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Antioxidant Enzymes in Rheumatoid Arthritis

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Abstract

Joint destruction in rheumatoid arthritis (RA) is due to tissue injury in the area caused by inflammatory reactions, release of MMPs and free radicals produced by neutrophils and macrophages. The control of free radical production may have therapeutic roles thus the study was done to check the status of lipid peroxidation product malondialdehyde (MDA) and a few antioxidant enzymes in RA patients. 45 RA patients and 40 controls were selected. Controls were asymptomatic and RA patients were selected according to ACR criteria. RA patients had significantly high MDA, SOD and ALP and reduced activity of catalase and GR as compared to controls. SOD showed positive correlation with ALP. GR was positively related with MDA, SOD and ALP. The study shows that MDA is involved in the pathogenesis of RA. The system is trying to quench free radicals by high SOD activity. Higher production of H_2O_2 or some other mechanism is responsible for inhibition of catalase and GR. However system is trying to reduce the damage by neutralizing superoxide anion. Therapeutic intervention of the oxidative stress may be considered for effective control of inflammation in RA patients.

Keywords: Malondialdehyde; Superoxide anion; Superoxide dismutase; Catalase; Glutathione S-transferase

Abbreviations

RA: Rheumatoid Arthritis; MDA: Malondialdehyde; SOD: Superoxide Dismutase; GST: Glutathione-S-Transferase

Introduction

Rheumatoid arthritis (RA) is the inflammatory disease which leads to progressive destruction of multiple synovial joints [1]. T-cells and cytokines play an important role along with oxygen radicals as superoxide and hydrogen peroxide released by activated macrophages in the progression of rheumatoid arthritis [2]. These reactive oxygen species (ROS) and reactive nitrogen species (RNS) which are thus produced have both beneficial and toxic effects. Oxidative stress is the condition when concentration of ROS and RNS becomes deleterious and damage the cells and biological macromolecules [3,4] and thus the body. Oxidative stress occurs due to disturbed balance between body's antioxidant mechanisms and oxidative stress production and has important role in the development of chronic disease as autoimmunity like RA, cancer etc. [5-7].

They are capable of damaging membrane lipids, connective tissue and nucleic acids of the cell. Free radicals and their byproducts are essential mediators of inflammation. Due to chemo-attractant property of synovial fluid, leukocytes accumulate with in the synovial tissue triggering a respiratory burst characterized by increased oxygen consumption and increased anaerobic glycolysis leading to generation of superoxide, hydroxyl, hypochloric radicals etc. [8]. Neutrophils have been shown to be very active in synovial fluid of patients with RA which leads to inflammation and damage [9,10].

In the body free radical generation and enzymes degrading them are in tight homeostasis which prevents damage. However, studies [11,12] show that enzymatic/non enzymatic antioxidant systems are highly deregulated and impaired in RA. Markers of protein and lipid oxidation have been found to be raised in arthritic animals. Therefore there are chances of free radical mediated damage to the body of RA patients due to their higher production or improper scavenging. Thus analysis of activities of different antioxidant enzymes like superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px) and glutathione reductase (GR) may have effective therapeutic potential [11,13,14]. There is more interest in roles of these in the clinical outcomes of disease like RA, therefore, we were interested in analyzing the level of MDA which is product of lipid peroxidation and level of enzymes of free radical scavenger system like superoxide dismutase (SOD), GR, catalase and levels of alkaline phosphatase (ALP) in RA patients treated with MTX, Folic acid Vit-C and occasional corticosteroids.

Materials and Methods

45 samples (30 females, 15 males) were randomly selected from the OPD of Orthopaedics from different centers during the study period. Patients were recruited who fulfilled 4 or above criteria of American College of Rheumatology (ACR) [15]. Out of 45 patients with RA, 38 were tested positive for RF factor and anti-cyclic citrullinated protein (CCP) antibody. 40 asymptomatic independent controls (24 females, 16 males) were recruited from local clubs, neighbourhood and volunteers. Controls were asymptomatic (painless, no criptation, no decrease of joint space on X-ray, nonobese and without any other systemic disease) and independent of the patients.

Patients were recommended MTX (15 mg once a week) along with folic acid (1 mg OD) and vitamin C to alleviate symptoms. Whenever

the patient complaints about swelling with the existing treatment (usually at the change of season) the local corticosteroid (triamcinolone acetonide 0.5 ml) was given at the swollen joints. The usual requirement was 4-6 times/year. The patients did not had any renal disease and were non hypertensive. Blood was drawn from overnight fasting patients for all the analysis. The study was started after approval from Institutional ethical committee and written informed consent was obtained from all the patients.

Laboratory analysis

MDA which is an indicator of oxidative stress was measured by the production of thiobarbituric acid reactive compounds (TBARS) [16]. Glutathione reductase activity was measured following the oxidation of nicotinamide adenine dinucleotide phosphate reduced (NADPH) in the presence of oxidized glutathione (GSSG). SOD estimation was done according to Mishra and Fridovich, [17]. Catalase activity was analysed according to method of Sinha [18]. Glutathione reductase activity was done as describe by [19]. Alkaline phosphatase level was measured by COGENT Kit, Span Diagnostic Ltd.

