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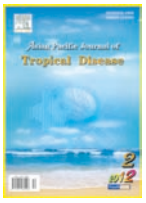

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Antioxidant gap and lipid peroxidation in patients with rheumatoid arthritis: Relationship to disease manifestations and activity

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ABSTRACT

Objective: To explore relationships between total antioxidant gap and lipid peroxidation with respect to the disease severity of Rheumatoid arthritis (RA), which may have further implications in understanding rheumatoid pathology and therapeutic management of the disease. **Method:** The present of study was designed to investigate the relationship between antioxidant gap and certain antioxidant parameters with disease activity in rheumatoid arthritis patients. A significantly increased lipid peroxidation, measured as malondialdehyde (MDA), was demonstrated in the plasma of rheumatoid arthritis patients. **Results:** MDA was observed in RA patients (0.98±0.34 μmol/L) than those found in controls (0.98±0.34 μmol/L). The antioxidant gap was significantly decreased. total antioxidant gap (0.34±0.14 mmol/L) and total antioxidant capacity (1.34 ±0.16 mmol/L) were significantly lower in RA patients as compared to healthy controls (0.76±0.33 and 1.78±0.35 mmol/Lrespectivel). **Discussion:** The excessive production of ROS disturbs redox status including antioxidant gap and can exacerbating inflammation and affecting tissue damage in RA, as exemplified by their strong association with disease activity.

1. Introduction

Citrullinated synovial antigens and the antibodies produced against them play a important role in the path physiology of RA[1]. Anti-CCP antibody positivity seems to be associated with increased synovial fluid oxidant activity (increased MDA and MPO levels) in patients with RA[2]. Excessive free radical production rather than impaired antioxidant enzymes activity due to autoantibody inhibition is been found in RA[3]. Antioxidant defenses protect the body from the detrimental effects of free radicals[4].

Blood contains many antioxidant molecules those prevent and/or inhibit harmful free radical reactions[5]. Albumin, the most abundant circulating protein in the plasma, exerts important antioxidant activities[6]. ROS, may cause a damage where they modify the antioxidant properties of albumin[7]. Uric acid formation may even provide a significant antioxidant defense mechanism against nitration by peroxynitrite during hypoxia[8]. The serum "antioxidant gap" reflects the antioxidant activity of ascorbate, alpha tocopherol, carotene, bilirubin and radical scavenging antioxidants other than albumin and uric acid[9]. There has been well documented the role of oxidative stress including redox state in autoimmune disease like RA[10-12]. However, relationship of antioxidant gap and total antioxidant capacity which present total antioxidant in the plasma has still to be illuminated. Further, There are no studies depicting the correlation of antioxidant gap and lipid peroxidation which is consider as a vital oxidant involved in the tissue damage in rheumatoid arthritis. Thus, the aim of this study was to explore relationships between total antioxidant gap and lipid peroxidation with respect to the disease severity of RA, which may have further implications in understanding rheumatoid pathology and therapeutic management of the disease.

2. Materials and methods

2.1. Cases and controls

Subjects for the study were selected from rheumatoid

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patients in Orthopaedic Unit of K.L.E's Dr Prabhakar Kore Hospital and Medical Research Centre, Belgaum, India. The study included 25 subjects with RA (19 females and 6 males) with mean age of 26.5±7.48 years, and the control group consisted of 25 healthy volunteers (19 females, 6 males) with mean age of 26.73±5.37 years. The subjects were diagnosed as RA according to the 1987 revised criteria of the American College of Rheumatology (Arnett *et al*., 1988).[13] Full history-taking, clinical examination and laboratory investigations were performed for calculating disease activity (DAS28 score) according to Prevoo *et al*.[14] . The study protocol was approved by the Institute Ethics Committee, Jawaharlal Nehru Medical College, Belgaum, India and informed consent was obtained from all the cases and healthy subjects. All the cases enrolled in the present study were non-smokers and non-alcoholics, not associated with any other autoimmune disease and were not undergoing any immunosuppressant drugs. However, cases that had been receiving ordinary dosages of nonsteroidal anti-inflammatory drugs (NSAID) and methotrexate were not excluded as almost all patients with RA had been undergoing this treatment.

2.2. Method

2.2.1. Collection of blood sample

5 mL of Blood will be collected from the patients and controls under aseptic precautionary measures using disposable syringe in plain tubes. Serum will be separated by centrifugation and kept at 4 ℃ and analyzed within 24 h. Samples were used for the preparation of plasma for the estimation of lipid peroxidation marker (MDA), antioxidant status and total antioxidant gap. Plasma albumin and Uric acid levels were determined by a colorimetric method with commercial kits Method of assay-The tests were done by Uric acid-modified Trinder Peroxidase method[15]. Albumin-BCG dye binding method[16]. Measurements were done using a semi automated analyzer.

