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Evaluating the Interleukin 17 Serum Level in Psoriatic Patients from Eastern Egyptian Population

KEYWORDS: Cytokines, Interleukin 17, psoriasis, psoriasis area severity score index (PASI).

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ABSTRACT ***Objectives:*** *Cytokines released by T helper 17 (Th17) cells maintain psoriatic inflammation. The aim of the study was to determine the serum level of IL-17 in psoriatic patient and to evaluate the effect of topical treatment on IL-17 level.* ***Materials and Methods:*** *Ninety subjects from both sexes with ages ranging from 20-40 years old were instructed to apply 0.64 mg of Betamethasone Dipropionate (equivalent to 0.5 mg (0.05%) of Betamethasone) and 30 mg (3%) of salicylic acid ointment twice per day for three weeks. For scalp psoriasis, patients were advised to use 0.64 mg of Betamethasone Dipropionate, equivalent to 0.5 mg (0.05%) of Betamethasone and 20mg (2%) of Salicylic acid lotion twice per day for three weeks. Serum IL-17 concentrations were determined by ELISA on day 0 and day 21.Psoriasis area severity score index(PASI) was evaluated.* ***Results:*** *A statistically significant higher level of IL-17 ($p< 0.05$) was observed in psoriatic patients versus control before therapy. On the other hand, a statistically significant lower level of IL-17 ($p< 0.05$) was observed in psoriatic patients after immunosuppressive therapy (at day 21) as compared to IL-17 levels before therapy. No significant difference ($p> 0.05$) in psoriasis area severity score index (PASI) in patients after treatment was observed. Further analysis indicated that there was no significant correlation between serum IL-17 level and PASI Score before therapy.*

Conclusion: *IL-17 is implicated in psoriasis immunopathogenesis and its level is reduced by local application of immunosuppressive drug for 3 weeks.*

1. Introduction

Psoriasis is a chronic inflammatory skin disease affecting about 2.5% of the worldwide population [1]. It is characterized by excessive proliferation, abnormal differentiation of epidermal keratinocytes, vascular proliferation, and leukocyte infiltration in the dermis and epidermis. For long time psoriasis has been considered as a cutaneous immune-mediated disease, however, there are increasing evidences that it is a systemic inflammation associates with active skin disease [2]. While the exact cause of psoriasis has not yet identified, it is clear that combination of heritable, environmental and immunologic factors contribute to its pathogenesis. Compiled statistical and pathogenic evidences link psoriasis with multiple comorbidities such as arthritis, obesity, smoking, hypertension, diabetes, cardiovascular disease, depression and suicide attempts [3-9]. Other factors that affect the severity of psoriasis include lower health-related quality of life (HRQoL), impaired social life and reducing working productivity [10].

Genetic basis of psoriasis is supported by family and twin studies, linkage studies and population based association studies [11]. Single nucleotide polymorphism (SNP) analyses of various loci of the immune system such as the Th17 pathway, innate immunity signaling pathway, and β defensin provide collective association between psoriasis and genetic factors (reviewed in [12]. Also, SNP analyses in genes encoding interleukin IL-12B, IL-23A, and IL-23 receptor have been identified in several psoriatic populations [13, 14].

Immunological basis of psoriasis is supported by several studies. Psoriatic lesion characterized by activation of both innate and adaptive immunity leading to a response by skin cells such as keratinocytes, fibroblasts and endothelial cells. Other studies conclude that the cutaneous and systemic overexpression of several pro-inflammatory cytokines such as interleukin (IL)-2, IL-6, IL-8, IL-12, IL-17, IL-23, interferon (IFN)-γ and tumor necrosis factor (TNF)-α are responsible for initiation, maintenance and recurrence of skin lesions [11, 15-17]. In addition, the immunological role in psoriasis is also supported by the use of many biologics that target these immune components especially IL-17 in psoriasis treatment (reviewed in [11].

