AJPS, 2017, Vol. 17, No.1 **Date of acceptance:3-5-2017**

The Role of IL-18, TNF-α and hs-CRP among Iraqi psoriatic Patients and their Correlation with Disease Severity

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Abstract:

Psoriasis is a chronic inflammatory disease characterized by number of immune regulatory abnormalities. Cytokines are small, biologically highly active proteins that regulate the growth, function, and differentiation of cells and help directing the immune response and inflammation, contribute to the induction or persistence of the inflammatory processes in psoriasis. Intensive research is ongoing to understand the mechanisms involved in the development of psoriasis and offer new treatment options for patients suffering from this disease.

This study was conducted to evaluate serum IL-18, TNF-α and hs-CRP levels among Iraqi psoriatic patients and to assess their relation to disease severity depending on Psoriasis Area and Severity Index (PASI). A 65 patients with psoriasis (42 females and 23 males), with mean age (35±13) years old, and 25 apparently healthy volunteers (15 females and 10 males) with no symptoms or history of psoriasis or other allergic or skin disorders (control group) were conducted in this study. Patients were divided into two groups according to the severity of their psoriasis, 20 cases diagnosed as moderate psoriasis (PASI < 15) and 45 cases diagnosed as severe (PASI ≥ 15).

The results demonstrated significant elevation of IL-18, TNF-α and hs-CRP levels in both groups of psoriatic patients compared with control group, and a significant positive linear correlation was found between IL-18, hs-CRP and severity of the disease.

These results identify IL-18 and hs-CRP as mediators in the pathogenesis of psoriasis and can be considered as useful markers to assess psoriasis and may be considered as useful follow-up markers for monitoring patients with psoriasis.

Key words: psoriasis, IL-18, TNF-α, hs-CRP, PASI.

مفاتيح الكلمات: الصدفية، الانترلوكين-18، عامل النخر الورمي- الفا، بروتين التفاعل-سي عالي الحساسية.

الخلاصة:

الصدفية مرض إلتهابي مزمن يتميز بتغيرات مناعية غير طبيعية. السايتوكينات عبارة عن بروتينات صغيرة عالية الفعالية تعمل على تنظيم النمو والوظيفة وتمايز الخلايا، كما تساعد في توجيه الإستجابة المناعية والإلتهاب وتلعب دور في حث وإدامة العملية الإلتهابية في مرضى الصدفية. توجهت العديد من الدراسات الى فهم ميكانيكية تطور مرض الصدفية ودراسة العلاجات الجديدة للمرضى الذين يعانون من هذا المرض.

هدفت الدراسة الحالية الى تقييم مستوى الانترلوكين-18، عامل النخر الورمي- الفا وبروتين التفاعل-سي عالي الحساسية بين المرضى المصابين بالصدفية ودراسة علاقة هذه المؤشرات مع شدة المرض بالاعتماد على معيار شدة الصدفية .

تضمنت الدراسة 65 مريض مصاب بالصدفية (42 أنثى و23 ذكر) و25 شخص (15 أنثى و10 ذكور) ليس لديهم تاريخ إصابة بالصدفية أو أمراض حساسية وجلدية أخرى حيث أعتبروا كمجموعة سيطرة ضمن الدراسة. قسم المرضى الى مجموعتين إعتماداً على شدة المرض حيث أعتبر 20 مريض متوسطي الشدة (بمعيار<10) و 45 مريض شديدي الإصابة (بمعيار>10)

سجلت النتائج إرتفاع معنوي في مستوى الإنترلوكين-18، عامل النخر الورمي- ألفا، وبروتين التفاعل-سي عالي الحساسية في مجموعتي المرضى مقارنة مع مجموعة السيطرة، كما أظهرت النتائج وجود علاقة خطية معنوية بين الإنترلوكين- 18 وبروتين التفاعل-سي عالي الحساسية مع شدة المرض.

بينت النتائج أن الإنترلوكين-18 ألفا وبروتين التفاعل-سي عالي الحساسية عوامل مهمة في إمراضية الصدفية ويمكن إعتبارها مؤشرات مهمة يمكن إعتمادها في تقييم الإصابة ومتابعة المرضى المصابين بهذا المرض.

