

# Measurement Serum Levels of IFN $\gamma$ , IL-17 and IL-4 of Psoriasis patients in Al- Najaf City.

قياس مستويات الانترفيرون كاما والانترليوكين والانترليوكين  
الصدفية في مدينة النجف

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**الخلفية :-** الصدفية هو مرض جلدي التهابي يتميز بتفعيل الخلايا اللمفية وزيادة انتاج الحركيات الخلوية على المستوى الموقعي والجهازى للجسم.  
**الاهداف:-** صممت هذه الدراسة لفحص مستوى الحركيات الخلوية لدى المرضى المصابين بداء الصدفية ودراسة الارتباط بين هذه الحركيات الخلوية ومدى شدة المرض.  
:- اخذت عينات دم من سبعين مريض لقياس مستوى الحركيات الخلوية ( IFN $\gamma$ , IL-17 او IL-4 ) بتقنية الايلايزا.

:- بنت الدراسة ارتفاع مستوى (IFN $\gamma$  و IL-17) لدى مرضى الصدفية، وتبين ان هناك فرق معنوي في معدل مستويات ( IFN $\gamma$ , IL-17 ) عند المصابين مقارنة مع مستوياتها عند مجاميع السيطرة وهناك علاقة ملحوظة بين ارتفاع مستوى هذه الحركيات الخلوية وشدة المرض. كذلك وجد ان مستوى (IL-4) لدى المرضى المصابين بالصدفية يقل معنويًا بشكل ملحوظ بالنسبة لشدة المرض. تم استخدام نظام SPSS لتطبيق اختبار T و اختبار الانوفا واختبار الفرق المعنوي  
:- يمكن الاستنتاج في هذه الدراسة ان مستوى الحركيات الخلوية (IFN $\gamma$  and IL-17) ربما لها دور كدليل في الامراضية المناعية لداء الصدفية وتعتبر كمعلمة لدرجة فعالية المرض.  
**التوصيات:-** استخدام حركيات خلوية اخرى و دلراسة تأثيرها على شدة داء الصدفية

## Abstract

**Background:** Psoriasis is an inflammatory skin disorder characterized by activation of lymphocytes, systemic and local over expression of pro-inflammatory cytokines.

**Objectives:-** The study was designed to investigate the level of cytokines in patients with psoriasis and study the correlation of the cytokines with disease severity.

**Patients and Methods:** Blood samples were collected from seventy patients with psoriasis for measurement of (IFN $\gamma$ , IL-17 and IL-4) by ELISA technique.

**Results:** High levels of IFN $\gamma$  and IL-17 were observed in patients with psoriasis. It was found that the mean levels of IFN $\gamma$  and IL-17 were significantly correlated to disease severity. Moreover, the mean level of IL-4 in psoriatic patient was found to decrease significantly according to graded severity of the disease. Statistical analysis was done by using SPSS (statistical package for social sciences) in which T-test and ANOVA test were used and chi- square ( $X^2$ ) test for categorical data.

**Conclusion:** The study concluded that the level of IFN $\gamma$  and IL-17 may play a key role as an indicator in the immunopathogenesis of psoriasis and markers of disease activity.

**Recommendation:-**Using another cytokines and study effect of these cytokines with disease severity.

**Keywords:-** Psoriasis, Cytokines, Disease severity

## INTRODUCTION

Psoriasis is characterized by hyper-proliferation and abnormal differentiation of epidermal keratinocytes which results in the characteristic scale or flakes of the lesions<sup>1</sup>. T-lymphocytes, cytokines and chemokines release appear to be the principal driver of lesion development and persistence, although endothelial cells, neutrophils, and natural killer cells may play an adjunctive role along with other cytokines and selectins such as intercellular adhesion molecule (ICAM)<sup>2</sup>. Many studies suggest that

cytokines released by activated T-lymphocytes, initiate, and maintain the psoriatic process by stimulating keratinocyte proliferation<sup>3</sup>.

