

# The role of serum Pro- and Anti-inflammatory cytokines as potential diagnostic markers for psoriasis

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## Abstract

**Aim:** Cytokines secreted by T helper cell subgroups are considered to play chief roles in the complex pathobiology of psoriasis. Herein, we aimed to reveal the effects of anti- and pro-inflammatory cytokines on the pathobiology of psoriasis disease and the correlation of these cytokines with severity of psoriasis.

**Materials and Methods:** Thirty-seven individuals diagnosed with psoriasis and 37 healthy control subjects were enrolled for the study. The levels of Tumor necrosis factor alpha (TNF $\alpha$ ), Soluble CD40 ligand (sCD40L), Interferon gamma (IFN $\gamma$ ) and Interleukin (IL) 1 $\beta$ , IL4, IL6, IL10, IL17F, IL17A, IL21, IL22, IL23, IL25, IL31, IL33 were determined by the Luminex method.

**Results:** The IL6, IFN $\gamma$ , TNF $\alpha$ , sCD40L, IL17F, IL17A, IL23, IL25, and IL31 levels were found to be markedly advanced in individuals with psoriasis in contrast to the control group (p=0.003, p=0.02, p<0.001, p=0.02, p=0.03, p=0.003, p=0.04, p=0.04, and p<0.001, respectively). No significant interrelation was found between the serum cytokine levels and severity of psoriasis (p>0.05).

**Conclusion:** IFN $\gamma$ , IL6, TNF $\alpha$ , sCD40L, IL17F, IL17A, IL23, IL31, IL25 and inflammatory markers were markedly advanced in patients with psoriasis, strongly supporting the notion that these cytokines are involved in the pathobiology of psoriasis disease. In conclusion, findings of the present study suggest that understanding of the functions of these cytokines in the complex pathogenesis of psoriasis disease is of great interest for the future therapeutic intervention.

**Keywords:** Cytokine; luminex; psoriasis; T helper

## INTRODUCTION

Psoriasis is a multifactorial inflammatory skin problem which is distinguished by chronic, recurrent, erythematous, squamous plaques (1). The most prevalent clinical form of psoriasis is chronic plaque psoriasis. The prevalence and severity of this disease varies from patient to patient. Sometimes it can occur in different clinical forms in the same patient in the time. It often progresses with lesions on the scalp, knee, elbow, sacral area and extensor face of the joints. Joint and nail involvement can also be seen in psoriasis (2). The treatment decision is made based on the scores of the psoriasis area severity index (PASI), body surface area, dermatology life quality index (DLQI). PASI is a method of grading the indications of psoriasis, such as scaling, erythema, and induration according to their anatomical localization (3).

In the last few decades, substantial improvement was made in elucidating the pathobiology of psoriasis through

research. It is considered that T cells play central roles in autoimmune condition of psoriasis disease and cytokines are associated with these cells (4). In recent studies, T helper (Th) cells, by stimulation of antigen presenting cells such as dendritic cells and macrophages, have been shown to be involved in psoriasis immunopathogenesis by differentiating into Th1 cells producing IFN $\gamma$  and Th17 cells producing IL17. The different populations of CD4 T cells are characterized in particular by their cytokine secretion profiles. Th1 and associated cytokines (IFN $\gamma$ , IL12, and TNF $\alpha$ ) are involved in the activation of the disease while Th2 and associated cytokines (IL4, IL5, and IL10) have protective roles in the disease immunopathogenesis (1,5,6).

Accordingly, herein we aimed to demonstrate the Th17 serum cytokine levels of plaque psoriasis patients and healthy controls and decipher the association between cytokine levels and PASI scores.

**Received:** 13.07.2020 **Accepted:** 23.11.2020 **Available online:** 22.12.2020

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## MATERIALS and METHODS

### Experimental and control groups

The experimental group consisted of 37 individuals that were diagnosed with plaque psoriasis in the Dermatological and Venereal Diseases Clinic of Tekirdağ Namık Kemal University Health Application and Research Center or that presented to this clinic with a pre-diagnosis of the same condition without having received any systemic or topical treatment within the last month. Furthermore, Age and gender-matched healthy volunteers (n=37) and patients with plaque psoriasis (n=37) were enrolled for the present study. The study was reviewed and approved by the Non-Interventional Clinical Research Ethics Committee of the Tekirdağ Namık Kemal University (Ethics Committee approval no: 2015/104/09/10). The participation was voluntary, and all the patients and the control subjects delivered written informed consent.

