Check for updates

# Comparison of Th1 and Th17 Inflammatory Cytokine Profiles Between Chronic Plaque and Acute Guttate Psoriasis

Hyun Jeong Ju, Hyo Jin Park[1], In Hye Choi[1], Kyung Ho Lee[1], Mi Yeon Kwon[2], Chul Jong Park[1]

Department of Dermatology, St. Vincent's Hospital, College of Medicine, The Catholic University of Korea, Suwon, [1]Department of Dermatology, Bucheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, [2]Institute of Clinical Medicine Research, Bucheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Bucheon, Korea

**Corresponding Author**
Chul Jong Park
Department of Dermatology, Bucheon
St. Mary's Hospital, College of Medicine,
The Catholic University of Korea, 327
Sosa-ro, Wonmi-gu, Bucheon 14647, Korea
Tel: +82-32-340-2115
Fax: +82-32-340-2118
E-mail: cjpark777smp@gmail.com
https://orcid.org/0000-0003-3099-4109

**Background:** The phenotypic heterogeneity of psoriasis is suspected to reflect differences in its pathogenesis, but not yet completely elucidated. Studies of the Th1 and Th17 cytokines associated with different phenotypes of psoriasis have yielded inconsistent results.
**Objective:** To investigate the tissue expression levels of Th1 and Th17 cytokines among patients with chronic plaque psoriasis, acute guttate psoriasis, and healthy control.
**Methods:** A total of 20 patients with psoriasis (10 with chronic plaque type and 10 with acute guttate type) and 5 healthy controls were enrolled. The tissue mRNA and protein levels of following cytokines were measured: interleukin (IL)-12, IL-2, interferon (IFN)-$\gamma$, IL-23, IL-17A, and IL-22.
**Results:** The tissue mRNA levels of IL-12, IFN-$\gamma$, IL-23, IL-17A, IL-22 and the protein levels of IL-12, IL-2, IFN-$\gamma$, IL-17A, IL-22 were significantly increased in the psoriasis patients compared with the healthy controls. In comparisons of the subtypes, the tissue mRNA level of IFN-$\gamma$ was increased in acute guttate psoriasis, whereas the protein levels of IL-12 and IL-17A were significantly increased in chronic plaque psoriasis. The cytokine ratios of IL-17A/IL-2 and IL-22/IL-2 were significantly higher in chronic plaque psoriasis than in acute guttate psoriasis.
**Conclusion:** We confirmed that the tissue levels of Th1 and Th17 cytokines were increased in psoriasis patients compared with healthy controls. The increased IFN-$\gamma$ mRNA level in acute guttate psoriasis and increased IL-12 and IL-17A protein levels in chronic plaque psoriasis suggest that an imbalance between Th1 and Th17 cytokines may play a role in the phenotypic transition of psoriasis.

**Keywords:** Cytokine, Immunity, Interleukins, Phenotype, Psoriasis

## INTRODUCTION

Psoriasis is a chronic immune-mediated inflammatory skin disease caused by interactions among multiple genetic, immunological, and environmental factors[1]. Classically, plaque psoriasis and guttate psoriasis are considered distinct phenotypic subtypes of psoriasis. However, phenotypic transition toward either subtype is often reported in psoriasis patients. Chronic plaque psoriasis often leads to inflammatory eruptive lesions, and acute guttate psoriasis may develop into the plaque type in 30%~70% of cases[2,3].

Psoriatic skin lesions exhibit increased infiltration of epidermal T cells secreting various inflammatory cytokines[4]. It has been suggested that activation of the Th1- vs. Th17-related pathway determines the plaque versus guttate phenotypes, respectively[2,5]. However, conflicting results have been reported regarding the serum cytokine profiles of the two subtypes[2,6,7], and a recent study proposed that directional changes between the Th1 and Th17 pathways lead to phenotypic transition[8]. In the present study, we investigated the tissue levels of Th1 (interleukin [IL]-12, IL-2, interferon [IFN]-$\gamma$) and Th17 (IL-23, IL-17A, IL-22) cytokines according to the psoriasis phenotypes.

