ESTIMATION THE SERUM CONCENTRATION OF INTERLUKINE 2 RECEPTORS IN PATIENTS WITH RHEUMATOID ARTHRITIS

Amina N. AL-Thwani¹  Mohammed I. Nadir¹  Hanaa N. AL-Okadi²

¹Genetics Engineering and Biotechnology Institute for Postgraduate Studies, University of Baghdad, Baghdad, Iraq
²Health and Medical Technology College, Foundation of Technical Education, Baghdad, Iraq

ABSTRACT

Serum soluble interleukin 2 receptors (sIL-2R) concentration reflect lymphocyte activation in vivo. The present study was conducted to determine if sIL-2R concentration correlate to disease activity parameters in patients with rheumatoid arthritis (RA) compared with healthy populations. One hundred blood samples were collected, from RA patients and thirty from apparently healthy groups. The mean age of RA patients was 46.75±12.82 and, the majority of patients were females (84%). The level of sIL-2R was estimated serologically by using enzyme linked immunosorbant assay. The result indicated that the sIL-2R was elevated significantly (mean 2101.67± 1899.21) in RA compared with healthy control group (mean16.76±5.75).

Key words: ELISA, Interlukine 2 receptors, Rheumatoid arthritis.
أسمه الهندسة الوراثية والتقنيات الإنجذبية للدراسات الطبية، جامعة بغداد، العراق، كلية التقنيات الصحية والطبية، هيئة التعليم التقني، بغداد، العراق.

INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory disease leading to joint destruction. The molecular mechanism of synovitis is associated with T-cell activation and an elevated production of proinflammatory cytokines, metalloproteinases, and adhesion molecules (1, 2). The interleukin-2 receptor (IL-2R) is part of a membrane receptor for interleukin-2, which can be localized on the cell surface of different lymphoid cell lines including activated T and NK cells, monocytes, eosinophils (3, 4) and on some tumor cells (5). This membrane receptor is important for cell stimulation with interleukin-2 (IL-2), which is one of the most significant interleukins in the immune system. IL-2R exists in three different forms: alpha (IL-2Rα), beta (IL-2Rβ), and gamma chains (IL-2Rγ). Three protein chains (α, β and γ) are non-covalently associated to form the IL-2R (6, 7). Some researches have demonstrated increased levels of sIL-2R in the sera of patients with a variety of autoimmune or immune-mediated diseases including such as systemic lupus erythematosus, rheumatoid arthritis, in most autoimmune diseases serum levels correlate with disease activity as defined by various clinical and laboratory parameters. These data suggest the usefulness of measuring serum sIL-2R levels in the management of autoimmune patients (8, 9). The aim of this study was to assess the clinical utility of serum sIL-2R concentration in rheumatoid arthritis.

MATERIALS AND METHODS

One hundred rheumatoid arthritis patients and thirty samples from apparently healthy control. All patients had RA as defined by the American Rheumatism Association (ACR). The diagnosis was made by the consultant medical staff in Baghdad teaching hospital from March 2008 to March 2009. Five milliliter of venous blood was collected from all subjects, and then sera were separated and freezes at -20°C until used. ELISA test was done according Biosource, Europe.

Laboratory indices measured included the Rheumatoid factor (RF) using latex agglutination test. The agglutination appears (positive result) when the serum contains approximately more than 10 IU/ml of RF.

Soluble Interleukin-2 Receptors

A commercially available enzyme linked immunosorbent assay (ELISA) (Immunotech, France) was used to assess the concentration of IL-2R in sera of RA patients and healthy control. It is a two immunological steps sandwich type assay. In the first step the IL-2R was captured by a monoclonal antibody bound to the well of microtiter plate. In the second step a biotinylated monoclonal antibody is added together with Streptavidine – peroxidase conjugate. The biotinylated antibody binds to the solid phase antibody – antigen complex, in turn, bind the conjugate. After incubation, the wells are washed and the antigen complex bound to the well detected by addition of a chromogenic (substrate). The intensity of the coloration is proportional to the IL-2R concentration in the sample or standard (10).

