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Serum Profile of Selected Oxidants and Antioxidants in Patients of Rheumatoid Arthritis

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Abstract
The level of selected markers of oxidative stress (protein carbonyls, nitrite, malondialdehyde) and antioxidants (ceruloplasmin and ascorbic acid) were measured in the serum of 40 patients with rheumatoid arthritis. 20 healthy individuals were taken as controls. All the markers of oxidative stress were seen to be significantly elevated in patients of rheumatoid arthritis as compared to controls ($p < 0.001$). Among the antioxidants, while serum ceruloplasmin was significantly raised in patients ($p < 0.001$) as compared to controls, there was no significant difference in serum ascorbic acid levels between the two groups. A significant positive correlation was observed between ceruloplasmin and all the measured indicators of oxidative stress in patients of rheumatoid arthritis ($r = 0.3$ $p<0.05$ for protein carbonyls, $r = 0.57$ $p<0.01$ for nitrite, $r = 0.64$ $p<0.01$ for malondialdehyde). No such correlation was observed in the control group.
Keywords: *Protein carbonyls, nitrite, malondialdehyde, ceruloplasmin, ascorbic acid.*

Introduction

Rheumatoid arthritis (RA) is a chronic multisystem disease of unknown cause. The characteristic feature of RA is persistent inflammatory synovitis, usually involving peripheral joints in a symmetric distribution. Activation of phagocytic cells within the inflamed joint gives rise to large amounts of reactive oxygen species (ROS) that in conjunction with cell peroxidases play a major role in the generation of tissue injury. Free radicals are now being implicated in the etiopathogenesis of RA[(1)].

The biological importance of oxidative damage to proteins has only recently been considered in detail. Oxidative modification of proteins occurs *in vivo* during aging and in certain disease conditions. Oxidative changes to proteins can lead to various functional consequences like inhibition of enzymatic activities, increased susceptibility to aggregation and altered immunogenicity. Protein oxidation serves as a useful marker for assessing oxidative stress. Protein carbonyls which are products of protein oxidation can be measured in biological samples[(2)]. Nitric oxide (NO) is a free radical which can react with superoxide anion to form highly reactive peroxynitrite. NO is produced in humans by a variety of cells, including synovial cells where they can cause

depolymerization of synovial fluid hyaluronate[3]. Peroxynitrites generated by NO are capable of oxidizing a wide variety of molecules and exerting cytotoxic effects[1]. Since determination of NO is difficult, nitrite which is a stable end product of NO is used as a measure of NO production[4]. Another target of ROS are the unsaturated fatty acids in membrane lipids. Oxidative stress can cause lipid peroxidation of membranes which is highly detrimental to the functioning of the cell and its survival. The detection of lipid peroxidation can be considered to be evidence of involvement of free radicals in human disease. Ceruloplasmin and ascorbic acid are considered to be important extracellular antioxidants. Ceruloplasmin is an important ROS scavenger while ascorbic acid protects lipid from peroxidative damage. The level of these antioxidants have been shown to be altered in various disease conditions [5].

Considering the increasing interest in the role of free radicals in RA, the objective of the present study was to evaluate the parameters of oxidative stress and antioxidants in patients of RA and to study the correlation, if any, between them.

Materials and Methods

This study included 40 patients of rheumatoid arthritis attending the outpatient department of Kaya Chikitsa at the Institute of Medical Sciences, BHU. 20 healthy, age and sex matched control subjects were also included in the study. Diagnosis of patients with RA was done on the basis of history, clinical examination, x-ray analysis and relevant laboratory investigations. All patients had active disease avoiding to the 1987, American College of Rheumatology revised criteria for the classification of RA. 5ml of blood was collected from each individual after an overnight fast. Serum was separated and sample stored at 4^{o}C. The following estimations were carried out on the samples:

Protein carbonyl content in serum was estimated by the method of Reznick and Packer[6]. The principle involves derivatization of the protein carbonyl group with dinitrophenyl hydrazine, which leads to the formation of a stable dinitrophenyl hydrazone product. The carbonyl content is calculated by peak absorbance at 370nm.

