

## Sera levels of Interleukin -6 in psoriatic patients in najaf city

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### الخلاصة

اجريت الدراسة الحالية لتقييم مستوى IL-6 ، العد الكلي لخلايا الدم البيضاء ، والعدلات والحمضات. شملت الدراسة مجموعة من (80) مريض صنفوا الى مجموعتين وفقا لشدة المرض ، الحالة الخفيفة والتي شملت 32 مريض تراوحت اعمارهم بين (9-40) عاما ، والحالة الشديدة والتي شملت 48 مريضا تراوحت اعمارهم ما بين (17-75) عاما اما مجموعة السيطرة تتكون من (30) متطوعين أصحاء تراوحت اعمارهم ما بين (9-75) سنة لم يشتكوا من أي أعراض وليس لهم تاريخ مرضي. وتم استخدام عدة Biosoure (EASIA) التي أجريت على المصل . اظهرت الدراسة أن متوسط تركيز IL-6 كان أعلى في مجموعة الحالة المرضية الشديدة (39.24 جزء من الغرام / مل) من مرضى الحالة المرضية الخفيفة (20.98 جزء من الغرام / مل) مع ارتفاع معنوي في حين أن مستويات IL-6 في الحالة المرضية الخفيفة لم تظهر فرق معنوي مقارنة مع مجموعة السيطرة تحت مستوى معنوية 5%. في حين مجموع خلايا الدم البيضاء كانت في مستوى معنوية عالي في الجلد التهابات ومرضى الصدفية نسبة العدلات في المجموعات المدروسة . وكانت مستويات القيمة المتوسطة من المرضى الدراسة الحالية هي مماثلة لتلك التي لعدد متزايد من الكريات البيض في الجلد والتهابات مرضى الصدفية أعلى بكثير من مجموعة السيطرة (32.0) جزء من الغرام / مل و (12.64) جزء من الغرام / مل على التوالي عند مستوى المعنوية ( $P < 0.01$ ).

### Abstract

This work was done to evaluate IL-6 level , Total white blood cells count , Neutrophils and Eosinophils. Study group included (80) subjects were classified to two groups according to the severity of the psoriasis , mild which include 32 patients, their ages ranged between (9-40) years and sever group which include 48 patients , their ages ranged between (17-75) years) . The control group consist of (30) healthy volunteers , their ages ranged between (9-75) years no symptoms and with no history of psoriasis. Il-6 were estimated by kit The Biosoure Il-6 (EASIA) using a solid phase enzyme amplified sensitivity immunoassay (EASIA) performed on microtiterplate for serum in both mild and sever subjects.

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The result revealed that The mean of IL-6 concentration was higher in sever psoriatic group (39.24 pg/ml) than mild psoriatic patients (20.98 pg /ml) with higher significantly at  $p < 0.01$  , while the levels of IL-6 in mild psoriatic group showed no significant differences as comparing with healthy control group ( $p > 0.05$ ); while Total white blood cells count of studied group were a significantly increased number of leukocyte in inflammatory skin and psoriasis patients, Neutrophils percentage of studied groups , the current study are similar to those of a significantly increased number of leukocytes in inflammatory skin and psoriasis patients , The mean value levels of patients were significantly higher than those of control s(32.0) pg/ml and (12.64) pg/ml respectively at ( $p < 0.01$ ).

## Introduction

psoriasis is a chronic , inflammatory skin disorder affecting up to 3% of the worlds population [1] .Is an immune mediated inflammatory skin disorder characterized by chronic , scaling erythmatous patches and plaques of skin[2] . Several findings immune regulation suggest that psoriasis is an immunologically mediated disease. Cellular infiltrates , included neutrophils in the dermis and epidermis while T-lymphocytes in the dermis. Cytokines including interleukin (IL-8) (IL-6) (IL-12) and gamma interferon are over expressed in individuals with psoriasis [3] . Psoriasis is characterized by epidermal hyper proliferation ; abnormal kerationcyte differentiation , angiogenesis with blood vessel. dilation and excess Th-1 and Th-17 inflammation [4]. Psoriasis is recognized as the most prevalent autoimmune disease caused by an appropriate activation of cellular immune system [5]. In the skin of patients with lesional psoriasis , there is an increase in the number of antigen – presenting cells that can activate T-cells [6]. T-cells are crucial mediators of the skin damage in psoriasis . A T-cell derived cytokine , is highly expressed in the skin of individuals with psoriasis [7]. Interactions between infiltrating T-cells and skin resident cells (keratinocytes , fibroblasts , endothelial cells) are often mediated by the synthesis and release of different