## MATERIALS AND METHODS

### Study population

A prospective study was conducted from January 2019 to June 2019. Patients newly diagnosed with psoriasis and healthy volunteers were enrolled. The diagnosis of psoriasis was confirmed based on clinical manifestations and histopathological criteria by two dermatologists. The histopathological criteria consisted of abnormal differentiation, including acanthosis, parakeratosis, loss of the granular layer, and neutrophil accumulation in the epidermis. Based on the clinical phenotype and disease activity, the psoriasis patients were classified into two groups: chronic plaque psoriasis and acute guttate psoriasis. The chronic plaque psoriasis group consisted of patients with at least one lesion >5 cm in diameter that had been stable for at least 6 months. The acute guttate psoriasis group consisted of those with eruptive papules <1 cm in diameter that began spreading within 1 month. Patients with any serious infections, an immunocompromised state, or diseases such as specific kidney, liver, cardiovascular, respiratory, rheumatic, or endocrine disease during the past 10 years were excluded. At the first visit, the psoriatic phenotype was classified, and the severity of psoriasis was assessed based on the body surface area and psoriasis area and severity index. Demographic information was also recorded. Approval for the study was obtained from the institutional review board of Bucheon St. Mary's Hospital, Bucheon, Korea (approval no. HC19TESI0034).

### Real-time reverse-transcription PCR

Skin biopsy samples (4 mm) were collected from psoriasis patients and healthy controls. The acquired samples were immediately frozen at –70℃ until use. Purifying RNA was collected using the RNeasy Plus Mini Kit (Qiagen, Valencia, CA, USA). Genomic DNA was eliminated by treatment with DNase 1 (Qiagen), and 100 ng purified RNA was reverse transcribed into first-strand cDNA using the CellScript cDNA Synthesis Master Mix (CellSafe, Suwon, Korea), which includes a genomic DNA elimination step. Real-time PCR amplification and relative expression quantification of IL-12, IL-2, IFN-γ, IL-23, IL-17A, IL-22, and GAPDH as a control were performed by TaqMan gene expression assays (Applied Biosystems, Foster City, CA, USA; Assay ID: CHT1, Hs01011518_m1, Hs00174114_

m1, Hs00174143_m1, Hs00372324_m1, Hs00174383_m1, Hs01574154_m1, Hs99999905_m1) on the Lightcycler 480 PCR system (Roche, Mannheim, Germany). All assays had a similar amplification efficiency, and relative expression was quantified using the ΔΔCt method. Each reaction was performed in triplicate in a volume of 20 μl using TaqMan probe Master Mix (Roche) with 20 ng cDNA. The results were analyzed using Lightcycler 480 instrument software 1.2 (Roche).

### Immunohistochemical staining

Tissues were fixed in formalin, embedded in paraffin, and cut into 3-μm-thick sections. Immunohistochemical staining (IHC) was performed using an automated immunohistochemical stainer (Ventana, Tucson, AZ, USA) according to the manufacturer's protocol. The sections were deparaffinized, pretreated with Cell Conditioning 1 solution (Ventana), and subjected to ultraviolet irradiation to abolish endogenous hydroperoxidase activity. The primary antibodies were diluted in Dako antibody diluent (Dako Cytomation, Glostrup, Denmark) with background-reducing components and were used at the following dilutions: IL-12 (rabbit, 1:100; Abcam, Franklin Lakes, NJ, USA), IL-2 (rabbit, 1:250; Abcam), IFN-γ (rabbit, 1:1,000; Abcam), IL-23 (rabbit, 1:200; Abcam), IL-17A (mouse, 1:100; Abcam), and IL-22 (rabbit, 1:300; Abcam). The sections were incubated with primary antibodies at room temperature for 32 minutes and then hybridized with an HRP-conjugated secondary antibody (Ventana) for 8 minutes. The reaction

**Table 1.** Demographic characteristics of the study patients

| Characteristic | Chronic plaque psoriasis | Acute guttate psoriasis |
|---|---|---|
| Total no. of patients | 10 | 10 |
| Age (yr) | 44 (25~59) | 41.5 (31~56) |
| Sex | | |
|     Male | 3 | 8 |
|     Female | 7 | 2 |
| Disease duration (mo) | 114 (6~600) | 1 (0.5~1) |
| Psoriasis area and severity index | 12.2 (8.5~22.9) | 10 (7.2~17.3) |
| Body surface area (%) | 10 (8.5~63) | 10 (8~33) |

Values are presented as number only or median (range).

was developed by incubating with diaminobenzidine (DAB; Dako Cytomation) for 5 minutes, and the slides were counterstained with hematoxylin II (Ventana) for 4 minutes and with bluing reagent (Ventana) for 4 minutes. The sections were observed under a light microscope (BX50; Olympus, Tokyo, Japan).

