Immunological Evaluation of Psoriatic Patients In Erbil City/Iraq

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ARTICLE INFO

Article History:
Received: 03/05/2017
Accepted: 10/09/2017
Published: 23/1/2018

Keywords:
Psoriasis, Neopterin, Tumor Necrosis Factor-α, Interleukin-10, Interleukin-22, High sensitive C-reactive protein, Malondialdehyde.

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ABSTRACT

Psoriasis is a common inflammatory skin disease with an incompletely understood etiology. The immunopathogenesis of this disease is complex and involve alternations in the innate and acquired immune system through cytokines that released by keratinocytes and inflammatory leukocytes contribute to the induction and persistence of inflammatory processes in psoriasis. This study was carried out to evaluate the immunological aspects in psoriasis patients attended consultation clinics of dermatology at Hawler Teaching Hospital in Erbil city/Iraq. Blood samples were obtained from 50 psoriatic patients and 30 healthy controls. Serum neopterin (NPT), tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10), interleukin-22 (IL-22), high sensitive C-reactive protein (hsCRP), and malondialdehyde (MDA) levels were estimated in the patients. The result showed that serum TNF-α, IL-22, NPT, hsCRP, and MDA were highly significant in the psoriatic patients compared to healthy controls (p< 0.05), while serum IL-10 showed no significant difference between psoriatic patients and healthy controls. The study revealed that elevation of serum NPT, TNF-α, IL-22, hsCRP, MDA in one hand, and impaired IL-10 expression, on the other hand, are involved in the pathogenesis of psoriasis by induction and maintenance of psoriatic lesion. The dysregulation of IL-10 is associated with enhanced immunopathology and increased risk for chronic non-healing infection in psoriasis patients.

1. INTRODUCTION

Psoriasis is a common skin disease with extracutaneous manifestations. It is characterized by chronic inflammation of the skin with changes in the maturation of keratinocytes (KCs), which manifested by the hyperproliferation of the epidermis. It is mediated by T lymphocytes, multigenic and environmental factors (Scarpa et al., 2010).

The Hyper-proliferation of epidermal KCs in psoriasis patients is driven by cytokines secreted from activated resident immune cells, an infiltrate of T cells, dendritic cells and other cells of the innate immune system, as well as the KCs themselves (Baliwag et al., 2015). Different factors including infections, trauma, medications and emotional stress can activate KCs to release cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) which in turn initiate the activation of resident cells.
skin macrophages and dendritic cells (DCs) (Nickoloff and Nestle, 2004). The cytokines produced by dendritic cells and effector T-cells stimulate KCs to proliferate and increase the migration of inflammatory cells into the skin, promoting epidermal hyperplasia and inflammation (Monteleone et al., 2011). These changes are accompanied by dermal angiogenesis leading to an increasingly complex underlying vascular system, giving the plaques their deep red coloration (Liu et al., 2007).

This increased vascularity allows for a greater influx of inflammatory cells into the skin, further driving the inflammation. Lesions are rich in activated CD4+ and CD8+ T cells that release proinflammatory cytokines and are typically distributed symmetrically on the scalp, elbows, knees and lumbosacral area. Thus, psoriasis is now generally regarded as a T cell-mediated immune disease with a mixed Th1/Th17 cytokine environment (Baliwag et al., 2015).

Acute phase reactants, cytokines and growth factors are known to play an important role in the pathogenesis of psoriasis and it is accepted that different cells are crucial in psoriasis at different stages (Coimbra and Santos-Silva, 2014). Neopterin, which is a non-specific marker for activation of cellular immune system (Mualla et al., 2010) is produced by human monocytes and macrophages upon stimulation with IFN-γ, increased levels indicate T cell activation and IFN-γ production (Huber et al., 1984) which plays an important role in psoriasis (Mualla et al., 2010).