Introduction:

Psoriasis is a chronic inflammatory disease characterized by erythematous papules and plaques with thick scales on the skin. The disease shows exacerbations and remission attacks [1].

As cytokines play an important role in inflammatory diseases, much attention has been directed towards the influence of cytokines in psoriasis. In addition, a number of studies have suggested that various cytokines released by keratinocytes and inflammatory leucocytes could contribute to the induction or persistence of the inflammatory processes in psoriasis; however, the precise mechanism of their involvement in psoriasis remains unclear [2].

Although the cytokine-mediated response is an essential part of the natural protective mechanism, excessive production of pro-inflammatory cytokines, or production of cytokines in the wrong biological context, are associated with the pathology in a wide range of diseases including psoriasis. At the present time, many researches in the psoriasis field concerns the role of cytokines in the pathogenesis of this disease. Different cytokines play a part in sustaining the two main characteristics of a psoriatic lesion; keratinocyte hyperproliferation and inflammation [3]. Psoriasis is associated with an overexpression of pro inflammatory cytokines produced by T helper1 (Th1) cells and under expression of Th2 cytokines [4].

Interleukin-18 (IL-18) is a proinflam-matory cytokine that stimulates T cells and natural killer cells (NK) and enhances innate immunity as well as specific Th1 immune responses. Human keratinocytes produce IL-18, like monocytes and macrophages do, being two major sources of this molecule[5,6]. IL-18 acts directly on NK cells to stimulate INF-γ synthesis and upregulate their killing capacity [7]. It is believed that IL-18 derived from keratinocytes might be involved in the cutaneous Th1- type immune response [5]. Certain publications have shown that IL-18 expression in psoriatic skin is higher than in normal skin [5, 8] .

Tumo necrosis factor-α (TNF-α) is a 17-kD polypeptide that plays a central role in the regulation of innate immune responses. It is involved in stimulating the production of inflammatory cyokines, inducing the expression of cell surface adhesion molecules, enhancing the phagocytic/bactericidal properties of macrophages, and activating apoptotic pathways. The TNF- α is produced by a wide variety of cells, ranging from lymphocytes and monocytes, to keratinocytes, mast cells and antigen presenting cells in the skin. It is believed to contribute to the pathogenesis of psoriasis through its ability to both promote immune cell trafficking to the skin and induce keratinocyte proliferation [9].

C- reactive protein (CRP) is an acute phase protein released in response to increased levels of cytokines, such as IL-6 and TNF-α . This protein has special importance for psoriasis due to its relation with cytokines which are responsible for skin inflammation [10].

This study aimed to evaluate the role of IL-18, TNF-α and high sensitive C- reactive protein (hs-CRP) in the serum of patients with psoriasis and compare them to healthy controls. Also, to investigate the association with disease severity to determine the use of these cytokines as markers of disease severity in patients with psoriasis

Materials and Methods:

This randomized clinical study was carried out during the period from June to December 2015, the study involved 65 patients (42 females and 23 males), their mean age (35±13) years old. A25 apparently healthy volunteers (15 females and 10 males) with no symptoms or history of psoriasis or other allergic or skin disorders were considered as a control group.

Informed consent was obtained from both patients and healthy individuals before venopuncture. Those patients were diagnosed and treated in Al-Kadhimiya Teaching Hospital, Baghdad. Clinical examination was performed under supervision of a specialist physician in dermatology. Only patients with psoriasis who had not received any local or systemic psoriatic treatment within one month were included in this study. Subjects with history of acute or chronic infections, liver disease, renal disease, or diabetes mellitus were excluded from the study.

Patients were divided into two groups according to the severity of their disease. Severity of psoriasis was determined according to the Psoriasis Area and Severity Index (PASI) which depends on the intensity of erythema, scaling, and thickening of the affected area, in addition to size of the psoriatic lesion. Accordingly, moderate psoriasis were defined as PASI <15, while severe psoriasis defined as PASI ≥15 [11]. Twenty cases diagnosed as moderate psoriasis and 45 cases diagnosed as severe one.