### Statistical analysis
Differences between groups were examined using the non-parametric Kruskal-Wallis rank sum test followed by the Mann–Whitney U test. In all analyses, $p < 0.05$ was taken to indicate statistical significance. All statistical analyses were performed using R software (ver. 3.6.1; R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

### Study population
A total of 25 adults (16 male and 9 female, median age 44 years), consisting of 20 psoriasis patients (10 with chronic plaque psoriasis, 10 with acute guttate psoriasis) and 5 healthy controls, were enrolled in this study. The median ages of the chronic plaque psoriasis and acute guttate psoriasis patients were 44 years (range, 25~59 years) and 41.5 years (range, 31~56 years), respectively. The median disease durations in the chronic plaque psoriasis and acute guttate psoriasis groups were 114 months (range, 6~600 months) and 1 month (range, 0.5~1 month), respectively. The median psoriasis area and severity index was 12.2 in the chronic plaque psoriasis group and 10.0 in the acute guttate psoriasis group (Table 1).

### Assessment of tissue levels of cytokines in psoriasis patients and healthy controls
The tissue mRNA levels of IL-12, IFN-γ, IL-23, IL-17A, and IL-22 were significantly higher in the psoriasis patients than healthy controls (Table 2). The tissue protein levels of IL-12, IL-2, IFN-γ, IL-17A, and IL-22 were significantly higher in the psoriasis patients than healthy controls (Table 3).

### Assessment of tissue levels of cytokines in relation to psoriasis phenotype
The tissue mRNA level of IFN-γ was significantly higher in the acute guttate psoriasis than chronic plaque psoriasis groups. The tissue protein levels of IL-12 and IL-17A were significantly higher in the chronic plaque psoriasis than acute guttate psoriasis groups (Fig. 1).

### Ratio of Th1/Th17 cytokines according to the psoriasis phenotype
As there were no distinct differences in tissue cytokine levels between the two psoriasis subtypes, we evaluated cytokine imbalance by comparing the relative ratios of cytokines rather than the absolute levels of each cytokine. All cytokine combinations were compared, and the results indicated that the tissue mRNA IL-17A/IL-2 ratio and IL-22/IL-2 ratio were significantly higher in the chronic plaque psoriasis than acute guttate psoriasis group.

**Table 2.** Comparison of tissue mRNA levels of cytokines among chronic plaque psoriasis patients, acute guttate psoriasis patients, and healthy controls

| Cytokine (median Target/Ref) | Chronic plaque | Acute guttate | Healthy control | Comparisons between groups (*p*-value) | | |
| --- | --- | --- | --- | --- | --- | --- |
| | | | | Chronic plaque vs. control | Acute guttate vs. Control | Chronic plaque vs. acute guttate |
| IL-12 | 0.135 | 0.136 | 0.011 | 0.008* | 0.008* | 0.912 |
| IL-2 | 0.075 | 0.103 | 0.179 | 0.165 | 0.141 | 0.326 |
| IFN-γ | 0.148 | 0.328 | 0.029 | 0.003* | 0.003* | 0.015* |
| IL-23 | 0.264 | 0.289 | 0.091 | 0.019* | 0.013* | 0.853 |
| IL-17A | 0.368 | 0.495 | 0.000 | 0.003* | 0.003* | 0.630 |
| IL-22 | 0.133 | 0.154 | 0.000 | 0.002* | 0.002* | 0.405 |

Kruskal–Wallis rank sum test was performed to compare tissue mRNA levels of cytokines between healthy controls and patients with chronic plaque or acute guttate psoriasis. IL: interleukin, IFN: interferon. *$p < 0.05$.

