A distinct inflammasome IL-1β gene expression profile in patients with psoriatic arthritis in Basra city

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Abstract---Psoriatic arthritis (PsA) is defined as a heterogeneous inflammation correlating with psoriasis, usually sero-negative inflammatory arthropathy and the factor genes essential for its expression and prediction. Therefore, current study aimed to determination whether IL-1β gene expression has a role in the development of psoriatic arthritis. A total of (128) individual participated in this study, (59) with psoriasis, and (29) others with psoriatic arthritis, in addition to (40) healthy subjects. All participants were from those who attended Al-Sadr Teaching Hospital or Al-Basra teaching hospital in Basra governorate, southern Iraq, from August 2020 to February 2022. After the samples were collected, peripheral blood was isolated and total RNA was extracted to estimate the expression of the genes of interest using q RT-PCR. Our results confirmed that the expression level of IL-1β in the PsA patients was up-regulated by 22.09-fold compared with the down-regulated psoriasis patients by 2.8. Thus, we concluded that IL-1β was significant in the development of psoriatic arthritis.

Keywords---psoriatic arthritis, IL-1β, psoriasis, gene expression.
Introduction

Briefly describes psoriatic arthritis (PsA) as a common chronic inflammation featured by joint pain and swelling accompanied by systemic manifestations and in some cases progressing to joint desolation with dysfunction (Chua-Aguilera, Möller, & Yawalkar, 2017; Schett et al., 2022; Umezawa, 2021). Besides, it is considered an autoimmune disease of a heterogeneous type and distinguishing it from rheumatoid arthritis is difficult due to their great similarity in clinical manifestations (Saalfeld, Mixon, Zelie, & Lydon, 2021). General clinical manifestations of psoriatic arthritis include synovitis with subsequent osteolysis, sacroiliitis, and extra-articular symptoms. It should be noted that PsA usually develops long period after psoriasis (Ps) in most patients (Mease et al., 2021; Zabotti et al., 2021). Estimates of PsA prevalence vary, indicating that environmental and genetic factors influence the risk of developing this disease (Stober, 2021). Inflammatory responses are identified by the abundant production of pro-inflammatory molecules that propagate recurrent inflammation (Livshits & Kalinkovich, 2018). In the case of psoriatic arthritis, activated T cells as well as macrophages stimulate product of certain inflammatory cytokines, including interleukin (IL)-1β (Veale & Fearon, 2018). IL-1β, which belongs to the IL-1 family, can be simply defined as a pleiotropic cytokine with immunomodulatory activities. It is highly regulated by RNA stability and translational control, and its release requires post-translational processing. As circulating initiates, its effects are further controlled by multiple IL-1 receptors (Stehlik, 2009; Strand & Kavanaugh, 2004). In recent years, IL-1β inflammasomes have been shown to have associations with several inflammatory diseases (Satoh, Otsuka, Contassot, & French, 2015). These inflammasomes consist of various proteins that, when assembled, activate pro-caspase-1 and the subsequent cleavage of pro-IL-1β into active IL-1β. Because IL-1β mediates pannus formation, it contributes to the destruction of both cartilage and bone, as well as obstructs its repair, thus directly affects the patient's physiology, causing disability for arthritis patients (Juneblad et al., 2021; Levescot et al., 2021; Netea et al., 2010). The purpose of the current study was to examine the levels of IL-1β gene expression in the peripheral blood of both Ps and PsA patients.

Materials and Methods

This prospective study was conducted on 128 participants who attended the rheumatology unit and biological treatment center of Al-Sadr Teaching Hospital and Al-Basra Teaching Hospital, located in Basra Governorate, southern Iraq, from August 2020 to February 2022, after obtaining the approval of the Ethics Committee in Basra health directorate to conduct this study. The current study included (59) patients with psoriasis (Ps) and (29) other patients with psoriatic arthritis (PsA) after their diagnosis was confirmed by skilled medical professionals, in addition to (40) healthy volunteers for control (HC), after written informed consent from all participants. The participants also included adults of both genders. In contrast, autoimmune diseases, chronic systemic diseases, non-adult, and pregnancy were excluded. Peripheral blood was collected from patients and healthy volunteers then total RNA was extracted to estimate the expression of the interested genes by qRT-PCR. Each sample was placed in an EDTA tube (5 ml) then the sample underwent RNA isolation. The total RNA was extracted using
Wizol™Reagent according to the manufacture instructions. The final concentration and the quality of elute RNA was measured by a Nano-drop spectrophotometer. All RNA samples were stored at -80°C until further analysis. Total RNA (400 ng) was transformed into cDNA using WizScript™ RT FDmix kit from (Wizbiosolution, Korea) according to the manufacture instructions. Primers for the IL-1β, in addition to β-acting as a housekeeping gene were obtained from Macrogen company (alpha DNA, Canada) and the sequences of primers used to detect marker mRNA expression are shown in Table (1).