Several cytokines form a complex and indicated that immunopathogenesis of the disease is T-helper-1 (Th1) mediated<sup>4</sup>. Interferon gamma (IFN $\gamma$ ) induces the expression of the adhesion molecule-1 (ICAM-1) on keratinocytes and endothelial cells, influencing the trafficking of T lymphocytes into lesional epidermis<sup>5</sup>. IFN- $\gamma$  activated keratinocytes secrete Interleukin (IL-1), (IL-6), (IL-8), IFN- $\gamma$ , tumor necrotic factor alpha (TNF- $\alpha$ ), and chemokines, which influence both themselves and other cell types including T lymphocytes<sup>6</sup>. The dominant role of Interleukin-23 (IL-23) involves the stimulation of T-helper-17 (Th-17) to produce Interleukin-17 (IL-17), which is a critical component in the establishment and perpetuation of autoimmune inflammation<sup>1</sup>.

## **OBJECTIVES:-**

The study was designed to investigate the level of cytokines in patients with psoriasis and study the correlation of the cytokines with disease severity.

## **MATERIALS AND METHODS**

Five ml of venous blood were collected from 70 patients with plaque psoriasis. These patients attended Al-Sadder Medical City, outpatient clinic of Dermatology in Al-Najaf city (Iraq) during the period from May 2011 to October 2011. The included patients had not received any treatment, topical or systemic for at least 3 weeks. Patients were diagnosed clinically and the disease severity was evaluated using the Psoriasis Area and Severity Index (PASI). The study also included 20 healthy individuals as a control group. Blood samples allowed for few minutes to form appropriate clot. Serum was separated by centrifugation at 1500 rpm and divided into 3 tubes (200  $\mu$ l) for each aliquots and stored at freeze (-20°C) to be used for different investigations. Serum levels of IFN $\gamma$ , IL-17 and IL-4 were measured by enzyme-linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay using Peprotech (USA) kit that contains the key components required for the quantitative measurement of natural and/or recombinant human IFN $\gamma$ , IL-17, and IL-4 within the range of 47-1500 pg/ml, 16-2000 pg/ml, and 16-2000 pg/ml, respectively.

## **RESULTS**

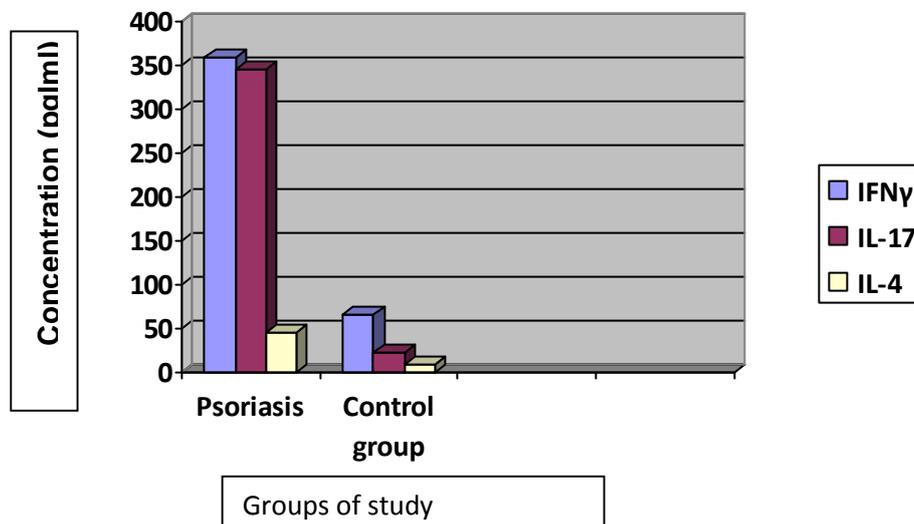
The patients comprised 36 (51.4%) females and 34 (48.6%) males and the ratio of female to male was 1.1:1. According to PASI score classification the severity of 70 patients with psoriasis was graded into mild, moderate and severe. The results were revealed that 29 (41.4%) patients had mild, 35 (50.0%) patients had moderate and 6 (8.6%) patients had severe grade (Table -1). There was no significant difference in the severity of the disease between males and females ( $P > 0.05$ ). High levels of IFN $\gamma$  and IL-17 were observed in patients with psoriasis ( $358.129 \pm 180.504$  pg/ml) and ( $346.026 \pm 273.469$  pg/ml), respectively compared with control group ( $64.984 \pm 21.357$  pg/ml) and ( $22.7 \pm 4.966$  pg/ml). The difference between the mean concentration of IFN $\gamma$  and IL-17 level of psoriasis patients and control group was significant ( $p < 0.05$ ). In the present work, IL-4 mean level ( $45.364 \pm 42.981$  pg/ml) of patients with psoriasis was found to be higher than the mean level of control group ( $8.063 \pm 3.96282$  pg/ml) (Figur-1). In addition, serum levels of both IFN $\gamma$  and IL-17

