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Research Article

Cumulative effect of systemic inflammation and oxidative stress in 40 known cases of active rheumatoid arthritis

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ABSTRACT

Background: Oxidative stress has been implicated in the pathophysiology of a number of diseases such as cancer, hypertension and inflammatory diseases. Although previous evidences provided extensive literature about the biological role of antioxidant enzymes in rheumatoid arthritis (RA), there is a paucity of satisfactory explanation regarding the alteration in the level of antioxidant enzymes along with marker of systemic inflammation in RA. The objective of present study was to estimate the level of C-reactive protein (CRP), Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSHPx) and Ceruloplasmin in active RA patients.
Methods: 40 patients of either sex (30-50 years age group) suffering from active RA and 40 normal healthy individuals served as control; were included in the study. Above mentioned parameters were estimated using standard methods and data from patients and controls were compared by using Student's t-test.
Results: Erythrocyte SOD, CAT and GSHPx activity were significantly low in RA subjects ($P<0.001$) whereas plasma Ceruloplasmin level was found to be significantly high ($P<0.001$) as compared to healthy controls.
Conclusions: These findings suggest that combined effect of inflammation and free radical generation is involved in the pathogenesis of active RA, characterized by imbalance in antioxidant enzyme status and enhanced CRP levels, which served as an excellent marker of oxidative stress and systemic inflammation in active RA.

Keywords: Superoxide dismutase, Catalase, Ceruloplasmin, C-reactive protein, Free radical

INTRODUCTION

Contrary to common belief rheumatoid arthritis is not a trivial illness but a major medical condition that affects the quality of human life of developed and developing countries as well.[1] Previous evidences suggest that reactive oxygen species derived from molecular oxygen (Superoxide anion, hydrogen peroxide and hydroxyl radical) contribute to the tissue injury, which accompanies inflammatory disorders including rheumatoid arthritis and osteoarthritis.[2,3] A number of sources of free radical generation have been identified in biological tissues, which may be mutually interactive and once triggered, they may lead to loss of antioxidant defense system. The defense system to combat the potentially damaging effects of free radical species includes antioxidant enzymes and antioxidants. Superoxide dismutase (SOD, EC: 1.15.1.1), Catalase (CAT, EC: 1.11.1.6), Glutathione peroxidase (GSHPx, EC: 1.11.19) and Ceruloplasmin (EC: 1.16.3.1). SOD catalyses the conversion of superoxide radicals to hydrogen peroxide and molecular oxygen. Hydrogen peroxide is further detoxified by either heme containing enzyme CAT or selenium containing enzyme GSHPx.

Ceruloplasmin, is a blue colored copper binding protein, function as antioxidant enzyme by virtue of its ferroxidase activity and scavenges superoxide anion radical.[4,5]

Alterations in the levels of these antioxidant enzymes with subsequent biomolecular deterioration via increased ROS production can cause cartilage collagen degradation, loss of homeostasis in chondrocytes leading to impaired chondrocyte function, destructive changes in extracellular matrix, synovitis and cartilage ageing, and thereby perpetuate arthritis development. Moreover, free radicals mediated oxidative stress has been described as an important mechanism underlying destructive proliferative synovitis in arthritic patients.[6] C-reactive protein (CRP), a marker of systemic inflammation and synthesized in liver, has been received considering attention in inflammatory disorders such as rheumatoid arthritis. In addition, previous studies have demonstrated an association between arthritis progression and inflammation as measured by plasma C-reactive protein.[7]

Although several evidences provided extensive literature about the role of antioxidant enzymes and inflammation in arthritis, there is a paucity of satisfactory explanation regarding, alteration in the level of these antioxidant enzymes and systemic inflammation in active RA. In addition, as best of our knowledge, previous studies on active RA patients have not included systemic inflammation and oxidative stress in a single setting Therefore, the objective of present study was to estimate the level of these antioxidant enzymes and systemic inflammation in active RA patients and statistically determine the variation in their level by comparing it with that of healthy subjects served as control.

METHODS

In the present study 40 patients of either sex with active rheumatoid arthritis belonged to age group 30-50 years and 40 age matched healthy individuals, served as control, were taken. A general information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination including blood pressure measurement was completed from all the subjects after taking their informed consent and approval of protocol by ethics committee of college. All patients had active RA, defined as the presence of at least three of the following criteria: six or more tender joints; three or more swollen joints; ≥ 30 min of morning stiffness; an erythrocyte sedimentation rate of ≥ 28 mm/h.

Inclusion criteria

Subjects, who gave informed consent for study, having no history of any type of arthritis, don't under any medical treatment (anti-inflammatory drug) or taking antioxidant supplement for at least 1 month prior to blood collection were included.

Exclusion criteria

Patients with diabetes mellitus, hepatic disease, hypertension, those taking antioxidant vitamin supplements or non-steroidal anti–inflammatory drugs and with other connective tissue disease like systemic sclerosis and osteoarthritis were excluded.

