

## A Multi Marker Evaluation of Cardiovascular Disease Risk in Patients with Rheumatoid Arthritis

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### Abstract

**Background:** Rheumatoid arthritis (RA), an inflammatory joint disease, is also associated with systemic effects. Patients with RA have an increased risk of morbidity and mortality that is mainly attributable to cardiovascular disease (CVD). Both traditional and novel risk factors are implicated in causing the increased CVD risk in RA patients.

**Materials and Method:** Forty six RA patients, diagnosed with RA as per 1987 revised ARA criteria and forty six age and sex matched healthy controls were studied. Fasting lipid profile (Total cholesterol, triglycerides, HDL cholesterol), inflammatory markers (high sensitivity C reactive protein [hsCRP] and fibrinogen), oxidant and antioxidant markers (malondialdehyde [MDA] and ferric reducing ability of plasma [FRAP]), uric acid, homocysteine and Nitric oxide (NO) were measured in all subjects.

**Results:** Among the traditional lipid parameters studied, triglycerides were increased in RA patients compared to controls ( $p=0.023$ ). Both hsCRP ( $p<0.001$ ) and fibrinogen ( $p=0.006$ ) were elevated in RA patients than in controls. MDA levels were increased ( $p<0.001$ ) and FRAP levels decreased ( $p<0.001$ ) in patients with RA compared to controls. Uric acid and homocysteine levels showed no significant difference between RA patients and controls. Nitric oxide levels were increased in RA patients when compared with controls ( $p=0.030$ ).

**Conclusion:** Patients with rheumatoid arthritis were found to have an increased risk of CVD as evidenced by increased triglyceride levels, increased inflammatory markers, presence of oxidative stress. Hence, management of these patients should also include evaluation of CVD risk besides treatment of joint symptoms.

**Keywords:** Cardiovascular Disease, Inflammation, Oxidative Stress, Rheumatoid Arthritis.

### Introduction

Rheumatoid arthritis (RA) is a prototypical chronic inflammatory disease affecting about 1-2% of world population.<sup>(1)</sup> The exact etiology of RA is not known, however genetic factors appear to be involved in the development of rheumatoid arthritis.<sup>(2)</sup> Although primarily a joint disease, RA is associated with extra articular and systemic effects as well. In spite of improved treatment strategies, patients with RA experience increased morbidity and mortality.<sup>(3)</sup> Moreover, while the mortality rates in general population are showing a decreasing trend, the same is not observed in patients with RA.<sup>(4)</sup> RA associated increased mortality is majorly attributed to cardiovascular disease (CVD), which is considered as an extra articular manifestation of RA.<sup>(1)</sup>

Several factors contribute to the increased CVD risk in RA patients.<sup>(5)</sup> An increased prevalence of traditional CVD risk factors such as obesity, hypertension, dyslipidemia, impaired physical activity is observed in RA patients,<sup>(1)</sup> these however do not fully explain the increased CVD related mortality in RA patients,<sup>(6)</sup> thus suggesting that mechanisms other than classical risk factors play important role in causing increased CVD risk in these patients. In this context, RA itself, with its inflammatory component might contribute to increased cardiovascular risk.<sup>(1)</sup> In fact, CVD may be considered as an extra articular manifestation of RA.<sup>(1)</sup> It is understood

that RA and atherosclerosis share common pathophysiological mechanisms.<sup>(1)</sup> The inflammation in RA and its interaction with other atherogenic mediators might be implicated in the pathogenesis of atherosclerosis in RA patients.

Vascular endothelium is actively involved in the maintenance of vascular tone and permeability, hemostasis, angiogenesis, inflammatory response through release of various factors.<sup>(7)</sup> Altered function of endothelium, known as endothelial dysfunction (ED) is considered to be a critical, early and a reversible event in the development of atherosclerosis and occurs as a result of various factors,<sup>(7)</sup> including RA. Evaluation of risk factors for CVD in RA patients helps in their early recognition and management, thereby minimising the risk of CVD in these patients. In this background, the present study was taken up to evaluate traditional (lipid profile) as well as novel (inflammation, oxidative stress, homocysteine, uric acid) risk factors and also to evaluate nitric oxide levels as an indicator of endothelial dysfunction in patients with rheumatoid arthritis.

