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Serum total sialic acid, lipid peroxidation, and glutathione reductase levels in patients with rheumatoid arthritis

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Aim: The changes in the serum sialic acid levels, total lipid peroxidation products (MDA), and glutathione reductase activity were estimated in patients with rheumatoid arthritis. Serum Sialic acid is known as a parameter of inflammation. This work was undertaken to assess the potential role of sialic acid as well as oxidative stress and antioxidant status in patients with rheumatoid arthritis.

Materials and methods: The levels of these parameters in serum were studied in 52 subjects with rheumatoid arthritis (study subjects) and 52 age and sex matched healthy subjects as controls. Serum total sialic acid levels were determined using the assay method described by Warren. Serum MDA was determined as the measure of thiobarbituric acid reactive substances (TBARS) and glutathione reductase activity was measured in the serum by the method described by Goldberg DM.

Results: It was observed that there was a significant increase in serum sialic acid, MDA levels, and glutathione reductase activity in patients with rheumatoid arthritis compared to controls.

Conclusion: The results of our study suggest higher oxygen free radical production, evidenced by increased MDA support to the oxidative stress in rheumatoid arthritis. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. The results suggest the antioxidant activity of sialic acid in response to the increased oxidative stress in rheumatoid arthritis.

Key words: Sialic acid, malondialdehyde (MDA), glutathione reductase, rheumatoid arthritis

Introduction

Arthritis, the joint inflammation, refers to a group of diseases that cause pain, swelling, stiffness, and loss of motion in the joints. Rheumatoid Arthritis (RA) is a chronic, systemic disease, in which various joints in the body are inflamed, leading to swelling, pain, stiffness, and a possible loss of function. It is an autoimmune disease in which the body's immune system attacks itself. RA affects approximately 1%-2% of the total world's population (1). Annual incidence rate of RA between 0.5%-1% of the total population is reported every year in both developed and developing countries (2). Lower incidences of RA are reported every year in East Asia (3). RA affects around 1 in 50 people and is more common in women than men. It is most common after the age of 40, but can happen at any age. Sialic acid is an acetylated derivative of neuraminic acid and it is attached to non-reducing residues of carbohydrate chains of glycoproteins and glycolipids. Serum sialic acid has been reported as a marker of the acute phase response; increased sialic acid concentrations have been observed in several diseases, such as myocardial infarction and diabetes. Serum sialic acid is

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also increased during inflammatory processes as a consequence of elevated concentrations of richly sialylated acute phase glycoproteins (4). Serum sialic acid was reported to be a useful parameter of inflammation (5). On the other hand some studies reported that the monomeric sialic acid is a potent defense molecule against oxidative damage and cell death caused by H_2O_2 (6). However, its significance has not been discussed in various pathological conditions. Since the oxidative damage caused by free radicals is a pivotal mechanism in the progression of RA, the relation between sialic acid levels and oxidant–antioxidant status was evaluated. Lipid peroxidation mediated by free radicals is considered to be a major mechanism of cell membrane destruction and cell damage. Free radicals are formed in both physiological and pathological conditions in mammalian tissues (7). The uncontrolled production of free radicals is considered as an important factor in the tissue damage induced by several pathophysiologies (8,9). Alteration in the oxidant–antioxidant profile is known to occur in rheumatic diseases (10,11). Oxidative stress due to damage brought about by free radicals is also known to influence the response of these patients to therapy. Moreover, the body's defense mechanisms would play an important role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions (12) and 2 main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated (13). They exist in both aqueous and membrane compartments of cells and can be enzymes or non-enzymes. The aim of our study was to investigate the changes in oxidant and antioxidant status and its relation with sialic acid in patients with RA.

Materials and methods

The study was conducted in the Department of Biochemistry, Saveetha Medical College & Hospital, Saveetha University, Chennai, T.N, India. Fifty-two clinically diagnosed patients from orthopedics department, who had not undergone any previous treatment for their arthritis, were chosen for the study. Out of the 52 patients included in the study 12 are females. An equal number of age and sex matched healthy subjects with a similar socio-economic status were also investigated**.** The females of the study group were matched with females of the control. As menopause and female sex hormones and their receptors play a major role in oxidant status and arthritis, menopause in females was considered for matching. Body weight was also taken into consideration. Due permission was obtained from the ethical committee of the institution before the start of the study. Written consents were also obtained from the patients prior to the study and the objectives of the study were fully explained. Four of the participants dropped out at the end of the selection as they did not like the idea of giving blood.