Results and Discussion

In our study lipid per-oxidation in terms of MDA production was significantly increased in RA patients (Table 1) which may be due to increased ROS during chronic inflammation. Lipid peroxides are generated at the site of tissue injury due to inflammation and diffuses into blood and can be estimated in serum or plasma [20]. Studies [21-24] have reported raised levels of MDA in the serum, plasma and erythrocytes of RA patients. In our study SOD levels are highly increased (Table 1). Superoxides anion (O_2-) has important role in pathogenesis of many diseases (7). It is neutralize by SOD to hydrogen peroxide (H_2O_2). H_2O_2 is further quenched by activity of catalase and glutathione peroxidase. Transformation of O_2- to H_2O_2 prevents the formation of aggressive compound as peroxynitrile (ONOO-) and hydroxyl radical (OH.) [25]. The patients showed significantly higher activity of SOD and ALP (Table 1). There was strong positive correlation between SOD and ALP activity (Table 2). They showed reduced activities for catalase and glutathione reductase (Table 1). The GR activity was positively correlated to MDA, SOD and ALP (Table 2).

	Control	Rheumatoid arthritis patients
	N=40	N=45
MDA	0.763 ± 0.02134	2.519 ± 0.11784 P**
SOD	484.96 ± 14.55467	1186.08 ± 31.87685 P**
Catalase	1.722 ± 0.0450	1.31 ± 0.0264 P**
GR	25.84 ± 0.7822	20.94 ± 0.3887 P**
ALP	151.17 ± 0.8482	182.86 ± 0.7321 P**

Table 1: The table shows the data for MDA, SOD, catalase, GR and ALP. The p is significant at *p<0.05, **p<0.01.

Reactive oxygen species and oxidative stress have a role in the pathogenesis of RA [22]. Free radicals and other reactive species play an important role of super oxidant leading to oxidation of biomolecules like proteins, amino acids, lipids and DNA [26], which are ultimately responsible for cell injury and death [27]. Prime targets of ROS attack are the polyunsaturated fatty acids in the membrane lipids causing lipid peroxidation (LPO) which may lead to disorganization of cell structure and function. Further decomposition of peroxidized lipids yields a wide variety of end-products, including malondialdehyde (MDA) [28]. Malon dialdehyde (MDA) is one of an important lipid peroxide which is high in RA patients [29,30]. Measurement of MDA is widely used as an indicator of LPO.

	MDA	SOD	Catalase	GR	ALP
MDA	1				
SOD	-0.049	1			
Catalase	-0.114	-0.111	1		
GR	0.328*	0.486**	-0.01	1	
ALP	-0.147	0.682**	0.001	0.326*	1

Table 2: Shows the correlation between various parameters in RA patients (N-45). The p is significant at *p<0.05, **p<0.01.

Many studies have reported high MDA in the serum, plasma and synovial fluid of RA patients [22,28,31]. MDA has an important role in pathogenesis of RA. There is growing awareness that reactive oxygen species and free radicals may play an important role in mediating cellular injury and tissue damage in rheumatoid arthritis. Thiele et al. [32] have reported malondialdehyde-acetaldehyde (MAA) adduct formation is increased in RA. They appear to result in robust antibody responses which are strongly associated with anti citrullinated protein antigens (ACPAs) suggesting that MAA formation may be a cofactor that drives tolerance loss, resulting in the autoimmune responses characteristic of RA.

Higher levels may be the result of respiratory burst triggered by leucocytes. A study has shown activation of neutrophilic myeloperoxidase-hydrogen peroxide system in RA synovial tissue which may contribute to cyclic self-perpetuating inflammation [10]. Methotrexate treatment has been reported to increase Zn-SOD activity but it has no effects on GSH-Px in rats [33,34].

But possibly increased activity of SOD [35,36] may be attributed to increased O_2- production by hyperactive cells leading to SOD induction [37,38]. Another possibility may be excessive free radical production through the xanthine-xanthine oxidase system is the primary factors in RA, rather than an impaired antioxidant system [36]. Else higher SOD levels may be a change to nullify excessive free radical production. Post treatment the antioxidants are increased which lead to lower plasma MDA and increased total antioxidant capacity (TAC) [3,39].

However lower SOD has also been reported in patients with RA on MTX therapy in comparison with RA without MTX therapy [40]. It has also being observed that MTX can suppress directly or indirectly the generation of active oxygen metabolites induced by IL-6, which is produced in response to TNF-α stimulation in synovial cells of RA [41] as well as in polymorphnuclear cells. The increased levels serum Cu/Zn SOD may support the hypothesis of radical- mediated injury.

Over expression of extracellular SOD leads to dismutation of superoxide resulting in H_2O_2 accumulation. Analysis of H_2O_2 in different settings is being done and authors conclude, more SOD does not mean more H_2O_2 [42]. The formation of H_2O_2 due to dismutation of superoxide is limited by the amount of superoxide, not by the rate it is converted to H_2O_2. Accumulation of superoxide leads to the

oxidation of NO forming peroxynitrite. There more H_2O_2 is unlikely to be toxic as this would amount to substituting a very mild cytokine (H_2O_2) for a potant (peroxynitrite) [43].