Table 1
The primers sequence of the genes used in the current study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>F: 5’- GCACGATGCACCTGTACGAT-3’</td>
<td>(Bhat et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>R: 5’-CACCAAGCTTTTTTGCTGTGAGT-3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F: 5’-CCACACTGTGCCCATCTACG-3’</td>
<td>(Ranjbaran et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>R: 5’-CCGTGGTGCTGAAGCTGTAG-3’</td>
<td></td>
</tr>
</tbody>
</table>

The reference volumes of a single PCR reaction of of (IL-1β) and (B-Actin) gene PCR. (2-6) μl of cDNA mixed with 10 µl master mix GoTaq® qPCR Master Mix (Promega, U.S.A), 1µ revers primer, 1, 1µl forward primer and the mixture was toped up by adding Nuclease-free water to 20 µl. The Applied CFX ManagerTM Software (Bio-Rad) was used to measure the expression. The qRT-PCR conditions were as in table (2). Primer sequences and assay characteristics are given in Table (1).

Table 2
Thermal cycler conditions for the (IL-1B) gene amplification

<table>
<thead>
<tr>
<th>Proceedings</th>
<th>Temperature (Cellulosic)</th>
<th>Time</th>
<th>Cycles (Frequents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>95˚ C</td>
<td>2 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95˚ C</td>
<td>15 seconds</td>
<td>50</td>
</tr>
<tr>
<td>Annealing</td>
<td>55˚ C</td>
<td>30 seconds</td>
<td>50</td>
</tr>
<tr>
<td>Extension</td>
<td>72˚ C</td>
<td>30 seconds</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72˚ C</td>
<td>10 minutes</td>
<td>1</td>
</tr>
</tbody>
</table>

The data was analyzed using the ∆∆CT method as follow:

\[ ∆\Delta CT(PsA) = ∆CT(T_{gene}) - ∆CT(HK_{gene}) \]
\[ ∆\Delta CT(HC) = ∆CT(T_{gene}) - ∆CT(HK_{gene}) \]
\[ \Delta CT = ∆CT(PsA) - ∆CT(HC) \]

Gene Exp. = \[2^{-\Delta \Delta CT}\]

Fold change (FC) = Gene Exp. PsA / Gene Exp. HC

-PsA= Psoriatic Arthritis , HC= Healthy Controls, T_{gene}= target gene, HK_{gene}= Housekeeping gene, Gene Exp.= gene expression

To analyze the data, statistical analyzes were performed using the (SPSS system) program, appropriate nonparametric tests were chosen to determine the differences between the three study groups.
Results and Discussions

Total RNA was extracted from samples. The quality of the samples ranged from 1.3-1.8 A260/A280 and 0.7-1.9 A260/A230. While the concentrations ranged from 14.4-244 ng/μl.

Estimation of gene expression level in study subjects

The expression of several genes were estimated by qRT-PCR in PsA patients and HC (Ps=29, PsA=59 and HC= 40) using β Actin as a reference gene. The genes of interested include IL-1β The binding of the SYBR green dye was specific to the target genes through one peak in the melting curve data and amplification curve is shown in Figure 1,2 respectively.

Figure 1. Melting curve for IL-1β gene using SYBR green chemistry qRT-P.

Figure 2. Amplification curve for IL-1β gene.
**IL-1β**

The current results from qRT-PCR showed that the expression level of IL-1β in PsA groups was up regulated 22.09-fold than Ps was down regulated 2.8, as shown in Figure (3).

![Figure 3. Gene expression levels of IL-1β in Ps, PsA and healthy controls](image)

**Discussion**

Generally, in the innate immune cells, specifically in the cytosol, inflammatory particles (inflammasomes) with complex molecules are established (Próchnicki & Latz, 2017), and during activation the sending signaling platforms attain proteolytic action followed by inflammatory proceedings including IL-1β maturation (Broz & Dixit, 2016; Rabolli, Lison, & Huaux, 2015). The physiological function of those inflammatory molecules is mainly to provoke an immune response and contribute to repair of cellular tissue homeostasis, and unregulated activation of inflammasomes can be detrimental (Meizlish, 2021; Nich et al., 2013). Excess inflammasomes response has been shown to contribute to autoimmune diseases and cancer (Man, Karki, & Kanneganti, 2016). Realization the mechanism that leads to activation of inflammasomes in pathological states will improve the identification and remediation of deviate inflammation such as osteoarthritis (Spel & Martinon, 2020). In this study, we found significantly increased gene expression at higher IL-1β serum levels in patients with psoriatic arthritis, but not in patients with psoriasis. This is evidence that IL-1β is among inflammasomes-related genes embroiled in the pathogenicity and developments of psoriatic arthritis. Polymorphisms in the IL-1β gene locus related with tendency to PsA (Monnet et al., 2012). Our founding was in agreement with the results of other similar studies. In a previous study conducted by Gu and colleagues, they found that inflammatory mediator gene expression (IL-1β) was greater in blood mononuclear cells of psoriatic arthritis patients, compared to normal individuals (Gu et al., 2002). In another study of 140 patients with PsA, Ravindran and
colleagues demonstrated that the IL-1 gene complex had a clear role in PsA development or acted as a sign for another gene on chromosome (2q12) to (2q13) (Ravindran et al., 2004). In addition, studies have reported high level of IL-1β in the peripheral blood cells of PsA patients, besides, active caspase-1 was detected in the patients' synovial fluid (Son et al., 2013; Yokose et al., 2018).

Conclusions

Results of current study concluded that IL-1β has a role in the development of psoriatic arthritis, and this can be exploited in developing an effective treatment for this disease.

References


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