Serum nitrite level was estimated by Griess reaction. The principle is a diazotization reaction. The absorbance of the magenta coloured azo product formed was measure at 540nm [4].

Serum malondialdehyde (MDA) level was estimated by the thiobarbituric acid (TBA) test using the method of Satoh K (7). The absorbance of the pink coloured adduct formed was measured at 530 nm.

Serum ceruloplasmin level was estimated by the method of Ravin[8] utilizing the oxidase activity of ceruloplasmin. Ceruloplasmin catalyzes the oxidation of a dye p-phenylene diamine to a violet coloured oxidation product, the absorbance of which is measured at 546 nm.

Serum ascorbic acid was estimated by the dinitrophenyl hydrazine method[9]. Ascorbic acid is oxidized to form dehydroascorbic acid which reacts with 2, 4 dinitrophenyl hydrazine to form a bis-hydrazone, which is measured spectrophotometrically at 520nm.

The results were analyzed statistically by students 't' test. Correlation coefficient was also calculated among relevant parameters.

Results and Discussion

The values of various parameters studied in patients and controls are given in Table 1. The difference in mean levels of serum protein carbonyls between patients and controls was statistically significant ($p < 0.001$). Among the various oxidative modifications of amino acids in proteins, carbonyl formation may be an early marker of protein oxidation. The findings in the present study were in accordance with the observation of various authors who have detected increased protein carbonyl levels in rheumatoid arthritis and other conditions like ischemia-reperfusion injury and exhaustive exercise[6]. Lunec *et al.*[10] and Jasin[11] have shown that

oxidation of synovial fluid immunoglobulins causes aggregation which may contribute to the etiology of RA. In the light of studies showing various functional consequences of protein oxidation in RA, raised serum protein carbonyl values in patients in our study assumes significance. Mean serum nitrite levels and serum MDA levels were also elevated in patients compared to controls and had statistically significant higher values ($p < 0.001$). Farrell *et al.*[12] reported increased concentrations of nitrite in synovial fluid and serum samples in rheumatoid arthritis, suggesting a role for nitric oxide as an inflammatory mediator in rheumatic diseases. Bhogade *et al.*[13] have also reported increased serum nitrite levels in patients with rheumatoid arthritis. Murrell *et al.*[14] have shown that nitric oxide activates metalloprotease enzymes in articular cartilage and may play an important role in cartilage damage in inflammatory arthritis. Significantly elevated levels of MDA in the serum of rheumatoid arthritis patients observed in the present study are indicators of increased ROS production. Similar findings have been reported by other authors like Lunec *et al*[10], Ozgunes *et al.*[15] and Gambhir *et al.*[16] emphasizing the role of lipid peroxidation in RA.

Of the two antioxidants measured, the level of serum ceruloplasmin was higher in patients than controls and statistically significant ($p < 0.001$). Serum ceruloplasmin also had significant correlation with serum protein carbonyls ($r = 0.3$; $p < 0.0$ S), nitrite ($r = 0.57$; $p < 0.01$) and MDA ($r = 0.64$; $p < 0.01$) in patients of RA (Fig 1, 2, 3). No such correlation was observed in controls ($r = -0.283$ p = not significant for protein carbonyls, $r = -0.022$ p = not significant for nitrite, $r = -0.039$ p = not significant for MDA). Conforti *et al.*[17] reported that serum ceruloplasmin level was elevated in RA patients. Ozgunes *et al.*[15] and Gambhir *et al.*[16] have reported similar findings. These findings show that ceruloplasmin, whose levels are consistently increased in rheumatoid arthritis may play a significant antioxidant role. It has been demonstrated that ceruloplasmin can scavenge free radicals, as well as, transition metals and thereby protect against lipid peroxidation. Biemond *et al.*[18] demonstrated that serum and synovial fluid from rheumatoid arthritis patients and controls were both protective against lipid peroxidation in an *in vitro* system. They also showed that ceruloplasmin was the factor responsible for this protection. The parallel increase in ceruloplasmin activity in response to oxidant stress, as is evident from the positive correlation between ceruloplasmin and markers of oxidative stress, also supports the protective role of ceruloplasmin.

