Relationship between some Immunological aspects and Psoriasis among Iraqi Psoriatic patients

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Abstract:
Psoriasis is a chronic inflammatory skin disorder, worldwide. The pattern of cytokine expression suggests that Th1 may mediate or maintain psoriasis disease. IL-12 plays a major role in the development of Th1-cell mediated immune responses.

Materials & Methods: Eighty cases of psoriatic patients have been studied. Patients were grouped as mild psoriasis group (32) and severe psoriasis group (48) according to severity, and (30) apparently healthy individuals used as control group. Sera samples of all groups were collected for estimation IL-12, immunoglobulines (IgM, IgA, IgG), and diagnosis serum positivity for streptolysin-O antibody and C-reactive protein tests.

Results & Conclusions: Serum IL-12 was significantly increasing among psoriatic patients as compared to healthy controls (46.9 pg/ml and 39 pg/ml) respectively. It was concluded that IL-12 has a significant role during the acute phase of disease. A significant difference between all psoriatic patients and healthy control as well as between two types of psoriatic patients could be detected in IgA, and IgG levels, while serum IgM levels were significantly increased in the both types of illness compared to healthy controls. It was concluded that IL-12 has a significant role during the acute phase of disease. Presence of A.S.O and CRP positivity in serum of some patients refer to that infection with Streptococcal bacteria could be it is the triggered to the psoriasis in those patients, and a positive correlation was founded between IL-12 and CRP.

Key words: IL-12, psoriasis, IgS, CRP, ASO.

Introduction:
Psoriasis, a common and enigmatic recurrent cutaneous disease, has for long been considered a hyper proliferation, with extremely increased rate of epidermal turnover, and an activated mononuclear infiltrate in the underlying dermis. It is afflicts up to 4.6% percent of population worldwide.

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Psoriasis is recognized as the most prevalent autoimmune disease caused by an inappropriate activation of cellular immune system (3). T-cell activation appears to be the primary event in development of the psoriatic lesion. T-cells are activated by a number of events that lead to enhanced IFN-[gamma] production and activation of the Th1 cell cytokine cascade. In addition, there is co-stimulation by membrane bound soluble factors such as interleukin (IL) -1, IL-6, and IL-12(4). The pattern of cytokine expression suggests that Th1 may mediate or maintain psoriasis disease. Th1 cytokines (TNF alpha, IFN gamma, and IL-12), and some proinflammatory cytokines such as (IL-6, IL-8, and IL-18) are found in high levels both in lesional skin and in the peripheral blood of psoriatic patients. Moreover significant correlation between severity of the disease and serum levels of INF-gamma, IL-12, IL-17, and IL-18 (5). Besides, in situ IL-12 may also have a role in the induction of new psoriatic skin lesions (6). Other interesting thing about IL-12; this cytokine has recently been shown to promote cellular responses characterized by production of the Th1 lymphokine gamma-interferon (7). IL-12 can be produced by various cell types. Interestingly, IL-12 was detected in free nerve endings in the epidermis and in dermal nerve fibers. The presence of IL-12 in neural tissue supports the notion that the nervous system can affect cutaneous immune responses (8). Increased immunoglobulin levels, including (IgA, IgG, IgM, and IgE) were reported in psoriasis specially that of IgA was observed in psoriasis (9). CRP is the major classic human acute phase protein which is a sensitive of inflammation occurring in the body. It is synthesized by hepatocytes and regulated to a large extent by the proinflammatory cytokine including IL-1, IL-6, and TNF(10). A prospective study was conducted to obtain more clarification about the impact of some immunological abnormalities on clinical expression of psoriasis disease.