The means ± SD were given difference between means of patients and healthy controls were assessed by Least Significant Differences (LSD). These statically analysis was done by using Pentium-four computer through the SSPS program, version 10 (11).
RESULTS

Demographical distribution

The demographical distributions of studied groups are shown in Table (1) which reveals that the mean of age for RA patients was 46.75±12.82. Moreover, the majority of patients were females (84%) with females: male ratio of 5.2:1.

The rheumatoid factor positivity among RA patients was 47%. The duration of disease was found varied among RA patients (8.072 ± 7.810 years).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RA Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (years)</td>
<td>46.75 ± 12.82</td>
</tr>
<tr>
<td>Female: Male</td>
<td>5.2: 1</td>
</tr>
<tr>
<td>RF Positivity (%)</td>
<td>47%</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>8.072 ± 7.810</td>
</tr>
<tr>
<td>Total No.</td>
<td>100</td>
</tr>
</tbody>
</table>

Soluble interlukine-2 receptor concentrations

Concentrations of IL-2Res were significantly higher in patients (mean 2101.67±1899.21) in RA compared with healthy control group (mean 16.76 ±5.75) (Table 2).

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Groups</th>
<th>No.</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>t-test P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2R</td>
<td>Healthy control</td>
<td>30</td>
<td>16.76</td>
<td>5.73</td>
<td>189.21</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>RA</td>
<td>100</td>
<td>210.67</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Rheumatoid arthritis is one of the most common autoimmune diseases, affecting 0.5% of the population (12). In this current study, the mean age for RA patients were observed to be at the fourth decade (46.75 ± 12.82 years) which is in agreement with previous studies (13). Moreover, the prevalence of RA among RA females was 84% and 16% for male, this frequency is higher to some extent, with that of local previous studies in Iraq which are mentioned by Abdul-Abas (14) and Al-Haidary (15) who noticed the lower percentage which reached to 70.7%, 79.7% respectively. This result could be explained to the environmental conditions beside the psychological situation of Iraqi people which results in high stress that enhance RA development. Also higher prevalence was noticed in North American families 76.8% (16). This result denoted a high frequency among females rather than males which may be due to the hormonal differences between them and in turn, their effects on the immune responses (17). The positivity of RF was observed in (47 %) of Iraqi patients, it seemed which done by Iraqi researchers which were (53.3%, 57.7%) respectively, while other study found high positivity of RF 85% 18. These differences may be due to most of the samples related to well-established RA patients who were undergone treatment which lower RF titer below the significant level. In patients with rheumatoid arthritis the total IL-2R expression is increased on lymphocyte obtained from synovial
In addition to membrane expression of IL-2R, activated T cells secrete a truncated soluble form of this subunit which retains the ability to bind IL-2R with roughly the same affinity as the membrane form (20, 21). The availability of reliable, commercially available assay for this molecule provides a robust and easy method to quantify T cell activation in vivo (21). Interleukin -2 receptor in this study was observed to be elevated in highly significantly difference between RA patients and apparently healthy control (P<0.001). This result is in agreement with previous studies that had shown the concentration of IL-2R were significantly higher in RA Patients compared with healthy control (P<0.001). The result indicated that serum levels may reflect the degree of immune activation within affected joints (19, 22). Other workers have shown that levels are higher in Synovial fluid from Patients with RA than in serum samples (23). Serum concentrations of IL-2R have been reported to be useful in assessing and monitoring the response to treatment in diverse range of disorders associated with immune activation and immune deficiency (12). However, the serum sIL-2R level in RA probably reflects activation of underlying immunopathogenic mechanisms and appears to be an excellent monitor of clinical disease activity. More importantly rising levels may also predict exacerbation of disease activity (24, 25).

REFERENCES