is insignificant numbers of studies of the serum level of IL23 and IL17 in cutaneous lichen planus. The results of the current study have demonstrated higher levels of IL23 and IL17 than the control group in accordance with previous studies **(13, 14).** The difference of the serum levels of IL23 and IL17 between psoriasis and lichen planus was not statistically significant but IL23 and IL17 were significantly higher in LP than AD. All these finding support the possible role of IL23 and IL17 in the pathogenesis of LP.

More focus on LP revealed that the patients who suffered from both cutaneous and oral LP had higher serum levels of IL23 and IL-17 than those presenting with cutaneous LP only. This was in accordance with the results reported by **Chen et al. (13)** who have found that IL23 expression level in the oral LP lesions was significantly increased compared with cutaneous LP.

No significant correlation was found between the serum levels of IL23 and IL17 and the severity of lichen planus as measured by VAS. Also, there was no significant difference of the serum levels of IL23

and IL17 between HCV positive and HCV negative patients. This finding was also observed by **Shaker and Hassan (15)** who have reported no significant difference of the serum levels of IL17 between patients with a positive history of underlying viral hepatitis infection and those with negative history of HCV infection.

In conclusion, the serum levels of IL23 and IL17 in psoriasis, atopic dermatitis and lichen planus were significantly higher than the control group but there was no correlation between the serum levels of IL23, IL17 and the clinical severity of the three diseases. From the result of this study we suggest that IL23 and IL17 play a role in the pathogenesis of these inflammatory diseases but they are not the sole cytokines in controlling these diseases.

Table (1): Demographic data of the studied patients and control group

Variable	Psoriasis	Atopic dermatitis	Lichen planus	Control	F/X2	P value
Age(years)						
Range	21-63	17-58	17-68	18-70	2.34	0.06 (NS)
X± SD	43.4±10.2	39.4±10.3	40.1±14.3	41.4± 6.4		
Gender						
Male	14 (66.7 %)	13 (63.3 %)	8 (40 %)	12 (60 %)	5.16	0.08 (NS)
Female	6 (33.3 %)	7 (36.7 %)	12 (60 %)	8 (40 %)		

NS: Non-significant (p>0.05)
F: Anova test, X2: Chi–square test

Table (2): Severity scores among the studied patients

Variable	Psoriasis n=20	Atopic dermatitis n=20	Lichen planus n=20
Score	**PASI**	**SCORAD**	**VAS**
Range	(3.40-40)	(20-61)	(0-9)
X ± SD	14±10.9	36.5±12.9	6.1±1.9
Sever	9 (44.8 %)	6 (29 %)	6 (26.7 %)
Moderate	7 (34.5 %)	11 (52.9 %)	10 (50 %)
Mild	4 (21.7 %)	3 (18.1 %)	4 (23.3 %)

Table (3): Serum levels of IL23 and IL17 among the studied subjects

Variable	Psoriasis	Atopic dermatitis	Lichen planus	Control	k	P
Il 23 (pg/ml)						
Range	(3.20- 242.7)	(1.90 - 251.1)	(15 - 196.3)	(2.2-20.4)	5.2	0.00*
X+SD	41.2	27	37.5	19		
Il 17 (pg/ml)						
Range	(15.20 - 30.1)	(16.3 - 26.5)	(17.9 - 28.7)	(0.5-13.1)	5.89	0.00*
X±SD	24.8±4.1	20.5±3.8	23.3±2.17	7.4± 5.3		

* Highly significant (p<0.01)
K: Kruskal Wallis test

Table (4): Comparison between serum interleukins levels of the studied patients

P value	Psoriasis/ Atopic dermatitis	Lichen planus/ Atopic dermatitis	Psoriasis/Lichen planus
Il23	0.02*	0.01*	0.3 (NS)
Il17	0.03*	0.00*	0.51 (NS)

* significant, NS: non significant

Table (5): Correlation between serum interleukins levels and PASI of the psoriatic patients

	Relation (r)	P value
PASl /IL23	0.09	0.63 (NS)
PASI /IL17	0.01	0.95 (NS)
IL23 /IL17	0.74	0.03*

Table (6): Correlation between serum interleukins levels and SCORAD of the atopic dermatitis patients.

	Relation (r)	P value
SCORAD /IL23	0.26	0.16 (NS)
SCORAD /IL17	0.20	0.28 (NS)
IL23 /IL17	0.54	0.02*

Table (7): Correlation between serum interleukins levels with VAS score of lichen planus patients

	Relation (r)	P value
VAS/IL23	0.26	0.16 (NS)
VAS /IL17	0.20	0.28 (NS)
IL23 /IL17	0.41	0.04*

Table (8): Serum interleukin levels of patients with cutaneous LP only and those with both cutaneous and oral LP

Variable	Cutaneous lichen planus	Combined Cutaneous and oral lichen planus	P-value
IL23(pg/ml)			
Range	(15-59.2)	(40-196.3)	
X±SD	37.4±13.4	150±41.1	0.01*
IL17(pg/ml) Range			
X±SD	(17.9-25.6)	(24.5-28.7)	
	22.6±1.9	25.6±1.45	0.01*

* Significant

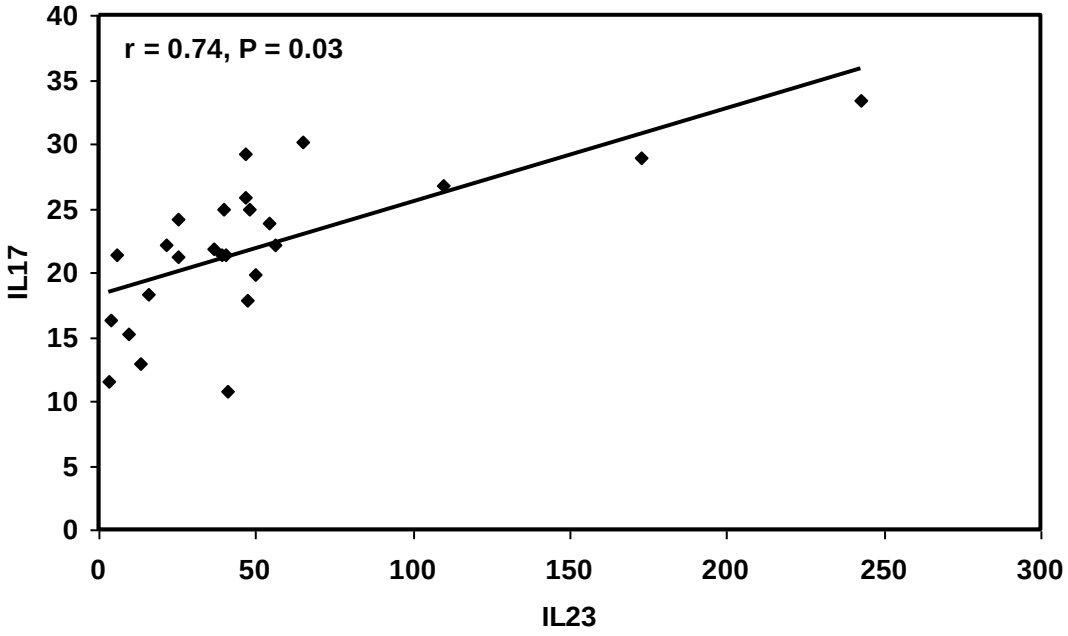

Fig. (1): Positive correlation between IL23 and IL17 in psoriatic cases.

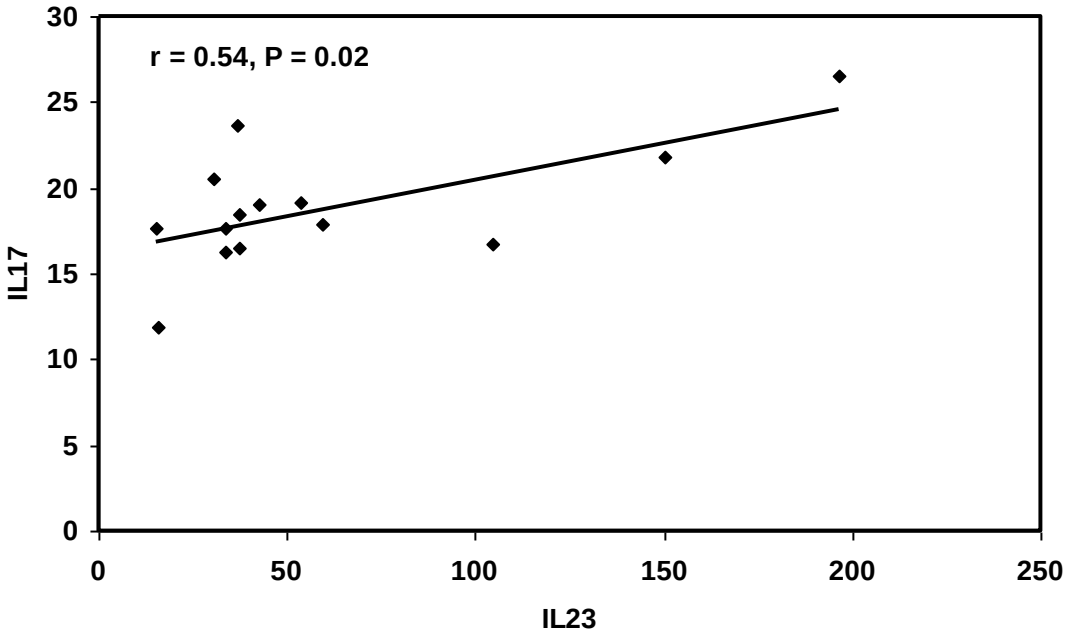

Fig. (2): Positive correlation between IL23 and IL17 in atopic dermatitis.

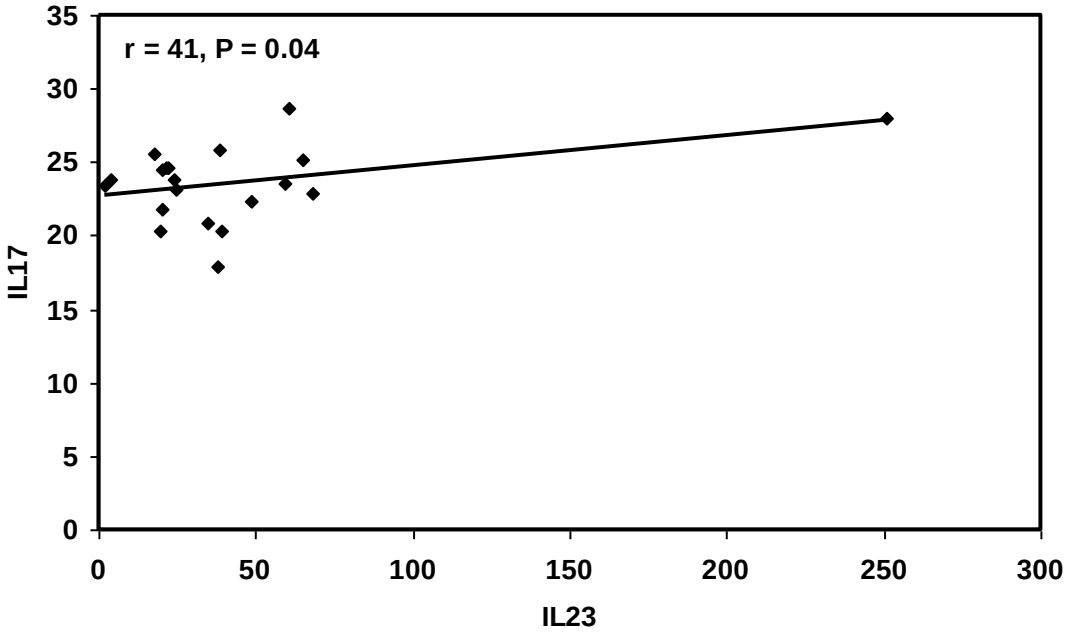

Fig. (3): Positive correlation between IL23 and IL17 in lichen planus.

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