

## Research article

## Serum level of certain cytokines associated with psoriasis progression in Iraqi patients

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## ABSTRACT

**Introduction and Aim:** Psoriasis is a skin ailment that typically affects the scalp, lower back, knees, elbows, and results in flaky skin patches. The purpose of this study was to compare cytokines, leptin, and adiponectin levels, as well as several physiological characteristics, in psoriasis patients with and without treatment to levels in healthy people in Iraq.

**Materials and Methods:** The study included 310 subjects, males and females aged (25-65) years. The patients were attending a dermatology consultant at Al-Yarmouk Teaching Hospital in Baghdad. Blood samples were collected by venipuncture from all individuals. The Sandwich ELISA was used to estimate serum levels of IL-2, IL-8, IL-22, TNF- $\alpha$ , IFN- $\gamma$ , ADP and Leptin.

**Results:** Patients with psoriasis had considerably decreased lymphocyte and RBC counts while having significantly higher neutrophil, platelet, NLR, and PLR counts. WBC mean values in females and males among control groups were  $7.16 \pm 3.09 \times 10^3/\mu\text{l}$  and  $8.6 \pm 3.62 \times 10^3/\mu\text{l}$  respectively. In psoriasis patients the WBC counts varied from  $3.69 \times 10^3/\mu\text{l}$  to  $12.8 \times 10^3/\mu\text{l}$  with a mean of  $7.27 \pm 4.05 \times 10^3/\mu\text{l}$  in female patients and from  $6.47 \times 10^3/\mu\text{l}$  to  $13.62 \times 10^3/\mu\text{l}$  in males. Serum levels of cytokines, leptin and adiponectin were significantly higher in Group I patients, while in Group II it was almost like levels in the control group.

**Conclusion:** Smoking is a risk factor for psoriasis. Lymphocytes, erythrocytes, and hemoglobin levels are decreased in patients with psoriasis, while platelets and cytokines are elevated, signifying a relationship between these immunological parameters and development and severity of psoriasis disease.

**Keywords:** Psoriasis; cytokines; adipokines; smoking; CBC.

## INTRODUCTION

Psoriasis is a chronic, autoimmune illness marked by elevated, abnormal-looking skin patches. These spots are scaly, dry, irritating, and red, pink, or purple (1). Psoriasis is considered a hereditary condition that is brought on by environmental factors and the prevalence rate of psoriasis is 2–3% which signifies the epidemiological burden (2). The symptoms of psoriasis vary from person to person; lesions can range in size from a few flakes on the scalp or elbow to the entire body (3). Psoriasis is an autoimmune disease that can start at any age, but most often develops in adults between 20 and 30 years old and between 50 and 60 years old, it affects men and women equally (4). The Psoriasis Area Severity Index (PASI) score is used to determine the severity and scope of psoriasis, which depends on intensity and area (5).

Psoriasis is a multifactorial skin condition with complicated pathophysiology (6). Antigen-presenting cells (APCs), T cells, keratinocytes, Langerhans' cells, macrophages, natural killer cells, a variety of Th1 cytokines, specific growth factors such as vascular endothelial growth factor (VEGF), keratinocyte growth factor (KGF), and others have all been implicated in the psoriasis pathogenesis. It has been

theorized that the disease begins with the activation of T cells by an unidentified antigen, which causes activated T cells, inflammatory cells, and keratinocytes to secrete a variety of cytokines (7).

Any physical or chemical injury to the defective keratinocytes could activate synthesis and release of cytokines, resulting in antigen-independent activation of T lymphocytes. This abnormal regulation of T cells is coupled with interaction between keratinocytes and complex cytokine networks (8). Additional cytokines were released as a result, which was followed by the growth of keratinocytes, T lymphocytes, and inflammation (9). Nevertheless, keratinocytes are a key source of pro-angiogenic cytokines (VEGF, IL-8), even though angiogenesis may not be the main event in the pathogenesis of psoriasis (8). Following T cell activation, activated T cells release a series of cytokines, including GM-CSF, EGF, IL-1, IL-6, IL-8, IL-12, IL-17, IL-23, TNF-, and others. These cytokines have effects on keratinocyte proliferation, neutrophil migration, potentiation of Th-1 type response, angiogenesis, up-regulation of adhesion molecules and epidermal hyperplasia. (10). In this study we aimed to compare cytokines, leptin, and adiponectin levels, as well as several physiological parameters in psoriasis patients with and without treatment to levels in healthy people in Iraq.

## MATERIALS AND METHODS

### Study design and setting

The study included 230 (112 male and 118 female) patients aged between 25-65 years attended by a dermatological consultant at Al-Yarmouk Teaching Hospital in Baghdad from September 1<sup>st</sup> to December 31<sup>st</sup>, 2022. The patients were further grouped into two: Group I which included a newly diagnosed untreated group consisting of 77 patients (36 males and 41 females), while Group II included 153 patients (76 male and 77 females) who were already on treatment for psoriasis. Group III included 40 healthy volunteers (21 males and 19 females) as controls. Patients in Group I and II were all psoriasis patients and had no other inflammatory or chronic diseases.

