Peripheral Whole Blood FOXP3 TSDR Methylation and Plasma IL-10, IL-17, IL-35, and IL-36 Levels in Psoriasis

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ABSTRACT

Recent studies have shown that methylation levels in regulator T-cells, which are characterized by high FOXP3 expression, pro-inflammatory, and anti-inflammatory cytokines are involved in the pathogenesis of psoriasis. Our aim was to determine the levels of FOXP3 TSDR methylation, FOXP3 mRNA expression, and levels of IL-10, IL-17, IL-35, and IL-36 in patients with psoriasis. In addition, we intended to investigate the relationship of these parameters with disease severity and treatment status. We performed a hospital-based case-control study on 38 patients with psoriasis and 20 controls. We performed HRM-PCR, real-time PCR, and ELISA to determine FOXP3 TSDR methylation status, FOXP3 mRNA expression, and interleukin levels, respectively. FOXP3 TSDR was found to be methylated both in the patient and control groups. There was no significant difference between the melting temperatures and FOXP3 mRNA levels between the groups. Among the cytokines, only IL-10 was found to be significantly lower in patients. Positive correlation was found between IL-35 levels and psoriasis area and severity index (PASI) scores in all patients; IL-17A levels and PASI scores in patients with mild psoriasis, and IL-36γ levels and PASI scores in patients with moderate-to-severe psoriasis. The production of IL-10 seems to be impaired in patients with psoriasis. Positive correlation between the levels of IL -35, IL-17A, and IL-36γ and disease severity supports the clinical implication of these cytokines in psoriasis.

KEY WORDS: psoriasis, regulator T-cell, FOXP3, methylation, interleukin

INTRODUCTION

Psoriasis is a common, immune-mediated inflammatory disease characterized by red-colored plaques with well-defined borders and silvery-white dry scales on the skin. Because of its chronic process, the disease often leads to a significant impaired quality of life and dissatisfaction with existing therapeutic approaches. Although it has been long known that genetic, environmental, and immunological conditions

have a triggering effect on the onset of psoriasis, the exact immunopathogenesis of the disease has not yet been fully understood (1).

Regulator T (Treg) cells are a subset of T-cells that are specialized to modulate the activity of effector T-cells and suppress immune responses via various signaling mechanisms and cytokines, such as transforming growth factor beta (TGFβ), interleukin (IL)-10, and IL-35 (2). Treg cells are characterized by high levels of FOXP3 (forkhead box protein 3) expression, the transcription factor that is required for their development and function (3). Epigenetic modifications in the highly conserved, non-encoded regions of the FOXP3 gene, such as the promoter, TGFβ-sensitive element, and Treg-specific demethylated region (TSDR), are important in the regulation of FOXP3 transcription. Stability of FOXP3 expression is associated with TSDR methylation, which is a CpG-rich and conserved intronic region (4). Since it is an enhancer region, TSDR hypomethylation is important for Treg cell lineage characteristics and works to enhance the effect of FOXP3 transcription by recognizing and binding transcription factors (5).

Epigenetic studies have demonstrated that DNA methylation in Treg cells has a role in the pathogenesis of psoriasis (6,7). Ngalamika *et al.* have shown that determining FOXP3 TSDR methylation status can be a useful tool in the diagnosis and monitoring of the severity of autoimmune diseases such as systemic lupus erythematosus and psoriasis. Based on the results of their study, they have suggested the use of high-resolution melt-polymerase chain reaction (HRM-PCR) to detect FOXP3 TSDR methylation status as a reliable and easy method to predict natural Treg cell levels in peripheral blood in different disease conditions (7).

Recent advances have shown that a complex network of pro-inflammatory and anti-inflammatory cytokines are involved in the pathogenesis of psoriasis, and serum concentrations of certain interleukins are also associated with the severity of the disease. The rapid progress in understanding the central role of these interleukins in the pathogenesis of psoriasis opened up new prospects in the therapy of this difficult-to-treat disease (8). It has been shown that the levels of IL-17 and IL-36, the two pro-inflammatory cytokines which increase the secretion of each other, are elevated in psoriasis (9-12). In contrast, as anti-inflammatory cytokines, IL-10 and IL-35 have immunosuppressive effects on cytokines and have been suggested as the potential targets for the treatment of autoimmune diseases (13,14). Even though pro-inflammatory cytokines and anti-inflammatory cytokines are thought to be related to the pathogenesis of psoriasis, there are contradictory results about circulating levels of IL-10 and IL-17, and only few studies have investigated circulating levels of IL-35 and IL-36 in psoriasis.

In this study, we aimed to investigate the levels of FOXP3 TSDR methylation, FOXP3 mRNA expression, and levels of IL-10, IL-17, IL-35, and IL-36 in patients with psoriasis. In addition, we intended to investigate the association between these factors and the severity of the disease.