**Table 3.** Comparison of tissue protein levels of cytokines among chronic plaque psoriasis patients, acute guttate psoriasis patients, and healthy controls

| Cytokine (median %) | Chronic plaque | Acute guttate | Healthy control | Comparison between groups (*p*-value) | | |
|---|---|---|---|---|---|---|
| | | | | Chronic plaque vs. control | Acute guttate vs. control | Chronic plaque vs. acute guttate |
| IL-12 | 82.47 | 69.06 | 52.45 | 0.001* | 0.005* | 0.043* |
| IL-2 | 74.68 | 81.37 | 55.64 | 0.019* | 0.001* | 0.315 |
| IFN-γ | 69.65 | 70.77 | 60.25 | 0.055 | 0.129 | 0.971 |
| IL-23 | 37.55 | 31.14 | 34.15 | 0.594 | 0.768 | 0.247 |
| IL-17A | 31.44 | 19.46 | 8.76 | 0.013* | 0.055 | 0.043* |
| IL-22 | 80.56 | 72.24 | 50.20 | 0.001* | 0.055 | 0.165 |

Kruskal–Wallis rank sum test was performed to compare tissue mRNA levels of cytokines between healthy controls and patients with chronic plaque or acute guttate psoriasis. IL: interleukin, IFN: interferon. *$p<0.05$.
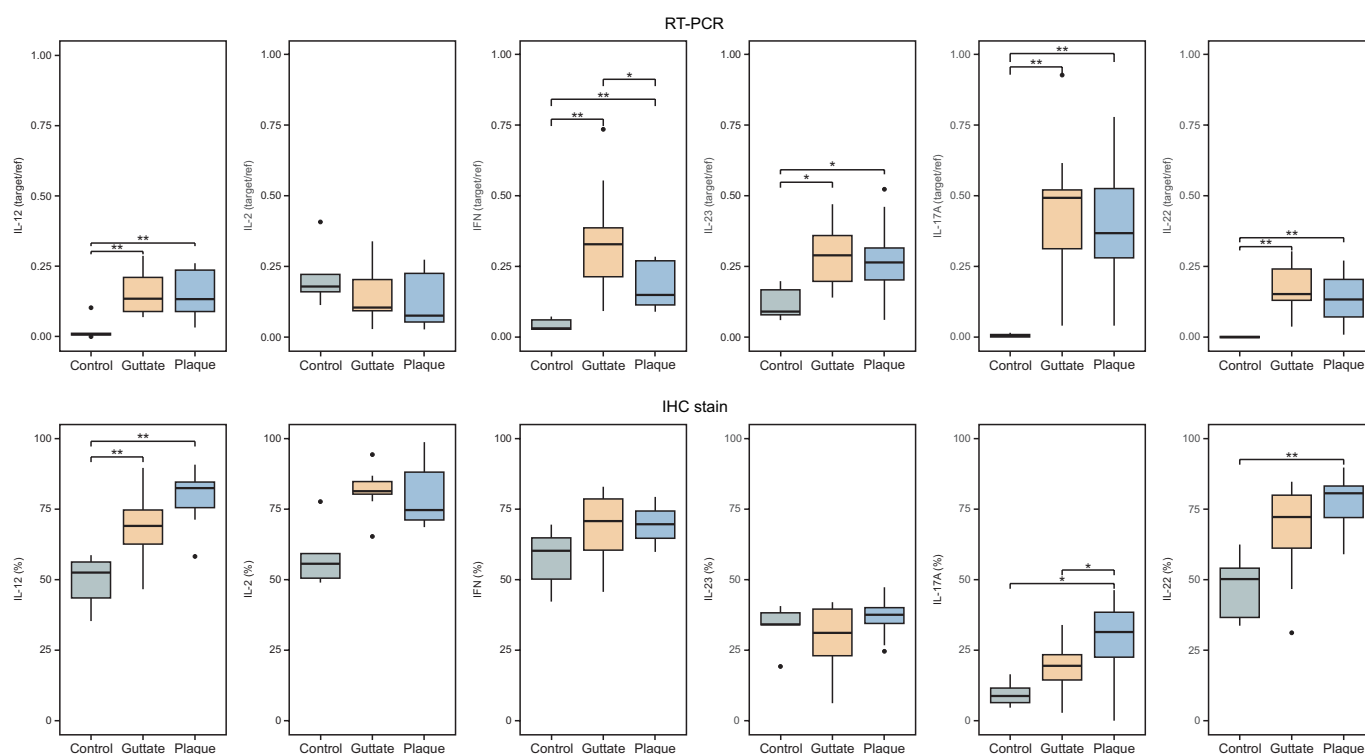


**Fig. 1.** Comparisons of tissue cytokine levels among chronic plaque psoriasis patients, acute guttate psoriasis patients, and healthy controls. IL: interleukin, IFN: interferon, RT-PCR: real-time reverse-transcription polymerase chain reaction. *$p<0.05$, **$p<0.01$.