### Materials and Method

**Subjects:** The present study included forty six (46) consecutive patients attending Rheumatology Outpatient Department, Sri Venkateswara Institute of Medical Sciences, Tirupati, India and diagnosed with Rheumatoid arthritis as per the revised 1987 American

Rheumatology Association criteria.<sup>(8)</sup> Forty six (46) age and sex matched healthy subjects were recruited as controls. Patients with other forms of arthritis, active infection, history of smoking, alcoholism, diabetes, hypertension, patients on lipid lowering drugs, antioxidants, history of coronary artery disease, liver, kidney and thyroid diseases were excluded from the study. Informed consent was obtained from all the subjects and the study was approved by Institutional ethics committee.

**Sample collection:** Venous blood samples were collected into plain and EDTA tubes from all the subjects after an overnight fast. The samples were centrifuged and the separated serum and plasma were transferred into appropriately labelled aliquots and stored at -80°C until the biochemical analysis was carried out.

**Laboratory analysis:** Total cholesterol (TC) (Aspen Laboratories Pvt. Ltd., India), Triglycerides (TGL) (Futura system S.R.L., Italy) and High density lipoprotein (HDL) cholesterol (Beckman system pack, USA) were measured using enzymatic methods. High sensitivity C reactive protein (hsCRP) and fibrinogen as inflammatory markers were assayed by immunoturbidimetry using commercial kits (APTEC diagnostics nv, Belgium for hsCRP and Tulip Diagnostics (P) Ltd., India for fibrinogen). Malondialdehyde (MDA), as a measure of lipid peroxidation was estimated using thiobarbituric acid reactive substances (TBARS)<sup>(9)</sup> method and ferric reducing ability of plasma (FRAP) was measured as an indicator of total antioxidant status using the method described by Benzie and Strain.<sup>(10)</sup> Uric acid (UA) (Crest Bio systems, India) and homocysteine (Hcy) (Dialab, Austria) were analysed by enzymatic methods. Nitric oxide levels were determined by kinetic cadmium reduction method using Griess reagent.<sup>(11)</sup> All the analyses were performed on Beckman Synchron CX5

auto analyser (for TC, TGL, HDL cholesterol, hsCRP, Hcy and UA), Chemwell auto analyzer (for fibrinogen) and Perkin Elmer Lambda 1.2 UV-visual double beam spectrophotometer (for MDA, FRAP and NO).

**Statistical analysis:** Distribution of data was tested using Kolmogorov Smirnov test. Data were expressed as mean  $\pm$  standard deviation or median (inter quartile range) for normally distributed and skewed data respectively. Statistical comparison of data was done using independent samples T test or Mann Whitney U test as appropriate. A p value less than 0.05 was considered statistically significant. Statistical analysis was done using Medcalc statistical software version 12.2.2 and SPSS version 16.0.

## Results

Demographic characteristics and lipid profile of controls and RA patients were shown in Table 1. The study groups were age and sex matched. Compared to healthy controls, patients with rheumatoid arthritis had significantly higher levels of triglycerides ( $p=0.023$ ).

Table 2 shows the levels of inflammatory markers, oxidant and antioxidant levels, uric acid, homocysteine and nitric oxide levels in controls and RA patients. Both hsCRP and fibrinogen were significantly elevated in RA patients compared to controls ( $p<0.001$  and  $0.006$  for hsCRP and fibrinogen, respectively). The extent of lipid peroxidation as indicated by MDA levels was significantly increased, and FRAP levels significantly decreased in patients with rheumatoid arthritis than in control group ( $p<0.001$  for both MDA and FRAP). Serum nitric oxide levels were significantly increased in RA patients compared to controls ( $p=0.030$ ). However, uric acid and homocysteine levels were similar between RA patients and controls.

**Table 1: Demographic characteristics and Traditional Cardiovascular risk factors in controls and RA patients**

Parameter	Controls n = 46 Mean $\pm$ SD	RA patients n = 46 Mean $\pm$ SD	p Value
Age (years)	40.15 $\pm$ 11.59	42.33 $\pm$ 12.62	0.392
M / F	5 / 41	5 / 41	-
BMI (Kg/m <sup>2</sup> )	20.25 $\pm$ 1.28	20.70 $\pm$ 2.66	0.298
Total cholesterol (mmol/L)	4.77 $\pm$ 0.90	4.62 $\pm$ 1.06	0.472
Triglycerides (mmol/L)	1.22 $\pm$ 0.79	1.59 $\pm$ 0.75	0.023*
HDL cholesterol (mmol/L)	0.97 $\pm$ 0.15	1.04 $\pm$ 0.31	0.177

