Interleukin-23 and its Receptor Expression (IL-23R) in Psoriatic and Psoriatic Arthritis Patients

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ABSTRACT

Introduction: Psoriasis (Ps) is a systemic autoimmune disorder that develops under the influence of environmental factors in a genetically susceptible person. The IL-23/IL-17 axis is the primary signalling pathway for cellular and molecular alterations in Ps.

Objective: The aim of the study was to investigate the levels of interleukin 23 (IL-23) in serum and its receptor expression (ILR23) in blood of patients with Ps and psoriatic arthritis (PsA), as well as to evaluate the possibility of using (IL23R) in blood as a marker for susceptibility of Psoriasis.

Patients and Methods: Medical data and clinical evaluation were done and blood samples from patients and control groups were collected. Serum IL-23 concentration was measured using enzyme-linked immunosorbent assay (ELISA) and the Expression of IL-23R in human peripheral blood mononuclear cells (PBMCs) was determined using Western blotting.

Results: Serum IL-23 concentration was significantly higher among Ps cases than in controls and it was significantly different between PsA and Ps groups. The protein expression of IL-23R was significantly greater in Ps group than in control group with no significant difference between Ps and PsA groups. Receiver operating characteristics (ROC) curve showed a diagnostic value for the increased blood IL-23R with a sensitivity of 83.3% and a specificity of 73.3% for psoriasis. Also, (ROC) curve showed a diagnostic value for the increased blood IL-23 with a sensitivity of 80% and a specificity of 73.3% for the diagnosis of psoriasis.

Conclusion: Serum IL-23 and its receptor expression measurements are helpful tools in the diagnosis of Ps as well as in the prediction of PsA. We hypothesized that there is a link between IL-23 and IL-23R and the risk of Ps in addition to PsA, with evidence that the expression of IL-23R is linked to a significantly greater risk of psoriasis.

Keywords: IL-23, IL-23R, Expression, Psoriasis, Psoriatic arthritis.

INTRODUCTION

Psoriasis is the most prevalent autoimmune, inflammatory and proliferative dermatosis which involves 2%-3% of the world's population and is frequently associated with other comorbidities, including inflammatory bowel disease and arthritis. Classical plaque psoriasis has focal inflamed, red and raised plaques due to overgrowth of epithelial cells. Other clinical subtypes include (guttate, pustular, and erythrodermic). Early onset psoriasis accounts for 75% of cases and occurs before the age of 40. Psoriasis is caused by the imbalance between innate and adaptive immune components of skin cells due to disturbance in cytokines. The skin inflammation increases the serum and local concentrations of many cytokines as TNF-α, IFN-γ, IL1, IL23 and IL17. Psoriasis is thought to be a TH1-driven disease because of the increased TH1-cell pro-inflammatory cytokines in relation to Th2-type cytokines.

Mice received IL-23 minicircle DNA in vivo developed psoriasis and arthritis. Furthermore, the interference with IL-23 signalling pathway by neutralizing antibodies showed effectiveness against several inflammatory conditions including psoriasis. In addition, intradermal injections of IL23 in mice result in a psoriasis-like disease with increased transcription of IL-23/Th17-related genes. Therefore, IL-23 and Th17 axis might have an important role in Ps pathogenesis.

Increased systemic level of IL23 is not an absolute necessity for skin or joint disease development as augmented by the transgenic K23 mice (mouse model of Ps and PsA caused by selective and conditional skin expression of IL-23). Thus, factors induced by IL-23 in the skin (IL-17 and IL-22) can be pathogenic and initiate joint disease.

IL-23 belongs to IL-12 cytokine family and is formed of two subunits; p19 is a unique for IL23 and p40 which is also a component of IL-12. It binds to a receptor complex that consists of IL-23R and the IL-12 receptor β1. IL-23 is released by keratinocyte and activated antigen-presenting cells (APCs), and its action is mainly mediated by the production of inflammatory mediators. IL-23 has a role in maintaining the immune responses through controlling the function of T-cell memory and affecting the proliferation and survival of IL-17-producing (TH)-17 cells. Recent data reveal that T lymphocyte expresses IL-23R and respond to IL-23 by releasing IL-17 and IL-22.