PATIENTS AND METHODS

Study design

This was a hospital-based case-control study that include 38 patients with psoriasis and 20 control subjects who were consecutively admitted to the Department of Dermatology, Dokuz Eylul University Faculty of Medicine, Turkey, between February 2017 and November 2017. The study was approved by the Non-interventional Ethics Committee of Dokuz Eylul University, which follows the guidelines set by the Helsinki Declaration (approval number: 2015/24-25).

The diagnosis of psoriasis was based on clinical findings confirmed with biopsy. Exclusion criteria were age <18 years old and pregnancy. Apart from psoriasis, neither patients with psoriasis nor control participants had any other chronic inflammatory dermatological disorder. Each participant provided written informed consent prior to the examinations. After signing the informed consent, all cases and controls were visited by a dermatologist (OO) who registered clinical and sociodemographic findings and the other relevant data on a case report form.

The severity of chronic plaque psoriasis was graded according to PASI. On the basis of the PASI score, patients were stratified into two groups: mild disease (PASI ≤10) and moderate-to-severe disease (PASI >10).

FOXP3 TSDR DNA methylation analysis

Genomic DNA was isolated from 200 µL whole blood using the GeneJET Genomic DNA Purification Kit (Thermo Scientific). The concentration and purity of DNA samples were measured using a NanoDrop 2000 Spectrophotometer (Thermo Scientific). Bisulfite conversion of 400 ng genomic DNA was performed with the EpiJET Bisulfite Conversion Kit (Thermo Scientific) according to the manufacturer's guidelines. After the bisulfite conversion, 20 ng bisulfite converted DNA was used in the HRM-PCR to detect methylation status of FOXP3 TSDR in samples. In order to check whether bisulfite conversion was performed properly, EpiTect PCR Control DNA Set (Qiagen) containing unmethylated, bisulfite converted methylated and bisulfite converted unmethylated human genomic DNA served as

the methylation control. HRM-PCR was performed on a Rotor-Gene Q real-time machine (Qiagen) using the Luminaris Color HRM Master Mix (Thermo Scientific) according to the manufacturer's instructions. Validated primers (Human FOXP3 Methylation Assay – ADS783, EpigenDx) were used for the amplification of the intron 1 TSDR of the FOXP3 gene. The 10 µL reaction volumes contained a 5 µL master mix, 0.5 µL of each primer, and 4 µL of template. All samples were analyzed in triplicate with an initial denaturation at 95 °C for 10 min, followed by 45 cycles at 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 20 s. Melting was performed by increasing the temperature from 70 to 95 °C in 0.1 °C increments.

FOXP3 expression analysis

Total RNA was isolated from 300 µL whole blood using the Total RNA Mini Kit (Blood/Cultured Cell) (Geneaid). The concentration and purity of RNA samples were measured using a NanoDrop 2000 Spectrophotometer (Thermo Scientific). Complementary DNA (cDNA) synthesis was performed using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's instructions. Total RNA (1 µg) was transcribed into cDNA with Oligo $(dT)_{18}$ primer.

Real-time PCR was performed on a LightCycler 480 real time machine (Roche) to determine the relative expression level of FOXP3. Maxima SYBR Green qPCR Master Mix (Thermo Scientific) was used to determine the relative fold change in the expression of FOXP3 as the target gene and *β-actin as* the reference gene. The following primer sequences were used: 5′-CTGACCAAGGCTTCATCTGTG-3′ (FOXP3 forward), 5′-ACTCTGGGAATGTGCTGTTTC-3′ (FOXP3 reverse), 5′-AGTTGCGTTACACCCTTTCTTG-3′ (β-actin forward), 5′-TCACCTTCACCGTTCCAGTTT-3′ (β-actin reverse) (15). The 20 µL reaction volumes contained 10 µL master mix, 0.6 µL of each primer, 5 µL (125 ng) cDNA, and 3.8 µL nuclease-free water. All samples were analyzed in triplicate with an initial denaturation at 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. The cycle threshold (C_t) values of FOXP3 were averaged and normalized against the C_t values of *β-actin*. The $2^{-\Delta\Delta C}{}_t$ method was performed for relative quantification of gene expression (16). PCR products were analyzed using agarose gel electrophoresis to confirm amplification of the expected size fragment.

Analysis of plasma IL-10, IL-17A, IL-35, and IL-36γ concentrations

Peripheral blood samples were collected into tubes containing EDTA and centrifuged at 1000 × g for 15 min at 4 °C within 30 min of collection. Quantification of human IL-10, IL-17A, and IL-36γ in the plasma samples was performed according to the manufacturer's instructions using the Human IL-10, Human IL17A, and Human IL-36G (IL-1F9) ELISA Kits (Thermo Scientific), respectively. IL-35 was measured using the Human IL-35 (Interleukin 35) ELISA Kit (Elabscience). Absorbance was measured at 450 nm using a Synergy HT Microplate Reader (BioTek). All samples were measured in duplicate.