In the present study there was no significant difference between serum ascorbic acid levels in patients and controls. Some authors have obtained different results. Schorah *et al.*[19] reported depletion of ascorbate levels in conditions of oxidative stress. Sahud *et al.*[20] reported decreased levels of ascorbic acid in patients of rheumatoid arthritis. Serum ascorbate plays a pivotal role in protecting plasma lipids from ROS attack; however, it is rapidly oxidized when challenged by oxidants released from activated polymorphonuclear leucocytes. The study of ascorbate/dehydroascorbate ratio might give a better insight into the role of ascorbic acid in this disease.

In conclusion, our study shows that there is a definite evidence of oxidative stress in patients of RA as evidenced by an increase in levels of all parameters of oxidative stress measured. This oxidative stress has further lead to a compensatory increase in serum ceruloplasmin levels in patients underlining the role of ceruloplasmin as an antioxidant in RA. Since in our study serum ascorbic acid was not found to be significantly elevated in patients of RA, the role of ascorbic acid as an antioxidant in RA needs further study.

Table1: Levels of oxidants and antioxidants in serum of patients with rheumatoid arthritis and control group (mean ± SD).

Parameters	Patients n = 40	Controls n = 20
Protein carbonyls (n mol/mg protein)	2.30* ± 0.52	1.59 ± 0.46
Nitrite (mol/l)	22.39* ± 5.21	14.51 ± 5.46
MDA (mol/l)	1.75* ± 0.73	0.87 ± 0.54
Ceruloplasmin (mg/dl)	39.94* ± 7.47	28.78 ± 6.09
Ascorbic acid (mg/l)	5.70* ± 0.97	5.68 ± 0.87

* = Significance level as compared to controls (p< 0.001).

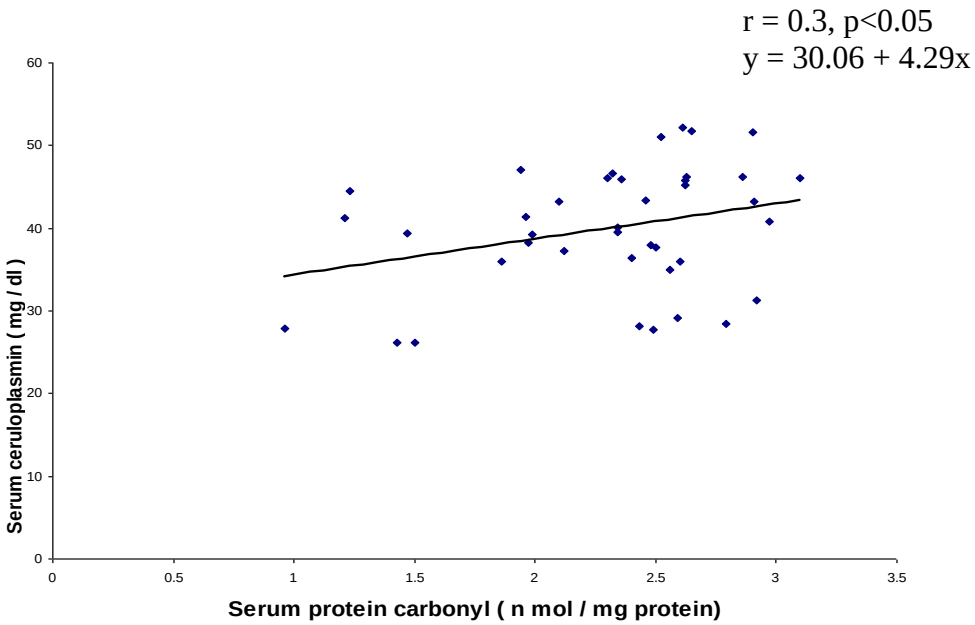

Fig 1 Correlation between serum ceruloplasmin and serum protein carbonyl levels in patients with rheumatoid arthritis (n = 40).