IL-23 stimulates IL-17 synthesis by natural killer cells and neutrophils to control the acute infections (innate) and by TH helper 17 (Th17) cell leading to the formation of autoreactive IL-17-producing T-lymphocytes that provoke chronic autoimmune inflammatory process (adaptive). Thus IL-23 links the innate with adaptive immunity. More than 160 unique genes were activated by IL-23, including new DNA-
binding proteins and a large number of expression sequence tags that are still of unknown functions. Also, IL23 promotes upregulation of the matrix metalloprotease MMP9 and stimulates the antigen presentation by dendritic cells(12).

Under the stimulation of various cytokines, naïve CD4+T cells undergo differentiation into Th1, Th2, Th17, or T follicle helper (TFH) cells. The Th17 cell has a significant role in the pathogenic process of PS as it releases IL-17A, IL-17F, IL-22, IL-21, TNF-α, and interferon-γ(13). TH17 cell differentiation is regulated by reciprocal association with CD4+ regulatory cells (Tregs) whose key function is to inhibit T-cell response against self and foreign antigens(14). Naïve CD4+ T cells in existence of TGF-β1 mature to Foxp3 + (Tregs) cells whereas in the existence of both IL-6 and TGF-β1, mature to ROR-γ+ TH17 cells, which when further exposed to IL23 become pathogenic TH17 cells(15). So, manipulation of the differentiation pathway in between both cells may result in novel therapeutic targets in chronic inflammatory diseases. Cytokines produced by TH17 recruit neutrophils and monocytes to the lesion site, which in turn produces multiple inflammatory factors that mediate the pathogenesis of psoriasis(16).

The IL-23R gene exists on chromosome 1 (1p31). IL-23R is a type I cytokine receptor that pairs with the receptor molecule IL12R β1, to confer IL-23 responsiveness probably through activation of the JAK-STAT signalling cascade on cells that express both subunits(17). IL23R mRNA transcript is approximately 2.9-kb. IL-23 R cDNA encodes a 629 aa type I transmembrane protein. IL-23R is a key determinant of the pathogenicity and of autoimmune of Th17 cells in general(18). IL23R has a significant role in the proliferation and survival of TH17 cells that are important for the host defence against bacteria, fungi, and viruses. IL-23R gene polymorphisms might affect IL-23 responses. Dysregulation of IL-23–IL-17 axis, causes a break in self-tolerance to tissues and antigens, resulting in severe autoimmune responses(19).

The aim of the study was to investigate the levels of interleukin 23 (IL-23) in serum and its receptor expression (ILR23) in blood of patients with Ps and psoriatic arthritis (PsA), as well as to evaluate the possibility of using (IL-23R) in blood as a marker for susceptibility of psoriasis.

PATIENTS AND METHODS

Patients and samples:

Sixty cases were obtained from patients attending Mansoura University Hospital, Dermatology Clinic, thirty were diagnosed to have PSA and thirty have PS. In addition, thirty persons from age and sex matched healthy volunteers were used as controls. Thorough medical and clinical evaluation were done. Pregnant women or women on hormonal contraception and subjects with immune-mediated comorbidities and systemic disease were excluded from the start. Psoriasis area and severity index (PASI) scores were recorded to quantify PS severity in each patient.

Ethical consent:

Signed consents were obtained from all subjects. The research protocol obtained its approval from University of Mansoura [Code number: R.21.10.1501]. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Preparation of samples:

Overall, 10 ml of venous blood samples was obtained from all subjects by venepuncture under aseptic condition. 5 ml of them were poured into a serum separator tubes. After clotting, centrifugation at 2000g for 10 minutes was performed, and the sera underwent separation and storage at −20°C until used for ELIZA. The remaining 5 ml blood samples were withdrawn into EDTA containing tubes and immediately placed on ice; isolation of white blood cells (WBCs) from all these samples using RBCs lysis buffer, then used for determination of IL23R protein by Western blotting.