Statistical analysis

The statistical analyses were performed with the SPSS/PC software (Version 20.0 for Windows; SPSS Inc., Chicago,Ill). The Kolmogorov-Smirnov test was used to examine the normality of the distribution of the data. Continuous variables were expressed as median and interquartile ranges, and categorical variables were expressed as frequency and percentage. The Mann Whitney U-test was used to determine the difference between the groups. The correlation between the results was determined using Spearman's correlation analysis. $P<0.05$ was considered significant in all analyses.

RESULTS

Clinical assessment

The median ages in patients and controls included in this study were 51 years (quartiles: 33.5-58.0) and 34 years (quartiles: 30-45), respectively. Seventeen (44.7%) of the patients with psoriasis and seven (35%) of the control subjects were female. The median time with psoriasis and psoriasis area severity index (PASI) scores were 9.2 years and 7.6 (min-max: 1.2-46.0), respectively. Thirty-six (94.7%) patients had chronic plaque psoriasis, and the remaining two (5.3%) had palmoplantar pustular psoriasis. Twenty-one of (58.3%) the patients with chronic plaque psoriasis had mild disease, while the remaining 15 (41.7%) had moderate to severe disease. With regard to treatment status, 12 (31.6%) patients were using topical corticosteroids and 4 (10.5%) patients were using systemic antipsoriatic medications (acitretin in two patients, cyclosporine in one patient, and methotrexate in one patient).

Methylation of FOXP3 TSDR

The melting temperatures of the methylation standards which were unconverted unmethylated human genomic DNA, bisulfite converted unmethylated human genomic DNA and bisulfite converted methylated human genomic DNA were 78.43, 78.36, and 81.14, respectively. The difference between the

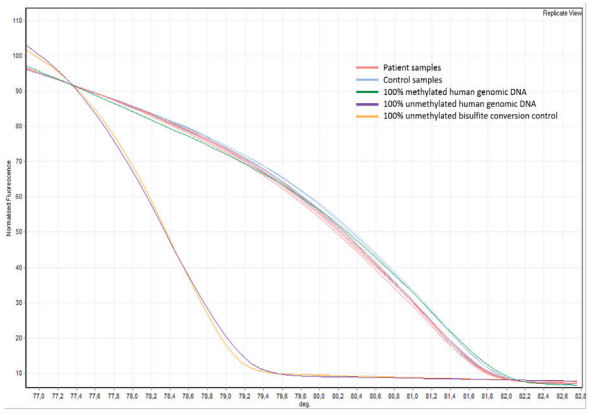

Figure 1. Normalized standard and sample curves from the high-resolution melting analysis.

melting temperatures of methylated and unmethylated human genomic DNA samples showed that the bisulfite conversion and HRM PCR were properly performed. All of the control and patient samples were completely methylated when they were compared with the methylation standards (Figure 1). There was no significant difference in the melting temperature between healthy controls (median 81.17, min-max 80.88-81.33) and patients with psoriasis (median 81.10, min-max 80.78-81.26) (*P*=0.174). Since all the samples were fully methylated, there were no statistical differences in the melting temperatures with regard to disease severity (*P*=0.222), treatment status (*P*=0.178), and associated diseases (*P*=0.118) between the groups.

Expression of FOXP3

There was no significant difference in the relative expression levels between the patients and controls (fold-change median 1.20, min-max 0.31-2.56 vs. median 0.96, min-max 0.64-1.54, *P*=0.242). With regard to disease severity, there was no significant difference between patients with mild psoriasis and moderate-to-severe psoriasis (*P*=0.665). Additionally, no significant differences were detected the use of antipso-