Interleukin IL-17 is a pro-inflammatory cytokine that belong to a family of six members (IL-17A-F) [18]. IL-17 is mainly produced by a subpopulation of T-helper-17 (Th17) cells that is distinct from Th1 and Th2 cells, although large amounts of IL-17 are also secreted by CD8+ cells (Tc17), mast cells, neutrophils, and natural killer cells [19-21]. Because of its pleiotropic activity on various tissue cells and innate immunity cells, in addition to the successful use of its monoclonal antibody in treatment of psoriasis, IL-17 is considered as a key player in the pathogenesis of psoriasis [13, 22]. The purpose of this study was to evaluate the level of IL-17 in serum collected from psoriatic patients attending Zagazig University Hospital, Egypt and the effect of local treatment on its level.

2. Materials and Methods

2.1. Patients

This study included sixty patients with chronic plaque psoriasisof both sexes with age ranging from 20-40 years oldwith mean age ± SD 30 ±10, male/female 36/24 and thirty healthy controlswith mean age ± SD 32 ± 8, male/female 17/13. All subjects were recruited from Dermatology Department, Zagazig University Hospitals.Written informed consent was obtained from all patients and control subjects.None of the psoriasis patients had received topical (e.g. corticosteroids, vitamin D analogue, etc.) or systemic (e.g. corticosteroids, methotrexate, etc.) therapy for three months time period before being involved in this study. Pregnant and lactating

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ladies or patients with other autoimmune diseases were excluded from the study.Subjects in this study were subjected to full history taking, clinical examination, liver and kidney function tests, and complete blood count. All patients were evaluated through Psoriasis Area Severity Score Index PASI [23] on day 0 before topical therapy applied. Then patients were instructed to apply 0.64 mg of Betamethasone Dipropionate (equivalent to 0.5mg (0.05%) of Betamethasone) and 30mg (3%) of Salicylic acid ointment twice/day for three weeks. For scalp psoriasis patients were advised to use 0.64 mg of Betamethasone Dipropionate (equivalent to 0.5mg (0.05%) of Betamethasone) and 20 mg (2%) of Salicylic acid lotion two times per day for three weeks.Again the PASI values were calculated at end of the therapy (day 21). After the course of the treatment any possible side effects of topical therapy (e .g skin atrophy, telangiectasia, hypo pigmentation, etc.) were evaluated.

2.2. Blood sample collection
Blood samples (3 ml) were collected under complete aseptic precautions from both control subjects and patients with chronic plaque psoriasis in plain tubes on day 0 and day 21. All subjects were asked to attend fasting.

2.3. Interleukin (IL)-17 detection
IL-17 was detected in serum by Enzyme Linked Immunosorbent Assay (ELISA) using IL-17 ELISA Kit (Catalog No.CSB-E08056h, CUSABIO, Newark, USA).

2.4. Statistical analyses
Numerical data were expressed as mean ± standard error. Statistical analysis was determined by ANOVA with paired t-test ($p< 0.05$). Data were entered, checked and analyzed using SPSS software package version (16) for windows. Results were expressed as means ± SE. T-test was used for statistical comparisons between parametric data from pairs of groups. The paired T-test was used for statistical comparison within the same group. p-values below 0.05 were considered significant.

3. Results
3.3. Plasma IL-17 assay
Blood samples were collected from psoriatic patients and control subjects at day 0 and after 21 days of treatment as well and IL-17 levels were determined in the serum using ELISA. Serum IL-17 levels were significantly higher in patients with chronic plaque type psoriasis (mean ± standard error SE is 225.2 ± 67.96 pg/ml, $p<0.05$, Table 1) compared to healthy controls (43.2 ± 23.4 pg/ml) at day 0 before the treatment. This result strongly suggests that IL-17 cytokine is a key factor in the pathogenesis of psoriasis. To investigate the correlation between IL-17 levels and the severity of the disease the psoriasis area severity score index (PASI) was calculated (Table 2 and 4). No significant correlation was found between the IL-17 level and the PASI where higher level of IL-17 was correspond to low PASI and vice versa (example subject number 6 has 503.32 IL-17 level and showed PASI of 11.6 compared to subject number 21 that had IL-17 level of 120.46 pg/ml IL-17 and showed PASI 29.7) (Table 4). This data suggests that the correlation between IL-17 and the PSAI depends on other factors such as age, sex, and health-related quality of life (HRQoL).