Materials & Methods:

A total of eighty patients with psoriasis were included in the present study. All patients attending to the Department of Dermatology in Al-Kadhemyh Educational Hospital, during the period between December 2007 and May 2008. None of the patients received corticosteroids, antihistamins, or other systemic therapy at the time of study. The clinical diagnosis of psoriatic patients was confirmed by dermatologists in the mentioned center above. Patients were divided into two groups according to the severity of the psoriasis: Mild group and Severe group. In addition, a control group consists of (30) healthy volunteers. Blood samples were collected by vein puncture. Blood was left to clot for (30min) at(37c). Serum was obtained by centrifugation for (10min) at (3000rpm) and kept at (-20c) for further batch analysis for Immune assays. Quentitation of (IL-12) had been performed using Enzyme Linked Immunosorbent assay (ELISA) technique revised by Biosource–Europe and measurement the concentrations of immunoglobulins (IgA, IgG, IgM) by Radial immunodiffusion test kits supplied by Biomaghreb- Tunisia, Anitstreptolysin-O test and C-reactive protein Latex kits.

Statistical Analysis:

All data were analyzed using the statistical package for social science (SPSS) 10.0 for Windows program on the computer. All data were given as mean ± standard deviation (SD). Chi-square test was used to compare differences between the frequencies. Serum cytokines levels were analyzed using the normality test. The Student t test was used to compare mean values between groups. Pearson correlation test was used for the assessment of correlation. The statistical significance was accepted as ( P value < 0.05 & <0.01) (11...
Results:
Number, age and clinical characteristics:
Some of the clinical feature and demographic picture of patients in comparison with controls

Table-1: Baseline clinical and demographic findings of study groups.

<table>
<thead>
<tr>
<th>Subjects Characteristics</th>
<th>Mild group* N=32</th>
<th>Sever group** N=48</th>
<th>Total groups N=80</th>
<th>Control group N=30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Range (mean) (years)</td>
<td>9-40 (23.5)</td>
<td>12-75(42.9)</td>
<td>9-75(35)</td>
<td>9-75(35)</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>24/8</td>
<td>24/24</td>
<td>48/32</td>
<td>17/13</td>
</tr>
<tr>
<td>Percentage of frequency</td>
<td>40%</td>
<td>60%</td>
<td>100%</td>
<td>---</td>
</tr>
<tr>
<td>CRP positivity (%)</td>
<td>16(50%)</td>
<td>27(56%)</td>
<td>43(53%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>A.S.O positivity (%)</td>
<td>5(8.33%)</td>
<td>11(23%)</td>
<td>16(20%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>Acute cases</td>
<td>11</td>
<td>3</td>
<td>14</td>
<td>---</td>
</tr>
<tr>
<td>Chronic cases</td>
<td>21</td>
<td>45</td>
<td>66</td>
<td>---</td>
</tr>
</tbody>
</table>

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

The results presented were based on total of blood samples were collected from (80) psoriasis patients and (30) healthy control subjects. Patients were divided into two groups according to the severity of their psoriasis, 32 case were diagnosed as mild psoriasis (prevalence = 40%) and (48) case were diagnosed as severe psoriasis (prevalence = 60%). The prevalence of severe psoriasis patients was high comparing to the mild patients. Furthermore a total group which represent the total number of patients. The mean age of psoriasis cases in general was (35) years old, which approximated to that of healthy controls (35) years old. According to gender distributions there were obvious variation between males and females frequency among the psoriasis patients the females are more affected by the disease, but these variation were non significantly. The frequency of C-reactive protein positivity 53% of total psoriatic patients. Results showed that the patients with severe psoriasis had increased inflammation, by made CRP-Latex test positive result in (56%) of severe group, while CRP-Latex test positive result in (50%) of mild group. Additionally, A.S.O.positivity was 14 (20%) among psoriatic patients.