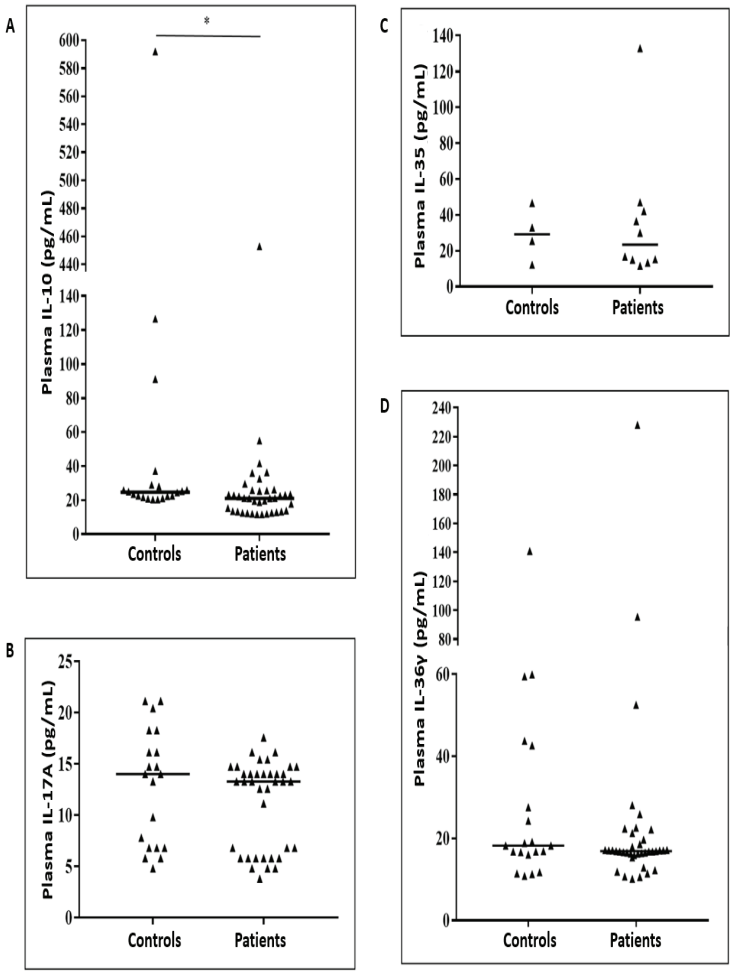

Figure 2. IL-10 (A), IL-17A (B), IL-35 (C), and IL-36γ (D) plasma levels of healthy controls and patients with psoriasis. Results are shown as individual values for each person (triangles) and median values (lines) (**P*=0.008).

riatic treatment (*P*=0.554) and associated diseases (*P*=0.872).

Plasma levels of IL-10, IL-17A, IL-35, and IL36γ

Plasma cytokine levels of patients with psoriasis and controls are presented in Figure 2. The median plasma IL-10 levels were significantly lower in patients with psoriasis compared with healthy controls (21.10 (11.63-452.88) vs. 24.70 (20.40-592.00), *P*=0.008). However, no significant differences were detected in plasma IL-17A (13.29 (3.80-17.57) vs. 14.00 (4.80-21.14), *P*=0.135) and in plasma IL-36γ concentrations (16.88 (10.14-227.96) vs. 18.23 (10.84-140.85), *P*=0.287) between the patients and controls. IL-35 was detectable in only 10 of the patients with psoriasis and in only four of the control subjects. The median values of IL-35 in these patients and controls were similar (23.41 (11.61-132.99) vs. 29.22 (12.23-46.58), *P*=1.000). Additionally, no significant differences were detected in the patient group regarding the disease severity, treatment status, and associated diseases.

Correlation between disease severity and interleukin levels

A positive correlation was found between plasma IL-35 levels and PASI score in patients with psoriasis (r=0.648, *P*=0.043). With regard to psoriasis severity, a positive correlation was found between the PASI score and plasma IL-17A levels in the mild psoriasis group (r=0.499, *P*=0.021), and a positive correlation was found between the PASI score and plasma IL-36γ levels in the moderate-to-severe psoriasis group (r=0.532, *P*=0.041) (Figure 3).

DISCUSSION

As a marker of circulating Treg cells, FOXP3 mRNA expression has been investigated in numerous previous studies. Some of these have revealed decreased FOXP3 mRNA expression in peripheral blood mononuclear cells in patients with psoriasis compared with healthy controls (15,17-19), whereas others have found no significant difference (20-22). In the present study, we found no significant difference in whole blood FOXP3 mRNA expression levels between pa-

