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Antioxidant status in rheumatoid arthritis and role of antioxidant therapy

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Abstract

Background: Oxygen free radicals have been implicated as mediators of tissue damage in patients of rheumatoid arthritis (RA). This study was designed to elucidate plasma oxidant/antioxidant status in rheumatoid arthritis, with the aim of evaluating the importance of antioxidant therapy in the management of this disease. *Methods*: The study included 40 patients of rheumatoid arthritis who were randomly divided into two subgroups of 20 each. One group received conventional treatment for 12 weeks and in the other group conventional treatment was supplemented with antioxidants for the same duration. Twenty age- and sex-matched normal individuals constituted the control group. Blood samples of controls and patients were collected at the time of presentation and analyzed for total thiols, glutathione, vitamin C and malondialdehyde (MDA-marker of oxidative stress). The investigations were repeated in the patients after 12 weeks. *Results*: The blood concentrations of total thiols, glutathione and vitamin C were found to be significantly lower in rheumatoid arthritis patients as compared to healthy controls, while the concentrations of MDA were much higher. There was a statistically significant increase in the posttreatment concentrations of these antioxidants, along with a decrease in the concentrations of MDA. *Conclusions*: The antioxidant defense system is compromised in rheumatoid arthritis patients. There is a shift in the oxidant/antioxidant balance in favor of lipid peroxidation, which could lead to the tissue damage observed in the disease. The results suggest the necessity for therapeutic co-administration of antioxidants along with conventional drugs to such patients. However, due to the limited number of cases included in this study, more studies may be required to substantiate the results and arrive at a definite conclusion, in terms of safety and efficacy of adding on antioxidant therapy for the treatment of RA.

Keywords: Oxidative stress; Antioxidants; Rheumatoid arthritis

1. Introduction

Rheumatoid arthritis (RA) is a chronic relapsing immuno-inflammatory multisystem disease with predominant synovial proliferation and destruction of articular cartilage. It is the most common inflammatory arthritis affecting approximately 1–2% of the general population worldwide [1]. Incidence increases with age, with women being affected three times more than men [2]. The exact etiology of RA remains unknown. It has been assumed that either a foreign

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agent or some alteration in control of cellular responses is involved, possibly genetically mediated, in the chronic persistent synovial inflammation [2].

The formation, behavior and scavenging of oxygen free radicals and other oxygen derived species in biological system have received much attention [3]. There is increasing evidence that they are closely connected with a variety of pathological conditions including atherosclerosis, cancer, arthritis and liver diseases. Fortunately, in healthy individuals the reactive oxygen species (ROS) production is low and lipid peroxidation is inhibited by the combined activities of various antioxidants present in the plasma.

It has been suggested that the pro-oxidant/antioxidant imbalance in RA may be either due to acceleration of some cellular reactions or insufficiency of the antioxidant defense system [4]. So, we considered it important to analyse the concentrations of lipid peroxides in terms of malondialdehyde (MDA), in association with certain antioxidant parameters like total thiols, glutathione and vitamin C [5,6]. The evaluation of these may prove to be of great importance in the management of RA. The study was also aimed to find out the effect of antioxidant supplementation on the oxidative stress in RA patients.

2. Materials and methods

The study was conducted in the Department of Biochemistry and the Department of Medicine, Pt.BDS, PGIMS Rohtak. Forty newly diagnosed patients of RA attending the Medicine OPD were the subjects of this study. The patients in the study group were diagnosed according to the criteria of American Rheumatism Association [7]. Twenty healthy age- and sex-matched individuals formed the control group (Group I). The study group was further randomly divided into two subgroups. Group IIa received treatment with conventional drugs like NSAIDS and steroids for 12 weeks, while Group IIb patients received antioxidants in the form of a fixed dose combination of vitamins A, E and C along with the conventional drugs for the same period. The two RA groups were comparable at the time of diagnosis with respect to duration and severity of symptoms.

The protocol was approved by the ethical committee of the PGIMS. Patients found to be suffering from renal disease, diabetes mellitus, hepatic disease, hypertension, ischemic heart disease, tuberculosis, HIV, alcoholics, smokers and patients already on antioxidant therapy were excluded from the study. Blood samples were taken twice, once at the time of presentation and next after 12 weeks of treatment. Disease activity of patients in the two RA groups was also measured twice, before and after treatment, using the Rheumatoid Arthritis Disease Activity Index (RADAI) [8]. Plasma was separated and stored at 4 °C until analysis.