Elevation of NPT, CRP, up-regulation of proinflammatory cytokines, may contribute to a cumulative increase in the risk of cardiovascular diseases (CVDs) in psoriasis (Balta et al., 2013, Yanchun and Zhidong, 2011). It has been also suggested that generation of reactive oxygen species (ROS) from neutrophils (Miyachi and Niwa, 1983), KCs (Turner et al.,1998), and fibroblast (Raynaud et al., 1997) can contribute to neutrophil activation, which plays an important role in the psoriatic process. Elevation in ROS result in malondialdehyde (MDA) production from the skin cells in the psoriatic lesion have a significant increase in arachidonic acid, which is the natural substrate for synthesis of malondialdehyde (MDA), an end product of lipid peroxidation (Kadam et al., 2010).

We aimed through this study to evaluate the role of acute phase reactants cytokines and growth factors, TNF-α, IL-10, IL-22, NPT, hsCRP, and MDA, in the pathogenesis of psoriasis to improve understanding of the mechanisms of immunopathology of the disease that eventually lead to developing therapeutic strategies against the impaired cytokine production.

2. MATERIALS AND METHODS
2.1. Patients

Patients were consented to take part in this study. Among 90 psoriatic patients, only 50 patients were willing to participate in the study, 26 males (52%) and 24 females (48%) with a mean of age of 30.46±14.612 years, attended consultation clinics of dermatology at Hawler Teaching Hospital in Erbil city-Iraq during September 2014 to April 2015.

2.2. Normal Controls

The control group consists of 30 volunteers, 17 males (56.66%) and 13 females (43.33%), with a mean of age of 26.50 ±11.33 years and from the same urban populations. The purpose and nature of the study were explained to all subjects. Exclusion criteria for the healthy control included a family history of psoriasis,
smoking, medication, pregnancy and any abnormalities in the body.

2.3. Blood Sampling and Storage

Seven milliliters of non-heparinized venous blood was drawn from patients and healthy controls using sterile disposable syringes then the 7ml was put into a clot-activator tube for serum separation. The serum was collected after centrifugation at 3000 round per minute (rpm) for 10 minutes and it was separated and stored at -80°C.

2.4. Cytokines detection

Serum TNF-α, IL-10, IL-22 levels were measured by quantitative enzyme-linked immunosorbent assay (ELISA) technique. The assay was achieved according to the manufacturing company instruction (Komabiotechinc Company Republic of Korea). Sample results were calculated by interpretation from the calibration curve that is performed in the same assay with samples. The standard curve was plotted using Graph Pad Prism (Version 6.01) then the optical density for each sample was plotted on the standard curve to obtain the concentration in pg/ml.

2.5. Detection of High sensitive C- reactive protein (hsCRP)

Quantitative determination was used for detection hsCRP. The test was achieved and data were analyzed according to manufacturer instruction (CobasC111, Roche, USA). Measuring range was 1-200 mg/L and 5 mg/L was the upper limit of the normal range.

2.6. Detection of Human Neopterin (Npt)

The serum of patients and normal controls were subjected to the levels of Human Neopterin using quantitative enzyme-linked immunosorbent assay (ELISA). The test was achieved and data were analyzed according to manufacture construction (My Biosource, USA).

2.7. Detection of Human Malondialdehyde (MDA)

Serum MDA was measured spectrophotometrically by a modified method described by (Muslih et al., 2002) using Trichloro Acetic Acid (TCA) and Thiobarbituric Acid (TBA).

2.8. Statistical Analysis

Data were processed and analyzed with Graph Pad Prism (Version 6.01). The data were not normally distributed and since the median is not affected by outliers like mean would do the results were expressed as Median-Interquartile Range and statistical differences between psoriasis patients and controls were determined using Mann-Whitney’s test. P-values below 0.05 were considered to be statistically significant.