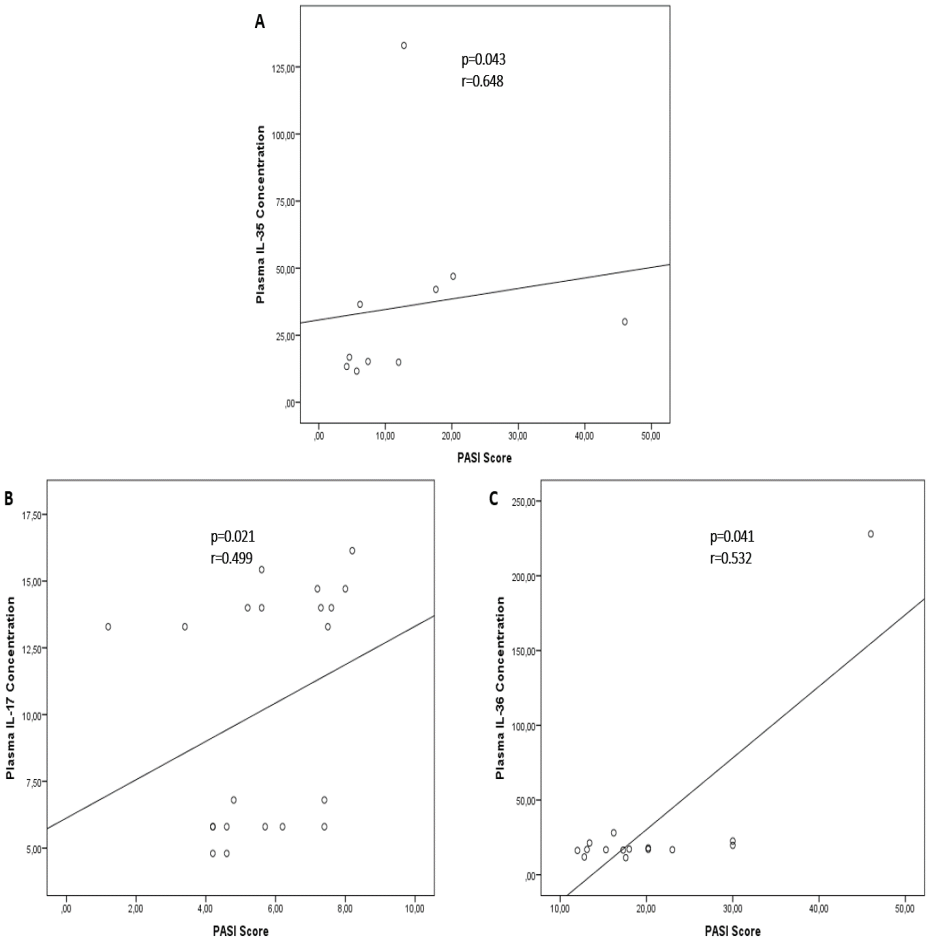

Figure 3. Correlation between psoriasis severity and plasma cytokine levels in the patient group (A), mild psoriasis group (B), and moderate-to-severe psoriasis group (C).

tients with psoriasis and controls. It has been suggested that the dysfunction of Treg cells with consequent loss of their inhibitory activity may be the leading factor in the pathogenesis of psoriasis, even in the absence of a decrease in the cell count or a decrease in the FOXP3 mRNA expression levels (20,21). In contrast, a decrease in Treg cell percentage and FOXP3 mRNA levels, as well as an increase in IL-17 and Th17 cell percentages in patients with psoriasis compared with healthy controls has also been suggested in some reports (15). Similarly, the Treg cell percentage and FOXP3 mRNA levels have been reported to be negatively correlated with PASI scores, while Th17 cell percentage and IL-17 mRNA levels have been reported to be positively correlated with PASI scores (19). Furthermore, it is possible to increase Treg cells and FOXP3 expression levels (17,18) and to restore Treg cell function with certain treatment methods (21). Thus, it seems reasonable to evaluate the relation between FOXP3 expression and the functionality of Treg cells in future studies, and our study may constitute a basis for this new area of research.

In recent studies, the methylation status of FOXP3 TSDR has been examined as a circulating Treg cell marker in various disease groups such as cancer (23), sepsis (24), allergy (25), and atopic dermatitis (26). FOXP3 TSDR methylation levels of DNA samples isolated from whole blood samples of patients with psoriasis were examined using the HRM-PCR in only one study up to date. The authors of the study stated that the level of Treg cells in circulation was found to be lower in patients with psoriasis compared with controls and was associated with higher methylation levels of FOXP3 TSDR. However, only the melting temperatures were compared between the groups in this study, rather than employing methylation controls, and no detailed information was given about the psoriasis group, such as the subtype and the severity of the disease (7). By contrast, in the present study, all of the patient and control samples were fully methylated, and there was no significant difference in the melting temperatures between the groups. Even though these two different results could be caused by the different sample sizes or the different disease stages among the patients with psoriasis, it would be useful to investigate whether the method is sensitive enough to reflect the FOXP3 TSDR methylation levels of the circulating Treg cells.

Over the past two decades, a wide variety of data have been obtained from studies investigating the circulatory levels of interleukins in patients with psoriasis. In this regard, IL-17 and IL-10 have been widely examined in their roles as pro-inflammatory and anti-inflammatory cytokines. Although no significant difference has been found in IL-10 and IL-17 levels between patients and controls in some studies (9,27,28), Takahashi *et al.* demonstrated decreased IL-10 and increased IL-17 levels in the serum of patients with psoriasis (29). They also detected an increase in IL-10 levels after treatment, and suggested the use of serum IL-10 levels as a marker for psoriasis (29). Similarly, Zhang *et al.* found higher numbers of circulating Th17 cells, Treg cells, and serum IL-17 levels in patients with psoriasis compared with controls, emphasizing that these parameters were also positively correlated with disease severity (30). They stated that the Th17/Treg cell ratio in circulation was positively correlated with the PASI scores, whereas the ratio in the lesions was negatively correlated with PASI scores. As a result, the authors suggested that Th17 and Treg cells affect the severity of the disease, but that IL-17 secretion in lesions may not be regulated by Treg cells, and that a different regulation mechanism may also be involved in this process (30). Furthermore, the presence of an immune imbalance in Th17/Treg through different pathways such as an increase in circulating Th17 cell percentage along with a decrease in Treg cell levels and FOXP3 mRNA expression in psoriasis has also been suggested. In addition to these results, the study found decreased IL-10 and increased IL-17 mRNA expression which were isolated from $CD4^+$ T-cells and similar serum cytokine levels in the patient group (31).