Serum levels of IgA, IgM and IgG immunoglobulines:

This study was undertaken with the aim of evaluating the levels of IgA, IgM and IgG in the serum of patients and compared with healthy control group. The present data showed that there was an increase in the levels of IgA, IgM and IgG in psoriatic patients as shown in table (2).
**Table-2 Immunoglobulins levels in sera of study groups.**

<table>
<thead>
<tr>
<th>Serum Immunoglobulin types</th>
<th>Mild group</th>
<th>Sever group</th>
<th>Total group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA Con. (mg/l) Range Mean ± SD</td>
<td>1344-1629 1431±153</td>
<td>1370-1860 1711±140**</td>
<td>1361-1859 1629±135.2*</td>
<td>1392-1519 1443±122</td>
</tr>
<tr>
<td>IgM Con.(mg/l) Range Mean ± SD</td>
<td>159-268 228 ± 20*</td>
<td>102-260 206 ± 23.4*</td>
<td>146-263 210 ± 35*</td>
<td>124-133 12.9 ±19.8</td>
</tr>
<tr>
<td>IgG Con. (mg/l) Range Mean ± SD</td>
<td>2070-2765 2485 ±120**</td>
<td>1510-2283 1885 ±191</td>
<td>1950-2387 2082 ± 156 *</td>
<td>1552-1690 1628 ±109</td>
</tr>
</tbody>
</table>

** Significant Difference at the (P < 0.01)**

**Serum Levels of IL-12:**

The serum level of IL-12 was assessed in three study groups. As shown in the table (3)

**Table-3 Sera levels of (IL-12) among study groups.**

<table>
<thead>
<tr>
<th>IL-12 concentration</th>
<th>Mild group</th>
<th>Sever group</th>
<th>Total cases group</th>
<th>Healthy control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range (pg/ml)</td>
<td>32.3-60</td>
<td>32.3-58</td>
<td>32.3-52.3</td>
<td>36.7-41.7</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>47.1 ±3.6</td>
<td>46.6±5.6</td>
<td>46.9±3.9</td>
<td>39±4.3</td>
</tr>
<tr>
<td>P value</td>
<td>0.032*</td>
<td>0.023*</td>
<td>0.030*</td>
<td>---</td>
</tr>
</tbody>
</table>

*The mean is differences significant at the (P < 0.05).

The mean of IL-12 was higher in psoriatic than healthy controls (TG 46.9 pg/ml and CG 39 pg/ml) respectively the significantly differences which were observed between different study groups at (P<0.05). The mean IL-12 concentration was higher in mild psoriatic group (47.1 pg/ml) than severe psoriatic patients (46.6pg/ml).

**Correlation between IL-12 & Other Parameters:**

Pearson Correlation has been applied to study the correlation between level of (IL-12) and other serological and hematological parameters. The results are listed in Table (4).

**Table 4: Correlation between (IL-12) level and some parameters**

<table>
<thead>
<tr>
<th>The Parameters</th>
<th>Psoriatic patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP positivity (%)</td>
<td>0.323</td>
<td>0.013**</td>
</tr>
<tr>
<td>A.S.O positivity (%)</td>
<td>0.365</td>
<td>0.223</td>
</tr>
<tr>
<td>WBCs count</td>
<td>0.003</td>
<td>0.983</td>
</tr>
<tr>
<td>IgA</td>
<td>-0.726</td>
<td>0.043*</td>
</tr>
<tr>
<td>IgM</td>
<td>-0.002</td>
<td>0.990</td>
</tr>
<tr>
<td>IgG</td>
<td>-0.862</td>
<td>0.005**</td>
</tr>
<tr>
<td>Acute cases</td>
<td>0.335</td>
<td>0.044*</td>
</tr>
<tr>
<td>Chronic cases</td>
<td>0.455</td>
<td>0.055</td>
</tr>
</tbody>
</table>

* = Significant Correlation at (p<0.05)

** = Significant Correlation at the (P < 0.01)
These data reveal that there is no significant correlation between (IL-12) and other parameters among the samples of psoriatic patients except for a significant proportional correlation with CRP positivity (\%)(P= 0.01). However, there are inverse relationship between IL-12 and IgA and IgG isotype with significant. While non-significant inverse relationship with IgM. Acute cases appeared positive significantly correlation with IL-12.

Discussion:
Psoriasis is a chronic recurrent skin disease involving 1% to 2% of human population world wide. It is widely accepted that genetic predisposition and environmental factors have a profound effect on the immune system and play a crucial role in triggering psoriatic lesion development. In this research, psoriatic patients were categorized into two main types according to severity, mild and severe psoriasis groups. The prevalence of severe psoriasis group compared with mild psoriasis group similar to the results reported by (12), these differences may results from the treatment carelessness, most of patients affected with infection skin and environmental factors such as stress, excessive alcohol consumption, smoking and obesity.