Fasting blood samples were collected in EDTA vials from the anticubital vein of the study group subjects and processed immediately. Erythrocyte SOD activity was measured by Marklund and Marklund's method. The enzyme SOD inhibits the auto-oxidation of pyrogallol by catalysing the breakdown of superoxide. The inhibition of pyrogallol oxidation by SOD is monitored at 420 nm and the amount of enzyme producing 50 % inhibition is defined as one unit of enzyme activity.[8]

Plasma ceruloplasmin levels were estimated by Ravins's method (1961). Ceruloplasmin due to its oxidase activity, catalyses the oxidation of substrate p- phenylenediamine chloride into purple coloured oxidation product, measured spectrophotometrically at 530 nm.[9]

Erythrocyte glutathione peroxidase (GSHPx) activity was estimated by Beutler's method (1971), after preparation of hemolysate. GSHPx catalyse the oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) by H_2O_2. The rate of formation of GSSG is measured by means of glutathione reductase reaction in which NADPH is oxidized and measured at 340 nm.[10]

Erythrocyte catalase activity was estimated by Goth's method which involves the enzymatic breakdown of H_2O_2 under optimized condition followed by spectrophotometric assay of H_2O_2 (405 nm) based on formation of its stable complex with ammonium molybdate.[11] Plasma CRP levels were measured using commercially available ELISA kits (R&D Systems, USA), according to manufacturer's instructions.

Statistical analysis

The data collected from study group subjects were entered separately in Microsoft Excel sheet of windows 2007 and values were expressed as Mean ± SD. The significance of mean difference between study group subjects was compared by using Student's t test. The distribution of t-probability was calculated depending on 'n' and significance of test was obtained. P value <0.05 and <0.001 were considered as significant and highly significant respectively.

RESULTS

The level of antioxidant enzymes and systemic inflammation in active RA patients and controls were depicted in Table 1. In the present study, erythrocyte SOD and GSHPx levels were significantly decreased in patients with active RA (P <0.001; 42.27% low and P

<0.05; 43.5% low respectively) as compared to controls. Plasma Catalase activity was also significantly low in active RA patients (P<0.001; 45.52% low) whereas plasma ceruloplasmin level was significantly elevated in active RA subjects as compared to controls (P<0.001; 30.65% high). Plasma CRP levels were found to be significantly high (P<0.001; 33.53% high) in patient group as compared to healthy controls which reflect the role of inflammation and oxidative stress in disease process.

Table 1: Marker of systemic inflammation and antioxidant enzymes in study group subjects (Mean ± SD).

Particulars	Control Group (n=30)	Patient group (n=30)	Level of Significance	% Increase	% Decrease
CRP (mg/L)	3.28 ± 0.14	4.38 ± 0.15	P < 0.001	33.53 %	
SOD (U/gm Hb)	1932.24 ± 231.53	1115.48 ± 315.36	P < 0.001	-	42.27
Ceruloplasmin (mg %)	24.57 ± 4.6	32.10 ± 6.2	P < 0.05	30.65	-
GSHPx (IU/ gm Hb)	30.8 ± 4.7	17.4 ± 3.2	P < 0.05	-	43.5
Catalase (KU/L)	52.5 ± 16.2	28.6 ± 5.8	P < 0.001	-	45.52

Where, *p<0.1: Non-significant; **p<0.05: Significant; **p<0.001: Highly significant

DISCUSSION

Reactive oxygen species have been implicated in the pathogenesis of many disease processes including rheumatological disorders.[12] Superoxide anion, which is believed to be one of the initiators of free radical mediated pathological alterations (such as cartilage degradation, synovitis and lipid peroxidation) leading to arthritic complication, is efficiently removed by antioxidant enzyme SOD.[1] In the present study, we observed that the SOD activity in active RA patients was significantly low (P<0.001) as compared to healthy controls, which direct towards its protective and superoxide radical scavenging action in active RA patients. Our findings were in concordance with the findings of Karatas et al. According to them, reduced activity of SOD could be the result of inter and intramolecular cross-linking of proteins and thereby causing conformational changes in SOD which leads to accumulation of H_2O_2 followed by induction of lipid peroxidation leading disease progression.[13]

Superoxide anion ($O_2^{\cdot-}$) scavenging action of SOD can be mimicked by other copper containing enzyme ceruloplasmin also has the capacity to scavenge $O_2^{\cdot-}$. In the present study, plasma ceruloplasmin level was found to be significantly increased (P<0.001) in active RA patients as compared to healthy controls which indicate that high ceruloplasmin level is associated with its antioxidant activity to protect the myocardial tissue against the deleterious effects of oxygen free radical and to compensate the loss of SOD activity, occur due to oxidative stress in RA patients.[14] Among the enzymatic systems of protecting the cell against free radical injury, GSHPx and CAT play a crucial role in the final detoxication of H_2O_2 to H_2O. In the present study, low GSHPx and CAT activity were observed (i.e. p<0.05 and p<0.001 respectively) in active RA patients as compared to controls. Aryaeian et al. also observed a direct relationship of free radical production with active RA progression and concluded that reduced level of CAT and SOD may be due to their consumption during metabolism of oxygen free radicals. However, it is unclear whether the reduced activity of antioxidant enzymes is the cause or the consequence of the increased oxidative stress in RA.[15]

Moreover, reactive oxygen species and their intermediates serve as mediators of inflammation in inflammatory and arthritic disorders by enhancing various culprit events such as inhibition of glycolytic enzymes, reduction of antioxidant reserves in synovial fluid and activation of proteolytic enzymes to degrade cartilage. [6] In the present study, plasma CRP levels were found to be significantly high (p<0.001) in active RA patient which clarify the combined role of inflammation and oxidative stress in the etiopathogenesis of active RA and its complications.[7,14,16]

On the basis of present findings and consistent findings of previous studies, it can be inferred that these antioxidant enzymes and CRP are excellent markers of oxidative stress and systemic inflammation in active RA patients. It also authenticate the fact that combined effect of inflammation and oxidative stress plays a significant role in the etiopathogenesis of RA and the patients are unable to counteract the augmented oxidative stress effectively. Thus, antioxidant supplementation in diet/drug regime regularly along with anti-inflammatory drug as prescribed by physician may reduce the RA progression and its complications.

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