Venous blood (5ml) was collected from each subject by venopuncture using disposable syringes. Blood was allowed to clot at room temperature for 30 min and then was centrifuged for 10 min at 4000 rpm, then sera was separated and divided into several aliquots, frozen at (-20C) and thawed immediately prior to analysis of IL-18, TNF-α and hs-CRP.

Both cytokines (IL-18, and TNF-α) and (hs-CRP) serum levels were measured by direct enzyme linked immunosorbent assay technique (ELISA) using a readymade kit for this purpose (Human company, Germany).

Statistical analysis:

The results were expressed as mean ± standard error (SE). P-values < 0.05 were considered significant and P-values < 0.01 were considered highly significant. Pearson's correlation coefficient (r) was used to assess the statistical significance strength and direction of linear correlation. To draw the graphs, Microsoft® Excel software 2010 was used.

Results:

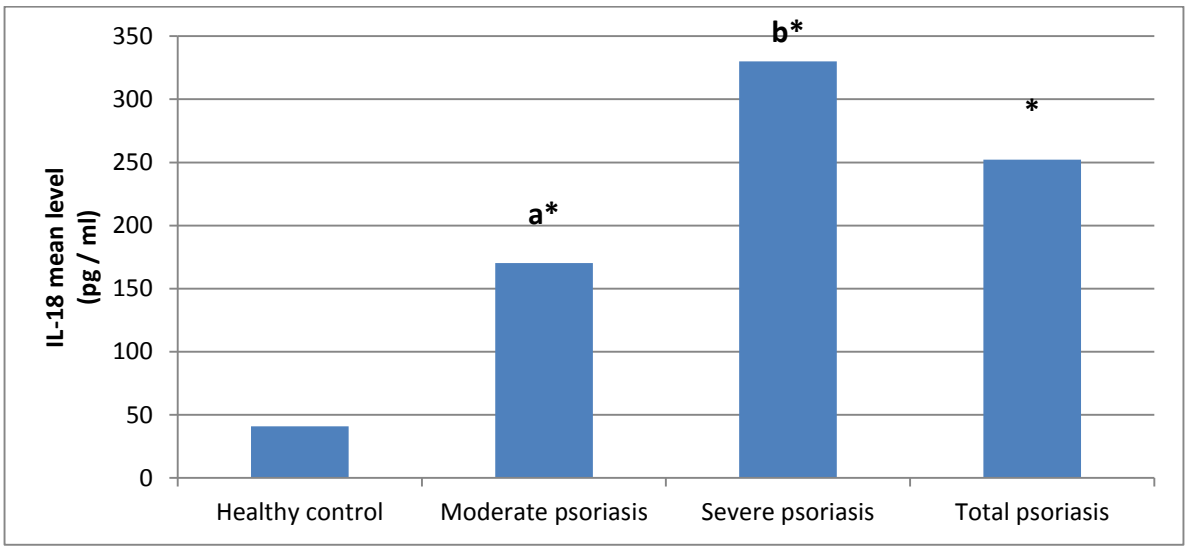
As shown in table-1, figure-1, 2 and 3, serum levels of IL-18, TNF-α and hs-CRP were elevated significantly ($p<0.05$) in both of the psoriatic groups, compared with control one. Also, mean levels of serum IL-18 and hs-CRP significantly increased ($p<0.05$) as the severity of psoriasis increased. The results also showed that there was positive highly significant correlation between serum levels of IL-18, hs-CRP, and severity of the disease ($p<0.01$) (Table- 2).

Table-1: Mean levels of IL-18, TNF-α and hs-CRP for control and psoriatic groups.

parameters	Healthy control n=25	Moderate psoriasis n=20	Severe psoriasis n=45	Total psoriasis n=65
IL-18 (pg/ml)	40.8±3.16	170.2±11.26 [a*]	330.0±38.50 [b *]	252.1±42.38*
TNF-α (pg/ml)	32.8±2.16	75.2±17.36 [a*]	82.2±18.60 [a*]	78.8±12.62*
hs-CRP µg/ml	2.05±1.5	6.2±1.2 [a *]	14.2±3.1 [b *]	10.65±2.2*