3. RESULTS AND DISCUSSION

Psoriasis is a chronic inflammatory cutaneous skin disease. The diseases characterized by red, scaly and well-demarcated skin lesions formed by the hyperproliferation of epidermal KCs. This hyperproliferation is driven by cytokines secreted by activated resident immune cells, an infiltrate of T cells, DCs and cells of the innate immune system, as well as the KCs themselves. (Baliwag et al., 2015).

Psoriasis can present at any age and has been reported at birth and in older people of advanced age. The majority of our cases, 72% (36 patients out of 50), present before the age of 40 years. The remaining cases, 28% (14 patients out of 50), present after the age of 40 years. A bimodal age of onset has been recognized in several studies. The mean age of onset for the first presentation of psoriasis can range from 15 to 20 years old before the age of 40 years, with a second peak occurring at 55–
60 years after the age of 40 years (Griffiths and Barker, 2007) and this was consistent with our results.

Familial clustering in psoriasis had been observed for many years (Naldi et al., 2001). The present study showed that sixteen patients (32 %) had a positive family history of psoriasis in one or more of their relatives. This confirmed the important role of genetics in the etiology of psoriasis especially in those with early onset (Ferrándiz et al., 2002).

Understanding the function of individual cytokine in psoriatic patients is complicated because their role can vary depending on the cellular source, target, and phase of the immune response. In fact, numerous cytokines have both proinflammatory and anti-inflammatory potential (Commins et al., 2008). Various cytokines released by KCs and inflammatory leukocytes could contribute to the induction or persistence of inflammatory processes in psoriasis (Ragab et al., 2010).

Serum TNF-α level was significantly elevated in patients with psoriasis when compared to healthy controls (figure 1). Tumor Necrosis Factor-alpha is a somewhat enigmatic cytokine with respect to psoriasis pathogenesis; although it is produced by most activated T cells and antigen presenting cell (APC), TNF-α alone does not evoke significant responses from KCs; however, in combination with another cytokine IL-17A (Chiricozzi et al., 2011), IL-17C (Johnston et al., 2013) it forms strong synergies and amplifying responses which play a major role in the pathogenesis of the disease through stimulating a cytokine storm in psoriasis.

Furthermore, our results indicated a significant elevation of serum IL-22 levels in patients when compared with healthy controls (figure 2). The over expressions in IL-22 disrupt the normal differentiation of KCs. Boniface and his colleagues (2005 and 2007) show that treatment of in-vitro reconstituted human epidermis with an IL-22 result in epidermal hyperplasia accompanied by hypogranulosis and parakeratosis that result in disruption of normal terminal differentiation of KCs.

Interleukin-22 is remarkably over-expressed most probably as a result of upregulated IL-23 (Nograles et al., 2010, Boniface et al., 2007, Zheng et al., 2007) and IL-6 levels. IL-6 is reported to be important for Th-22 differentiation (Duhen et al., 2009), and its expression is also elevated in psoriatic skin.

![Figure 1. Serum levels of TNF-α in patients with psoriasis compared to healthy controls.](image1)

![Figure 2. Serum levels of IL-22 in patients with psoriasis compared to healthy controls.](image2)
Thus, the psoriatic skin provides an environment that favors the expansion/generation of IL-22-producing Th-22 cells as well as the enhanced response of keratinocytes to IL-22.

Few data have been available to reveal the serum levels of IL-10 in patients with psoriasis and the results are contradictory. Our results show no significant difference in the serum IL-10 concentrations between the psoriatic patients and control group (figure 3). The possible point that might be involved is a total lack of IL-10R expression on KCs in psoriatic lesions, and this phenomenon inevitably results in the lack of IL-10 activity (Seifert et al., 2003) in psoriatic plaques and thus activation of Th1 cells (Asadullah et al., 1998)

The immunological pathogenesis of psoriasis is a complex interaction, however, our result was came up compatible with Mussi et al. (1993) and Nickoloff et al. (1994) results which indicate the key role of IL-10 in psoriatic disease. The low level of serum IL-10 or the same levels as controls will not inhibit stimulated Th-1, Th-22, and Th-17 which lead in turn to an increased IL-22 level which has a direct effect on disruption of KCs differentiation (Wolk and Sabat, 2006).

