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Assessment of Serum Paraoxonase and Its Relation with Endothelial Dysfunction in Rheumatoid Arthritis Patients

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Abstract **Original Research Article**

Background: Free radicals mediated various sorts of destructive events along with systemic inflammation overwhelm protective action of antioxidants and responsible for disease development including rheumatoid arthritis (RA). Howe only few studies have been documented to enlighten the biochemical mechanism involved in pathophysiology of RA al with endothelial dysfunction and altered activity of serum paraoxonase. ***Aim:*** The present study was carried out to as serum Paraoxonase (PON), plasma Nitric oxide (NO), marker of lipid peroxidation and systemic inflammation in the bl samples of RA patients and to determine their relation in the development of CVD risk. ***Material & method*:** Serum P oxidative stress markers (malondialdehyde , MDA), NO and CRP levels were estimated in 30 RA subjects (35-50 years using standard methods and statistically compared with 30 age matched healthy controls. ***Result:*** Marked depletio plasma NO level and serum paraoxonase activity ($p<0.05$) were observed in RA subjects as compared to healthy cont whereas serum CRP and malondialdehyde levels (MDA) were increased significantly ($p<0.001$) in RA subjects. In addit serum PON activity was directly correlated with endothelial dysfunction, and inversely related to lipid peroxidation systemic inflammation. ***Conclusion:*** Thus, assessment of serum paraoxonase along with NO in RA patients plays a cru role for early interpretation of future cardiovascular complications. Therefore, treatment of RA should include not adoption of antioxidant rich diet along with anti-inflammatory drugs but also monitoring of cardiac markers on regular b for early prediction and to reduce the burden of CVD risk in RA patients.

Key words: Nitric oxide, lipid peroxidation, free radicals, inflammation, vasodilation.

INTRODUCTION

Rheumatoid arthritis (RA) is a cumulative effect of enhanced free radicals production mediated biomolecular deterioration and systemic inflammation. This lethal convergence initiates a cascade of events leading to altered cellular homeostasis followed by disease process [1]. It has been documented in several studies that RA patients are susceptible to develop future cardiovascular complications [2]. Moreover, it has been predicted that by the year 2020 there would be an almost 75% increase in the global cardiovascular disease burden [3].

Increased production of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$) and its metabolites along with reduced bioavailability of antioxidant defenses leads to the development of oxidative stress (OS) [4]. Moreover, ROS may act through several mechanisms to mediate disease process, which include major interrelated derangements of cell metabolism such as damage to endothelium, cartilage, membrane ion transporters, DNA strand breakage, other specific proteins and oxidative modification of lipoproteins [5].

Amongst various culprit events, ROS mediated lipid peroxidation has been implicated in the development of RA and its related cardiovascular complications. The prime targets of peroxidation by ROS are the polyunsaturated fatty acids (PUFA) in the membrane lipids. As a result, various sorts of end products are produced, including reactive aldehydes (malondialdehyde) and lipid hyroperoxides. The levels of malondialdehyde (MDA) indicate the extent of lipid peroxidation in general and serve as markers of oxidative damages due to free radicals leading to cellular destruction and disease process [6].

Free radicals production is efficiently controlled by antioxidant defense system which includes antioxidant enzymes and non-enzymic antioxidants. In this context, assessment of Paraoxonase (PON), a HDL-associated enzyme carried on apo A-I that protects lipoproteins against oxidative modification, has received much attention. In addition, PON is a glycoprotein, synthesized mainly in the liver and hydrolyzes organophosphates like pesticides, neurotoxins, and arylesters [7]. Previous studies have shown that PON level alters in various age related complications such as cardiovascular diseases, musculoskeletal and neurological disorders [8, 9]. However, alteration in PON activity in RA patients and in determining future risk of CVD complications is still in obscure, and has received much attention in order to explore hidden facts related to commencement of secondary complications in RA.