proinflammatory cytokines [8]. IL-6 is a component of normal human skin and it was immunologically detected in basal keratinocytes, endothelial cells many mononuclear cells , fibroblasts and sudoriparous ducts [9]. IL-6 has been assumed to play an important role in the path physiology of psoriasis [10] . Cytokines attack large number of other large WBC known as neutrophils [11]. IL-6 is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine. It is secreted by T-cells and macrophages to stimulate immune response to trauma , especially burns or other tissue damage leading to inflammation [12]. Eosinophils are recognized as proinflammatory cells implicated in protection against parasitic infection , and is likely to play a major role in allergic disease such as bronchial asthma and other dermatologic disease including psoriasis [13]. The clinical picture of psoriasis is highly variable with regard to lesion characteristics and the severity of disease [14]. The degree of severity is generally based on the proportion of body surface area affected ; disease activity and the degree of plaque redness, thickness and scaling [15]. Psoriasis often persist for life and the patient has an increase risk of cardiovascular disease and their complications [16]

### **Aims of study**

- Determine the levels of IL6 in disease etiology & provide further insight into psoriasis severity ,& differentiation between the two main types of psoriasis mild & sever.
- Detection some hematological parameters like total WBC count , percentage count Neutrophils & Eosinophils

### **Materials and methods**

Blood samples (5ml) of venous blood were collected. Blood was divided into two parts, first part (1 ml) of blood was put in EDTA tube for hematological tests (Total white cells count, Neutrophils and Eosinophils percentage) , the second part (3ml) of blood was separated by centrifugation and immediately stored at -20°C for

further batch analysis for immune assays . Eighty patients with psoriasis , 32 males and 48 females were included in the present study. All patients attending the department of dermatology in education hospital , in najaf city , during the period between July 2010 and January 2011 (mean age years , 9-75) . All these patient had not been treated with any systemic or tropical drug at the time of study. The patients group were sub classified to two groups according to the severity of the psoriasis , mild which include patients (8 male and 24 females) their ages ranged between (40) years and sever and moderate group which include 48 patients (25 male and 24 female) , their ages ranged between (17-75 years) . The control group consist of (30) healthy volunteers , (15 males and 15 females) their ages ranged between (9-75) years no symptoms and with no history of psoriasis.

Blood samples (5ml) of venous blood were collected. Blood was divided into two parts, first part (1 ml) of blood was put in EDTA tube for hematological tests (Total white cells count, Neutrophil and eosinophil percentage) , the second part (3ml) of blood was separated by centrifugation for (10) min at (3000 rpm) and immediately stored at -20°C for further batch analysis for immune assays (?).

## **Method**

The Biosoure Il-6 (EASIA) :- is a solid phase enzyme amplified sensitivity immunoassay (EASIA) performed on microtiterplate . The assay is based in an oligoclonal system in which ablend of monoclonal antibodies (mAbs) directed against distinct epitopes of Il-6 are used. Antibody-producing cells are immortalized using the myeloma cell fusion method of kohler and Milstein.

The total WBCs (white blood cells count) were estimated by using neubaur Haemocytometer.

Neutrophils and Eosinophils were estimated by differential leukocyte count. The differential count is expressed as a percentage of the total number of leukocyte (WBC).

**Statistical method**

All variable data were shown as the mean ± SD. The data between control and test groups was compared using unpaired students t test. Correlation was determined by Pearson's correlation coefficient. The level of significance used was p less than 0.05 .

**Results**

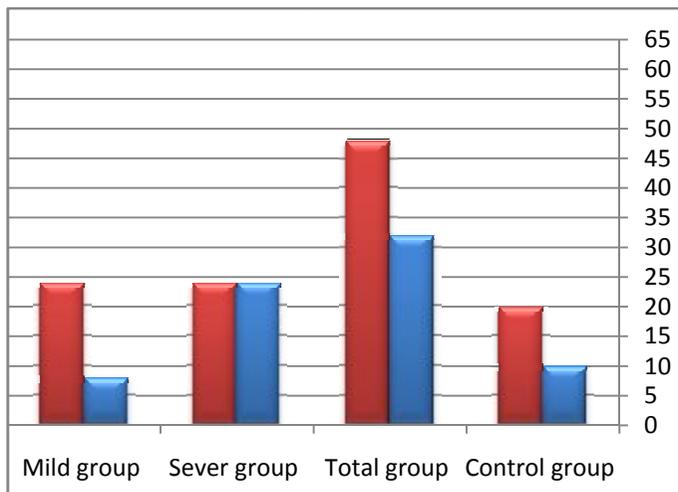
**Table (1):- Number , age and clinical characteristic of study groups**