### Ethical consideration

The protocol of the study was approved by the Ethics Committee of the Ministry of Higher Education and Scientific Research – University of Anbar (No. 81 dated 5/6/2023). Consent was obtained from all participants in the study and their personal information gathered through a questionnaire under the supervision of the consultant.

### Sample collection

Blood (5 ml) was collected from each participant by venipuncture using a disposable syringe. Each blood sample was separated into two aliquots; 3 ml transferred into gel disposable tubes and let to stand at room temperature (20–25°C) for clotting, while the remaining 2 ml was transferred into an EDTA tube for the relevant hematological test. Serum separated by centrifugation at 3000 rpm for 5 min. was distributed equally into Eppendorf tubes and kept at -20°C until assayed (11).

The Sandwich ELISA, which is highly specific, flexible, and sensitive, was used to estimate serum levels of IL-2, IL-8, IL-22, TNF- $\alpha$ , IFN- $\gamma$ , ADP and

Leptin. The Sandwich ELISA technique employs two antibodies specific for different epitopes of the antigen. While one of the antibodies, known as a capture antibody, is coated on the surface of the multi-well plate, and utilized as a capture antibody aiding in antigen immobilization, the other antibody is conjugated and facilitates the detection of the antigen (12).

### Statistical analysis

All obtained results in the current study were subjected to various biometrical analyses such as student (t) test to testify the differences between means of examined variables with P value of  $\leq 0.05$  and  $\leq 0.01$  considered to indicate statistical significance. Analysis was performed using the statistical package for social science (SPSS) software program version 25.

## RESULTS

According to the information form directed to patients and healthy people, it was observed that 86.6% of the male patients were long time smokers while, 13.4% of male patients and all female patients were recorded as non-smokers. Among healthy individuals 92.5% were non-smokers while the remaining (7.5%) were smokers.

Investigations into the blood profiles of the patient and control groups revealed that psoriasis patients had significantly reduced lymphocyte and RBC counts but significantly higher neutrophil, platelet, and NLR and PLR counts (Table 1). The results of WBC as shown in Table 1 varied from  $3.69 \times 10^3/\mu\text{l}$  to  $12.8 \times 10^3/\mu\text{l}$  with a mean of  $7.27 \pm 4.05 \times 10^3/\mu\text{l}$  in females' patients and from  $6.47 \times 10^3/\mu\text{l}$  to  $13.62 \times 10^3/\mu\text{l}$  in males with a mean of  $12.55 \pm 3.62 \times 10^3/\mu\text{l}$ . The mean value for WBC in females and males among control groups was  $7.16 \pm 3.09 \times 10^3/\mu\text{l}$  and  $8.6 \pm 3.62 \times 10^3/\mu\text{l}$  respectively.

**Table 1:** Complete blood profile of participants in patient and control group

Variables	Patient Group		Control Group	
	Females (n=118)	Males (n=112)	Females (n=19)	Males (n=21)
WBC $\times 10^3/\mu\text{l}$	$9.27 \pm 4.05$	$12.55 \pm 3.62$	$7.16 \pm 3.09$	$8.6 \pm 3.62$
Neutrophils	$5.04 \pm 2.61$	$4.91 \pm 2.27$	$3.2 \pm 2.71$	$4.48 \pm 2.44$
Lymphocytes	$2.23 \pm 0.68$	$2.19 \pm 0.65$	$3.06 \pm 0.6$	$3.49 \pm 1.95$
Monocytes	$0.49 \pm 0.03$	$0.83 \pm 0.14$	$0.71 \pm 0.02$	$2.88 \pm 0.14$
RBC	$4.22 \pm 0.21$	$4.54 \pm 0.3$	$5.1 \pm 2.1$	$5.67 \pm 0.13$
HB	$10.17 \pm 2.97$	$11.55 \pm 1.48$	$12.31 \pm 0.71$	$14.4 \pm 1.48$
Platelets	$284.2 \pm 49.86$	$303.5 \pm 51.9$	$198.6 \pm 47.56$	$262.7 \pm 67.4$
Eosinophils	$0.17 \pm 0.01$	$0.19 \pm 0.02$	$0.17 \pm 0.02$	$0.17 \pm 0.04$
Basophils	$0.02 \pm 0.0$	$0.03 \pm 0.0$	$0.0 \pm 0.0$	$0.01 \pm 0.0$

Values expressed as Mean  $\pm$  S.D.