Complete clinical and personal history of the subjects was recorded. The subjects were 35–60 years old. All patients in the study were clinically diagnosed as patients with RA. The presence of RA in patients was diagnosed via X – ray analysis of joint destruction, rheumatoid factor test, C–reactive protein, and antinuclear anti bodies test. None of these subjects was alcoholics or chronic smokers and did suffer from any systemic diseases like hypertension or any diabetic complication. Patients suffering from a disease of any origin other than osteoarthritis were excluded from the study. Subjects with normal nutritional habits without supplementing any vitamins during the prior 6 months were included. Subjects with history of receiving anti-inflammatory drugs in the prior 6 months and history or present symptoms of any other stress induced disorder were excluded.

The controls and patients were divided into 2 groups.

Group 1: Fifty-two healthy age and sex matched controls with similar a

socio economic status.

Group 2: Fifty-two patients with clinically diagnosed RA.

The venous blood samples obtained from these subjects in the morning after an overnight fasting were used for analysis. Serum was separated by centrifugation at 1000 g for 15 min at +4 °C. Separated serum was used for the estimation of sialic acid,

MDA, and glutathione reductase. Serum total sialic acid levels were determined using the thiobarbituric acid assay method described by Warren (14). Serum MDA was determined as the measure of thiobarbituric acid reactive substances (TBARS) (15) and glutathione reductase activity was measured in the serum by the method described by Goldberg DM (16). Necessary care was taken during sample collection, storage, and assay.

Chemicals

All reagents used were of analytical reagent grade or the highest grade available. Glutathione and thiobarbituric acid were obtained from sigma chemicals, St.Louis, MO, USA. Statistical analysis between group 1 (controls) and group 2 (patients) was performed by the Mann Whitney U test. The data are expressed as mean ± SD. $P < 0.05$ was considered as significant.

Statistical analysis

Statistical analysis between group 1 (controls) and group 2 (patients) was performed by the Independent Student's t test using SPSS for Windows version 15. The data are expressed as mean ± SD. $P < 0.05$ was considered as significant.

Results

Mean ± SD of total sialic acid, malondialdehyde (MDA) levels, and activity of glutathione reductase in controls and patients with RA described in Table1 and Figure 1. There was a statistically significant increase in the serum total sialic acid and malondialdehyde (MDA) levels, and glutathione reductase activity in patients with RA compared to controls.

Table 1. Mean ± SD values of total sialic acid and malondialdehyde (MDA) levels, and glutathione reductase activity in controls and patients with RA.

Parameter	Group 1 (controls) n = 52	Group 2 (Patients) n = 52
Sialic Acid (mg/dL)	25.33 ± 0.81	59.98 ± 0.58***
MDA (micromoles/L)	4.89 ± 0.89	6.61 ± 0.54***
Glutathione Reductase (U/L)	35.05 ± 1.62	57.92 ± 1.22***

***P < 0.001 compared to controls

Discussion

In the present study the lipid peroxidation product, i.e. MDA, levels were increased significantly in the serum of the patients with RA. Rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids. Similar reports of elevated MDA levels have been reported in patients with rheumatic disease (11,17). In contrast to our study, Kajanachumpol et al. reported no significant change in MDA levels in patients with RA compared to controls (18).

We observed a significant increase in the serum total sialic acid levels in patients with RA compared to controls. In our study total serum sialic acid levels were significantly increased parallel to oxidative stress. So, we suggest that increased levels of sialic acid levels might be considered as a defense molecule against the increased oxidative stress in RA. Antioxidant property of Sialic acid as a H_2O_2 scavenger has been reported by Tanaka et al. (19).

In our study the erythrocyte antioxidant enzyme, i.e. glutathione reductase activity, has been increased significantly in patients with RA. Similar reports of raised antioxidant enzyme activities have been reported in patients with RA (10,20). 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 (21). The rise in the activity of GR could be due to its induction to counter the effect of increased oxidative stress.

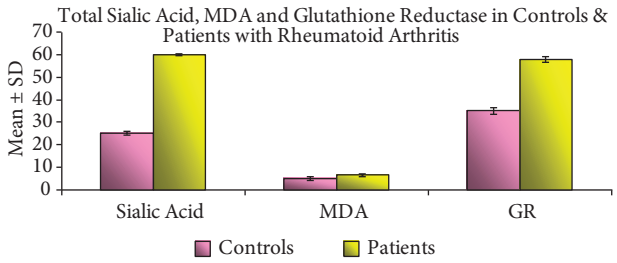

Figure 1. Mean ± SD values of total sialic acid and malondialdehyde (MDA) levels and glutathione reductase activity in controls and patients with RA.

In conclusion, oxidative stress may be involved in RA. There is a shift in the oxidant–antioxidant balance in favor of lipid peroxidation, which could lead to the tissue damage observed in this disease. The results of our study suggest higher oxygen free radical production, as evidenced by significant increase in malondialdehyde levels, supports the higher oxidative stress hypothesis in RA. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. Furthermore, serum total sialic acid levels increased in parallel to oxidative stress, supporting the role of sialic acid as an antioxidant.

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