In the present study, there was no significant difference between plasma IL-17A levels in the patient and control groups. However, IL-17A levels and PASI scores were positively correlated in the mild psoriasis group, which is in accordance with the literature (9,12,32,33). There are different possibilities that would explain this result: IL-17 alone may not be sufficient to cause psoriasis pathogenesis, IL-17 may be active in the circulation in the early stages of the disease, or it might be present only in the lesions (34,35). Contrary to our finding, high circulatory IL-17 levels showing statistically significant positive correlation with disease severity were reported in some studies (17,30,36). On the other hand, whereas serum IL-17A levels were found to be significantly higher in patients than in controls in the study by Fotiadou *et al.*, they found no correlation between IL-17A levels and PASI scores. In the same study, due to the higher serum IL-17A levels in patients with active psoriasis compared with patients with stable psoriasis, the authors suggested that IL-17A may be important for the early stage of the active disease (37).

In the literature, there is a limited number of studies mentioning circulatory IL-35 levels in psoriasis. Cardoso *et al.* found undetectable serum IL-35 levels

both in patients and in controls (38), whereas Li *et al.* found significantly higher serum IL-35 levels only in patients with psoriatic arthritis, as opposed to patients with psoriasis and controls (39). Based on this result, they suggested that IL-35 might be associated with psoriatic arthritis only, but not psoriasis directly (39). In another study, no significant difference was found in serum IL-10 and IL-35 levels, whereas the levels of IL-10 and IL-35 in lesions were significantly lower in patients (40). Consistent with these studies, IL-35 was undetectable in most of the samples and there was no significant difference in plasma IL-35 levels between the patients and controls in our study. In contrast to these findings, lower plasma IL-35 levels and higher plasma IL-10 and IL-17 levels were found in patients with psoriasis compared with healthy controls (41). In this study, plasma IL-35 levels were positively correlated with IL-10 levels and negatively correlated with IL-17 levels and PASI scores. According to these data, the authors suggested IL-35 as a target in psoriasis because of the potential mediator effect on the expression of related cytokines in psoriasis pathogenesis (42).

Although some studies have found higher levels of IL-36 in the psoriatic skin, so far only two studies have examined the relationship between serum IL-36 levels and psoriasis. These studies found significantly higher IL-36α and IL-36 levels, showing positive correlations with PASI scores (39,42). Moreover, a detailed analysis showed that IL-36α levels were significantly higher in patients with pustular psoriasis than in patients with chronic plaque psoriasis. As a result, the authors emphasized that IL-36α may be associated with different types of psoriasis, especially with the pustular subtype of the disease (39). Our study is the first in literature examining plasma IL-36γ concentration in psoriasis. We found no significant difference in plasma IL-36γ concentration between patients and controls. However, we found that plasma IL-36γ levels showed a strong positive correlation with PASI scores in moderate-to-severe psoriasis. The correlations of PASI scores with IL-17A in the mild psoriasis group and IL-36γ in the moderate-to-severe psoriasis group may indicate increased circulating levels of different pro-inflammatory cytokines in association with disease severity. To confirm our results, we suggest further studies with a larger patient population to examine the relationship between these cytokines and disease severity.

CONCLUSION

This study examined the levels of FOXP3 TSDR methylation, FOXP3 mRNA expression, and levels of IL-10, IL-17, IL-35, and IL-36 in patients with psoriasis, and investigated their relationship with disease severity and treatment status. We found that FOXP3 TSDR was methylated both in patients and controls. Plasma IL-10 levels were found to be significantly lower in patients with psoriasis. In addition, we found a positive correlation between IL-17A levels and PASI scores in patients with mild psoriasis and a positive correlation between IL-36γ levels and PASI scores in patients with moderate-to-severe psoriasis.

Although we suggest the use of simple and practical methods to investigate whole blood FOXP3 TSDR methylation status and plasma levels of related cytokines in psoriasis, as performed in our study, they may also be analyzed using more precise methods in future studies. However, our study is the first in literature to investigate the selected epigenetic and inflammatory parameters together in patients with psoriasis. The cytokines examined in this study, especially IL-17 and IL-36, may be important indicators of disease severity. On the other hand, future studies should also clarify whether the FOXP3 mRNA expression and FOXP3 TSDR methylation levels reflect the circulating Treg cell levels or not. There is a need for large-scale studies with more detailed grouping and larger sample sizes in order to achive a better understanding of the molecular pathogenesis of psoriasis.