Enzyme-linked immunosorbent assay (ELIZA):

IL23 was quantitated by ELISA method using a commercially available Human IL-23 ELISA Kit (BioLegend, San Diego, CA, USA). Measuring absorbance was at a wavelength of A450 nm.

Detection of IL23R protein by Western blotting:

Total protein from cells underwent extraction utilizing the QIAzol Reagent (Qiagen, Germany Cat. No.79306), based on the manufacturer’s specification and measured by the BCA assay (Bosterbio, Canada). Separation of pre-stained protein molecular weight marker (Thermo Scientific, USA) and equal amounts of proteins (10 mg) was performed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10%). Then transferred to nitrocellulose membrane (Abcam, USA) utilizing Eco-Line Biometra apparatus (Gottingen, Germany), incubated in 5% non-fat milk (a blocking agent), over 60 minutes at 37°C. The membranes underwent incubation overnight at 4°C with anti-human IL23R unconjugated primary antibody (Santa Cruz Biotechnology, USA, Cat. No. sc-293485), and a rabbit antihuman glyceraldehyde 3-phosphate dehydrogenase (GAPDH) polyclonal antibody as internal control (Santa Cruz Biotechnology, USA, Cat. No. sc-25778) in blocking buffer. After incubation with goat anti-rabbit IgG antibody conjugated to horseradish peroxidase (HRP) (Santa Cruz Biotechnology, USA, Cat. No. sc-2030) at 37°C for 120 minutes, bound proteins were visualized utilizing colorimetric immuno-detection by 1-Step TMB-Blotting (Thermo Scientific, US). The membrane bands were digitally photographed and the resulted photographs were analysed using ImageJ.
software for calculation of the relative protein concentrations depending upon the ratio between peak areas of the IL23R proteins in relation to GAPDH.

**Statistical analysis**

Data were analysed by the Statistical Package of the Social Sciences (SPSS) program for Windows (version 21). At first, one-sample Kolmogorov-Smirnov test was utilized to test for normality of data. Qualitative data were represented as numbers and percent. Correlation between categorical variables was tested utilizing Chi-square test whereas Fischer exact test was applied when expected cell count less than 5. Continuous variables were represented as means ± SDs (standard deviations) for normally distributed data and medians (min-max) for non-normal data. The 2 groups underwent comparison by Student t test for normal data and Mann Whitney test for non-normal data. Sensitivity and specificity at different cut off points were tested by ROC curve. P< 0.05 was considered significant.

**RESULTS**

The patient’s medical history and PASI score were recorded. Ninety persons (40 males and 50 females) were enrolled in this study in three groups, with 30 individuals for each group (PsA, Ps and control). The female: male ratio was 17:13 for PsA, 13:17 in Ps, and 20:10 in control groups.

There was a highly significant difference in BMI in PsA and Ps when compared with the BMI of the control group. Also, there was a significant difference in BMI between PsA and Ps. As regard other demographic and some clinical data of the studied group, the difference was insignificant (Table 1).

<table>
<thead>
<tr>
<th>Table (1): Demographics and some clinical data of the study groups</th>
</tr>
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<tbody>
<tr>
<td><strong>Psoriatic arthritis (n=30)</strong></td>
</tr>
<tr>
<td>Age (years) Mean ± SD</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
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<tr>
<td>BMI Mean ± SD</td>
</tr>
<tr>
<td>Duration of PS. Median (Min-Max)</td>
</tr>
<tr>
<td>PASI Score Mean ± SD</td>
</tr>
<tr>
<td>No of swollen / Tender Joint Median (Min-Max)</td>
</tr>
<tr>
<td>Rheumatoid factor (RF): Positive</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>RF value Mean ± SD</td>
</tr>
</tbody>
</table>

P1: Comparison between psoriatic arthritis and control groups, P2: Comparison between psoriasis and control groups, P3: Comparison between psoriatic arthritis and psoriasis groups. Z: Mann Whitney test, FET: Fischer exact test. χ²: Chi square test, t: student t test, *significant P

As demonstrated in table 2, the biochemical parameter of inflammation investigated (CRP) was significantly higher in PsA and Ps in comparison to controls. Also, the mean serum uric acid concentration was significantly greater among PsA cases and insignificantly different among Ps cases when compared with controls. Also, a significant difference existed in uric acid levels between PsA and Ps patients. Again, in table 2, the serum concentrations of IL-23 were significantly greater among Ps cases and PsA than in control subjects. Also, a significant difference existed in IL-23 level between PsA and Ps patients.
The presence of IL-23R in PBMCs was quantified by western blotting (Figure 1). Western blotting demonstrated increased IL-23R protein expression in both Ps and PsA groups, as compared with the control. Protein bands were digitized, and the net band intensities were recorded and expressed as pixels.