Apart from oxidative stress, systemic inflammation has been implicated in the pathogenesis of various age related complications including RA and CVD [10]. C-reactive protein (CRP), a marker of systemic inflammation and synthesized in liver, has been received considering attention in inflammatory disorders such as CVD, osteoarthritis and cancer etc [10-12]. It is well documented in previous studies that there is an association between RA pathophysiology and systemic inflammation as measured by plasma C-reactive protein [13]. In addition, emerging concepts reveal its relation with markers of oxidative stress, vascular injury and endothelium dysfunction, and attract the researchers to clarify its role in RA related secondary complications[13-15].

Moreover, assessment of Nitric oxide (NO), a marker of endothelial dysfunction, has clinical relevance due to its versatile role in regulating both intracellular and extracellular signaling mechanisms and in maintaining the cellular homeostasis. NO takes part in blood pressure control, inhibits mast cells degranulation, possess antioxidant and anti-aggregant properties, and regulates vascular tone [16]. Augmented oxidative stress leads to alteration in the levels of NO which in-turn exerts culprit effect in inducing hypertension and other pathophysiological complications in inflammatory disorders [5, 6]. Therefore, considering the role of aforesaid parameters in the pathophysiology of RA, the objectives of present study was to determine the relation of serum paraoxonase with endothelium dysfunction along with markers of lipid peroxidation and systemic inflammation in RA patients and to determine their role in focusing early prediction of cardiovascular complication in RA patients.

MATERIAL AND METHODS

In the present study, 30 patients of both the sex with rheumatoid arthritis (belonged to age group 35-50 years) and 30 age matched healthy individuals, served as control, were taken. In each group, 15 male and 15 female (1:1 ratio) were included. These subjects were selected randomly from Delhi-NCR region, after taking their informed consent and approval of protocol by ethics committee of college. Objective oriented information including demographic information, family history of CVD, RA or both and limited physical examination i.e. blood pressure measurement was completed from all the subjects. Height and weight were measured with subject barefoot and light dressed. The body mass index (B.M.I.) was calculated as [B.M.I. = *weight (Kg) / Height (metre*^{2})].

Inclusion criteria

Criteria recommended by the American Rheumatism Association were used for the diagnosis and recruitment of RA.[17] Subjects who gave informed consent for study, having positive rheumatoid factor, and not under any medical treatment including supplementation of antioxidants or non-steroidal anti–inflammatory drugs were included.

Exclusion criteria

Patients with concomitant diseases such as diabetes mellitus, hypertension, renal insufficiency, hepatic disease or under any medicinal treatments were excluded. Pregnant and lactating women, obese (B.M.I > 25), hypertensive (B.P. >120/80 mmHg), smokers and subjects who did not follow study instructions were excluded from the study.

Taking aseptic precautions, blood samples (approximately 6 ml) were collected in sterile plain vecutainer (4 ml) and EDTA vecutainer (2 ml) by venous arm puncture after overnight fasting for serum and plasma preparation. Plasma nitric oxide, serum CRP levels, markers of oxidative stress i.e. erythrocyte lipid peroxidation and paraoxonase activity were estimated in controls as well as in RA subjects. Serum C-reactive protein (CRP) level was measured by using commercially available ELISA kits (R&D Systems, USA). Serum paraoxonase activity was estimated by Gan et al method using p-nitrophenyl acetate (5.5 mM/L) as a substrate [18]. Serum malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, by the method of Satoh *et al*. [19].

The measurement of plasma NO is difficult because this radical is poorly soluble in water and has a short half-life in tissue (10-60 s), but its half-life may be as long as 4 minutes in the presence of oxygen. For these reasons, the end products of the phenomenon, nitrate and nitrite, are preferentially used in clinical biochemistry. Plasma total nitrate and nitrite levels were measured with the use of Griess reagent as described earlier [20].

STATISTICAL ANALYSIS

The data obtained form study group subjects were expressed as Mean ± SD. The significance of

mean difference between groups was compared by using Student's t-test and relationship between aforesaid parameters was analyzed by linear regression analysis and Pearson correlation test.

RESULT

Demographic profile of the study group subjects was depicted in Table 1. BMI measurement revealed insignificant increase ($p<0.1$) in RA patients as compared age matched healthy controls. In addition, RA patients had significant variation in blood pressure ($p<0.05$) with respect to healthy controls indicating that RA patients were more susceptible to develop future CVD risk.

Marked alteration in serum PON activity and in the levels of markers of oxidative stress (MDA), systemic inflammation (CRP) and endothelial dysfunction (NO) were observed in the study group subjects, as represented in Table 2. Serum paraoxonase activity and plasma NO levels were found to be significantly low ($p<0.05$) in patient group as compared to controls. Conversely, serum MDA levels were 58.5% high ($p<0.001$) in RA patient as compared to healthy controls. Similarly, serum CRP levels, as a marker of systemic inflammation, were 46.06% high ($p<0.001$) in RA patient as compared to healthy controls.

Moreover, negative correlation of serum PON activity with serum CRP and MDA levels was observed in RA patients whereas marker of endothelial dysfunction (NO levels) was positively correlated with PON activity, as shown in Table 3. These results clarify the role of reduced PON activity along with endothelial dysfunction in enhancing the CVD risk in RA patients most probably due to rise in oxidative stress and systemic inflammation with disease process.

Table-1: Demographic profile of Rheumatoid arthritis patient and Control groups (Mean ± SD)

S.No.	Parameter	Control Group (n=30)	Patient group (n=30)
1	Age (years)	42.2 ± 4.8	45.6 ± 3.4 *
2	M:F ratio	1:1	1:1
3	Height (meter)	1.58 ± 0.025	1.57 ± 0.03
4	Weight (Kg)	57.5 ± 1.6	62.2 ± 2.3
5	BMI (Kg/m^2)	22.7 ± 1.1	24.6 ± 1.3 *
6	Systolic blood pressure (mm Hg)	107.6 ± 3.40	112.35 ± 4.25*
7	Diastolic blood pressure (mm Hg)	74.5 ± 2.4	77.8 ± 2.18*

Where,
* p < 0.1 : Non-significant;
** p < 0.05 : Significant,
BMI: Body mass index

Table-2: Serum PON activity and markers of oxidative stress, systemic inflammation and endothelial dysfunction in Rheumatoid arthritis patients and control group. (Mean ± SD)

S.No.	Particulars	Control group (n=30)	Patient Group (n=30)	% increase	% decrease
1)	NO (µ mol/L)	8.36 ± 1.58	5.75 ± 1.87**		31.20%
2)	Paraoxonase (U/ml)	234.4 ± 9.4	157.25 ± 8.27**		32.91%
3)	Malondialdehyde (µmolMDA/ml)	2.68 ± 0.16	4.25 ± 0.21***	58.5%	
4)	CRP (mg/L)	3.30 ± 0.14	4.82 ± 0.20***	46.06%	

Where, * p< 0.1: Non-significant; ** p<0.05: Significant; *** p<0.001 : Highly significant

Table-3: Correlation coefficient between serum PON activity and other variables in RA patients

Particulars	NO	MDA	CRP
PON activity	0.654*	- 0.552*	-0.458*

Where, * p<0.05: Significant

DISCUSSION

Reactive oxygen species have been implicated in a number of chronic diseases such as rheumatoid arthritis, osteoarthritis etc [21]. Emerging concepts reveal the relation of RA pathology with circulating markers of oxidative stress, inflammation, and endothelium dysfunction, and receives much attention among researchers. It has been well documented that in RA patients, neutrophils migrate into the synovium and release large amount of reactive oxygen species eg. Superoxide radical ($O_2^{\cdot}$), hydrogen peroxide and highly reactive hydroxyl radical ($OH^{\cdot}$). These ROS attack and

damage the hyaluronic acid and cartilage of synovial fluid, and at confluence, contributing to the destruction of joints [22].

