Serum biochemical markers in rheumatoid arthritis

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Rheumatoid arthritis (RA) characterized by local and systemic effects of inflammation has a wide range of biochemical markers implicated directly or indirectly to its pathogenesis. In the present study, homocysteine, cortisol, adenosine deaminase (ADA), ferritin, malondialdehyde (MDA) and α-tocopherol in serum of RA patients and healthy individuals were estimated to assess if they contribute to the disease process. The markers of disease activity such as erythrocyte sedimentation rate (ESR) and rheumatoid factor (RF) were also measured. The study group included a total of 45 subjects, including 30 RA patients and the rest being healthy individuals. RA group showed a significant increase in the levels of homocysteine, ADA and MDA, and a significant decrease in α-tocopherol compared to the healthy individuals. However, cortisol and ferritin levels did not show any significant change. Also, there was no significant correlation between the studied serum markers and markers of disease activity. Our results indicate that these biochemical markers contribute independently to the pathogenesis of RA.

Keywords: Homocysteine, Adenosine deaminase, Cortisol, Oxidative stress, Rheumatoid arthritis

Rheumatoid arthritis (RA), whose etiology still remains obscure is one of the most common chronic systemic and articular inflammatory joint diseases, affecting approximately 0.75% of adult Indian population¹. Persistent synovitis, leading to joint destruction represents dysregulated control of the inflammatory process². A defective response of neuroendocrine system to inflammatory stimuli influenced by a non-major histocompatibility genetic factor may also contribute to the pathogenesis of RA³.

Homocysteine, an intermediate product of methionine catabolism is considered as an independent risk factor for cardiovascular disease (CVD) and has also been implicated in other clinical conditions including RA⁴. RA patients are more vulnerable to CVD in comparison to osteoarthritis (OA) patients as well as general population. The mortality rate from CVD is also higher in RA patients⁵.

An enzyme adenosine deaminase (ADA, adenosine aminohydrolase E.C.3.5.4.4), predominantly found in T-lymphocytes⁶, catalyzes the conversion of adenosine to deoxyadenosine and inosine to deoxyinosine with the release of ammonia. ADA has been attributed to the degree of stimulation of T-lymphocytes, as a response to cell-mediated immunity to mycobacterial antigens⁷.

The glucocorticoids, the end-products of hypothalamic-pituitary axis are the most potent endogenous inhibitors of immune and inflammatory process and play a role in the pathogenesis of RA⁸. Similarly, ferritin which is found in macrophage and areas of lymphocytic infiltrate is responsible for iron storage and detoxification⁹. Increased level of ferritin within the synovial membrane has been reported in RA patients¹⁰.

Evidence suggests that RA has characteristics of a free-radical produced disease¹¹. Malondialdehyde (MDA) is a major reactive aldehyde used as an indicator of free-radical induced tissue damage¹² and is found to be increased in RA patients¹³. The plasma lipophilic antioxidant α-tocopherol protects all the cell membrane lipids from oxidative damage initiated by reactive oxygen metabolites¹². Decrease levels of α-tocopherol reported in RA patients indicates the role of oxidative stress in the disease¹³.

Rheumatoid factor (RF), an autoantibody is commonly used as a diagnostic indicator for RA¹⁴. Erythrocyte sedimentation rate (ESR) is a widely used marker of acute phase response¹⁵ as an indicator of inflammation.

The biochemical markers homocysteine, ADA, cortisol, ferritin, MDA and α-tocopherol, are known to contribute to the disease pathogenesis. In this

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Abbreviations: ADA, adenosine deaminase; CVD, cardiovascular disease; DMARDs, disease modifying anti-rheumatic drugs; ESR, erythrocyte sedimentation rate; MDA, malondialdehyde; NSAIDs, non-steroidal anti-inflammatory drugs; OA, osteoarthritis; RA, rheumatoid arthritis; RF, rheumatoid factor.
study, serum levels of these biochemical markers have been estimated to assess their influence in the RA patients and the correlations between these parameters.

**Materials and Methods**

**Chemicals**

Standards for α-tocopherol and malondialdehyde (MDA) were procured from Sigma Chemicals, St. Louis, USA. Other laboratory chemicals used were of analytical grade, purchased either from Sisco Research Laboratories Pvt. Ltd, Mumbai, India or Loba Chemie Pvt. Ltd, Mumbai, India.

**Subjects**

About 30 RA patients (3 males, 27 females) in the age range 25-65 yrs, undergoing treatment at the Rheumatology Clinic, Sri Ramachandra Hospital were selected for the study. Pregnancy, other types of arthritis, and chronic disorders of bone, liver and endocrine were the exclusion criteria for selection of patients. RA patients fulfilled the American Rheumatism Association (ARA) criteria and were suffering from the disease for a minimum of 6 months. Among the selected group of RA patients, 4% were only on non-steroidal anti-inflammatory drugs (NSAIDs), 7% on sulfasalazine + NSAIDs, 67% on methotrexate + NSAIDs, and 13% were on other disease modifying anti-rheumatic drugs (DMARDs) + NSAIDs. Seventeen of the 30 RA patients were found to be RF positive. For control, 15 healthy age- and sex-matched subjects were chosen for the study. Informed consent was obtained from all the study participants.