**Table 2:** Serum levels of IL-2, IL-8, IL-22, TNF- $\alpha$ , IFN- $\gamma$ , leptin and adiponectin among patient and control groups

Immunological parameters	Mean $\pm$ SD			P-value
	Group I patients	Group II patients	Group III Control	
IL-2	29.61 $\pm$ 2.1	17.1 $\pm$ 2.93	15.88 $\pm$ 1.92	0.00001
IL-8	45.19 $\pm$ 12.3	18.71 $\pm$ 14.8	14.56 $\pm$ 5.12	0.00001
IL-22	29.1 $\pm$ 6.88	14.47 $\pm$ 11.37	12.04 $\pm$ 2.53	0.005997
TNF- $\alpha$	58.46 $\pm$ 14.8	28.53 $\pm$ 1.32	24.56 $\pm$ 6.18	0.00001
IFN- $\gamma$	31.57 $\pm$ 5.24	16.9 $\pm$ 1.28	19.72 $\pm$ 2.6	0.00001
Leptin	195.75 $\pm$ 19.6	138.85 $\pm$ 38.4	159.27 $\pm$ 27.2	0.00003
Adiponectin	2.93 $\pm$ 0.0	1.19 $\pm$ 0.14	1.5 $\pm$ 0.101	0.00001

The study also investigated the immunological parameters and compared the levels of circulating cytokines (TNF- $\alpha$ , IFN- $\gamma$ , IL-2, IL-8I and L-22), leptin and adiponectin levels in the three groups (Table 2). Table 2 shows that serum levels of cytokines, leptin and adiponectin were significantly higher in Group I patients who received no treatment. In Group II where patients were on regular treatment, the mean levels of these parameters were observed to be significantly lower than that seen in Group I and being almost like levels recorded in healthy individuals.

## DISCUSSION

The disease psoriasis is known to affect 2- 3% of the world's population. Although psoriasis can manifest itself at any age, the onset peaks have been between 20-30 years and 50-60 years, with males and females being equally impacted (13-16). Some individuals are predisposed to having psoriasis, particularly if a family member has the disease. Environmental factors, obesity and metabolic syndromes are some of the other risk factors for developing psoriasis (14). Infection, smoking, and some drugs can all aggravate psoriasis in certain patients (17).

In this study most of the psoriasis patients were smokers. This assumes significance as smoking is known to increase the risk of psoriasis or worsen psoriatic symptoms (18). Nicotine in cigarettes has been demonstrated to raise the level of cytokines, which impact the immune system, causing increased inflammation, allergic reactions, and the risk of autoimmune illnesses, decreasing the benefits of therapeutic drugs (19,20).

Compared to the healthy controls, psoriasis patients in this study had lower lymphocyte and red blood cell counts, but higher total white blood cell, neutrophil, platelet, NLR and PLR ratios, which agrees with a previous study (21). A study also showed that the neutrophil-to-lymphocyte ratio could be possibly used as a biomarker in psoriasis diagnosis (22). Platelets, which are frequently elevated during infectious and inflammatory disorders, store a variety of inflammatory cytokines and chemokines, are important in playing an important role in severity of psoriasis disease (21). Platelets, in this study, were observed to be more abundant in psoriasis patients

than in healthy individuals, which could be most likely due to persistent inflammation (21).

An association study between circulating leukocytes and psoriasis has revealed that high numbers of monocytes, neutrophils, and eosinophils increase the risk of psoriasis (23).

Psoriasis is an immune-mediated condition characterized by both local and systemic inflammation. Numerous chemotactic substances, including IL-8, NAP-2, NAP-3, and LPS, can cause neutrophils to infiltrate the dermis and epidermis layers of psoriatic lesions causing micro-abscesses. Previous research demonstrated that most neutrophils in psoriatic lesions expressed IL-8 (21). Skin adipose tissue is considered an important source of bioactive mediators such as adipokines (adiponectin or resistin), chemokines, and cytokines (TNF-, IL-6, and IL-8), which when affected could cause systemic inflammation contributing to the pathogenesis of psoriasis (24). In the inflammatory state underlying psoriasis, resistin produced by adipose tissue could stimulate the secretion of pro-inflammatory cytokines such as TNF- $\alpha$  and IL-12, which in turn enhances the production of chemokines in endothelial cells contributing to immune cells infiltration (13). Enhanced expression of IL-1 $\beta$  and TNF- $\alpha$  is known to increase the production of Chemerin in the white adipose tissue, which stimulates chemotaxis and recruits inflammatory cells to sites of inflammation (25).

## CONCLUSION

Smoking is a potent risk factor for the development of psoriasis. Psoriasis patients had significantly lower lymphocyte and RBC counts and Hb levels, but significantly higher neutrophil and platelet counts, NLR, and PLR. Chemokines and cytokines were seen to impact the skin immune system and have a role in the development of psoriasis.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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