**Figure (1):** Blotting membrane for IL-23R expression in human PBMCs. MW of IL-23R is 55 kDa and of GAPDH is 37 kDa. GAPDH: glyceraldehyde 3-phosphate dehydrogenase (GAPDH). IL-23R: interleukin-23 receptor.

Finally, (IL-23R/GAPDH ratio) was significantly higher in PsA and in Ps than control group. The protein expressions of IL-23R were significantly greater among patients than in control subjects but non-significant difference exists between Ps and PsA groups. In contrast, no significant difference was observed for the levels of IL-23 or its receptor according to the disease severity or duration.

**Table (2):** Biochemical parameters of studied groups

<table>
<thead>
<tr>
<th></th>
<th>Psoriatic arthritis (n=30)</th>
<th>Psoriasis (n=30)</th>
<th>Control (n=30)</th>
<th>Test of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric Acid mg/dL</td>
<td>8.73±2.10</td>
<td>5.78±1.90</td>
<td>5.07±1.15</td>
<td>t=7.76, p≤0.001*</td>
</tr>
<tr>
<td>CRP ng/mL</td>
<td>10.31±2.15</td>
<td>9.50±1.73</td>
<td>2.07±0.66</td>
<td>p≤0.001*</td>
</tr>
<tr>
<td>IL-23/pgm/ml</td>
<td>35.15±7.15</td>
<td>25.36±5.12</td>
<td>3.12±0.62</td>
<td>Z=6.65, p≤0.001*</td>
</tr>
<tr>
<td>IL-23R relative</td>
<td>0.31±0.05</td>
<td>0.31±0.04</td>
<td>0.0±0.0</td>
<td>Z=5.06, p≤0.001*</td>
</tr>
<tr>
<td>quantification</td>
<td></td>
<td></td>
<td></td>
<td>Z=0.799, p=0.425</td>
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<tr>
<td>(IL-23R/GAPDH ratio)</td>
<td></td>
<td></td>
<td></td>
<td>Z=4.06, p≤0.001*</td>
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<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td>Z=2.74, p=0.006*</td>
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</table>

Z: Mann Whitney test, t: student t test, *significant P

ROC analyses were used to define the diagnostic profile of IL-23 and its receptor was evaluated by ROC curve analysis. Results demonstrated an AUC of 0.79 for IL-23 in the diagnosis of psoriasis with 80% sensitivity and 73% specificity, whereas the best IL-23 cut-off value was > 0.122. The area under the curve (AUC) of IL-23/GAPDH ratio was 0.873 for the diagnosis of psoriasis with a sensitivity of 83.3%, a specificity of 73.3% and the best IL-23/GAPDH cut-off value was > 0.123 as shown in (Figure 2)

**Figure (2):** ROC for diagnosis of psoriasis using IL-23R/GAPDH ratio (a) and (b) IL-23
DISCUSSION

The pathologic consequences of increased IL-23 signalling have been associated with its capacity to enhance inflammatory mediators’ synthesis. These mediators recruit macrophages and granulocytes to damage tissue, cause chronic inflammation and, finally, development of psoriasis[21-22]. IL-23 is mainly released by APCs to induce and maintain Th17 and Th22 cells’ differentiation. Both cell lines are the primary cellular source of pro-inflammatory cytokines, which mediate epidermal hyperplasia, induction of keratinocytes and inflammation in Ps. IL-23 has a pivotal role in the activation of pathogenic Th17 cells resulting in tissue damage in different organs including joints[23].