The present work revealed that both sexes were affected the higher percentage of females were affected but no significant differences in both sex distribution among psoriatic patients. Recent studies have revealed that sex hormones manifest a variety of biological and immunological effects in the skin, Pregnancy, menstruation and the menopause modulate the natural course of psoriasis, indicating a female hormone-induced regulation of skin inflammation. Estrogen in vitro down-regulates of the production of neutrophil, Type 1 T-helper-cell and macrophage –attracting chemokines, by keratiocytes, and suppresses IL-12 production and antigen-presenting capacity while enhancing anti-inflammatory IL-10 production by dendritic cells, this indicate that estrogen may attenuate inflammation in psoriatic lesions (13,14).

The variations between the present results comparing with recent studies may be related to the differences in sample size, to the specific morphological structure of their skin, or the specific Iraqi nature which increased the stress in the subjective nature and that related to adverse life events. The diagnostic feature may not all be present at the same time on in every case and are some times obscured or evanescent (15).

Reactivity of CRP, the current study showed that CRP reactivity is 53% of psoriatic patients, this results were agreement with (16) who noted that in the active stage of disease highly increased plasma levels of CRP and alpha 2-macro globulin, also other finding revealed patients with moderate to severe plaque psoriasis had increased inflammation, as exhibited by elevated CRP levels. However, CRP seem to provide a marker for worsening of psoriasis (17). An acute phase of psoriasis can be induced by cytokines involved in psoriatic pathogenic phenomena. Activation of the acute phase reaction by proinflammatory cytokines may account for systemic symptoms in severe psoriasis. C-reactive protein (CRP) a marker of inflammation, in patients with moderate to severe plaque psoriasis (16). New findings showed ENBREL is a type of protein blocker that blocks the action of a substance body’s immune system makes called TNF, ENBREL improved the symptoms of patients with severe psoriasis by reduced the levels of CRP (17). Finally this result it refers to the that patients with severe psoriasis had been increased inflammation, as exhibited by elevated CRP levels so this reinforces that psoriasis is not only a skin disease, but in some patients could be a serious medical condition.
The streptococcal infections in the upper respiratory caused by streptococcus bacteria are known to trigger guttae psoriasis in children and young adults. The infections may also worsen ordinary plaque psoriasis. Dependent on this hypothesis we were detect about present of Anti streptolysin O antibodies which are produce due to the presence streptolysin O antigen liberated by the bacteria (18). Our results were in agreement with (19) Who study 70 chronic psoriasis patients and found an significant elevated antibacterial titres (A.S.O) in 2070 of cases. Streptococcus pyogenes releases a number of proteins, including several virulence factors, Streptolysin O and S, these are beta – hemolytic property. Streptolysin O is toxins which are the basis of the organism potent cell poison affecting many types of cell including neutrophils, plateletes, and sub-cellular organelles. It causes an immune response and detection of antibodies to it ;antistreptolysin O (ASO) can be clinically used to confirm a recent infection (20). Finally the presence of A.S.O positivity in serum of some patients refer to that infection with Streptococcal bacteria could be it is the triggered to the psoriasis in those patients. A significant differences between each psoriatic patients and controls could be detected especially in IgA and IgM. Serum concentration of Igs particularly in severe psoriatic patients in accord with (21, 22) they were reported that amounts of IgA, IgM, and IgG in psoriasis are higher than normal and increases have been observed in severely affected persons. Humoral immunity (IgA, IgM and IgE) was raised in psoriasis, increased immunoglobulin levels specially that of IgA were observed in psoriasis (9). It is suggested that in psoriasis stratum corneum antigens either exposed or activated by epidermal trauma which provokes an inflammatory response, and therefore IgG antibody production is triggered (22). However, it was demonstrated the presence of serum IgA within psoriatic stratum corneum and have speculated that serum IgA might initiate chemotaxis via the alternate pathway which turn could un mask the antigenicity of the stratum corneum and lead to an IgG auto antibody reaction (23) Some workers have suspected cell mediated immune pathomechanism playing an active role in psoriasis and this highlighted by the increased antigen presenting capacity of Langerhans cells isolated from psoriatic skin compared to normal skin (24). Psoriasis can be described as T-cell mediated disease, with complex role for a variety of cytokines and other factors. Interaction between T-lymphocytes and keratinocytes, via cytokines, is likely to play a pivotal role in the pathogenic process in psoriasis (1). The pattern of cytokine expression suggests that Th1 cells may mediate or maintain disease (5). T-cell activation appears to be the primary event in development of the psoriatic lesion. T-cells are activated by a number of events that lead to enhanced IFN- [gamma] production and activation of the Th1 cell cytokine cascade. In addition, there is costimulation by membrane bound soluble factors such as IL-1, IL-6 and IL-12. Our data confirmed previously published data (25, 1) that Th1 cytokine (IL-12) was influenced in the serum of psoriatic patients. IL-12 plays a major role in the development of Th1-cell mediated immune responses. Besides, in situ IL-12 may also have a role in the induction of new psoriatic skin lesions (6). Contrary to present results, (26) reported that a serum mean level was decreased in patients with psoriasis. This different results may stem from various laboratory used. We measured p40 subunit of IL-12. IL-12 and IL-23 are heterodimers that share a common p40 chain. The origin of circulating cytokine in the blood serum in psoriatic patients is not clear, and the serum cytokines concentrations are altered by several processes like production, tissue, cellular deposition, degradation, and elimination of these molecules. To achieve the cytokine
concentration that can induce biological responses at distant skin lesions, huge amount of free cytokine, that induce generalized inflammation are requires. Thus, the receptors on psoriatic keratinocytes may be more sensitive to this cytokine.