\*Statistically significant

RA=Rheumatoid arthritis; M/F=male/female ratio; BMI=Body mass index; HDL cholesterol=High Density Lipoprotein cholesterol

**Table 2: Inflammatory markers, Oxidant-antioxidant levels, Uric acid, Homocysteine and Nitric oxide in controls and RA patients**

Parameter	Controls n = 46 Mean ± SD	RA patients n = 46 Mean ± SD	p Value
†hsCRP (mg/L)	2.1 (0.8-5.9)	9.4 (2.1-19.5)	<0.001*
†Fibrinogen (µmol/L)	2.91 (1.94-7.92)	5.85 (3.83-9.85)	0.006*
MDA (µmol/L)	1.02 ± 0.18	4.92 ± 0.67	<0.001*
FRAP (mmol/L)	0.85 ± 0.21	0.64 ± 0.11	<0.001*
Uric Acid (µmol/L)	258.14 ± 64.83	241.49 ± 76.13	0.264
Homocysteine (µmol/L)	17.71 ± 6.43	15.11 ± 6.42	0.055
NO (µmol/L)	16.56 ± 9.26	20.93 ± 9.43	0.030*

\*Statistically significant, †Median (inter quartile range)

RA=Rheumatoid arthritis; hsCRP=high sensitivity C reactive protein; MDA=malondialdehyde; FRAP=ferric reducing ability of plasma; NO=nitric oxide

## Discussion

Cardiovascular disease represents a major cause of mortality in patients with rheumatoid arthritis. Studies have shown that the increased CVD related morbidity and mortality is not completely explained by traditional risk factors. The chronic systemic inflammation in general population and also in patients with rheumatoid arthritis can lead to increased cardiovascular events independent of the traditional cardiovascular risk factors.<sup>(1)</sup> Thus, the key factor explaining the increased CVD risk in RA patients appears to be inflammation, which contributes to the atherogenic process and also accentuates the established CVD risk factors.<sup>(12)</sup>

Consistent with inflammatory nature of the disease, patients with rheumatoid arthritis in the present study had significantly increased levels of inflammatory markers hsCRP and fibrinogen when compared to controls ( $p < 0.001$  and  $p = 0.006$  for hsCRP and fibrinogen, respectively). Similar findings were reported earlier.<sup>(13-15)</sup> CRP and fibrinogen are acute phase reactants whose levels increase in inflammation. CRP, belonging to pentraxin family is a sensitive marker of inflammation<sup>(16)</sup> and is synthesized by liver in response to stimulation by IL-6, which in turn is released as result of pro inflammatory response.<sup>(17)</sup> hsCRP testing is used to detect even low grade of inflammation.<sup>(18)</sup> Fibrinogen, which is a clotting factor is also synthesized by liver in response to IL-6<sup>(19)</sup> and is used as an indicator of inflammation. CRP levels are known to be independent predictors of cardiovascular risk in general population as well as in rheumatoid arthritis patients.<sup>(16)</sup> Similarly, increased fibrinogen levels have also been shown to be associated with cardiovascular disease.<sup>(20)</sup>

Dyslipidemia is an established risk factor for cardiovascular disease and atherogenic lipid profile has been reported in patients with rheumatoid arthritis.<sup>(21)</sup> In the present study, among the lipid parameters studied, triglyceride levels were found to be significantly increased in patients with rheumatoid arthritis, compared to controls ( $p = 0.023$ ), whereas total cholesterol and HDL cholesterol levels showed no significant difference

between RA patients and controls ( $p = 0.472$  and  $0.177$  for TC and HDL-cholesterol, respectively). Earlier studies have reported similar findings.<sup>(14,22)</sup> Studies evaluating lipid profile in RA patients reported varied findings that can be attributed to the differences in study population, disease activity<sup>(5)</sup> or an effect of treatment. The inflammatory cytokines released from synovial tissue enter systemic circulation and exert multiple effects, including increased triglyceride synthesis by the liver,<sup>(23)</sup> which can cause elevated triglyceride levels as observed in RA patients in the present study.