### PASI scoring

The prevalence-severity of the disease was determined using the PASI scoring method. In the evaluation of severity, a four-point scale was used (0=no symptom, 1=mild symptoms, 2=moderate symptoms, 3=severe symptoms, 4=most severe symptoms).

### Determination of serum cytokine levels

The peripheral blood samples (5-10 ml) were obtained from the individuals with psoriasis and the control subjects and sera was separated. The samples were maintained at -86°C until use. The levels of sCD40L, TNF $\alpha$ , IL1 $\beta$ , IL4, IL6, IL10, IL17F, IL17A, IL21, IL22, IL23, IL25, IL31, IL33, and IFN $\gamma$  were analyzed with the Luminex method using a Bio-Plex Pro Human Th17 Cytokine Panel kit (BIO-RAD laboratories, USA) following the manufacturer's recommended protocols.

### Statistical analysis

The data was evaluated bi-directionally at the 95% confidence interval. In this process, first, the descriptive statistics, including the frequency, mean and standard deviation values were calculated. Afterwards, when the parametric test assumptions were met, a Student's t-test was employed to compare the two groups (patient and control), chi-square analysis for the comparison of categorical data, and Pearson correlation test for the assessment of the relationship between the variables. All statistical analysis was performed on a computer using SPSS-PASW Statistics v. 18.0 software package.

## RESULTS

Of the 37 patients with plaque psoriasis, 11 (29.7%) were females and 26 (70.3%) were males. The mean age of the psoriasis patients was 40.46 $\pm$ 14.64 (age range 14-65). Since the age and gender of the individuals in the control group (n=37) were matched with those of the patient group, there was no statistically marked alteration between the groups in terms of these variables (p>0.05) (Table 1).

The average PASI score of patients was 5.77 $\pm$ 3.65 (1-15.7). The protein expression levels of IL6, TNF $\alpha$ , IFN $\gamma$ , sCD40L, IL17F, IL17A, IL25, IL23 and IL31 were markedly elevated

in the patients with psoriasis in comparison to the control group. However, there was no marked alteration between the two groups concerning the mean serum values of IL1 $\beta$ , IL10, IL4, IL21, IL22 and IL33 (Table 2).

**Table 1. Demographic data in experimental and control group**

Variables	Experimental Group (n=37)	Control Group (n=37)	P
Age	40.46 $\pm$ 14.64	41.67 $\pm$ 14.43	
Sex (F/M)	11/26	11/26	p>0.05
PASI	5.77 $\pm$ 3.65	-	

The values are presented as mean $\pm$ SD; F: Female M: Male

**Table 2. The serum cytokines levels in psoriasis patients and healthy controls**

Cytokines (pg/ml)	Experimental Group (n=37)	Control Group (n=37)	p
IL6	40.03 $\pm$ 15.28 (22-88, 36.0)	31.23 $\pm$ 8.80 (20-56)	0.003*
IFN $\gamma$	8.82 $\pm$ 1.13 (7-11, 9.0)	8.22 $\pm$ 1.10 (6-11, 8.0)	0.02*
TNF $\alpha$	32.34 $\pm$ 9.68 (21-59, 29.0)	24.96 $\pm$ 5.86 (17-46, 24.0)	<0.001*
IL17A	40.66 $\pm$ 5.26 (33-53.5, 40.0)	38.09 $\pm$ 5.04 (31-54, 37.0)	0.03*
IL17F	18.88 $\pm$ 3.64 (14-34, 18.0)	16.54 $\pm$ 2.73 (12-22, 16.0)	0.003*
IL23	33.89 $\pm$ 8.02 (18-48, 35.0)	29.38 $\pm$ 10.82 (13-54, 28.0)	0.04*
IL25	11.05 $\pm$ 1.18 (9-13, 11.0)	10.49 $\pm$ 1.24 (8-13, 10.0)	0.04*
IL31	17.54 $\pm$ 3.19 (13-24, 17.0)	14.42 $\pm$ 2.23 (10-19, 14.0)	<0.001*
sCD40L	1064.20 $\pm$ 410.52 (238-2032, 1078.0)	506.86 $\pm$ 280.22 (118-1308, 471.0)	0.02*
IL1 $\beta$	29.57 $\pm$ 33.09 (11-151, 18.0)	19.51 $\pm$ 11.48 (11-65, 15.2)	0.10
IL4	44.27 $\pm$ 30.91 (14-184, 35.0)	35.38 $\pm$ 21.42 (14-93, 26.0)	0.15
IL10	15.46 $\pm$ 6.19 (11-49, 14.0)	15.43 $\pm$ 5.95 (10-42, 14.0)	0.98
IL21	14.39 $\pm$ 3.00 (10-20, 14.0)	13.14 $\pm$ 3.62 (9-21, 12.0)	0.11
IL22	15.11 $\pm$ 1.93 (11-19, 15.0)	14.15 $\pm$ 2.42 (10-22, 14.0)	0.06
IL33	32.49 $\pm$ 28.03 (10-138, 23.0)	26.36 $\pm$ 17.88 (9-74, 18.5)	0.32