were found to be significantly correlated with PASI score. Serum level of IFN $\gamma$  was increased in severe degree of psoriasis and the level found to be (701.1167  $\pm$  91.7329 pg/ml) as compared with that in moderate (250.3960  $\pm$  83.92543 pg/ml) and in mild degree (122.8759  $\pm$  36.44595 pg/ml). The mean levels of IL-17 in psoriatic patients were also found to increase according to severity of disease (Table -2). Furthermore, the mean serum levels of IFN $\gamma$  and IL-17 in psoriatic patient were found to be correlated to disease severity ( $r = 0.849$ ,  $P < 0.001$ ) and ( $r = 0.433$ ,  $P < 0.001$ ) respectively (Figur-2) and (Figur-3). According to IL-4 serum levels the study was found that the mean level in severe group of psoriatic patients (3.0167  $\pm$  2.17522 pg/ml) was lower than the mean levels of mild (79.9759  $\pm$  97.82719 pg/ml) and moderate groups (15.3784  $\pm$  23.88903 pg/ml) (Table-2). The mean concentration of IL-4 in psoriatic patient was found to be inversely proportional with the severity of the disease. Patients with severe grade have lower mean concentration level than those with moderate and mild grades. The correlation of these cytokine mean concentration between grads severity of disease was appeared ( $r = 0.416$ ,  $P < 0.001$ ) (Figure-4).

**Table (1): Number of psoriasis patients according to severity grades of disease**

| Severity | Male | Female | Total (%) | P value |
|----------|------|--------|-----------|---------|
| Mild     | 13   | 16     | 29 (41.4) | 0.858   |
| Moderate | 17   | 18     | 35 (50.0) |         |
| Sever    | 4    | 2      | 6 (8.6)   |         |
| Total    | 34   | 36     | 70        |         |

This table indicate that no significant difference between severity of the disease and the gender.



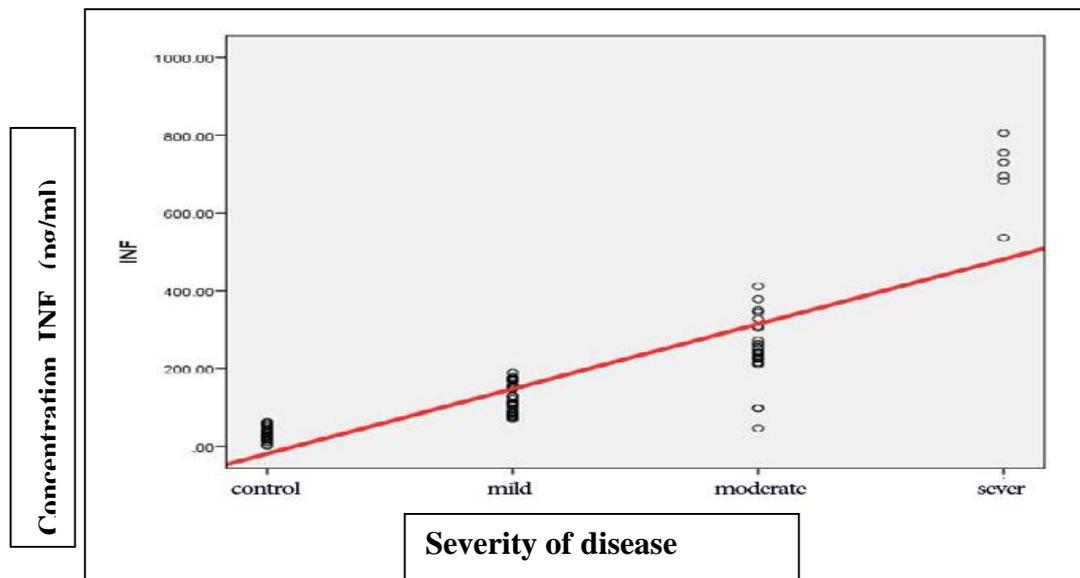
**Figure-1: Mean distribution of IFN $\gamma$ , IL-17 and IL-4 levels (pg/ml) among psoriatic patients and healthy control group**

This figure indicates that the levels of cytokines increase significantly in serum of psoriasis patients compare with control group.