Furthermore, the therapeutic trials done by Asadullah and his colleague (1998) confirm the key role of IL-10 through the injection of recombinant human IL-10 (rhIL-10) subcutaneously three time weekly decrease Th1-type cytokines and induced remission of psoriasis however, recombinant IL-10 therapy doesn’t show strong therapeutic potential in all psoriatic patents, as systemic administration of rhIL-10 will not be sufficient to deliver IL-10 to inflammatory sites to exert its function as anti-inflammatory and the blocked IL-10R will not be solved based on systemic administration (Murai et al., 2009). Thus, the potential therapeutic role will be through gene therapy that targets the impaired IL-10 gene expression and signaling.

Moreover, normal level of IL-10 will not inhibit stimulated Th-1, Th-22, and Th-17 which lead in turn to an increased IL-22 level which has a direct effect on disruption of KCs differentiation (Wolk and Sabat, 2006).

Our results showed the mean values of serum NPT levels were significantly higher p=0.0006 in the patient group compared to healthy controls (figure 4). Sepp et al. (1993) have reported that only a minority of patients had elevated NPT levels either in urine or serum. These inconsistent results may be due to the different cytokine response, different environmental factors, genetic factors, or infections.
Increasing concentrations of CRP have been widely reported in mild, moderate and severe forms of active psoriasis (Chodorowska et al., 2004, Arias-Santiago et al., 2012, Balta et al., 2013, Zhao et al., 2014). Our result revealed that hsCRP levels were significantly elevated p=0.001 in psoriasis patients when compared to healthy control (figure 5).

![Figure 5](image)

**Figure 5.** Serum levels of hsCRP in patients with psoriasis compared to healthy controls.

However, Few studies did not observe significantly increased values (Laurent et al., 1981, Romaní et al., 2012), most likely because the patients that were not at the active phase of psoriasis, or were under treatment with anti-psoriatic therapies. IL-17 is known to play an important role in the pathogenesis of psoriasis (Lin et al., 2011). It stimulates CRP expression in hepatocytes, coronary artery, and smooth muscle cells, independently of IL-1 and IL-6 (Patel et al., 2007) and this is the possible mechanism, which may lead to up-regulation of CRP levels in psoriasis patients.

Plasma membranes of the skin cells in the psoriatic lesion have a significant increase in arachidonic acid, which is the natural substrate for synthesis of MDA, an end product of lipid peroxidation (Kadam et al., 2010). The present study shows that serum MDA levels were significantly increased p=0.025 in psoriasis patients when compared to healthy persons (figure 6). A rise in MDA could be due to increased generation ROS due to the excessive oxidative damage generated in these patients. These oxygen species, in turn, can oxidize many other important biomolecules including membrane lipids (Kadam et al., 2010).

![Figure 6](image)

**Figure 6.** Serum levels of MDA in patients with psoriasis compared to healthy controls.

On the other hand, TNF-α, which is the major proinflammatory cytokine in psoriasis, may activate cells in a positive feedback loop producing other inflammatory mediators, including ROS and various other cytokines (Gottlieb et al., 2005, Lowes et al., 2005, Pelle et al., 2005). Thus it is thought that an insufficient antioxidant system, together with increased levels of ROS, contributes to the pathogenesis of psoriasis (Antille et al., 2002, Baz et al., 2003, Bickers and Athar, 2006).

4- CONCLUSIONS

The elevation of serum NPT, TNF-α, IL-22, hsCRP, MDA in one hand, and impaired IL-10 expression, on the other hand, are involved in the pathogenesis of psoriasis by induction and maintenance of psoriatic lesion. The dis-regulation of IL-10 is associated with enhanced immunopathology and increased risk for chronic non-healing infection in psoriasis patients.
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