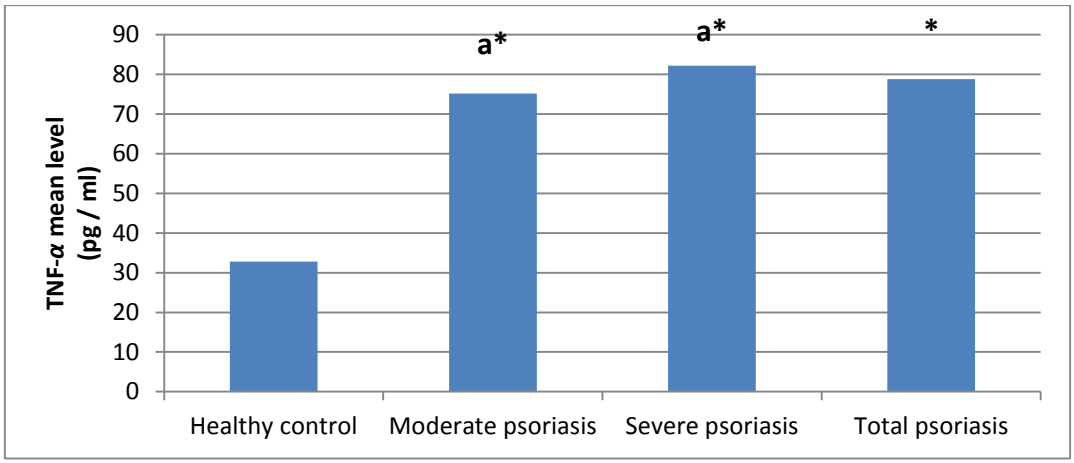
Data expressed as (Mean±SE).

n: number of subjects for each group.
* Significant difference as compared with healthy control group (P<0.05).
Results with non identical superscripts (a, b) within different psoriatic groups were considered as a significant difference (P<0.05).



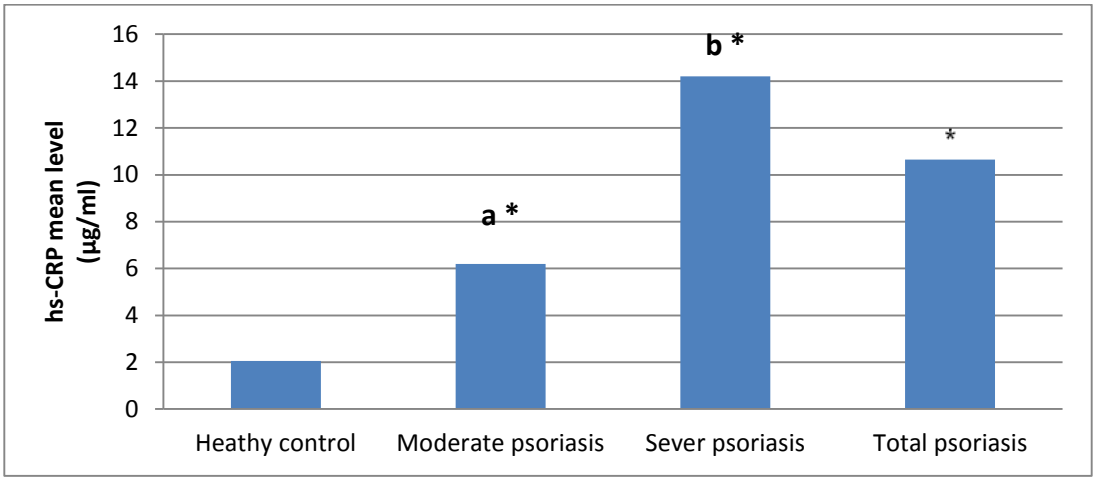
* Significant difference as compared with healthy control group (*P*<0.05).
Results with non identical superscripts (a, b) within different psoriatic groups were considered as a significant difference (P<0.05).

Figure-1: Serum levels of IL-18 for control and psoriatic groups.



* Significant difference as compared with healthy control group (P<0.05).
Results with identical superscript (a) within different psoriatic groups were considered as a non significant difference (P>0.05).

Figure-2: Serum levels of TNF-*α* for control and psoriatic groups.