**Table (2): Relationship between cytokine levels (INF , IL-17 and IL-4) with the severity of psoriasis patients**

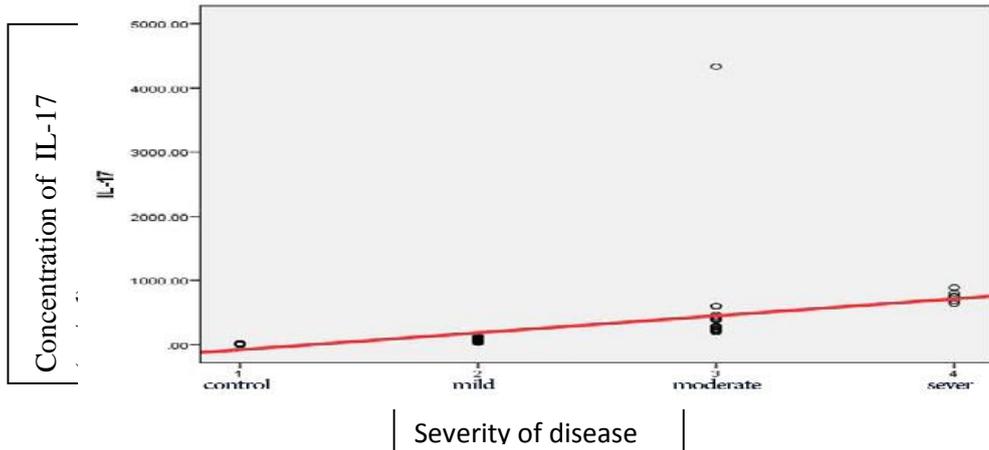
| Cytokine type | Study group   | Mean     | Std. Deviation | Std. Error | P value |
|---------------|---------------|----------|----------------|------------|---------|
| INF           | Mild          | 122.8759 | 36.44595       | 6.76784    | <0.001  |
|               | Moderate      | 250.3960 | 83.92543       | 16.78509   |         |
|               | Sever         | 701.1167 | 91.73290       | 37.44980   |         |
|               | control       | 64.984   | 21.3571        | 6.7537     |         |
| IL-17         | Mild          | 72.6862  | 26.39197       | 4.90087    | <0.001  |
|               | Moderate      | 265.3920 | 806.76233      | 161.35247  |         |
|               | Sever         | 702.2500 | 878.6832       | 35.87209   |         |
|               | control group | 22.7     | 4.96662        | 1.57058    |         |
| IL-4          | Mild          | 79.9759  | 97.82719       | 18.16605   | <0.001  |
|               | Moderate      | 15.3784  | 23.88903       | 4.77781    |         |
|               | Sever         | 3.0167   | 0.46655        | 0.19047    |         |
|               | control group | 8.063    | 3.96282        | 1.25315    |         |

This table indicates that serum level of IFN $\gamma$  was increased in severe degree of psoriasis as compared with that in moderate and in mild degree also the mean levels of IL-17 in psoriatic patients were also found to increase according to severity of disease