An increased secretion of IL-12 may account for the ability of bacterial products or autoantigens to aggravate psoriasis. Infection with group A, beta-hemolytic streptococci has been associated with the initiation and exacerbation of guttate and chronic plaque psoriasis. In one study, after culture with group A streptococci, T-cells from the psoriatic patients and control groups showed a significant increase in the mean percentage of cutaneous lymphocyte antigen (CLA+) expression (27). (CLA+) which activates CD4 lymphocytes in the infiltrates of inflammatory lesional skin, and evidence for continuous T-cell activation (28). IL-12 promotes IFN-[gamma] secretion by T cells and IFN-[gamma] in turn stimulates IL-12 production in dendritic cells and macrophages, thereby allowing for maintenance of a reverberating feedback that permits persistence of the pathological entities required for formation and non-regression of the psoriatic lesion (29).

Interleukin-12 (IL-12), also known as natural killer cell stimulatory factor (NKSF) or cytotoxic lymphocyte maturation factor (CLMF) is a cytokine that is produced primarily in antigen-presenting cells such as monocytes, macrophages, and dendritic cells, the physiologic effects of IL-12 are exerted by the intact p70 heterodimer that is produced after coexpression of both the p35 and p40 genes in the same cell. IL-12 plays a major role in mediating T1-cell responses and promotes induction of interferon gamma production. IL-12 enhances cytotoxicity of resting and activated T and NK cells, and acts as a co-mitogen to stimulate proliferation of resting T-cells along with IL-2. These activities of IL-12 are antagonized by IL-4 and IL-10, factors that are associated with the development of uncommitted T helper cells into Th2 cells and the mediation of humoral immune responses (30). Finally, this result is reinforcing the hypothesis that the psoriasis is an immunological disorder described by abnormal keratinocyte proliferation mediated through T-lymphocytes like IL-12, and data confirm the hypothesis that psoriasis might considered as a true systemic disease with particular immunologic pathways. IL-12 may be considered as useful follow up markers for monitoring psoritic patients and optimizing therapeutic strategies in daily medical practice.

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