The values are presented as mean $\pm$ SD; \* Statistically significant Interleukine (IL) 6, 17A, 17F, 23, 25, 31, 1 Beta ( $\beta$ ), 4, 10, 21, 22, 33; Interferon gamma (IFN $\gamma$ ), Tumor necrosis factor alpha (TNF $\alpha$ ), Soluble CD40 ligand (sCD40L)

In this work, no marked interrelation was determined between the serum cytokine levels and PASI scores ( $p>0.05$ ). Additionally, using the PASI score as a cut-off point, no difference was observed in the serum cytokine levels between the patients with a PASI score of  $<10$  ( $n=32$ ) and those with a PASI score of  $\geq 10$  ( $n=5$ ). Lastly, no correlation existed between PASI scores and cytokine levels ( $p>0.05$ ).

## DISCUSSION

Th cells responsible for inflammation in psoriasis, a T cell-dependent autoimmune disease, are known to consist of two subgroups, Th1 and Th2. However, the presence of new Th cell subtypes, Th17 and Th22, has also been reported to have an active role in the molecular pathogenesis of psoriasis disease through the cytokines they secrete (7).

In a previous study conducted by Buyukkara Yilmaz et al. with 70 psoriasis patients, the serum IL17 levels were found to be high in patients having plaque psoriasis and a PASI score of  $\geq 10$  compared to other psoriasis groups and the control group (8). In consistent with these results, we observed that the serum levels of IL17F and IL17A in patients with psoriasis were markedly enhanced in contrast to control group ( $p=0.03$  and  $p=0.003$ , respectively). In contrast, in the current study, when serum cytokine levels were compared according to the patients' PASI scores being  $<10$  ( $n=32$ ) or  $\geq 10$  ( $n=5$ ), no statistically marked difference was found ( $p>0.05$ ).

Ma and colleagues reported higher serum IL23 levels in patients with psoriasis disease compared to healthy control subjects (9). Brito-Luna et al. found no marked alteration in the IL23 and IL22 levels between 55 psoriasis patients and the healthy control group and the authors also determined no interrelation between the cytokine levels and PASI scores (10). In the current study, the serum IL23 levels of the patient group were markedly increased in psoriasis patients in contrast to healthy controls ( $p=0.04$ ), but no significant alteration was found in the serum IL22 levels ( $p=0.06$ ). These results support the knowledge that IL23 is a critical cytokine in the pathobiology of psoriasis disease and underlines the importance of undertaking further advanced molecular studies to elucidate the role of IL22.

Contradictory results have also been obtained in many studies investigating the relationship between TNF $\alpha$  levels and psoriasis severity. In the study with 34 psoriasis patients, Bulur et al. showed increased IFN $\gamma$ , TNF $\alpha$ , IL23 and IL17 levels in lesional serum samples (11). In the current study, the serum TNF $\alpha$  and IFN $\gamma$  levels were found markedly elevated in the psoriasis patient group in contrast to the controls ( $p<0.001$  and  $p=0.02$ , respectively). These results further support the notion that these cytokines are an important contributor to chronic inflammation in psoriasis (12).