* Significant difference as compared with healthy control group (*P*<0.05).
Results with non identical superscripts (a, b) within different psoriatic groups were considered as a significant difference (P<0.05).

Figure-3: Serum levels of hs-CRP for control and psoriatic groups.

Table-2: Correlation between studied markers and psoriasis severity depending on PASI.

Correlated parameters	Correlation coefficient (r-factor)	p-value
PASI and IL-18	0.8	p<0.01
PASI and TNF-*α*	0.07	p>0.05
PASI and hs-CRP	0.5	p<0.01

P<0.01 mean highly significant correlation.
P>0.05 mean non significant correlation.

Discussion:

Psoriasis is a chronic inflammatory disease characterized by number of immune regulatory abnormalities. The lesions in psoriasis develop secondary to T-cell mediated hyperproliferation of keratinocytes which is induced by antigen-presenting cells on the skin[12] .

Cytokines contribute to the induction or persistence of the inflammatory processes in psoriasis [13]. A number of cytokines and chemokines that either activate or suppress immune responses were secreted by Keratinocytes[14]. However, precise mechanism of their involvement in psoriasis remains unclear. Any local or systemic stimulus may stimulate keratinocyte cytokines production [15]. The pattern of cytokine expression suggests that Th1 cells may mediate or maintain disease [16].

Interleukin-18, a defined member of IL-1 cytokine family, was known as IFN-γ stimulating factor. Later studies clarified that this cytokine has wide range effects other than lymphocyte activation. As it is an important regulator in the production of both innate and acquired immune responses, IL-18 directly regulates the effects of T and B cells, NK cells, macrophages and dendritic cells; it acts usually synergistically with IL-12[17].

In the current study, serum IL-18 levels of psoriatic groups were significantly higher than healthy control group. This result was similar to the results reported by previous study which showed that serum IL-18 levels were higher in psoriatic patients than healthy control [18].

The present study also showed a significant correlation between severity of the disease depending on PASI and serum IL-18 levels. The correlation between IL-18 level and PASI may be an objective parameter for psoriasis activity and clinical severity. These data confirm the hypothesis of considering psoriasis as a true systemic disease with particular immunologic pathways [17,18].

Interleukin-18 play an important role in cellular adhesion, act as a pathway used by IL-1 and TNF-*α* that leads expression of ICAM-1 (intercellular adhesion molecule 1)[19]. The expression of IL-18 receptor is upregulated by IL-12, and thereby these two cytokines stimulate the release of IFN-*γ* [20,21].

Tumor necrosis factor-*α* together with IFN-*γ* induce IL-6, IL-8, IL-12, and IL-18 and constitute an important link in the cytokine network in the pathogenesis of psoriasis [22].

The role of TNF-*α* in the pathophysiology of psoriasis is still unclear, but anti-TNF-*α* therapy is highly effective in psoriasis, indicating that this cytokine has, together with IFN-*γ*, a central role in the pathogenesis [23].

The results also demonstrate a significant increase in TNF-*α* levels and this was in agreement with other studies which recognized the high level of TNF-*α* in psoriatic patients [24, 25, 23].

The results of this study support the important proinflammatory role of TNF-*α* in the clinical manifestations of psoriasis, but could not explain the absence of correlation between serum levels of TNF-*α* and PASI scores in patients with psoriasis. This finding was in accordance with another study which record that the significantly high levels of TNF-*α* were not correlated with severity of psoriasis [26].

Psoriasis can be described as a T-cell-mediated disease, in which a variety of cytokines and other factors play a complex role. The interaction between T lymphocytes and keratinocytes, by the help of cytokines, is likely to play an important role in the pathogenic process of psoriasis [27]. In the present study, serum IL-18, and TNF-*α* level were significantly higher in the psoriasis patients than in healthy controls.

C - reactive protein may increase in all kinds of infectious and tissue damage processes [28, 29]. It stimulates the release of inflammatory cytokines from monocytes like IL- 1, IL- 6 and TNF-α [30].

