Non-Enzymatic Antioxidants, Malondialdehyde, and Total Antioxidant Activity as Markers of Oxidative-Stress in Arthritis and Rheumatoid Arthritis

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ABSTRACT:

Generation of reactive oxygen species is an important factor in the development and maintenance of Arthritis and Rheumatoid arthritis (RA) in humans. This study was undertaken to investigate the interplay between antioxidants and pro-oxidants in pathogenesis of these conditions. Serum Ascorbate and Alpha-Tocopherol (Non-enzymatic antioxidants), Malondialdehyde (MDA, the major lipid peroxidation product), Total Antioxidant Activity (TAA) and Uric acid levels along with Rheumatoid Arthritis (RA) Factor were measured in 51 patients afflicted with Arthritis and Rheumatoid Arthritis and results were compared with a group of 51 normal subjects. Mean serum levels of MDA and Uric acid were found to be significantly higher (p < 0.01) and that of Ascorbate and Alpha-Tocopherol were found to be significantly lower (p < 0.01) while the level of TAA was found to be not significantly lower (p = 0.05) as compared to normal subjects. Data suggests that the decreased levels of serum Ascorbate, Alpha-Tocopherol and TAA and elevated levels of serum MDA and Uric acid in Arthritis and Rheumatoid Arthritis may be as a result of disruption of homeostatic balance between the entire gamut (range) of antioxidants and pro-oxidants causing an increase in oxidative burden, one of the many etiological causes of chronic Inflammation.

Keywords: Ascorbate, Alpha-Tocopherol, Malondialdehyde, Total Antioxidant Activity, Uric acid, Arthritis, Rheumatoid Arthritis.

INTRODUCTION:

Ascorbate and Alpha-Tocopherol are potent water-soluble and fat-soluble antioxidant vitamins which interact and work synergically to form a non-enzymatic antioxidant system operating in the aqueous phase (cytosol) and the lipid phase (plasma membrane and membrane of cell organelles) of aerobic cell respectively. OH⁻ (Hydroxyl radical) and O₂⁻ (Superoxide radical) are potentially neutralized by Ascorbate while lipid peroxides are neutralized by Alpha-Tocopherol. H₂O₂ (Hydrogen peroxide) is neutralized by anti-oxidants like Ascorbate, Glutathione, β-Carotene, Tocopherols and Tocotrienols. The main function of Alpha-Tocopherol is as a chain-breaking, free radical trapping antioxidant in cell membranes and plasma lipoproteins by reacting with lipid peroxide radical formed by peroxidation of poly unsaturated fatty acids. The tocopheroxyl radical product t is relatively unreactive, and ultimately forms non-radical compounds. Commonly, the tocopheroxyl radical is reduced to tocopherol by reaction with Vitamin C from plasma.

Free radicals are highly reactive chemical species which are conveniently classed as reactive oxygen species (ROS) include OH⁻, O₂⁻ and H₂O₂. As there are number of ways in which ROS may induce damage, it is difficult to define in terms of simplistic models how this is translated into loss of cellular integrity or tissue function. However, early studies showed that increase in oxidative stress can be assessed in terms of increased concentration of conjugated dienes or thiobarbituric acid reactive substances (TBARS: markers of lipid per-oxidation) as suggested by Kukreja et al. Malondialdehyde, the major lipid peroxidation product, is used as the best available measure of global reactive oxygen species and is substantially elevated in chronic inflammation.

Currently there is great emphasize on measuring “Total Antioxidant Activity” in biological fluids for the co-ordination among anti-oxidants. Total Antioxidant Activity represents total strength of different anti-oxidants to combat ROS attack in many ways and serum/plasma level is representative of cell/tissue status. However, it was observed that the entire gamut of non-enzymatic and enzymatic anti-oxidants and other relevant anti-oxidant bio-molecules come into play, trying to circumvent the Oxidative-Stress caused by Malondialdehyde or conjugated dienes or thiobarbituric acid reactive substances (TBARS: markers of lipid peroxidation).

Antioxidative role of uric acid is seen by its ability to scavenge carbon-centered and peroxyl radicals and its inhibitory effect on lipid peroxidation. Uric acid seems to scavenge free radicals in hydrophilic conditions to inhibit lipid peroxidation on the lipid-aqueous boundary, and the antioxidation is only little in lipophilic conditions.
MATERIALS AND METHODS:

The study was conducted in the Department of Biochemistry, Smt. NHL Municipal medical college attached to Sheth V.S general Hospital, Ahmedabad. 102 subjects were included in this study. Out of which 51 subjects were healthy normal controls comprising Group 1 (30 males & 21 females in the age group of 24 to 58 years with mean ± S.D. of 38.09 ± 8.40 years) and 51 were subjects afflicted with arthritis and rheumatoid arthritis comprising Group 2 (26 males & 25 females in the age group of 38 to 74 years with mean ± S.D. of 56.09 ± 11.10 years). Both the groups were matched according to socio economical status and dietary habits.

Patients with trauma, infection, inflammation of the eye, myotonic dystrophy, kidney pathology, emphysema and other cardiovascular diseases where free radical damage has been incriminated were excluded from the study.

Present and past history of every patient was recorded. The habits of subjects like smoking and tobacco chewing had been kept in mind while interpreting the results.

Blood samples were collected in plain vials and serum was obtained after centrifugation for the estimation of various biochemical parameters viz. Ascorbate, Alpha-Tocopherol, Malondialdehyde, Total Antioxidant Activity, Uric acid, and R A Factor (commercial kit) by chemical manual methods.

The instrument used for the estimation of various biochemical parameters was ERMA Colorimeter.