IL1 $\beta$ , also known as the Th1 cytokine, have important roles in the pathobiology of the psoriasis disease (13). Bonifati et al. also reported increased levels of IL1 $\beta$  in the lesional

extracts of the skin than in the non-lesional samples (14). However, in our study group, no marked alteration was determined in the IL1 $\beta$  serum levels between the psoriasis patients and the control group ( $p=0.10$ ). The low profile of IL1 $\beta$  in our study can be attributed to the predominance of patients diagnosed with mild plaque psoriasis.

Also, in our study, there was no marked alteration in the IL21 serum levels between the experimental and the healthy volunteers ( $p=0.10$ ). Our observations related to IL1 $\beta$  and IL21 are supported by the results of a recently published meta-analysis study (15).

In a previous study by Erturan et al. in 2014, the plasma sCD40L levels in psoriasis patients were found to be markedly advanced as compared to healthy controls (16). In line with the previous observations, in the current study, the sCD40L levels were markedly increased in patients with psoriasis disease.

IL4, a Th2-related cytokine, plays a protective role in the complex pathogenesis of psoriasis by reducing the effect of IFN $\gamma$  and inhibiting cell-mediated immunity (6). Verghese et al. reported higher serum levels of IL4 in a study group consisting of 30 psoriasis patients as compared to the control group, but this elevation was not significant (17). In the present work, supporting the earlier studies in the literature, no significant change was determined between the patient and control groups in terms of the mean of IL4 serum levels ( $p=0.15$ ).

Although many studies showed elevated IL10 levels in patients with psoriasis than in the healthy groups, Borska et al. showed that these levels were increased in psoriasis patients than in controls and suggested that this unexpected increase in IL10 levels may result from the interplay between anti- and pro-inflammatory cytokines during the inflammatory response (18). In the current study, the IL10 levels in psoriasis patients were only slightly higher when compared to the healthy controls, with no marked change between the two groups ( $p=0.98$ ).

Arican et al. found that the IL6 serum levels of psoriasis patients were statistically higher than those of the control group (19). Similarly, in our study, we determined that the IL6 levels were statistically higher in patients with psoriasis in contrast to the members of the control group ( $p=0.003$ ). These results in the literature explain the role of IL6 in the disease pathogenesis.

Senra et al. reported that keratinocytes in the psoriasis plates were the most important source of IL17E, and this keratinocyte-derived cytokine played a proinflammatory role by activating macrophages (20). In the current study, the IL17E levels of the patients were markedly higher in comparison to the control group ( $p=0.04$ ).

Narbutt et al. found significantly higher concentrations of IL31 in psoriasis patients compared to controls and suggested that this cytokine was involved in the pathogenesis of psoriasis (21). Similarly, we found that the mean serum levels of IL31 were markedly higher in the psoriasis patients in contrast to the control group ( $p<0.001$ ).

A growing mass of indication suggest that serum IL33 levels elevated in Th1/Th17-mediated diseases. Mitsui et al. demonstrated that the serum IL33 levels were markedly higher in psoriasis patients than in the healthy control group (22). In contrast, we found no marked alteration in the serum levels of IL33 between the experimental and control groups ( $p=0.32$ ).

## CONCLUSION

In the current study, the serum levels of IL6, IFN $\gamma$ , TNF $\alpha$ , IL17F, IL17A, IL23, IL25, IL31 and sCD40L cytokines to be higher in the psoriasis patients in contrast to the control subjects indicates that cytokines, especially those secreted by Th1 and Th17 cells, may be associated with this disease. The similar findings obtained from the psoriasis patients with a PASI score of <10 and  $\geq 10$  suggest that this study should be repeated with larger groups of patients.

In conclusion, our findings revealed, to a certain extent, the role of Th1/Th2/Th17 cytokines in the pathogenesis of psoriasis, which is considered to be a T-lymphocyte-mediated autoimmune inflammatory disease; however, further more comprehensive studies involving big cohorts are needed to better understand the association of these cytokines with PASI.

*Conflict of interest : The authors declare that they have no competing interest.*

*Financial Disclosure: This work was supported by the Tekirdag Namik Kemal University Scientific Research Projects Commission (NKUBAP.02.GA.16.016).*

*Ethical approval: The study was reviewed and approved by the Non-Interventional Clinical Research Ethics Committee of the Tekirdag Namik Kemal University (Ethics Committee approval no: 2015/104/09/10).*

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