The current model of Ps pathogenesis considers that it is a crosstalk between a complex network of dendritic cells in skin, T cells (predominantly Th17) and resident keratinocytes, which mediate inflammatory and immune responses resulting in development, progression and persistence of Ps[24]. It was found that injection of xenotransplant mouse model by neutralizing monoclonal antibody against IL-23 showed IL-23-dependent inhibition of psoriasis[25], and this is agreed with our finding. Also, another study revealed that the blood levels of IL-23 were normal in transgenic mice K23 but skin lesions precede the development of arthritis. So, high serum IL-23 concentrations, is not mandatory for the development of Ps, dactylitis and enteritis in K23 mice and IL-23 might have a role in initiation and early stages of skin and joint involvement rather than in disease progression and late stages[26]. Thus, factors triggered by IL-23 in the skin (IL-17 and IL-22) can be pathogenic and cause joint pathology[27]. IL-22 was highly elevated in the sera of K23 mice while its lack aggravated the arthritis like disease but not skin lesion[7]. In current study, when comparing Ps patients with controls regarding serum IL-23 level there was a statistically significant higher level in serum in Ps patients with much higher levels in those with PsA.

The aetiology of psoriatic arthritis is not yet clear. Whether, it is genetic, immunologic, or environmental but it seems all play a role. Up to 30% of Ps cases have PsA. The pathogenesis of PsA is complex[28]. First, high serum IL-23 concentrations can promote PsA via acting on an IL-23R positive cell population in patient’s skin and in the entheses[29]. Secondly, Ps is a characterized by rapid epidermal cell turnover, which can cause hyperuricemia[30], and this was supported by our finding as regard increase the level of uric acid in PsA as compared to controls.

Interestingly, our study demonstrated that participants with either Ps or PsA have a statistically significant higher expression of IL-23R than controls. However, we found no statistically significant difference as regard IL-23R expression between Ps and PsA patients. By other words, no significant IL-23R expression difference was detected among Ps and PsA suggesting that the direct impact of IL-23R expression is linked to Ps but it has a limited role as regard PsA.

Also, the inflammation involves a large body area of psoriatic patients leading to increase in CRP. CRP causes elevation of blood pressure via decreasing nitric oxide (NO) in endothelial cells with subsequent vasoconstriction that impairs blood flow to the joint. Vasoconstriction results in hypoxia and impaired joint function leading to arthritis[31]. Moreover, cutaneous manifestations of Ps are because of impaired differentiation and proliferation of keratinocytes due to altered interaction between these cells and immune cells. Apart from skin, similar inflammatory reaction occurs at the joint causing PsA[32]. In addition, IL-23 induces the production, development and differentiation of functional and mature osteoclasts, signifying that it is a potent initiator of bone erosion and loss[33]. Furthermore, there might be shared pathogenic pathways between primary skin disease and PsA. This was augmented by the detection of identical T cell clones in skin and synovial tissues of PsA cases proposing that a shared antigen may be a driving immune response in both sites[34]. Finally, evidence exists regarding a partial overlap between Ps and PsA susceptibility genes, such as HLA-Cw6 and other genes including IL-13, and IL-23R[35]. Interleukin-23 has been studied in a variety of systemic diseases including cancer, inflammatory and autoimmune diseases. Nonetheless, IL-23R expression in PsA remains unclear. To confirm the role of IL-23R expression in PsA, more research with large number of enrolled patients is required. It is crucial to investigate the involvement of IL-23 and its receptor in pathophysiology of Ps and PsA.

This study has numerous strengths. We are the first to analyse IL-23R expression in Ps and PsA. Moreover, a relatively large proportion of psoriatic patients having PsA.

We have some limitations such as small number of patients, additionally it was a single-centre study; this is simply explained by shortage of financial support.

Finally, our findings declared the importance of serum IL-23 in diagnosis of Ps and prediction of PsA. Moreover, our data suggest a link between expression of IL-23R and the risk of Ps.

CONCLUSION

IL23 and its receptor have been proved to be significantly elevated and over-expressed in blood of psoriatic patients and may be used as diagnostic or therapeutic targets in psoriatic patients.

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Author contribution: Authors contributed equally in the study.
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