Original Research Article

Serum Adenosine Deaminase and IL 6 as Inflammatory Marker in Rheumatoid Arthritis

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ABSTRACT

Background & Objectives: Rheumatoid arthritis (RA) is a prototypical inflammatory joint disease. The degree of inflammation is reflected in the extent of joint damage, which further has influence on the quality of life of patients with RA. Serum adenosine deaminase (ADA) and IL-6 have been previously proposed to predict disease activity in patients with RA. The aim of this study was to investigate the level of serum ADA and IL-6, and the relationship between ADA and IL-6 as disease activity markers, in a group of patients with RA and compared with healthy subjects.

Methods: Sixty two RA patients and forty six age and sex matched healthy controls were included in the study. ADA activity and IL-6 levels in serum were measured in all the subjects and correlated.

Results: Serum ADA level was found significantly higher among RA subjects with respect to controls (43.73 ± 19.74 versus 18.96 ± 4.83 U/L; P < 0.0001). Moreover, serum IL 6 levels were also increased in RA cases as compared to controls and were statistically significant (19.48 ± 9.81 versus 8.33 ± 4.89 U/L; P < 0.0001). However, no correlation was observed between serum ADA with serum IL 6 level (r = 0.528; P = 0.237) among RA subjects as well as in controls (r = 0.071; P = 0.615).

Conclusion: Study showed a correlation study in which serum ADA does not correlate with IL 6 levels which means that they are not inter-dependent on each other and their levels rise individually. However, further larger and well controlled studies are needed to establish its role as inflammatory marker.

Keywords: Adenosine deaminase, IL-6, rheumatoid arthritis, serum.
INTRODUCTION

Rheumatoid arthritis (RA) is a systemic inflammatory disease which affects the joints of hands and feet as an erosive, symmetrical polyarthritis. The etiology of rheumatoid arthritis is completely unknown, though it is measured as an autoimmune disorder involving other joints and organs. Approximately 1% of the total population worldwide are prevalent from RA and it is more commonly seen in 7th decade of an individual life.\(^1,2\)

The pathogenesis of RA involves the impaired function of immune system and it is mainly thought as a T cells-mediated disease characterized by accumulation of chronic inflammatory cells including T and B lymphocytes, monocytes, and macrophages. T cells (CD4+) activate B cells and macrophages via cell-surface receptors and secrete cytokines such as interferon (IFN) and various interleukins.

The diagnosis of RA is clinical. The autoantibodies and high level of inflammatory markers help in diagnosis while they are also important for prognosis and diagnosis of treatment.\(^3\) The biological markers such as acute phase reactants, rheumatoid factor (RF) etc. acts as an indicator of the disease progression besides the number of swollen and tender joints. Moreover, the genetic markers like polymorphisms in HLA-DR or candidate genes may be beneficial in diagnosis of RA.\(^4\) The Disease Activity Score (DAS) and its modifications such as the DAS28 (based on 28-joint counts), the DAS28-CRP, the Simplified Disease Activity Index (SDAI), and Routine Assessment of Patient Index Data (RAPID) also indicates the progression of the disease.\(^5,6\) The levels of acute phase reactants such as CRP and ESR are the best predictors of RA progression as they are the biomarkers of pro-inflammatory cytokine production.\(^7,8\)

Adenosine deaminase, one of the key enzymes of purine metabolism that catalyzes the deamination of adenosine to inosine, is vital for the differentiation and proliferation of T lymphocytes and monocytes, macrophage system, was considered as a suitable marker of cell-mediated immunity.\(^9\) It is present in mostly in lymphoidand fatty tissues and considered as one of the main components for maturation and function of lymphocytes and formation of macrophages from monocytes. The activity of ADA in RA patients as a result of cell-mediated immune response increases.\(^8,10\)