The results of Group 2 were compared with Group 1. The Values (Mean ± S.D.) with statistical significance (p' Values) & percentage increase or decrease of various biochemical parameters in normal subjects (Group 1) and Patients afflicted with arthritis and rheumatoid arthritis (Group 2) are given in Table 1.

RESULTS AND DISCUSSION:

The values (Mean ± S.D.) with statistical significance (p' values) and % increase or decrease of various biochemical parameters in normal subjects and patients afflicted with Arthritis and Rheumatoid Arthritis as in Table 1 and indicated in Fig. 1.
The level of non-enzymatic antioxidants Ascorbate and Alpha-Tocopherol were found to be significantly lower (p < 0.01) in Group 2 patients than normal subjects. Decreased circulating levels of non-enzymatic antioxidants supports the concept that ROS play an important role in Arthritis and Rheumatoid Arthritis. It may be due to the attempts of the body’s defense system to reduce the detrimental effects of ROS and their products in order to maintain pro-oxidant-antioxidant homeostasis. These findings are supported by Halliwell et al.14, 15, 16, 17.

The MDA level was significantly higher as compared to normal subjects (p < 0.01) thereby confirming that the load was decidedly higher in patients afflicted with Arthritis and Rheumatoid Arthritis. Indirectly it gives inkling that the chances of PUFAs oxidation were higher in these patients and could be one of the etiological factors in inflammatory process in these patients. These presumptions were well supported by findings of workers like Blake et al.18, 19, 20.

**Table 1: Mean ± S.D., 'p' values and % increase or decrease of various biochemical parameters.**

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Normal Subjects (Group 1) (n = 51)</th>
<th>Patients of Arthritis and Rheumatoid Arthritis (Group 2) (n = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbate (mg/dl)</td>
<td>0.648 ± 0.117</td>
<td>0.48 ± 0.112</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.92 % decrease</td>
</tr>
<tr>
<td>Alpha-Tocopherol (mg/dl)</td>
<td>0.989 ± 0.151</td>
<td>0.866 ± 0.148</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.43 % decrease</td>
</tr>
<tr>
<td>Malondialdehyde (nmol/ml)</td>
<td>2.521 ± 0.487</td>
<td>3.242 ± 0.448</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22.24 % increase</td>
</tr>
<tr>
<td>Total Antioxidant Activity (μmole/l)</td>
<td>78.885 ± 8.715</td>
<td>75.566 ± 6.382</td>
</tr>
<tr>
<td></td>
<td></td>
<td>**p &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.20 % decrease</td>
</tr>
<tr>
<td>Uric Acid (mg/dl)</td>
<td>5.125 ± 0.880</td>
<td>6.248 ± 0.939</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21.91 % increase</td>
</tr>
<tr>
<td>Rheumatoid Arthritic Factor (Qualitative)</td>
<td>94.11 % -ve (48 cases) 5.88 % +ve (3 cases)</td>
<td>96.07 % +ve (49 cases) 3.92 % -ve (2 cases)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>97.95 % increase</td>
</tr>
</tbody>
</table>

* - Statistically indicates Highly Significant change as compared to Healthy Normals
** - Statistically indicates significant change as compared to Healthy Normals

The Total Antioxidant Activity in Group 2 patients was found to be significantly lower (p < 0.05) than normal subjects. This can be attributed to the oxidative burden generated due to increased reactive oxygen species and body’s attempt to circumvent this oxidative insult. Lastly, the level of Uric acid which was considered for the patients afflicted with Arthritis and Rheumatoid Arthritis was found be significantly elevated (p < 0.01) than that of normal subjects. These findings co-relate very well with the study of Katie Payne.17

**SUMMARY AND CONCLUSIONS:**

With the increasing acceptance of ROS as commonplace in pathology and clinical biochemistry, either due to their motivated or provoked generation or weak antioxidant defense, they have been focus of attention in the recent decades or still more recently they were found to have implications in Arthritis and Rheumatoid Arthritis in humans. A crucial and causative role in the pathogenesis of these conditions is played by the free radical process known as lipid per-oxidation and is involved in the oxidative modification of cellular and sub-cellular structures. However, it was observed that the entire gamut of non-enzymatic and enzymatic antioxidant systems along with other relevant antioxidant bio-molecules like Albumin, Transferin, and Ceruloplasmin come into play, trying to circumvent the oxidative stress. It is quite clear that the patients of Arthritis and Rheumatoid Arthritis are subject to oxidative stress and decreased Total Antioxidant Activity and increased Uric acid levels are due to the high oxidative burden on these patients. This study has examined some of these aspects.

Many studies show that ROS play an important role in pathogenesis of Arthritis and Rheumatoid Arthritis.22, 31 Macrophages, neutrophils and lymphocytes are present in synovial fluid in high levels which produce ROS.26 The increased ROS generation is due to oxidative stress and lipid peroxidation. Also, other studies show that Arthritic and Rheumatoid Arthritic patients have lower serum levels of Ascorbate and Alpha-Tocopherol27 and there is an inverse association between serum antioxidant levels and inflammation in these patients.36 Therefore, because of roles of these nutrients as antioxidant and probability of their deficiency, it seems that there is increased lipid peroxidation in these patients and thus supports the need for further studies evaluating the role of antioxidant as free radical scavengers in Arthritic and Rheumatoid Arthritic patients. Also, therapeutic efficacy, dose, duration and appropriate timing of administration of antioxidant supplementation to derive best possible results are...
still not established. Hence there is need of conducting larger, adequately powered clinical trials in this direction to find out answers for such unsolved questions.

REFERENCES: