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RESEARCH ARTICLE

BIOCHEMISTRY

DYSLIPIDEMIA WITH ALTERED OXIDANT-ANTIOXIDANT STATUS IN RHEUMATOID ARTHRITIS

Corresponding Author

KOWSALYA R

[1]Department of Biochemistry, Institute of Nephrourology, Bangalore, India.

Co Authors

SREEKANTHA [2], VINOD CHANDRAN[2] AND REMYA[3]

[1]Department of Biochemistry, Institute of Nephrourology, Bangalore, India.
[2]Department of Biochemistry,Manipal University, Mangalore,India.
[3]Department of Anatomy,Nitte University,KSHEMA, Mangalore,India.

ABSTRACT

Rheumatoid arthritis is a clinical condition accompanied by inflammation and oxidative stress. In this study, we compared levels of antioxidants vitamin-E and C along with lipid profile between rheumatoid arthritis patients and healthy controls. We also looked into presence of any correlation between dyslipidemia, the antioxidants vitamins and lipid peroxide product among rheumatoid arthritis patients. We found a significant fall in vitamin E and C along with raised MDA in patients compared to controls. A highly significant positive correlation was found between MDA and LDL-cholesterol ($r = 0.781, P < 0.004$),whereas vitamin E and C were negatively associated with MDA level ($r = -0.70$, $P < 0.01$ and $r = -0.75$, $P < 0.001$ respectively).Thus the assessment of the lipid profile,along with other cardiovascular risk factors should be actively determined and appropriate treatment along with sufficient antioxidants supplementation should form part of the standard treatment protocol in rheumatoid arthritis patients.

KEY WORDS

rheumatoid arthritis, antioxidants, dyslipidemia,lipid peroxidation.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic disease, usually manifesting as inflammation of multiple joints. It is characterized by a number of extra-articular manifestations, including rheumatoid nodules, vasculitis, heart or lung disease, anemia, and peripheral neuropathy. Although the cause of RA is unknown, it is generally considered an autoimmune disease [1.]

The focus of RA is the synovial lining, a highly vascularised tissue which surrounds the bones of the joint. The inflammatory cells like the neutrophils and macrophages attack the affected joint and experience a “respiratory burst" which is characterized by an increased generation & release of reactive oxygen species. Excessive production of ROS leads to oxidative stress, loss of cell function, and ultimately apoptosis or necrosis. The level of ROS is also a function of the antioxidants such as vitamins A, C, E and of metabolites like uric acid, bilirubin, etc that are capable of either scavenging these reactive oxygen species directly or indirectly. However, a fine balance exists that may be disrupted in favor of oxidants (oxidative stress), leading to cellular damage, which in turn may play a role in major diseases such as cancer, rheumatoid arthritis and atherosclerosis.

In general, increased premature mortality has been noted in RA patients compared to general population which has been attributed to an excess of cardiovascular morbidity. The majority of cardiovascular deaths result from accelerated atherosclerosis [2,3,4.] Recently increased oxidative stress and impaired antioxidant defense have been suggested as a contributory factor for initiation and progression of complications in disease like rheumatoid arthritis [5.] It has been postulated that dietary intervention with vitamins may reduce oxidative stress and thereby reduce the disease related morbidity and mortality [6.]

So this study was undertaken to evaluate the lipid profile along with the oxidant/antioxidant status in patients with rheumatoid arthritis.

MATERIAL AND METHODS

This study involved 40 patients with RA, diagnosed according to the 1987 revised criteria of the American College of Rheumatology[7.] There were 27 women and 13 men (mean age 41.7 ± 6.5 year) with a mean disease duration of 6.8 ± 4.4 year. Informed consent was obtained from each individual. The patients were chosen for the study after having a preliminary evaluation consisting of a brief medical history, smoking and alcohol habits and physical examinations. Patients with smoking and alcohol habits, those suffering with systemic disease like diabetes mellitus, hypertension and those on corticosteroids, statins, immunosuppression and vitamins supplementation were excluded from the study.

Fasting venous blood samples were collected in vacutainers with anticoagulant EDTA and in plain vacutainers without any anticoagulant from both the groups. Blood collected without anticoagulant was centrifuged at 3000g for 10 minutes. Serum was collected carefully and used for estimation of Vitamin E, C and lipid profile. Blood collected in EDTA was also centrifuged at 3000g for 10 minutes. The separated cells were washed thrice with 0.9% cold saline. The RBCs were then suspended in an equal volume of 0.9% saline and used for the estimation of Malondialdehyde (MDA).

The carefully separated serum was analyzed for lipid profile (serum cholesterol, triglycerides, high density lipoprotein (HDL) and low-density lipoprotein (LDL) and Very Low density Lipoprotein (VLDL) on Clinical

Chemistry Autoanalyser (Beckman) using standard reagents and kits.

Lipid Peroxidation (MDA): The susceptibility of erythrocytes to undergo oxidation in the presence of 0.44 mol/L hydrogen peroxide was assessed. Lipid peroxidation was studied as Thiobarbituric acid (TBA) reaction products. The method of Stocks and Dormandy was followed with certain modifications [8].

Serum Vitamin-E: Determined by Baker and Frank Method which is based on reduction of Ferrous ions which forms a red colored complex with α−α1 dipyridyl[9].