Many studies recorded the elevation levels of CRP in psoriatic patients and its association with disease severity, which in accordance with the present study that recorded a significant elevation of hs-CRP levels in psoriatic patients compared with healthy control. Additionally, hs-CRP levels significantly correlated with PASI score. These studies reported that CRP levels were high in patients with acute psoriasis. They also found that CRP levels were significantly higher in patients with active psoriasis lesions than those who were not in active stage [10, 31]. Another study matched the current study correlation between hs-CRP and severity of the disease [32].

In conclusion, the present study showed that IL-18 and hs-CRP is up regulated in psoriatic patients and significantly correlated with severity. These results identify these parameters as mediators in the pathogenesis of psoriasis and can be considered as useful markers to assess psoriasis and may be considered as useful follow-up markers for monitoring patients with psoriasis and optimizing the strategies of therapies in daily medical practice. Studying the cytokines role is crucial for the development of biological therapies. Blocking these cytokines activities, by either antibodies or specific inhibitors, may open new therapeutic aspects have yet to be demonstrated.

References:

1 - Nestle FO, Kaplan DH, Barker J. PSORIASIS. N.Engl. J. Med. 2009. Vol. 361. Pp: 496-509.

2 - Kristina C, Krueger G. Genetic Variations in Cytokines and Cytokine Receptors Associated with Psoriasis Found by Genome-Wide Association. J. of Invest. Dermatol. 2009. Vol. 129. Pp: 827-833.

3- Stephen K, Gelfand M. Update on the Natural History and Systemic Treatment of Psoriasis. Adv Dermatol. 2008. Vol. 24. Pp: 171-196.

4 - Gudjonsson JE, Johnston A, Sigmundsdottir H, Valdimarsson H. Immunopathogenic mechanisms in psoriasis clin. Exp.Immunol. 2004. Vol.135 (1)· Pp:1-8.

5 - Ohta Y, Hamada Y, Katsuoka K. Expression of IL-18 in psoriasis. Arch Dermatol Res. 2000. Vol.293. Pp: 334-342.

6 - Mee JB, Alam Y, Groves RW. Human keratinocytes constitutively produce, but do not process interleukin-18. Br J Dermatol. 2000. Vol.143. Pp: 330-336.

7 - Modrzyn´ ski M, Rapiejko P. The place of IL-18 in allergic diseases. Terapia. 2001. Vol.1. Pp: 43-44.

8 - McKenzie RC, Boyce F, Forsey R, Gracie A, Szepietowski J, Weller R. Psoriatic skin expresses high levels of interleukin-18 (IL-18) and IL-18 receptor (IL-18R). Br J Dermatol. 2000. Vol.142. Pp: 618.

9 - Gottlieb A, Masud S, Ramamurthi R, Abdulghani A, Romano P, Chaudhari U, DooleyL, FasanmadeA, Wagner, C. Pharmacodynamic and pharmaco-kinetic response to anti-tumor necrosis factor-alpha monoclonal antibody (infliximab) treatment of moderate to severe psoriasis vulgaris. J Am Acad Dermatol. 2003. Vol.48 (1) Pp:68-75.

10 - Chodorowska G, Wojnowska D, Juszkiewicz-Borowiec M. C-reactive protein and alpha 2-macroglobulin plasma activity in medium-severe and severe psoriasis. J Eur Acad Dermatol Venereol. 2004. Vol.18. Pp: 180-183.

11 - Ashcroft Li, Wan Po, Griffiths. Clinical measures of disease severity and outcome in psoriasis: a critical appraisal of their quality. British Journal of Dermatol. 1999. Vol.141. Pp: 185-191.

12 - Valdimarsson H, Thorleifsdottir RH, Sigurdardottir SL, Gudjonsson JE, Johnston A. Psoriasis-as an autoimmune disease caused by

molecular mimicry. Trends Immunol. 2009. Vol.30. Pp: 494-501.

13 - Kristina C, Krueger G. Genetic Variations in Cytokines and Cytokine Receptors Associated with Psoriasis Found by Genome-Wide Association. J.of Inv. Dermatol. 2009. Vol.129. Pp: 827-833.

14 - Bonifati C, Ameglio F. Cytokines in psoriasis. Int. J. Dermatol. 1999. Vol.38 [4]. Pp: 241-251.

15 - Nickoloff BJ, Karabin GD, Barker JN. Cellular localization of interleukin-8 and its inducer, tumor necrosis factor-alpha in psoriasis. Am. J.Pathol. 1991. Vol.138[1]. Pp:129-140.

16 - Uyemura KY, amamura M, Fivenson DF, Modlin RL, Nickoloff BJ. The cytokine network in lesional and lesion-free psoriatic skin is characterized by a Thelper type 1 cell-mediated response. J. Invest. Dermatol. 1993. Vol.101 (5). Pp:701-705.

17 - Nakanishi K, Yoshimoto T, Tsutsui,H. Interleukine 18 regulates both Th1and Th2 responses. Annu.Rev.Immunol. 2001. Vol.19. Pp:423-474.

18 - Gangemi S, Merendino RA, Guarneri F. Serum levels of interleukin-18 and s-ICAM-1 in patients affected by psoriasis: preliminary considerations. J. Eur. Acad. Dermatol. Venereol. 2003. Vol.17 (1). Pp: 42-46.

19 - Borish LC, Steinke JW. Cytokines and chemokines. J. Allergy Clin. Immunol. 2003. Vol. 111. Pp: S460-S475

20 - Yoshimoto T, Takeda K, Tanaka T. IL-12 upregulates IL-18 receptor expression on T cells, Th1 cells, and B cells: synergism with IL-18 for IFN-γ production. J. Immunol. 1998. Vol.161 (7). Pp:3400-3407.

21 - Fantuzzi G, ed DA, Dinarello CA. IL-12-induced IFN-gamma is dependent on caspase-1 processing of the IL-18 precursor. J. Clin. Invest. 1999. Vol.104 (6). Pp:761-767.

22 - Valdimarsson H. Immunopathogenic mechanisms in psoriasis. Clin. Exp. Immunol. 2004. Vol.135 (1). Pp:1-8.

23 - Abanmi A, Al Harthi F, Al Agla R, Khan HA, Tariq M. Serum levels of proinflammatory cytokines inpsoriasis patients from Saudi Arabia. Int. J. Dermatol. 2005. Vol. 44 [1]. Pp: 82-83.

24 - Mussi A, Bonifati C, Carducci M. Serum TNFalpha levels correlate with disease severity and are reduced by effective therapy in plaque-type psoriasis. J. Biol. Regul. Homeost. Agents. 1997. Vol.11 (3). Pp:115-118.

25 - ChodorowskaG.Plasma concentrations of IFNgamma and TNF-alpha in psoriatic patients before and after local treatment with dithranol ointment. J. Eur. AcadDermatol. Venereol. 1998. Vol.10 (2). Pp:147-151.

26 - Ozer A, Murat A, Sezai S, Pinar C. Serum levels of TNF-α, IFN-γ, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis & correlation with disease severity. Mediators of Inflammation. 2005. Vol.5. Pp: 273-279.

27 - Williams JD, Griffiths CE. Cytokine blocking agents in dermatology. Clin. Exp. Dermatol. 2002. Vol.27 (7). Pp:585-590.

28 - Jaye DL, Waites KB. Clinical applications of C-reactive protein in pediatrics. Pediatr. Infect. Dis. J.1997. Vol.8. Pp: 735-746.

29 - Kono T, Otsuka M, Ito M, Misawa M, Hoshioka A. Negative C-reactive protein in children with bacterial infections. Pediatr. Int.1999. Vol.41. Pp: 496- 499.

30 - Mahdi MR. Relationship between plaque psoriasis and atherosclerosis in iraqi patients. Inter. J.of Develop. Res. 2014. Vol.4. Pp:191-194.

31 - Rocha-Pereira P, Santos-Silva A, Rebelo I, Figueiredo A, Quintanilha A. The inflammatory response in mild and in severe psoriasis. Br. J. Dermatol. 2004. Vol.150. Pp: 917-928.
32 - Coimbra S, Oliveira H, Reis F, Belo L, Rocha S. C-reactive protein and leucocyte activation in psoriasis vulgaris according to severity and therapy. J. Eur. Acad. Dermatol. Venereol. 2010. Vol.24. Pp: 789-796.