Decreased activity of SOD in RA patients has also been reported [44]. However our study is in line with [13,22] who have reported increased SOD levels in RA patients. Mazetti et al. [13] have reported higher serum copper/Zn superoxide in patients with RA. Igari et al. [45] have reported correlation between the overall synovial SOD activity and both the clinical severity of the disease and the CRP levels. Mazetti et al. [13] have concluded that exercise induced hypoxic reperfusion mechanism with in the inflamed joint in RA may lead to increased production of Cu/Zn SOD. Mateen et al. [46] have shown that increase of oxidative stress increases with the progression of RA.

H_2O_2 formed due to activity of superoxide dismutase need to be detoxified by glutathione peroxidase and catalase activity. Catalase plays an important role in preventing ROS mediated damage by using H_2O_2 and converting it to water and oxygen. In our RA patients, catalase activity is significantly decreased as compared to control. Lower catalasae activity may be due to interaction of catalase by hydrogen peroxide [44]. Lowered activities of their enzymes may lead to conversion of H_2O_2 to hydroxyl radical by iron released from hemoglobin of lysed erythrocytes [47]. However unaltered catalase activity in RA patients has been reported [48].

Catalase activity was not found in serum of RA patients. Decreased erythrocytes catalase activity is also being reported [47]. Our study is in accordance with 28,36 and shows lower catalase activity in serum of RA patients. Catalase expression affects expression of genes which influence inflammation [49]. Lower levels of catalase may be responsible for high inflammation in RA. Cimen et al. [36] have reported higher SOD activity and MDA levels and unchanged catalase and GSH-Px activities in RA patients. The study by Gonzalez et al. [50] observed the positive correlation between antioxidant GPx and lipid peroxidation levels. Their results suggest that GPx activity is involved in the primary mechanisms against oxidative stress in RA patients. Both GPx and catalase use H_2O_2 as substrate where catalase acts in the presence of high concentration of the substrate while GPx acts at lower concentrations. They also suggested that H_2O_2 concentration may be lower than in other chronic inflammatory diseases, with oxidative damage being mediated possibly by HO· [51].

Glutathione reductase (GR), an oxidative stress inducible enzyme, plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes.

Glutathione reductase is a flavoenzyme dependent on NADPH that catalyzes the reduction of GSSH to GSH. Feijoo et al. [52] observed that myeloperoxidase levels are elevated in patients with chronic inflammatory disease, especially those with active disease, and that high myeloperoxidase levels are related to an increase in oxidative damage and the inflammatory response, for myeloperoxidase and GR seem to show a similar activity pattern based on the availability of NADPH. Erythrocyte GSH and glutathione reductase levels rise in healthy individuals exposed to chronic oxidative stress [53]. These findings suggest that GSH levels may be inappropriate in patients with active rheumatoid arthritis, perhaps reflecting impaired glutathione reductase activity as observed in our study. The study by Aryaein et al. [54] showed that GR, vitamin E, Beta-carotene was lower and MDA was higher in the patient group than in controls. Kamanli et al. [22] observed significantly lower GSH-Px, catalase, levels of GSH in plasma of RA patients. However higher GR activity have also been reported in RA [55]. Kerimova et al. [56] also reported decreased catalase and unaffected GR activities in RA subjects. Low GR activities in the red blood cells and polymorphonuclear leucocytes of patients with RA was reported by Mulherin et al. [57]. Vanella et al. [58] described reduced EGR activity in 15 patients with rheumatoid arthritis and Tarp et al. recorded a similar finding in nine patients with rheumatoid arthritis [59].

In our patients alkaline phosphatase (ALP) activity is higher relative to control. ALP showed strong positive and significant relationship with SOD. ALPs role is implicated in osteoid formation and mineralization and expression of its isoform is in osteoblasts, leucocytes, liver, kidney, breast and brain [60,61]. The bone formation markers are measured in serum and about half of ALP in serum comes from bone. Several studies [62-64] have reported high serum ALP levels in RA patients. The increased activity may be due to inflammatory cytokines as interleukin-1 (IL-1) which has been correlated with the acute phase reactants [62] and CRP levels. The role of T-cells is well documented in the pathogenesis of RA. Raised ALP may be due to its leakage from injured or killed cells.

Alkaline phosphatase has been implicated as marker in RA patients. It can provide diagnostic information by determination of isoform of ALP derived from liver or bone [65]. Thus MDA and antioxidants systems work reciprocally to keep oxidative stress mediated damage in control. An inverse association between serum antioxidant levels and inflammation have been reported [66].

Study by Jalili et al. [67] showed that antioxidants may significantly improve disease activity but do not affect the number of painful and swollen joints. Thus antioxidants may be helpful in control of clinical outcomes and oxidative stress in RA patients. In conclusion oxidative stress management may be considered a therapeutic option for RA along with DMARD. Supplementation of antioxidants along with catalase and/or GPX may confer more protection.

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Disclosures

The authors have no conflict of interest.

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