Serum Vitamin-C: was estimated by the dinitro phenyl hydrazine method [10]. Ascorbic acid is oxidized by Cu+2 to form dehydro ascorbic acid, which reacts with acidic 2, 4 dinitro phenyl hydrazine to form a red bis-hydrazone which was measured spectrophotometrically at 520 nm.

Statistical Analysis: Statistical analysis was done by using Kruskal – Wallis test and Mann Whitney U- test for the comparison of different parameters. Correlations between the variables were estimated by Pearson's Correlation coefficients.

RESULTS

The demographic and clinical data in the both groups of RA patients and healthy controls are listed in Table 1.

Table 1

Mean Age	**41.7 ± 6.5 years**
Sex (females/males)	27/13
Mean disease duration	6.8 ± 4.4 years
Mean ESR	34.5±12.4 mm/hour
Mean hemoglobin	10.3 ± 2.6 g/dl

The levels of antioxidant vitamin C and E were all significantly lower in RA patients compared to healthy controls, whereas the level of erythrocyte MDA was significantly elevated in RA patients compared to controls (data shown in table-2).

Table – 2

Parameters	**RA patients n=40**	**Controls n=40**	**pvalue**
MDA(nmoles/dL)	465.32±202.1	265.86± 57.12	<0.0001
Vitamin E(mg/L)	7.67± 1.66	9.95± 0.75	<0.0001
Vitamin C	0.73 ± 0.35	0.95 ± 0.45	<0.01

n = number of subjects; Values are mean±SD; 'P'<0.05 was considered significant.

A significant decrease in serum levels of HDL-C along with increase in of TC and LDL-C were found in RA patients compared to controls. The data is shown table-3 as mean ± SD levels.

Table - 3

Parameters	**RA patients n=40**	**Controls n=40**	**pvalue**
TC	230.607±52.98	183.38±55.278	<0.01
TAG	168.092±28.23	120.676±19.787	<0.0001
HDL-C	30.23±5.38	51.461±7.007	< 0.001
LDL-C	142.269±25.54	111.89±18.96	<0.002
VLDL-C	46.76±9.776	33.15±6.619	<0.0004

n = number of subjects; Values are mean±SD; 'P'<0.05 was considered significant.

Strongly negative correlation was noted between the antioxidant vitamins and MDA level ($r = -0.706$, $P < 0.01$ and -0.753, $P < 0.001$ respectively).A very significant positive correlation between the serum LDL-C and MDA ($r = 0.781$, $P < 0.0045$) was noted. Additionally a positive correlation between total cholesterol, triglycerides and the lipid peroxide product MDA was noted.

DISCUSSIONS

In the present study we investigated the plasma levels of antioxidants vitamin C & E along with lipid profile and lipid peroxidation product Malondialdehyde in patients with rheumatoid arthritis. Studies suggest that free radical mediated oxidative stress can influence the plasma lipid concentrations and generate potent proatherogenic mediators. These free radicals also play an important role in the development of atherosclerotic vascular disease and other complications seen in rheumatoid arthritis [11, 12.]

In the present study, a significant increase in MDA along with significant decrease in antioxidant vitamin C & E was observed in RA patients compared to controls, revealing that there is an increased oxidative damage in these patients. This is similar to work of V Chaturvedi et al [13] and Gutteridge etal [14]who showed a decrease in antioxidant enzyme activities and increase in lipid peroxidation products in patients with rheumatoid arthritis. Significant lower levels of vitamin E and C were seen in patients with RA than in healthy control subject. Vitamin E helps to trap free radicals and interrupt the chain reaction that damage the cells whereas regeneration of vitamin E depends on Vitamin C. Due to increased oxidative stress in RA there may be increased consumption of both of these vitamins [15.]

There is substantial evidence indicating that a low antioxidant status is associated with a higher risk of developing RA. Furthermore, the disease process is associated with an increased generation of oxidant, which plays an important role in the inflammatory process and contributes to tissue destruction [16.] Antioxidant defenses limit the damages caused by oxidants, such as those formed during inflammation. In addition, in vitro-studies and animal studies have shown that antioxidants also possess anti-inflammatory properties [17.] This implies that antioxidative defense mechanisms are of particular importance for patients with RA.

The present study showed significant decrease levels of HDL cholesterol along with hypercholestermia and hypertrigercidemia in RA patients when compared with controls. Our results are in accordance with reports from Lakatos J et al who have shown presence of significant dyslipidemia in patients with RA in their study [18.] Hypercholestermia is an important factor in development of atherosclerosis whereas increased HDL cholesterol has atheroprotective function. Another substantial finding was presence of significant increase of LDL-cholesterol in RA patients. Low density lipoprotein has a positive relationship to the risk of coronary heart disease and also has been shown undergo oxidation forming oxidized LDL. Ox-LDL leads to phospholipid release, activating endothelial cells, thereby initiating an inflammatory process which leads to the formation of foam cells and subsequent fatty streaks. Under normal condition HDL exerts its antiatherogenic role by protecting LDL from oxidation[19.]Thus these altered lipoproteins along with an increase in lipid peroxidation products suggests that RA patients are at a high risk for the development of coronary heart disease.

CONCLUSION

Substantial evidence suggests that chronic systemic inflammation contributes significantly to excess cardiovascular disease in RA and that effective suppression of RA-associated inflammation appears to reduce mortality. Thus management of dyslipidemia should consider as a part of cardiovascular risk management in RA patients. In addition, cardiovascular risk factor screening and appropriate treatment in form of antioxidant supplementation may be necessary for

reducing the morbidity and mortality in these patients.

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