Oxidative stress, which occurs as a result of imbalance between pro oxidants and antioxidants is one of the important causative factors of atherosclerosis.<sup>(24)</sup> Several studies were conducted to evaluate the oxidant/antioxidant status in rheumatoid patients. In the present study, RA patients showed significantly elevated levels of MDA when compared to controls ( $p < 0.001$ ). These findings are in accordance with earlier studies which have reported increased MDA levels in plasma<sup>(25)</sup> or erythrocytes<sup>(26)</sup> of RA patients. Synovial inflammation in RA results in the migration of phagocytes and other leukocytes which generate reactive oxygen species (ROS) during phagocytosis.<sup>(27)</sup> These ROS cause toxic damage to cell membrane lipids, resulting in lipid peroxidation. Malondialdehyde is a product of lipid peroxidation and hence, increased MDA levels in RA patients in the present study could be a result of free radical induced tissue damage. The harmful effects of free radicals are effectively countered by body's antioxidant defense mechanism. In the present study, FRAP levels, measured as an index of total antioxidant capacity were significantly decreased in RA patients, compared to controls ( $p < 0.001$ ). Similarly, decreased antioxidants in RA patients have been reported earlier.<sup>(25,27)</sup> In inflammatory conditions such as RA, the ROS generated as a result of the inflammatory process may deplete antioxidants, leading to their decreased levels; however other causes such as decreased intake, decreased absorption or transport of antioxidant micro nutrients may also be responsible.<sup>(28)</sup>

Thus, the finding of increased MDA and decreased FRAP point towards the presence of oxidative stress in RA patients in the present study.

Uric acid, a metabolic product of purine metabolism is yet another novel cardiovascular risk factor and hyperuricemia has been shown to be associated with CVD in RA patients.<sup>(29)</sup> In the present study, serum uric acid levels in RA patients were similar to those of controls ( $p=0.264$ ). Similar findings were observed earlier.<sup>30</sup> Rheumatoid arthritis is not traditionally associated with hyperuricemia and the existence of gout in RA although reported, occurs at a lower rate compared to general population.<sup>(31)</sup>

Homocysteine is an intermediate in methionine metabolism and elevated homocysteine levels were found to be important predisposing factors for atherosclerosis.<sup>(32)</sup> In the present study, serum Hcy levels were measured in RA patients and compared to controls. The levels were lower in RA patients than controls, but the difference was not statistically significant ( $p=0.055$ ). Earlier studies reported similar findings in RA patients.<sup>(13,28)</sup> Rheumatoid arthritis patients in the present study who were undergoing treatment were receiving methotrexate which can result in increased homocysteine levels through inhibition of homocysteine-methionine pathway. However, all the patients receiving methotrexate in the present study were also supplemented with folic acid that can prevent increase in homocysteine levels. Van Ede AE et al.,<sup>(33)</sup> showed that folic acid supplementation in methotrexate treated RA patients resulted in significant decrease in homocysteine levels from their baseline values. The low Hcy levels observed in RA patients in the present study could be an effect of folic acid supplementation. However, clinical trials have shown that decreased homocysteine levels with Vitamin B or folic acid supplementation did not lower the risk of CVD in general population.<sup>(34,35)</sup>

Endothelial dysfunction is the earliest detectable sign of atherosclerosis and hence is considered as a good predictor of CVD risk.<sup>(36)</sup> ED occurs due to decreased biosynthesis or a reduced bioavailability of nitric oxide,<sup>(36)</sup> which is an important endothelial vasodilator. RA patients in the present study had significantly elevated NO levels than controls ( $p=0.030$ ). Similar findings were reported earlier.<sup>(37,38)</sup> Inflamed synovium may act as a source of increased NO levels.<sup>(37)</sup> The endogenous production of NO is increased due to the activation of inducible form of nitric oxide synthase (iNOS).<sup>(39)</sup> Accordingly, increased activity of iNOS has been reported in circulating monocytes<sup>(40)</sup> and ex-vivo cultures of inflamed synovium and cartilage,<sup>(41)</sup> thus suggesting that NO production is up regulated by cytokines in inflamed joints.

Findings of the present study show that patients with RA are at an increased risk of CVD as evidenced by hypertriglyceridemia, increased hsCRP and fibrinogen levels. Although NO levels were increased, the

concomitant oxidative stress present, as indicated by elevated MDA and decreased FRAP levels make NO biologically unavailable since the OS converts NO to peroxy nitrites, ultimately leading to ED and subsequent atherosclerosis. Thus, the atherogenic milieu that is present in these patients predisposes them to enhanced CVD risk. The inflammatory cytokines through alteration of function of various tissues are responsible for generation of a spectrum of proatherogenic changes that in turn promote atherogenesis in these patients.<sup>(23)</sup> These findings point towards the need for evaluating RA patients for CVD risk besides management of joint symptoms.

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