Interleukin 6 (IL-6) is a pleiotropic cytokine with a pivotal role in the pathophysiology of rheumatoid arthritis (RA). It is found mostly in the synovial fluid and serum of patients with RA. IL-6 can advance synovitis and joint destruction by stimulating neutrophil migration, osteoclast maturation and vascular endothelial growth factor (VEGF)-stimulated pannus proliferation.\(^11\) IL-6 may also be mediating many of the systematic manifestations of RA including inducing the acute-phase reaction [including C-reactive protein (CRP)], anaemia through hecipidin production, fatigue via the hypothalamic—pituitary—adrenal (HPA) axis) and osteoporosis from its effect on osteoclasts.\(^12,13\) In addition, IL-6 may contribute to the induction and maintenance of the autoimmune process through B-cell maturation and TH-17 differentiation.\(^14\)

Thus the aim of our study was to assess the ADA activity and IL6 in RA patients and correlate with the severity of progression of the disease.

MATERIALS AND METHODS

The case control study consists of 62 patients presenting rheumatoid arthritis and 46 control subjects attending the outpatient department of orthopaedics in ICARE Institute of Medical Sciences and Research, Haldia. The diagnosis of RA was established by clinical analysis, ESR, and rheumatoid factor tests. The subjects were age, sex and BMI matched for RA as well as controls. Routine biochemical parameters were done for both cases as well as controls. Informed consent were taken by all individuals in both the groups. The study was approved by the Institutional Ethics committee.
10 ml of venous blood was taken by arm venous puncture in sterile vials. 6ml of blood was collected without anticoagulant and serum was separated by centrifugation at 3500 rpm for 15-20 mins and was used for measurement of ADA and IL 6. Serum ADA was measured by spectrophotometer using adenosine as the substrate and expressed as U/L [14]. Also, the serum level of IL 6 was measured by ELISA Kit (Raybiotech, USA). The rest of the blood sample was collected in sterile tube containing potassium-EDTA anticoagulant for measurement of ESR by Westergren method. For the detection of CRP in serum, CRP kit was used which is a rapid latex agglutination test.

Statistical analysis of different biochemical parameters was performed by Students’ t-test. All variables were expressed as mean ± SD (standard deviation). Means obtained from two normally distributed sample groups were compared by Student’s unpaired two-tailed “t” test and for non-parametric Mann-Whitney “U” test. To find out the correlation between two variables, Pearson’s product moment correlation coefficient was used. A value of P < 0.05 was considered as statistically significant. All statistical analyses were performed by using Graph Pad prism software (version 5, 2007, San Diego, California, USA).

RESULTS

The demographic and biochemical profile of the RA subjects and healthy controls is presented in Table 1. There was no significant difference in age, sex distribution or BMI in either of the two groups between RA and control subjects (Table 1). ESR was elevated in RA cases as compared to controls which were found statistically significant (Table 1). CRP positive cases were seen in RA patients as depicted in Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (n = 46)</th>
<th>RA (n = 62)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td>51.68 ± 6.1</td>
<td>52.09 ± 6.9</td>
<td>0.7492</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>10/36 [21.74%/ 78.26%]</td>
<td>16/46 (25.81%/74.19%)</td>
<td>0.4612</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.72 ± 1.64</td>
<td>25.46 ± 1.92</td>
<td>0.4575</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>92.23 ± 8.52</td>
<td>93.7 ± 11.17</td>
<td>0.3421</td>
</tr>
<tr>
<td>Serum total CHL (mg/dl)</td>
<td>176.3 ± 23.44</td>
<td>182.9 ± 42.3</td>
<td>0.3421</td>
</tr>
<tr>
<td>Serum HDL (mg/dl)</td>
<td>44.98 ± 6.12</td>
<td>35.22 ± 4.66*</td>
<td>≤0.0001</td>
</tr>
<tr>
<td>Serum TG (mg/dl)</td>
<td>119.7 ± 19.34</td>
<td>122.8 ± 29.2</td>
<td>0.5332</td>
</tr>
<tr>
<td>ESR</td>
<td>10.2 ± 2.34</td>
<td>49.4 ± 22.34*</td>
<td>≤0.0001</td>
</tr>
</tbody>
</table>

[FPG, fasting plasma glucose; CHL, cholesterol; TG, triacylglyceride; HDL, high density lipoprotein cholesterol. Age, BMI, and serum levels of biochemical parameters were expressed as the means ± SD. Statistically significant, * p < 0.0001 vs Control.]