**Biochemical analysis**

Peripheral blood (5 ml) was collected in vacutainers containing potassium-EDTA anticoagulant or no anticoagulant. ESR was measured in potassium-EDTA sample by Westergren procedure using disposable EaSeR tubes (ICSH recommendations, 1993). RF in the serum was quantitatively measured by enzyme immunoassay using a commercially available kit AESKULISA RF-Check (AESKU Diaganostics GMBH, Germany). Serum (homocysteine) (ferritin) and (cortisol) were measured using kits from Bayer Healthcare (LLC, USA) based on competitive immunoassay using direct chemiluminiscent technology. (ADA) levels were estimated spectrophotometrically as described earlier. One unit of ADA (U) was defined as the amount of enzyme required to release 1 µg of amino nitrogen/h/ml at 37°C from adenosine at standard assay conditions. MDA and α-tocopherol levels were estimated as described earlier.

**Statistical analysis**

Statistical analysis was performed by Mann-Whitney test and the correlation between these markers and test of disease activity was assessed by Pearson correlation. The significance was defined at p value of 0.05 and all tests were two-tailed.

**Results and Discussion**

A significant increase (p<0.001) in the levels of homocysteine, MDA and ADA was observed in RA patients, compared to healthy individuals (Table 1). The levels of antioxidant α-tocopherol showed a significant decrease (p<0.001), compared to healthy individuals (Table 1). No significant changes were observed in the serum ferritin and cortisol levels. No significant correlations were observed between the measured serum biochemical markers and RF and ESR (markers of disease activity in RA).

RA is associated with increased co-morbidity and mortality resulting from CVD. A significant component in the pathogenesis, prevention and treatment of heart disease involves homocysteine. Elevated homocysteine levels observed in our study were in agreement with a previous study, where higher HC levels were associated with inflammatory markers. Though folic acid treatment reduces HC in RA patients, a combination therapy with methotrexate and folic acid has been shown to be associated with a reduced incidence of CVD in patients with RA. Homocysteine facilitates the generation of hydrogen peroxide and causes oxidative damage to low density lipoproteins.

**Table 1** — Serum biochemical markers in RA patients and controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RA patients (n = 30)</th>
<th>Controls (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR (mm/h)</td>
<td>65.4 ± 26.1</td>
<td>35.1 ± 11.4</td>
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<tr>
<td>α-Tocopherol (mg %)</td>
<td>0.42 ± 0.29**</td>
<td>0.91 ± 0.18</td>
</tr>
<tr>
<td>Malondialdehyde (nmol %)</td>
<td>116.47 ± 24.36**</td>
<td>100.79 ± 26.35</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>15.51 ± 5.078**</td>
<td>12.51 ± 6.13</td>
</tr>
<tr>
<td>Cortisol (ng/ml)</td>
<td>14.78 ± 4.34</td>
<td>10.67 ± 2.18</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>97.81 ± 70.97</td>
<td>48.05 ± 46.53</td>
</tr>
<tr>
<td>Adenosine deaminase (U/ml)</td>
<td>27.67 ± 8.74 *</td>
<td>19.79 ± 5.63</td>
</tr>
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**p < 0.001; *p < 0.05**
this marker can be attributed only, if other risk factors which mediated CVD events in RA are also assessed.

The studies of pituitary, adrenal and adrenogenic hormones in RA are often difficult to interpret and contradictory, and the presence of chronic inflammation itself may obscure the initial hormonal abnormalities. An earlier study reported a significant increase in serum cortisol level in premenopausal RA women, compared to controls. A significantly low level of glucocorticoid receptor expression and a low serum cortisol level were observed in female early RA patients. In the present study, no significant change was observed in serum cortisol levels of RA patients compared to healthy individuals. The hormonal role in RA can be understood better if the entire hypothalamus-pituitary-adrenal axis is studied, including the time of sampling, serial estimations and interference of drugs.

Increased levels of immuno-inflammatory marker ADA in RA patients indicated activation of cell-mediated immunity and the results were in accordance with an earlier study. Altered levels of MDA and α-tocopherol were observed in RA patients, compared to healthy individuals, indicating an altered pro-oxidant/antioxidant status, as reported in our previous study. Free iron released from various sources enhances free-radical generation. An earlier study reported no significant difference in serum ferritin levels between RA and OA patients and our study also did not show any significant change between RA patients and healthy individuals.

Absence of correlation between the measured serum biochemical markers and traditional markers of disease activity (ESR and RF) indicated that all these markers contributed independently to the disease process. The RA patients being on treatment and at different stages of the disease were the major limitations of our study.

In conclusion, this study has demonstrated that the serum biochemical markers (homocysteine, cortisol, ferritin, MDA, α-tocopherol) play a definite role in the pathogenesis of RA. However, the exact relevance of these markers can be evaluated in further controlled studies, including more number of patients with early and established RA.

References