Total thiols were estimated by the method of Ellman [9]. Glutathione was measured by the method of Beutler et al. [10]. The method of Roe was followed for the estimation of vitamin C [11]. MDA was

Table 2
Malondialdehyde (mean ± S.D.) and RADAI score in Group I (controls), Group IIa (on conventional treatment) and Group IIb (with antioxidant supplementation) before and after treatment

	Before treatment		After treatment	
	MDA	RADAI	MDA	RADAI
Group I	3.17 ± 0.68			
Group IIa	14.56 ± 2.14	64 ± 13.7	10.97 ± 1.70	48.3 ± 7.2
Group IIb	14.83 ± 1.92	67 ± 12.8	5.63 ± 1.09	16.1 ± 6.5

Table 1
Total thiol (mean ± S.D.), glutathione (GSH) and vitamin C in Group I (controls), Group IIa (on conventional treatment) and Group IIb (with antioxidant supplementation) before and after treatment

	Before treatment			After treatment		
	Total thiol (mmol/l)	GSH (mmol/l)	Vitamin C (mg/dl)	Total thiol (mmol/l)	GSH (mmol/l)	Vitamin C (mg/dl)
Group I	4.28 ± 0.46	1.94 ± 0.15	1.17 ± 0.28			
Group IIa	2.94 ± 0.26	0.95 ± 0.10	0.40 ± 0.15	3.66 ± 0.35	1.41 ± 0.15	0.65 ± 0.15
Group IIb	2.88 ± 0.33	0.99 ± 0.13	0.42 ± 0.14	4.16 ± 0.42	1.84 ± 0.16	3.87 ± 0.75

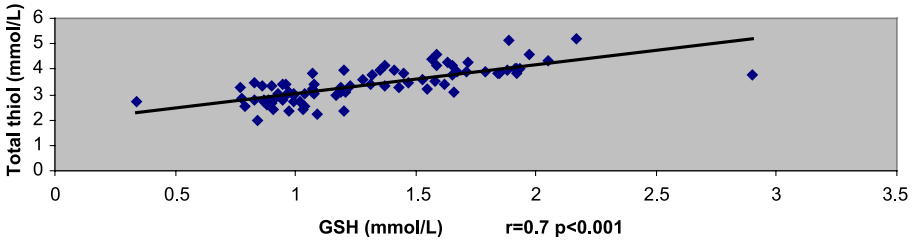

Fig. 1. Correlation between blood glutathione and total thiol levels.

measured by the modified method of Kumar et al. [12]. The coefficient of variation of different analytes was $<5\%$.

The results of the study group were compared with control group and with pre- and post-antioxidant therapy findings. The significance between various groups was arrived at using Student's t test. Results were expressed as mean $\pm$ S.D. for 20 individuals in the control group and 10 individuals in each of the two test groups. For statistical analysis, Group IIa and Group IIb were compared with Group I (control group) and Group IIa with Group IIb.

3. Results

Table 1 shows the concentrations of whole blood total thiols in the control group and the study groups before and after therapy. These values were significantly lower ($p<0.001$) in the study groups as compared to be control group. The pretreatment concentrations in the two study groups were comparable, but a highly significant increase ($p<0.001$) was observed in the concentrations after treatment. This increase in the concentrations of total thiols was much more in patients of Group IIb who received antioxidants along with conventional drugs.

Table 1 compares the concentrations of whole blood glutathione between the two groups. The concentrations of glutathione were significantly lower ($p<0.001$) in patients of RA of both the study groups when compared to normal controls. However, the difference in the pretreatment concentrations between the two study groups was not significant. A highly significant increase ($p<0.001$) in the concentrations of whole blood glutathione was observed in both the study groups after treatment, although the increase was much more in patients of Group IIb who received antioxidant supplementation.

A comparison of the concentrations of vitamin C in normal controls with patients of RA before and after treatment is also depicted in Table 1. The plasma vitamin C concentrations in the cases of RA in Group IIa and Group IIb were found to be statistically lower ($p<0.001$) than those in the normal controls. However, the pretreatment concentrations in the study groups were comparable. The concentrations increased significantly ($p<0.001$) after treatment in both groups, however, the increase was much more in patients on antioxidant therapy.

The comparison of concentrations of plasma MDA and disease activity (RADAI Score) in RA patients before and after treatment is given in Table 2. The concentrations of MDA and RADAI Score were

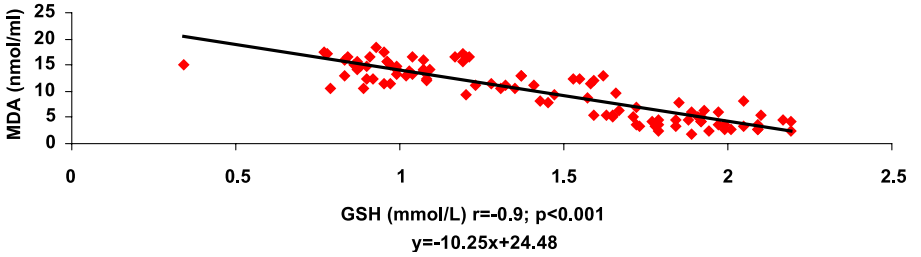

Fig. 2. Correlation between GSH and MDA levels.

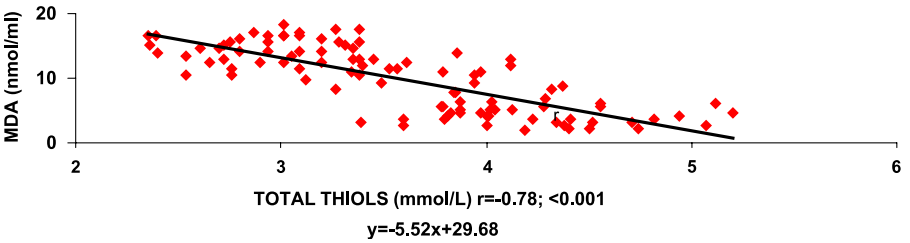

Fig. 3. Correlation between total thiols and MDA levels.

significantly higher in patients of RA ($p<0.001$) as compared to normal controls. These concentrations declined significantly ($p<0.001$) after treatment in both the study groups, though the fall was much more marked in patients of Groups IIb.

The correlations between total thiol concentrations and blood glutathione concentrations, between blood glutathione and MDA concentrations and between total thiols and MDA concentrations have been depicted in Figs. 1–3, respectively. A significant positive correlation was observed between blood gluthathione and total thiol concentrations ($r=0.7$, $p<0.001$). On the other hand, a statistically significant negative correlation was found between glutathione concentrations and MDA ($r=0.9$, $p<0.001$) and between total thiols and MDA ($r=0.8$, $p<0.001$).

4. Discussion

RA is a chronic relapsing immuno-inflammatory multisystem disease with predominant synovial proliferation and destruction of the articular cartilage [13]. Etiopathogenesis of RA still remains obscure despite extensive research. The pathogenesis of RA is multifactorial and recent research has implicated oxygen free radicals as mediators of tissue damage [4].

Several different pathways can lead to increased formation of reactive oxygen species in inflamed joints [14]. This enhanced oxidation plays a significant role in the tissue damage and inflammation perpetuating process in rheumatoid synovium. Oxygen free radicals lead to lipid peroxidation, which is defined as the oxidative deterioration of unsaturated fatty acids [15]. Lipid peroxidation is thus a process which is determined by the peroxide forming mechanisms and peroxide removing antioxidants [16]. Although lipid peroxidation affects many cellular components, the primary site involves membrane-associated polyunsaturated fatty acids and protein thiols [14]. During health, when reactive oxygen species production is low, lipid peroxidation is inhibited by the combined activities of various antioxidants present in the plasma. The failure of antioxidant defense mechanism to keep pace with oxidant generation may either be due to decrease in antioxidant defense or increased generation of oxidants as is the case in Rheumatoid arthritis. From the literature reviewed, it is apparent that patients of RA are exposed to oxidative stress and are more prone to lipid peroxidation [5]. Accordingly, altered concentrations of some antioxidants have also been reported [6].

As a consequence of the present understanding of the etiopathogenesis of RA, exogenous antioxidants, i.e., vitamins and other nutrients, appear to be potential agents for therapeutic management [17]. The present study was thus undertaken to observe the relationship, if any, between some antioxidant parameters in patients of RA and concentrations of MDA which is an indicator of oxidative stress. The study also aimed to find out the effect of antioxidant supplementation on the oxidative stress after 12 weeks of therapy.

The concentrations of whole blood glutathione and total thiols were found to be significantly lower in patients of RA, as compared to healthy controls. Total thiols play a vital role in the structure, activity and transport function of proteins, membranes and enzymes. They have been implied to decrease the damage provoked by oxidative stress [18]. Glutathione, on the other hand, is the most important non-protein sulfhydryl molecule present in both prokaryotes and eukaryotes and is an important component

of the antioxidant defense system in the living cells [19]. Glutathione protects the cellular constituents from the damaging effects of hydroperoxides formed during normal metabolism [19]. Decreased tissue glutathione has been associated with cell damage, depressed immunity and progression of ageing.

Low concentrations of sulfhydryl groups have been found in the sera of patients of RA [20]. Haataja and Kalliomaki [21] reported a marked decrease in the concentrations of serum sulfhydryl groups in patients as compared to controls, the decrease being greater in patients with active disease. Similar results have been reported by Banford et al. [22] and Taraza et al. [23]. The protective effect of thiols can be brought about directly (e.g., by scavenging radicals) or indirectly by elevating glutathione concentrations. As expected, a significant positive correlation between glutathione and total thiol concentrations was observed.

A significant increase in total thiols and glutathione concentrations was observed after treatment in both the study groups. However, the increase was much more marked when the routine treatment was supplemented with antioxidants.

Plasma vitamin C was also estimated as an antioxidant parameter. The concentrations in patients of RA were found to be significantly lower than normal controls. Plasma vitamin C plays a pivotal role in protecting plasma lipids from peroxidative damage initiated either by acquous peroxyl radicals or by activated polymorphonuclear cells [24]. It has been reported that plasma ascorbate is the first antioxidant to become oxidised immediately upon leukocyte stimulation [24]. This explains the low concentrations of plasma vitamin C in RA patients. The estimation of vitamin C may also be of diagnostic use as an early indicator of oxidative stress.

Blake et al. [25] estimated total ascorbate concentration in plasma and synovial fluid of patients of RA and found them to be at the lower end of the normal range. In separate studies conducted by Feri et al. [24] and Merry et al. [26], concentrations of vitamin C in patients of RA were found to be significantly lower than the controls. Situnayake et al. [27] studied 20 RA patients and found the concentrations of vitamin C to be significantly lower in the controls.

We further evaluated the alteration in the concentrations of vitamin C in the patients of RA after 12 weeks of treatment with conventional drugs in one group and with antioxidants along with conventional drugs in the other group. There was a marked rise in the concentrations of vitamin C in both the groups, but supplementation with antioxidants resulted in a much higher increase. Cerhan et al. [28] also found that greater intakes of supplemental vitamins C and E were inversely associated with RA.

The concentrations of MDA estimated before treatment in the two study groups were observed to be significantly higher than those of the normal controls. This extensive lipid peroxidation may be the cause of inflammatory arthropathy in RA. In a study conducted by Kalavacherla et al. [29], the concentrations of plasma MDA in cases of RA were significantly higher than the concentrations estimated in controls. Gambhir et al. [30] also reported markedly increased concentrations of MDA in patients as compared to controls. Similar results have been observed by others [23,31,32].

Increased serum MDA concentrations in RA suggests the role of free radicals in the pathogenesis of this inflammatory arthropathy and thus supports the need for studies assessing the therapeutic role of free radical scavengers is RA. In support of the above statement, we found a highly significant decrease in the concentrations of plasma MDA after treatment in both the study groups, although this decrease was much more in patients of Group IIb, who received antioxidant supplementation along with routine drugs.

The results of our study indicate that the antioxidant defense system is compromised in patients of RA, as evidenced by increased MDA concentrations (most potent marker of oxidative stress) and decreased concentrations of glutathione, total thiols and vitamin C. The oxidant/antioxidant imbalance is further revealed by the negative correlation between MDA and total thiol concentrations and also between MDA and glutathione concentrations. This derangement, in turn, leads to the inflammatory reaction and tissue damage observed in RA. Glutathione is probably the primary antioxidant which counters the effect of various ROS. Depletion of glutathione set in the oxidative damage to membrane lipids and the resultant inflammatory response is evident from a significant negative correlation between MDA and glutathione concentrations.

Improvement of the antioxidant parameters associated with decreased MDA concentrations after treat-

ment suggests that the treatment reduces the oxidative stress in those patients. Use of antioxidants as supplements to conventional drugs yields even better results as revealed by higher increase in total thiols, glutathione and vitamin C concentrations and higher decrease in MDA concentrations. Few clinical trials have also been forwarded which support the use of antioxidant vitamins as a complementary intervention to help manage RA [33,34]. The observations of better recovery in the patients treated with antioxidant supplemented drug regimen suggest that antioxidants may have an important role to play in this inflammatory disorder, as they lower the oxidative stress and the resultant inflammatory damage. More clinical trials may be required to evaluate the safety and efficacy of adding on antioxidant therapy for the treatment of RA. This treatment may be an important therapeutic modality in future management of RA patients.

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