Table 2. Serum CRP levels (mg/liter) of patients with Rheumatoid Arthritis and Controls

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (n = 46)</th>
<th>RA (n = 62)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Cases positive for CRP</td>
<td>0</td>
<td>56 (90.32%)</td>
</tr>
</tbody>
</table>

Serum ADA level was found significantly higher among RA subjects with respect to controls (43.73 ± 19.74 versus 18.96 ± 4.83 U/L; P < 0.0001) (Figure 1). Moreover, serum IL 6 levels were also increased in RA cases as compared to controls and were statistically significant (19.48 ± 9.81 versus 8.33 ± 4.89 U/L; P < 0.0001) (Figure 2). However, no correlation was observed between serum ADA with serum IL 6 level (r = 0.528; P = 0.237) among RA subjects as well as in controls (r = 0.071; P = 0.615).

Figure 1: Serum ADA levels in RA subjects and Control subjects. [Significantly higher among RA subjects with respect to controls (43.73 ± 19.74 versus 18.96 ± 4.83 U/L; P < 0.0001)]
DISCUSSION
The important compartments of management of RA patients are prevention of joint damage and decreasing the morbidity as well as an early measurement of appropriate diagnosis and treatment is required. The biochemical markers also act as indicators of progression of the disease. The estimation of CRP has been suggested to be crucial for estimating short term changes in RA patients. Some studies have shown elevated CRP levels in patients with RA. This may be due to synthesis of CRP in liver triggered by pro inflammatory cytokines released from monocyte and macrophages. The pro inflammatory response leads to secretion of IL-1β and TNF α which further results in the release of Interleukin-6, a messenger cytokine which stimulates liver to secrete CRP. Few studies reported the elevated levels of ADA along with CRP in RA patients.

A study by Sari et al reported that the serum total ADA activity is associated with RA and may provide a useful adjunct to assess inflammation besides traditional indices. Similarly, Surekha Rani et al., also observed that measurement of ADA besides CRP levels helps in the better management RA patients. Moreover, Demir G et al., reported that although ADA levels were higher in RA patients than in controls and also concluded ADA was not related to any of the disease activity parameters and thus traditional parameters and not ADA activity form the reliable markers to determine the disease activity in RA. On the other hand, Haque SS and colleagues found significantly higher levels of ADA in RA patients compared to controls and a positive CRP test in a significant number of RA patients and reported that measuring ADA activity helps in a better understanding of some of the pathophysiological aspects of the disease.

Our study has also observed a significant rise in serum ADA levels in RA subjects as compared to controls which are in agreement with other studies. However, a rise in serum IL 6 levels in RA patients are also similar to many studies. Moreover, we have showed a correlation study in
which serum ADA does not correlate with IL 6 levels which means that they are not interdependent on each other and their levels rise individually. Nevertheless, it is important to mention some limitations in our study. The sample size in our study is less. We have not observed serum uric acid levels in RA patients. Some of the patients includes other comorbidities along with RA. The drug history of the patients are also not taken into account as it might interfere with serum ADA or IL 6 levels. Despite these limitations, we have observed a significant rise in IL 6 and serum ADA levels. Moreover, a large cross-sectional study needs to be done to conclude the fact.

CONCLUSION
Study showed a correlation study in which serum ADA does not correlate with IL 6 levels which means that they are not inter-dependent on each other and their levels rise individually. However, further larger and well controlled studies are needed to establish its role as inflammatory marker.

Conflict of Interest
None declared.

REFERENCES
11. Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic