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References:

1. Perera GK, Di Meglio P, Nestle FO. Psoriasis. Annu Rev Pathol Mech Dis. 2012;7:385-422..
2. Buckner JH. Mechanisms of impaired regulation by CD4+ CD25+ FOXP3+ regulatory T cells in human autoimmune diseases. Nat Rev Immunol. 2020;10:849-59.
3. Morikawa H, Ohkura N, Vandenbon A, Itoh M, Nagao-Sato S, Kawaji H, *et al.* Differential roles of epigenetic changes and Foxp3 expression in regulatory T cell-specific transcriptional regulation. Proc Natl Acad Sci USA. 2014;111:5289-94.
4. Huehn J, Polansky JK, Hamann A. Epigenetic control of FOXP3 expression: the key to a stable regulatory T-cell lineage? Nat Rev Immunol. 2009;9:83-9.
5. Kitagawa Y, Ohkura N, Sakaguchi S. Epigenetic control of thymic Treg-cell development. Eur J Immunol. 2015;45:11-6.
6. Polansky JK, Kretschmer K, Freyer J, Floess S, Garbe

A, Baron U, *et al.* DNA methylation controls Foxp3 gene expression. Eur J Immunol. 2008;38:1654-63.

7. Ngalamika O, Liang G, Zhao M, Yu X, Yang Y, Yin H, *et al.* Peripheral whole blood FOXP3 TSDR methylation: a potential marker in severity assessment of autoimmune diseases and chronic infections. Immunol Invest. 2015;44:126-36.
8. Baliwag J, Barnes DH, Johnston A. Cytokines in psoriasis. Cytokine. 2015;73(2):342-50.
9. Choe YB, Hwang YJ, Hahn HJ, Jung JW, Jung HJ, Lee YW, *et al.* A comparison of serum inflammatory cytokines according to phenotype in patients with psoriasis. Br J Dermatol. 2012;167:762-7.
10. Clavel G, Thiolat A, Boissier MC. Interleukin newcomers creating new numbers in rheumatology: IL-34 to IL-38. Jt Bone Spine. 2013;80:449-53.
11. Gresnigt MS, Van de Veerdonk FL. Biology of IL-36 cytokines and their role in disease. Semin Immunol. 2013;25:458-65.
12. Michalak-Stoma A, Bartosińska J, Kowal M, Juszkiewicz-Borowiec M, Gerkowicz A, Chodorowska G. Serum levels of selected Th17 and Th22 cytokines in psoriatic patients. Dis Markers. 2013;35:625-31.
13. Tian G, Li JL, Wang DG, Zhou D. Targeting IL-10 in Auto-immune Diseases. Cell Biochem Biophys. 2014;70:37-49.
14. Collison LW, Chaturvedi V, Henderson AL, Giacomin PR, Guy C, Bankoti J, *et al.* Interleukin-35-mediated induction of a novel regulatory T cell population. Nat Immunol. 2011;11:1093-101.
15. Ma L, Xue H-B, Guan X-H, Shu CM, Wang F, Zhang JH, *et al.* The Imbalance of Th17 cells and CD4(+) CD25(high) Foxp3(+) Treg cells in patients with atopic dermatitis. J Eur Acad Dermatol Venereol. 2014;28:1079-86.
16. Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods. 2001;25:402-8.
17. Quaglino P, Bergallo M, Ponti R, Barberio E, Cicchelli S, Buffa E, *et al.* Th1, Th2, Th17 and regulatory T cell pattern in psoriatic patients: Modulation of cytokines and gene targets induced by etanercept treatment and correlation with clinical response. Dermatology. 2011;223:57-67.
18. Wang X, Wang G, Gong Y, Liu Y, Gu J, Chen W, *et al.* Disruption of Circulating CD4+T-Lymphocyte Subpopulations in Psoriasis Patients is Ameliorated by Narrow-Band UVB Therapy. Cell Biochem Biophys. 2014;71:499-507.
19. Wang X, Chen X, Li J, Zhang HY, Liu J, Sun LD, *et al.* MiR-200a expression in CD4 + T cells correlates with the expression of Th17/Treg cells and relevant cytokines in psoriasis vulgaris: A case control study. Biomed Pharmacother. 2017;93:1158-64.
20. Sugiyama H, Gyulai R, Toichi E, Garaczi E, Shimada S, Stevens SR, *et al.* Dysfunctional blood and target tissue CD4+CD25high regulatory T cells in psoriasis: mechanism underlying unrestrained pathogenic effector T cell proliferation. J Immunol. 2005;174:164-73.
21. Furuhashi T, Saito C, Torii K, Nishida E, Yamazaki S, Morita A. Photo(chemo)therapy Reduces Circulating Th17 Cells and Restores Circulating Regulatory T Cells in Psoriasis. PLoS One. 2013;8:1-9.
22. Kubin ME, Kokkonen N, Palatsi R, Hägg PM, Väyrynen JP, Glumoff V, *et al.* Clinical efficiency of topical calcipotriol/betamethasone treatment in psoriasis relies on suppression of the inflammatory TNFα – IL-23 – IL-17 axis. Acta Derm Venereol. 2017;97:449-55.
23. Sherston SN, Vogt K, Schlickeiser S, Sawitzki B, Harden PN, Wood KJ. Demethylation of the TSDR is a marker of squamous cell carcinoma in transplant recipients. Am J Transplant. 2014;14:2617-22.
24. Tatura R, Zeschnigk M, Adamzik M, Probst-Kepper M, Buer J, Kehrmann J. Quantification of Regulatory T Cells in Septic Patients by Real-Time PCR-Based Methylation Assay and Flow Cytometry. PLoS One. 2012;7:1-9.
25. Paparo L, Nocerino R, Cosenza L, Aitoro R, D'Argenio V, Del Monaco V, *et al.* Epigenetic features of FoxP3 in children with cow ' s milk allergy. Clin Epigenetics. 2016;8:1-6.
26. Roesner LM, Floess S, Witte T, Olek S, Huehn J, Werfel T. Foxp3 + regulatory T cells are expanded in severe atopic dermatitis patients. Allergy. 2015;70:1656-60.
27. Anderson KS, Petersson S, Wong J, Shubbar E, Lokko NN, Carlström M, *et al.* Elevation of serum epidermal growth factor and interleukin 1 receptor antagonist in active psoriasis vulgaris. Br J Dermatol. 2010;163:1085-9.
28. Deeva I, Mariani S, De Luca C, Pacifico V, Leoni L, Raskovic D, *et al.* Wide-spectrum profile of inflammatory mediators in the plasma and scales of patients with psoriatic disease. Cytokine. 2010;49:163-70.
29. Takahashi H, Tsuji H, Hashimoto Y, Ishida-Yamamoto A, Iizuka H. Serum cytokines and growth factor levels in Japanese patients with psoriasis. Clin Exp Dermatol. 2010;35:645-9.

30. Zhang L, Yang XQ, Cheng J, Hui RS, Gao TW. Increased Th17 cells are accompanied by FoxP3+ Treg cell accumulation and correlated with psoriasis disease severity. Clin Immunol. 2010;135:108-17.

31. Ma L, Xue H, Gao T, Gao M, Zhang Y. Notch1 Signaling Regulates the Th17/Treg Immune Imbalance in Patients with Psoriasis Vulgaris. Mediators Inflamm. 2018;2018:3069521.

32. Arican O, Aral M, Sasmaz S, Ciragil P. Serum levels of TNF-α, IFN-γ, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. Mediators Inflamm. 2005;2005:273-9.

33. Buyukkara Yilmaz S, Cicek N, Coskun M, Yegin O, Alpsoy E. Serum and tissue levels of IL-17 in different clinical subtypes of psoriasis. Arch Dermatol Res. 2012;304:465-9.

34. Nakajima K, Kanda T, Takaishi M, Shiga T, Miyoshi K, Nakajima H, *et al.* Distinct Roles of IL-23 and IL-17 in the Development of Psoriasis-Like Lesions in a Mouse Model. J Immunol. 2011;186:4481-9.

35. Nakajima H, Nakajima K, Tarutani M, Morishige R, Sano S. Kinetics of circulating Th17 cytokines and adipokines in psoriasis patients. Arch Dermatol Res. 2011;303:451-5.

36. Yoo IS, Lee JH, Song ST, Kim JH, Lee HJ, Kang SW. T-helper 17 cells: The driving force of psoriasis and psoriatic arthritis. Int J Rheum Dis. 2012;15:531-7.

37. Fotiadou C, Lazaridou E, Sotiriou E, Gerou S, Kyrgidis A, Vakirlis E, *et al.* IL-17A, IL-22, and IL-23 as markers of psoriasis activity: A cross-sectional, hospital-based study. J Cutan Med Surg. 2015;19:555-60.

38. Cardoso PR, Lima EV, Lima MM, Rêgo MJ, Marques CD, Pitta Ida R, *et al.* Clinical and cytokine profile evaluation in Northeast Brazilian psoriasis plaque-type patients. Eur Cytokine Netw. 2016;27(March):1-5.

39. Li J, Liu L, Rui W, Li X, Xuan D, Zheng S, *et al.* New Interleukins in Psoriasis and Psoriatic Arthritis Patients: The Possible Roles of Interleukin-33 to Interleukin-38 in Disease Activities and Bone Erosions. Dermatology. 2017;233:37-46.

40 Owczarczyk-saczonek A, Czerwińska J, Orylska M, Placek W. Evaluation of selected mechanisms of immune tolerance in psoriasis. Adv Dermatol Allergol. 2019;XXXVI:315-24.

41. Li T, Gu M, Liu P, Liu Y, Guo J, Zhang W, *et al.* Clinical significance of weak interleukin-35 expression in patients with psoriasis. Microbiol Immunol. 2018;62:454-61. doi:10.1111/1348-0421.12605.

42. Sehat M, Talaei R, Dadgostar E, Nikoueinejad H, Akbari H. Evaluating Serum Levels of IL-33, IL-36, IL-37 and Gene Expression of IL-37 in Patients with Psoriasis Vulgaris. Iran J Allergy, Asthma Immunol. 2018;17:179-87.