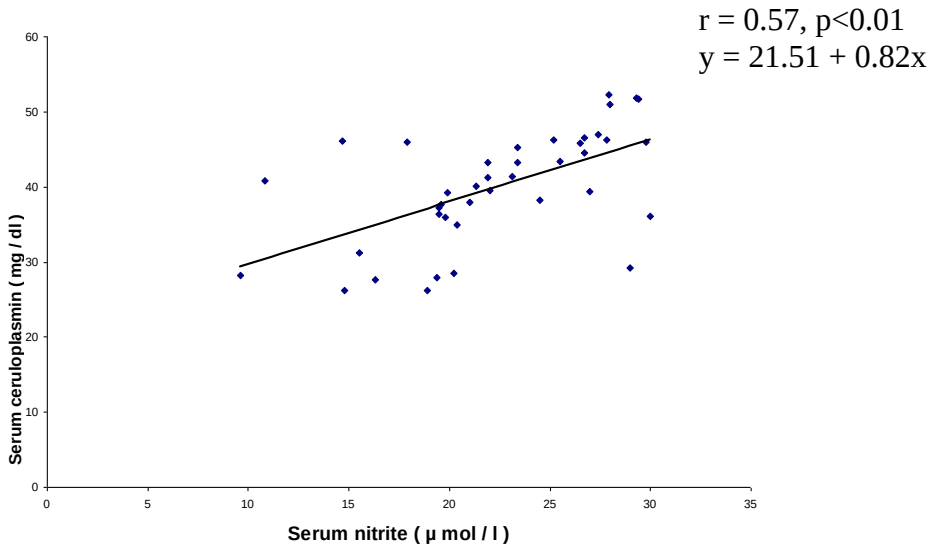

Fig 2 Correlation between serum ceruloplasmin and serum nitrite levels in patients with rheumatoid arthritis (n = 40).

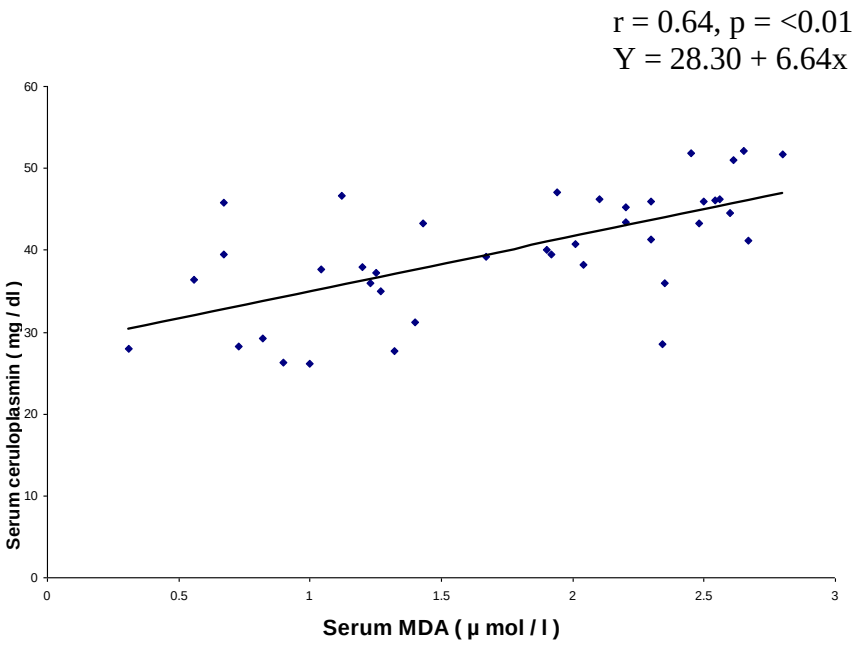

Fig 3 Correlation between serum ceruloplasmin and serum MDA levels in patients with rheumatoid arthritis (n = 40).

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