To test the effect of local application of the immunosuppressant drug on IL-17 level and then on the severity of the disease the psoriatic patients were subjected for Betamethasone Dipropionate topical treatment for 21 days and the IL-17 levels were estimated at the end of the treatment (Table 1). IL-17 levels were significantly reduced in patients in response to topical treatment (145.9 ± 49.4 pg/ml, $p>0.05$) compared to 225.2 ± 67.96 at day 0 suggesting that the topical corticosteroids application reduces the cytokine IL-17 level. Statistical analyses have shown that no significant correlation between the IL-17 level and the PASI Score in psoriasis patient (Table 2) was observed. To further find out if the reduction of IL-17 level affects the degree of the disease the PSAI was also calculated at the end of the therapy (Table 3 and 4). Although the levels of IL-17 were decreased significantly with the treatment the PSAI did not change significantly suggesting that either the three weeks treatment period is not enough or the dose is not sufficient or combination with other medication or interleukin suppressant is needed.

4. Discussion
Psoriasis is a chronic, relapsing inflammatory disease potentially affecting all areas of the skin, nails, and mucous membranes. The cause of psoriasis still unknown and because psoriasis affects the epidermis it was long regarded as an epidermal disease. It results from an interaction between an individual's genetic susceptibility, specific environmental factors and immunogenic response [24]. It is characterized by hyperproliferative epidermis and cutaneous lymphocyte infiltrate.T-cells involved in psoriasis pathogenesis were initially thought to be the differentiated Th1 because of the presence of elevated level of IFN-γ in psoriatic patients. Also, psoriasis is characterized by increased activation of CD4+ T lymphocytes, and systemic and local over expression of pro-inflammatory cytokines such as interleukin 2 (IL-2), gamma interferon (IFN-γ), IL-6 and tumor necrosis factor alpha, indicating that immunopathogenesis of the disease is T helper 1 (Th1)-mediated. T helper cell precursors (Thp) can be skewed towards mutually exclusive Th1, Th2, Th17 and T regulatory cell (Treg) phenotypes on the basis of the cytokine environment [25].Recent studies have showed that the Th17 cells are involved in the pathogenesis of psoriasis [26-30]. T-helper 17 cells are inflammatory CD4+ T cells that do not produce interferon IFN-γ or interleukin-4 (IL-4) but they produce IL-6, 17, 21, and TNF-α[31].

Progress in understanding of psoriasis has shown that both local and systemic cytokines collaboratively play a role in psoriasis pathogenesis.This study was designed to investigate the correlation between the serum level of IL-17 in chronic plaque psoriasis patients recruited from El-Sharkia governorate, Egypt and the severity of the disease as compared to sex and age matched control subjects. Data analysis have shown that psoriatic patients have highly significant level of IL-17 as compared to healthy subjects suggesting that cytokine might have essential pathogenic role in psoriasis pathogenesis. Similar results were obtained in other studies where high level of IL-17 were detected in psoriatic lesions.

In our study serum level of both these cytokines were significantly elevated and significantly correlated to PASI score, approving that Th17 and its cytokine might have essential pathogenic role in psoriasis pathogenesis [32, 33]. Application of the topical therapy for three weeks significantly reduced the IL-17 level in patient serum. This is in agreement with earlier results that a decrease in serum IL-17 level was obtained after topical therapy[33].On the other hand no significant changes in PASI and disease severity was observed among patients at the end of the topical corticosteroid therapy (day 21).This could be attributed to several reasons. First 21 days of topical therapy might not be enough and longer period of corticosteroid topical therapy is needed. Another possible reason is that combination with other topical treatment (e.g. Pimecrolimus or vitamin D analogue) and / or systemic treatment (e.g. methotrexate) therapy might give better improvement in the disease therapy. A more prolonged suppression of IL-17 together with other cytokines suppressors (e.g. IL-22) could have induced overt suppression in PASI. A suppression of other interleukins (e.g. IL-22 & IL-23) could have also induced more suppression of PASI [23].