## DISCUSSION

Major advances in the treatment of psoriasis have been made over the past 20 years, along with the development of biologics targeting inflammatory cytokines. Despite the high treatment response of plaque psoriasis, there are insufficient data on the response of guttate psoriasis. Moreover, phenotypic transition of psoriasis from the plaque to guttate type and vice versa has been reported often[9,10]. However, the differences in cytokine profiles between the phenotypes have not clarified.

Previous studies have yielded conflicting results regarding the serum levels of cytokines according to the clinical phenotype of psoriasis. Liu et al.[11] reported that the serum IL-23 level was higher in severe plaque psoriasis than guttate psoriasis.

Hwang et al.[8] revealed no significant difference in antimicrobial peptide and serum inflammatory cytokine levels between guttate and plaque psoriasis. Christophers[2] suggested that a bidirectional change from IL-12/IFN-γ to IL-23/IL-17 signaling could lead to the phenotypic switch in psoriasis patients. Therefore, we examined the tissue expression level of cytokines according to clinical phenotype and disease activity.

The results of the present study indicated that the tissue mRNA levels of inflammatory cytokines, including Th1 (IL-12, IL-2, IFN-γ) and Th17 (IL-23, IL-17A, IL-22) cytokines, were significantly increased in psoriasis patients compared with healthy controls, which supported our previous findings obtained using blood samples[7]. We confirmed that upregulated Th1 and Th17 cytokines play a pathogenic role in psoriatic skin.

Comparison of cytokine gene expression between psoriasis phenotypes revealed a significantly higher level of IFN-γ in acute guttate psoriasis than chronic plaque psoriasis. With regard to protein expression, chronic plaque psoriasis was characterized by higher levels of IL-12 and IL-17A compared with acute guttate psoriasis. The differences in gene and protein expression suggest the possibility of posttranscriptional modifications[12].

A previous study have suggested that chronic plaque and acute guttate psoriasis were involved with the Th1 and Th17 pathways, respectively[2], but our data suggest no clear distinction of Th1 and Th17 cytokines between two phenotypes. IFN-γ might play a role in the acute eruptive phase as several studies[13] showed that IFN-γ induced dendritic cells play a critical role in the initiation and maintenance of psoriasis[14]. Kurtovic and Halilovic[15] also showed that the level of IFN-γ was correlated with the severity of psoriasis. Yan et al.[16] reported that the proportion of regulatory T cells, which play a role in IFN-γ inhibition, was increased in plaque psoriasis while significantly decreased in guttate psoriasis. Meanwhile, IL-12 was overexpressed in psoriatic plaques, which is known to be involved in balancing Th1/Th17 immune response[17]. We suspect IL-12 might contribute to form and maintain psoriasis independent of IFN-γ[18]. Taken together, more complex factors may be involved in the pathogenesis of both subtypes of psoriasis, rather than being clearly classified into distinct pathways.

We hypothesized that skewed cytokine production or imbalance could be involved in the phenotypic heterogeneity of psoriasis. Comparison of Th1/Th17 cytokine ratios also showed that chronic plaque psoriasis was skewed to Th17 phenotype and acute guttate psoriasis toward the Th1 phenotype, which was contrary to previous reports. These results indicated that the phenotype and disease activity may vary according to whether Th1 or Th17 is more dominant. In addition, many clinical trials have been conducted on plaque psoriasis exclusively, and IL-17 blocking agents are effective toward plaque psoriasis but not guttate psoriasis. These therapeutic observations support our findings. Therefore, the Th1/Th17 ratio may represent a useful marker predicting the transition to other subtypes of psoriasis.

Our study was limited by the small sample size and heterogeneity of the sample with regard to disease duration, recurrence state, and severity. In addition, confounding variables, such as previous infection history or previous treatments including phototherapy or biologics, were not considered. However, we investigated the ratio of key pathogenic cytokines in the tissue according to phenotype, and suggest that a shift in the Th1/Th17 pathway could induce a phenotypic transition of psoriasis.