Groups	No. of subjects	%	Age in years	
			Range	Mean
a) Mild (MG)	32	40	9-40	23.5
b) sever (SG)	48	60	12-75	42.9
Total (TG)	80	100	9-75	35
Control (CG)	30	100	9-75	35

**a) Mild group : (<10% surface area involvement)**

**b) Sever group : (> 10% surface area involvement)**

According to gender distribution there were obvious between males and females frequency among the psoriasis patients the females are more affected by the disease , but these variation were non significantly.

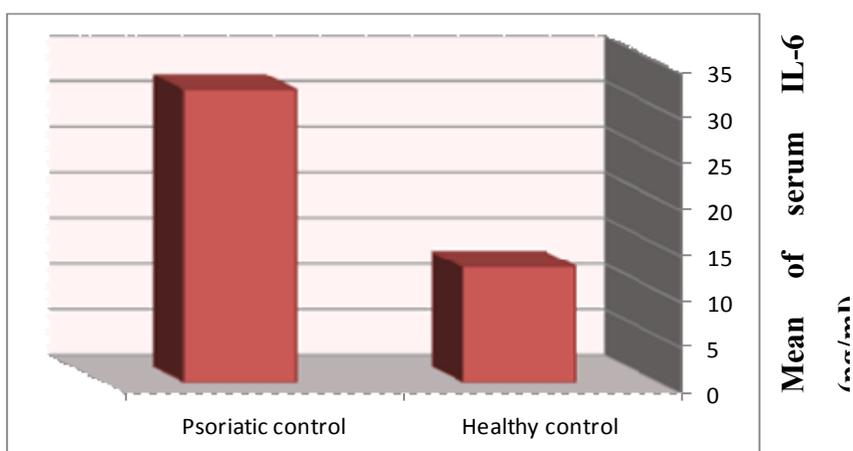


**Figure (1) :- Number , age and clinical characteristic of study group.**

**Table (2) :- level of IL-6 among studied groups .**

	N	Mean	Std. Deviation	Std. Error	Rand		T-test (p.value)	sig
					Minimum	Maximum		
Healthy control	30	12.640	9.730	2.176	0	40.5	000	Highly sig. (p<0.01)
Psoriatic control	80	32.004	19.199	2.521	30	70.9		
Total	110							

The mean value levels of patients were significantly higher than those of control s(32.0) pg/ml and (12.64) pg/ml respectively at (p<0.01).

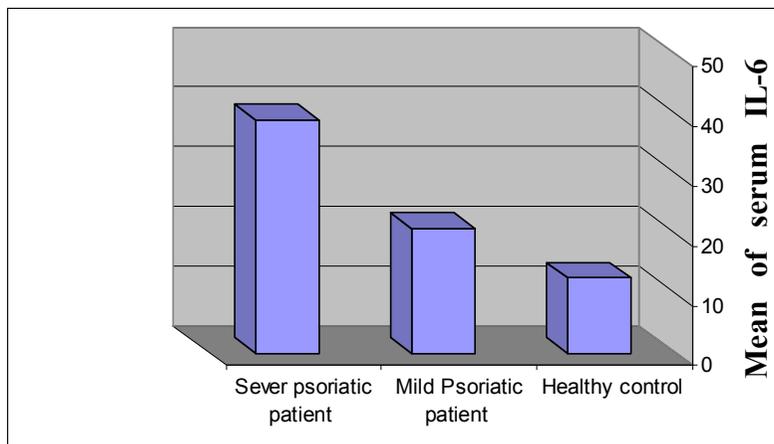
**Figure (2) :- levels of IL-6 in studied group**

**Table (3) : - serum levels of IL-6 (pg/ml) among studied groups .**

	N	Mean	Std. Deviation	Std. Error	Rand		Anova-test (p.value)	sig
					Minimum	Maximum		
Healthy control	30	12.640	9.730	2.176	0.0	40		highly
Mild Psoriatic patient	32	20.987	17.969	3.747	3.0	60.7	0.000	sig
Sever psoriatic patient	48	39.243	16.522	2.793	6.5	70.9		p<0.01
					p.value	sig		
Healthy control x mild psoriatic patient					0.084	NS		
Healthy control x sever psoriatic patient					0.000	HS		
Mild psoriatic patient x sever psoriatic patient					0.000	HS		

**NS: non significant , HS : Highly significant**

The mean of IL-6 concentration was higher in sever psoriatic group (39.24 pg/ml) than mild psoriatic patients (20.98 pg /ml) with higher significantly at p<0.01 , while the levels of IL-6 in mild psoriatic group showed no significant differences as comparing with healthy control group (p>0.05).