The strategies to modulate the IL-17 pathway include blocking of mechanisms upstream or downstream of IL-17 or blocking IL-17 per se. With respect to targeting IL-17 per se, so far only a monoclonal anti-IL-17 antibody under investigation in patients with Crohn's disease and psoriasis that is resistant to current therapies has been reported. However, the final and detailed results of these clinical trials are yet to be published [34]. Selective inhibition of the pro-inflammatory cytokine interleukin-17A using a novel fully human monoclo-

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nal antibody (AIN457) showed considerable early promise for treatment of psoriasis. Other alternatives for achieving upstream inhibition of IL-17 are the utilization of etanercept, a TNF-receptor-fusion protein, retinoid acid or its analogs, resolvin E1, simvastatin or blocking IL-15[35].

In conclusion, in this study we showed higher level of IL-17 in psoriatic patients compared to controls before treatment. On the other hand, we found lower level of IL-17 in serum of patients with psoriasis following local treatment with corticosteroids without effect on severity of the lesion as determined by PASI. Further studies are needed to elucidate the implications of local treatment for longer time and to study the effect of combination treatment with other cytokines suppressors.

Table 1. Determination of serum IL-17 (pg/ml) in psoriatic patients and control group before and after the treatment. Data represent means ± standard error (SE). "*" represent significant (p value < 0.05)

	IL-17 (pg/ml)	
Subjects	Psoriatic group	Control group
Day 0	225.2 ± 67.96 *	43.2 ± 23.4
Day 21	145.9 ± 49.4*	39.7 ± 16.7

Table 2. Correlation between serum Interleukin (IL)-17 (pg/ml) and PASI Score in psoriasis patient group (n=60).

	T value	P value	Significance
Serum IL-17 and PASI score	0.2	> 0.05	Non Significant

Table 3. Changes in psoriasis area severity index (PASI) score before and aftertherapy in psoriatic group

Psoriasis Area Severity Index (PASI)	
Before therapy (day 0)	After therapy (day 21)
15.71 ± 5.56	14.3 ± 6.9

Table 4. Correlation between serum IL-17 (pg/ml) and Psoriasis Area Severity Index (PASI) in psoriatic patients before and after the treatment.

	Serum IL-17 (pg/ml)		PSAI score	
Subjects	Before therapy (Day 0)	After therapy (Day 21)	Before therapy (Day 0)	After therapy (Day 21)
1	359.75	125.1	20.4	20.1
2	147.96	86.54	7.8	6.9
3	372.25	279.39	5.4	4.9
4	235.46	115.46	6.8	6.2
5	274.04	234.75	51	49
6	503.32	254.75	11.6	11.2
7	344.04	196.89	10.5	10
8	250.82	97.39	10.2	10
9	164.04	156.54	9.7	8.9
10	160.82	110.46	9.2	8.9
11	231.89	210.82	23.1	22.5
12	247.96	15	19.2	18.9
13	362.96	79.75	2.7	2.3
14	189.39	165.1	4.1	3.9
15	134.75	134.39	7.4	7.1
16	167.96	23.32	7.7	7.2
16	167.96	23.32	7.7	7.2
18	445.1	53.68	27	26.6
19	497.96	388.32	15.4	15
20	216.89	92.61	8.5	7.9
21	120.46	114.75	29.7	28.9
22	133.68	107.96	22.8	21.3
23	254.39	118.32	7.1	6.8
24	178.32	67.96	15.2	14.8
25	110.64	95.11	35.6	35
26	116.18	107.96	11.7	11
27	196.89	108.32	9.3	8.9
28	226.18	216.89	5.1	5
29	108.32	70.1	36	35.9
30	196.89	59.75	10.2	10

Data from thirty patients is represented here.

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