In conclusion, our findings confirmed that the tissue levels of Th1 and Th17 inflammatory cytokines were higher in psoriasis patients than healthy controls. In terms of cytokine expression in tissue, the mRNA level of IFN-γ was higher in acute guttate psoriasis, whereas the protein levels of IL-12 and IL-17A were higher in chronic plaque psoriasis. Further studies are needed to elucidate underlying pathogenesis of different phenotypes of psoriasis.

## CONFLICTS OF INTEREST

The authors have nothing to disclose.

## FUNDING SOURCE

## DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ORCID

Hyun Jeong Ju, https://orcid.org/0000-0003-2711-3112
Hyo Jin Park, https://orcid.org/0000-0001-8861-2236
In Hye Choi, https://orcid.org/0000-0001-5065-2080
Kyung Ho Lee, https://orcid.org/0000-0002-0108-3376
Mi Yeon Kwon, https://orcid.org/0000-0002-4462-7660
Chul Jong Park, https://orcid.org/0000-0003-3099-4109

## REFERENCES

1. 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;167:762-767. Erratum in: Br J Dermatol 2012;167:1400.

2. Christophers E. Explaining phenotype heterogeneity in patients with psoriasis. Br J Dermatol 2008;158:437-441.

3. Martin BA, Chalmers RJ, Telfer NR. How great is the risk of further psoriasis following a single episode of acute guttate psoriasis? Arch Dermatol 1996;132:717-718.

4. Lowes MA, Bowcock AM, Krueger JG. Pathogenesis and therapy of psoriasis. Nature 2007;445:866-873.

5. Christophers E, Metzler G, Rocken M. Bimodal immune activation in psoriasis. Br J Dermatol 2014;170:59-65.

6. Roh NK, Han SH, Youn HJ, Kim YR, Lee YW, Choe YB, et al. Tissue and serum inflammatory cytokine levels in Korean psoriasis patients: a comparison between plaque and guttate psoriasis. Ann Dermatol 2015;27:738-743.

7. Chun JH, Yoon YH, Choe YB, Kang LH, Paik SY, Park CJ. Gene expression analysis of inflammatory cytokines in Korean psoriatic patients. Ann Dermatol 2017;29:422-426.

8. Hwang YJ, Jung HJ, Kim MJ, Roh NK, Jung JW, Lee YW, et al. Serum levels of LL-37 and inflammatory cytokines in plaque and guttate psoriasis. Mediators Inflamm 2014;2014:268257.

9. Kim B, Maverakis E, Raychaudhuri SP. Is it possible to discontinue tumor necrosis factor antagonists after psoriasis remission? Ann Dermatol 2019;31:495-501.

10. Ko HC, Jwa SW, Song M, Kim MB, Kwon KS. Clinical course of guttate psoriasis: long-term follow-up study. J Dermatol 2010;37:894-899.

11. Liu XY, Zhao Y, Zhang H, Cai L, Zhang JZ. Comparison between guttate and plaque psoriasis in terms of serum inflammatory cytokines and antimicrobial peptides. Chin Med J (Engl) 2020;133:3004-3006.

12. Perl K, Ushakov K, Pozniak Y, Yizhar-Barnea O, Bhonker Y, Shivatzki S, et al. Reduced changes in protein compared to mRNA levels across non-proliferating tissues. BMC Genomics 2017;18:305.

13. Kim TG, Kim DS, Kim HP, Lee MG. The pathophysiological role of dendritic cell subsets in psoriasis. BMB Rep 2014;47:60-68.

14. Benezeder T, Wolf P. Resolution of plaque-type psoriasis: what is left behind (and reinitiates the disease). Semin Immunopathol 2019;41:633-644.

15. Kurtovic NO, Halilovic EK. Serum concentrations of interferon gamma (IFN-γ) in patients with psoriasis: correlation with clinical type and severity of the disease. Med Arch 2018;72:410-413.

16. Yan K, Han L, Deng H, Fang X, Zhang Z, Huang G, et al. The distinct role and regulatory mechanism of IL-17 and IFN-γ in the initiation and development of plaque vs guttate psoriasis. J Dermatol Sci 2018;92:106-113.

17. Baliwag J, Barnes DH, Johnston A. Cytokines in psoriasis. Cytokine 2015;73:342-350.

18. Kulig P, Musiol S, Freiberger SN, Schreiner B, Gyülveszi G, Russo G, et al. IL-12 protects from psoriasiform skin inflammation. Nat Commun 2016;7:13466.