**Figure (3) :- serum levels of IL-6 (pg/ml) among studied groups**

**Table (4) :- Total white blood cells count of studied group**

W.B.C	Mild group	Sever group	Total group	Healthy group
No. x 1000 range	6.2-8.8	5.2 – 7.9	5.2 – 8.8	4.8 – 6.4
Mean±SD	7.1±0.535	6.5 ± 0.304	6.7 ± 0.44	5.8 ± 0.449
p. value	*p = 2.43			

**\* significant Difference at (0.05)**

Total white blood cells count of studied group , these results were a significantly increased number of leukocyte in inflammatory skin and psoriasis patients.

**Table (5) :- Neutrophils percentage of studied groups**

Neutrophil percentage	Mild group	Sever group	Total group	Healthy group
Range	57 – 61.3	48.7 – 56	48.7 ± 61.3	48 – 59.3
Mean±SD	58.8 ± 3.5	53 ± 3	55.9 ± 3.3	54 ± 3.56
p. value	p = 0.108			

**\* significant difference at (0.05)**

Neutrophils percentage of studied groups , the current study are similar to those of a significantly increased number of leukocytes in inflammatory skin and psoriasis patients.



**Table (6) :- Eosinophils percentage of studied groups**

Eosinophils percentage	Mild group	Sever group	Total group	Healthy group
Range	4 – 7	2 – 12	2 – 12	2 – 4
Mean±SD	5.75 ± 0.63	5.5 ± 0.735	5.7 ± 0.623	2.7 ± 0.94
p. value	**p = 0.912	*p = 0.75	*p = 0.89	

**\*\* significant difference (0.01)**

**\* significant difference at (0.05)**

Eosinophils percentage of studied groups which represented that the differential count for leukocyte revealed a characteristic blood eosinophilia among all groups of patients (SG , MG & TG) , that significantly differ from CG (p<0.01 & 0.05)

**Discussion**

Recently abnormal cytokines production is being studied as a possible mechanism in psoriasis. Cytokines are small , biologically highly active proteins that regulate the growth , function and differentiation of cells and help steer the immune response and inflammation [17]. Keratinocytes secrete a number of cytokines and chemokines that either activate or suppress immune responses [18] .The pattern of cytokine expression suggests that Th-1 cells may mediate or maintain disease [19] . The recently data confirmed previously published data that the IL-6 one of the proinflammatory cytokines is influenced in serum of psoriatic patients [9, 17 , 20] and contrary to other tested Th-2 type cytokine IL-6 , had serum levels comparable with normal controls [21]. Deeva had investigated in their study patients affected by very severe forms of psoriasis and they were characterized by increased plasma levels of IL-6 . Also , in mild to moderate psoriasis patients , they had showed higher levels of IL-6 when compared to healthy control while who found that there is no correlation between psoriasis severity assessed by PASI (psoriasis Area and Severity Index) and levels of these mediators [22]. Mohammad demonstrated a significant increase in levels of serum IL-6 in Saudi psoriasis patients as compared with healthy controls. And he has been suggested that proinflammatory cytokines not only play a

fundamental role in the worsening of the disease or activating its pathogenetic mechanisms , but are also directly related to the clinical symptoms and disease evolution after effective therapy [23]. Ozer et al. have demonstrated that serum IL-6 is significantly higher in active psoriatic patients than in controls. Furthermore , higher levels of this parameter have been correlated with clinical severity and activity of psoriasis [24].

Takahashi et al have shown that serum levels of IL-6 are significantly increased in patients with psoriasis compared with those of healthy controls [25]. IL-6 mediates T-cell activation , stimulates proliferation of Keratinocytes , and at the beginning of acute inflammation mediates the acute phase responses [17]. Anna Zaleski et al were reported the patients plasma IL-6 are significantly increased when comparing with healthy controls and subsequently they fell down parallel with successful treatment in all the treatment groups [9] .

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