In the present study, serum malondialdehyde levels (marker of lipid peroxidation) were also found to be significantly high in RA subjects ($p<0.001$, Table 2) in association with significantly altered NO levels which indicate that excessive ROS generation takes place in RA patients which leads to not only lipid peroxidation but also associated with oxidative stress mediated endothelial dysfunction due to inhibition of NO and thereby enhances their susceptibility to develop hypertension. NO also inhibits both proliferation of smooth muscle cells and adhesion of leukocytes and platelets i.e. a key step in the prevention of atherosclerotic plaque formation. Depletion of NO makes the RA patients susceptible for later stages of atherosclerosis also. Interestingly, in previous studies on rheumatoid arthritis and osteoarthritis patients, increased levels of MDA were also reported [22, 23]. In addition, oxidative stress mediated lipid peroxidation initiates a complex cascade such as enhancement of cytosolic free calcium, and leakage of lysosomal hydrolases via breakdown of lysosomal membrane which cause dystrophic changes in cardiac muscle fibers leading to weakness of cardiac muscles and difficulty in performing normal functioning of heart. Furthermore, lipid peroxidation mediated electrolyte imbalance and production of protein radical in lipid membranes affects the normal ion transport, and thereby enhances hypertension risk followed by CVD in RA patients [24, 25].

In order to combat with oxidant mediated injury, various sorts of antioxidant enzymes are present in the body. Among them, serum PON contributes to anti-atherogenic and antioxidant activity by regulating oxidation of LDL, by hydrolyzing specific oxidized phospholipids, cholesterol linoleate hydroperoxides, and by neutralizing hydrogen peroxide[7,8,26,27]. Alteration in PON activity may have significant effect in inducing hypertension with advancing of disease. In the present study, serum PON activity was found to be decreased significantly in RA patients which reflects toward its utilization in preventing ROS mediated lipid peroxidation and its inactivation due to interaction of oxidized lipids with the PON free sulphydryl group. Similar findings have been documented by Gupta *et al*. in elderly knee osteoarthritis patients and implicated the role of reduced PON activity with the future CVD risk [28].

Amusingly, oxidative stress and inflammation fed each other and together contribute in inflammatory disease process. ROS, RNS and their intermediates serve as mediators of inflammation by reducing antioxidant reserves in synovial fluid and activating proteolytic enzymes to degrade cartilage [29]. C-reactive protein (CRP) is a phylogenetically highly conserved plasma protein that participates in the systemic response to inflammation. It is an excellent biomarker for acute-phase response and has emerged as an important, predictor of future cardiovascular disease [30]. Serum CRP levels were found to be increased significantly in RA patients which reflects toward the role of excessive ROS production mediated systemic inflammation and its related CVD risk development. Correlation studies also reveal that CRP levels were negatively associated with PON activity which indicates that oxidative stress and inflammation co-exist together in RA and its related secondary complications. Consistent findings have been observed in elderly arthritis patients with respect to elevated levels of CRP and its relation with inflammatory disease development [23].

CONCLUSION

On the basis of findings of present study and consistent findings of previous studies, it is concluded that oxidative stress in combination with systemic inflammation plays a crucial role in the pathogenesis of RA. Serum PON activity is directly associated with endothelial dysfunction and inversely associated with systemic inflammation and lipid peroxidation in RA patients. Therefore, assessment of PON activity along with NO and HDL-cholesterol is an important diagnostic marker to predict CVD risk. In addition, adoption of antioxidant rich diet, incorporation of daily normal exercise and life style modification can prevent oxidative stress and inflammation mediated RA and its related CVD complications. Furthermore, identification of therapeutic molecular markers at gene expression level is required not only to target oxidative stress and inflammation but also to reduce the prevalence of RA and CVD complication as well.

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