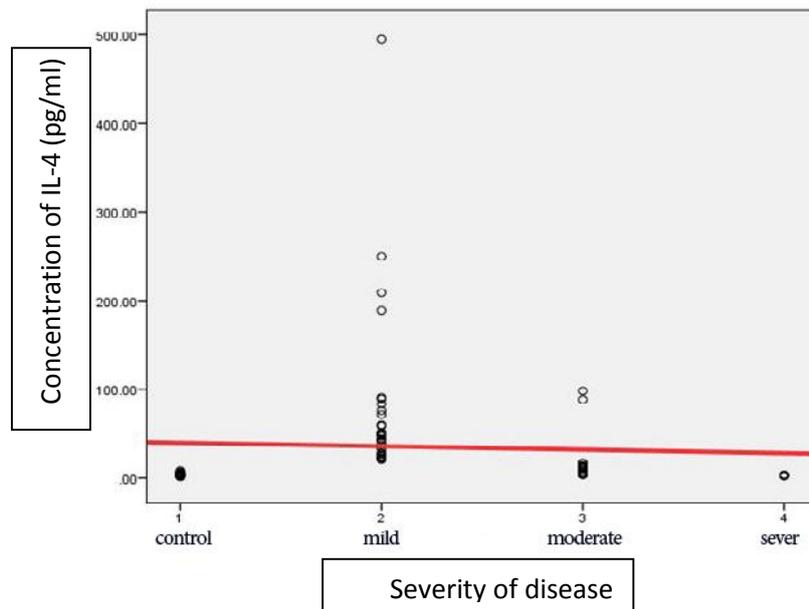
**Figure-2: The correlation between mean serum levels of IFN and the severity of psoriasis patients**

This figure shows the significant difference in increase level of IFN $\gamma$  in sever stage compare with mild, moderate, and the correlation the level of this cytokine with disease severity.



**Figure-3: The correlation between mean serum levels of IL-17 and the severity of psoriasis patients**

This figure indicates the significant increase level of IL-17 with disease severity of psoriasis patients.



**Figure-4: The correlation between mean serum levels of IL-4 and the severity of psoriasis patients**

This figure indicates that level of IL-4 not effect with severity of psoriasis patients.

## DISCUSSION

Several researchers reported that psoriasis occurs in any gender or race with equal male to female ratio<sup>7</sup>. The results of this study showed a slightly more female predominance (approaching 1:1) which yet again may reflect a sex-related preference in psoriatic patients. Until recently, T cells were classified as Th1 or Th2 cells by production of defining cytokines, IFN $\gamma$  and IL-4, respectively. However, a new type of T cell, Th17, has been linked to autoimmune inflammation<sup>8,9</sup>. Stimulated T cells play an important role in the expansion of psoriatic plaques<sup>10</sup>. However, the present study showed that the mean concentration of IFN $\gamma$  was significantly higher in patients with psoriasis compared with control groups. Similar result was shown by other studies<sup>11,12,13,14</sup> who found that IFN $\gamma$  and other some cytokines were elevated in

psoriatic patients. It was agreed with the hypothesis that psoriasis considered as a T-cell-mediated inflammatory skin disease. Th1-lymphocytes produce IFN $\gamma$  and induce cellular reactions, resulting in marked increases of keratinocyte proliferation, abnormal patterns of keratinocyte differentiation, concomitant inflammation, and dermal proliferation of small vessels<sup>3</sup>.

The study reveals that the level of IL-17 was higher in patients with psoriatic than that of control group. This observation supports the hypothesis that the high level of IL-17 may be critical mediators of the persistently altered epidermal growth and differentiation and local inflammation that was characteristic of psoriasis and Th17 cells may be proximal regulators of psoriatic skin inflammation<sup>18</sup>. However, many other studies were support this result, they found that the IL-17 level was increased in lesional tissue and serum of psoriatic patients<sup>15,16,17</sup>. The mean level of IL-4 in psoriatic patients was higher than control group in our work. This result was agreed with another study that showed the higher percentage of mean concentration of IL-4 in patients with psoriasis as compared with volunteers<sup>18</sup>. On the other hand, other study reported that serum levels of IL-4 in psoriatic patients were comparable with normal control<sup>17</sup>. The present study revealed that serum levels of the studied cytokines (IFN $\gamma$ , IL-4 and IL-17) of psoriatic patients were significantly elevated in comparison with control groups. In addition, the mean serum levels of both IFN $\gamma$  and IL-17 in patients found to be significantly correlated to disease severity index PASI of psoriasis. This result was supported by similar study that found a significant correlation between the level of IFN- $\gamma$  and psoriatic disease severity<sup>18</sup>. However, the mechanisms by which T cells, keratinocytes, and macrophages achieve and maintain their activated state in psoriatic skin lesions were poorly understood.

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