OXIDATIVE STRESS AND CALCIUM-PHOSPHORUS LEVELS IN RHEUMATOID ARTHRITIS

S. D. Walwadkar, A.N. Suryakar, R.V. Katkam, K.M. Kumbhar and R.D. Ankush

Department of Biochemistry, Dr. V. M. Govt. Medical College, Solapur (Maharashtra)

ABSTRACT

Generation of reactive oxygen species is an important factor in the development and maintenance of rheumatoid arthritis (RA) in humans. This study was undertaken to investigate interplay among oxidants, antioxidants and pathogenesis of Rheumatoid arthritis. Serum levels of lipid peroxides, nitric oxide, vitamin E and ratio of calcium/phosphorus in RA patients were determined and compared with normal healthy controls. Significant increases in lipid peroxides ($p < 0.001$) and nitric oxide ($p < 0.001$) levels were found in patients presenting with RA as compared to controls. Whereas significant decrease in vitamin E ($P < 0.001$) and calcium/phosphorus ratio ($p < 0.001$) were found in Rheumatoid arthritis patients as compared to controls. Positive correlation was found between lipid peroxides and nitric oxide as well as between vitamin E and calcium. While lipid peroxides and nitric oxide were correlated negatively with vitamin E. whereas negative correlation was observed between MDA and Calcium/Phosphorus ratio in patients with rheumatoid arthritis. Our findings suggest that there is a close association between bone loss and oxidative threat in patients presenting with Rheumatoid arthritis.

KEY WORDS

Rheumatoid arthritis, oxidative stress, lipid peroxidation, vitamin E, Calcium/Phosphorus

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic progressive autoimmune disorder characterized by symmetric erosive synovitis and some times shows multisystem involvement (1). Exact reason behind bone erosion and joint deformities is not fully understood. Formation of reactive oxygen species and lipid peroxides as a result of disease activity may play an important role in RA. While lowered concentrations of antioxidants in the blood considerably increase the probability of the occurrence of RA (2). Many investigators have focussed on oxidative stress since last few years and suggest that RA patients are more prone to lipid peroxidation (3). Growing evidences implicate nitric oxide (NO) in immune regulations, inflammation, autoimmunity and arthritis (4, 5). Antioxidants such as vitamin E and vitamin C are highly effective at mopping up free radicals (6).

In the formation of bones, calcium/phosphorus ratio is very important. It is possible that, generation of reactive oxygen species may be particularly important factor for bone

resumption in inflammatory process (7). Hypoxic conditions also disrupt an intracellular ionic environment and alter calcium and phosphorus level (8). In the light of these explanations, the present study was designed to investigate interrelationships among oxidants, antioxidants and pathogenesis of Rheumatoid arthritis.

MATERIALS AND METHODS

Present study was conducted in the Department of Biochemistry, Dr. V. M. Govt. Medical College Solapur (MS). This study included 50 rheumatoid arthritis patients and 50 normal healthy control subjects matched for age, sex, and socioeconomic status. Selection of controls was carried out by usual standard method. Whereas those having clinical history of the disease such as diabetes mellitus, cardiovascular diseases, inflammatory diseases, infectious diseases, etc. where free radical damages has been commonly found to be increased were strictly excluded.

Diagnosis of RA patients was done on the basis of clinical history such as morning stiffness in and around joints, serological investigation like serum rheumatoid factor and radiographic changes viz. erosions or unequivocal bony decalcification on postero-anterior hands and wrists. Only those patients who fulfill the American Rheumatism

Address for Correspondence:

Dr. A.N. Suryakar
Professor and Head,
Department of Biochemistry,
Dr. V. M. Govt. Medical College, Solapur (Maharashtra)

Association (ARA) (9) criteria for RA were included in the study. All these patients were between 30-70 years of age.

Control subjects as well as RA patients were not on vitamin, minerals, any drug or such supplementation, which may alter antioxidant and calcium/phosphorus, levels.

Fasting venous blood samples were collected and nonhemolysed sera were processed for all biochemical parameters. All the assays were carried out by well-established and sensitive methods for lipid peroxides (10), Nitric oxide (11), Vitamin E (12), calcium (kits from Starchem diagnostics) albumin correction for calcium (kits from Starchem diagnostics) and phosphorus (13).

Statistical analysis was done by using students 't' test and P values <0.05 were considered as statistically significant.

RESULTS

Table 1 shows comparison of MDA, NO⁻ and vitamin E levels in RA with control subjects. Statistically significant increase in levels of lipid peroxide ($P < 0.001$) and nitric oxide ($P < 0.001$) whereas statistically significant decrease in vitamin E ($P < 0.001$) was found in patients of RA as compared to control subjects. Table No.1 also depicts comparison of calcium, phosphorus and calcium/ phosphorus ratio in RA with control subjects. Serum calcium levels were found to be significantly decreased ($P < 0.001$) whereas phosphorus level was increased significantly ($P < 0.001$) in RA patients as compared to control subjects. On the contrary, calcium/phosphorus ratio was decreased significantly ($P < 0.001$) in RA patients as compared to control subjects.

As shown in Table 2, pairwise correlation analysis in RA patients showed following results. Lipid peroxide levels showed significant positive correlation with nitric oxide ($r = + 0.91$) and similarly vitamin E also showed significant positive correlation with calcium ($r = + 0.66$). On the contrary, lipid peroxide was negatively correlated with vitamin E ($r = - 0.66$) as well as with calcium/phosphorus ratio ($r = - 0.76$). Furthermore, a significant negative correlation was found between vitamin E and nitric oxide ($r = - 0.66$).

DISCUSSION

Present study indicates increased oxidative stress, which is reflected by increased lipid peroxidation in peripheral blood of patients with RA. These results are also in accordance with

TABLE 1
Serum levels of total lipid peroxide (MDA), nitric oxide, vitamin E, calcium, phosphorus and calcium/phosphorus in control subjects and rheumatoid arthritis patients.

Sr. No.	Parameters	No. of Subjects	Control Subjects	Rheumatoid Arthritis patients
1.	Serum total lipid peroxide (MDA) $\mu\text{mol/L}$	50	1.92 ± 0.63	$4.62 \pm 1.46^*$
2.	Serum nitric oxide (nitrite) $\mu\text{mol/L}$	50	38.3 ± 9.1	$98.8 \pm 26.3^*$
3.	Serum vitamin E mg/L	50	13.61 ± 2.40	$8.66 \pm 2.25^*$
4.	Serum Calcium mg %	50	10.18 ± 1.47	$7.79 \pm 1.38^*$
5.	Serum inorganic phosphorus mg %	50	2.91 ± 0.40	$4.63 \pm 0.91^*$
6.	Calcium/phosphorus ratio	50	3.52 ± 0.45	$1.79 \pm 0.63^*$

Values are expressed as mean $\pm$ SD; *P < 0.001

TABLE 2
Pairwise correlation analysis in RA patients

Sr. No.	Pairwise correlation of serum parameters	'r' value
1.	Positive correlation between lipid peroxide and nitric oxide	+ 0.91
2.	Positive correlation between vitamin E and calcium	+ 0.66
3.	Negative correlation between lipid peroxide and vitamin E	- 0.66
4.	Negative correlation between lipid peroxide and calcium/phosphorus ratio	- 0.76
5.	Negative correlation between vitamin E and nitric oxide	- 0.66

the earlier studies (14). MDA, the product of lipid peroxidation reacts with lysine residues in protein to produce immunogenic molecules, which can exacerbate inflammation. The longer chain polyunsaturated fatty acids are especially potent at increasing lipid peroxidation and causing cell damage by oxidative stress (15). Depolymerization of hyaluronic acid in synovial fluid results into loss of lubricating property of the fluid. It has been reported that, it is very important consequence of exposure of synovial fluid to super oxide (O_2^-) and hydrogen peroxide (H_2O_2) (16).

In the present study, increased Nitric oxide level in RA patients is similar with those previously reported (17, 18). But we found a significant positive correlation between Nitric oxide and lipid peroxides ($r = +0.91$) in RA patients. This finding shows extensive cohesiveness that, generation of Nitric oxide in excess may also trigger lipid peroxidation process. Cytokines induce nitric oxide synthesis by endothelial cells, macrophages

and polymorphonuclear leukocytes, indicating a role in inflammatory process (19). Articular cartilage and synovial fibroblasts have recently been found to synthesize substantial amounts of nitric oxide suggesting joints as a potential source of nitric oxide. Nitric oxide can induce a tissue damage especially after conversion into peroxynitrite radical (ONOO⁻) and also affects physiological process within joints including modulation of interleukin-1 (IL-1) induced bone resorption and cartilage metabolism. Therefore all these findings strongly suggest that, the cause of inflammation in RA is a result of cytotoxic and cytostatic effects of nitric oxide.

Vitamin E is a major chain breaking antioxidant, which is a crucial for proper antioxidant protection. Decreased levels of vitamin E in RA patients, in a present study, are comparable with previous reports (14, 20). Whereas negative correlation of vitamin E with lipid peroxides ($r = -0.66$) and nitric oxide ($r = -0.66$) shows a possibility that, in the prediagnostic stage, serum antioxidants are low because they have been used in reducing inflammatory products (21). This leads to increased potential for oxidative damage. Vitamin E may affect the immunity of the organism by decreasing H₂O₂ concentration and causing decrease in lymphocyte proliferation. It results in mild anti-inflammatory effect. It may play a role in controlling chronic inflammation of any cause. Proper functioning of polymorphonuclear leukocytes depends to a large extent on the proper concentration of vitamin E in these cells (2).

A significantly decreased calcium/phosphorus ratio in RA patients as compared to controls in our study clearly indicates that, there is an altered calcium and phosphorous metabolism in RA. As calcium and phosphorous are important constituent of bone, ultimately bone metabolism is altered in RA, the event observed by many workers (22, 23). Negative correlation between calcium/phosphorus ratio and lipid peroxide ($r = -0.76$) suggests that the generation of reactive oxygen species in excess may be particularly important in the bone resorption that occurs in association with inflammatory diseases (24). Decrease in calcitriol level may contribute to a negative calcium balance and acceleration of immunomodulatory effects (23, 25). Significant positive correlation between vitamin E and calcium ($r = + 0.67$) suggest that vitamin E may also be an important for immunity of an organism along with calcium.

It is postulated that the elevation of phosphorous was related to tissue hypoxia with an increase in ATP degradation resulting in the release of inorganic phosphorous from cells. Hypertrophy and hyperplasia creates a hypoxic environment in synovial joints. It is corroborated by the reports of low glucose and

high lactate levels in rheumatoid synovial fluid (8). Acidosis is another factor that may acts to promote shifts of phosphate from the intracellular to extracellular pool. The rise in serum inorganic phosphate may parallel increase in blood lactate levels suggesting that a state of partial anaerobic metabolism may be contributory factor (26). In RA, hypoxic environment triggers oxygen free radicals generation and alters oxidative metabolism within the cell. It leads to disruption in intracellular ionic environment and altered calcium and phosphorus levels. Some authors suggest that hypocalcemia results due to drugs used in RA, but decreased mean total body calcium levels in RA patients who did not received that corticosteroid drugs strongly suggest that, this is an integral feature of RA (27).

In summary, biochemical alterations in present study reflect the pathogenesis of RA. Increased oxidative threat in rheumatoid arthritis is evidenced by raised lipid peroxides, nitric oxide and decreased vitamin E and calcium/phosphorus ratio. We postulate that, lipid peroxidation is a giant distracter in rheumatoid arthritis and further studies on dietary management of antioxidant and minerals may helps to wind up the notorious oxidants.

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