2.2.2. Determination of MDA

The quantitative measurement of lipid peroxidation (LPO) was a measure of total malondialdehyde (MDA) in the plasma by Thiobarbiturac acid (TBA) method[17]. The amount of malondialdehyde (MDA) formed was measured by the reaction with thiobarbituric acid at 532 nm. The results were expressed as μmol/L using molar extinction coefficient of MDA-thiobarbituric chromophore ($1.56\times10^5\ M^{-1}\ cm^{-1}$).

2.3. Anti-oxidant system

2.3.1. Determination of Total Anti-oxidant capacity

The assay measured the capacity of the biological fluids to inhibit the production of thiobarbituric acid reactive substances (TBARS) from sodium benzoate under the influence of the free oxygen radicals derived from Fenton's reaction. A solution of 1 mmol/L uric acid was used as standard[18].

2.3.2. Determination of total anti oxidant gap

The plasma levels of total antioxidant gap were estimated by the method of Miller *et al*[9]. The antioxidant gap was calculated using the following equation: antioxidant gap = total antioxidant capacity-(albumin x TEAC) + (uric acid x TEAC). This indicator consists of evaluating the antioxidant activity of in plasma of ascorbic acid, α-tocopherol, bilirubin, transferrin and other minority antioxidant compounds, excluding albumin, uric acid and Trolox equivalent.

3. Result

The study included 25 subjects with RA (19 females, 6 males) with mean age of 48.2±10.45 years and the control group of 25 healthy volunteers (19 females, 6 males) with mean age of 48.1±9.04 years. The demographic and clinical characteristics of RA patients and healthy controls are summarized in the Tables 1. The disease activity of RA patients was calculated as DAS28 score according to the method of Prevoo *et al*[1,14].

Table 1
Demographic and clinical characteristics of patients with rheumatoid arthritis and controls.

	RA patients	Controls
Number (n)	25	25
Female/male	19/6	19/6
Age (yrs)	48.2±10.45	48.1±9.04
Duration of disease	3.58±1.50	NA
Allbumin (g/dL)	4.1±0.78	4.4±0.4
Uric acid (mg/dL)	5.79±1.80	5.4±1.02
DAS-28	5.2 ±1.20	NA
LPO (μmol/L)	0.98±0.34	0.60±0.29
Total antioxidant Gap (mmol/L)	0.34±0.14	0.76±0.33
Total antioxidant capacity (mmol/L)	1.34±0.16	1.78±0.35

Values are expressed as Mean±SD, NA: not applicable

MDA levels were studied in patients with RA and healthy controls. A significant ($P<0.01$) increase in the level of lipid peroxidation measured as MDA was observed in RA patients (0.98±0.34 μmol/L) than those found in controls (0.98±0.34 μmol/L). Antioxidant gap and total antioxidant capacity.

The plasma antioxidant gap reflects the total antioxidant activity of ascorbate, alpha-tocopherol, beta-carotene and other radical scavenging antioxidants. The levels of antioxidant gap and total antioxidant capacity were shown in Table 1. In plasma, levels of total antioxidant gap (0.34±0.14 mmol/L) and total antioxidant capacity (1.34±0.16 mmol/L) were significantly lower in RA patients as compared to healthy controls (0.76± 0.33 and 1.78±0.35 mmol/L respectively).

3.1. Correlation studies

To evaluate together the relationship of antioxidant gap and antioxidant status in the severity of RA, correlations among these parameters were studied. Further, to appraise together the role of antioxidant gap and antioxidant system in the severity of RA, correlations of these parameters were studied with disease activity of RA (Figures 1 and 2).

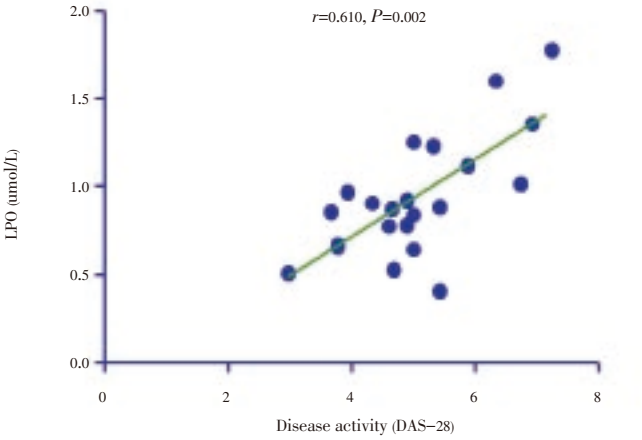

Figure 1. Correlation between LPO and disease activity of RA calculated as DAS28 score.

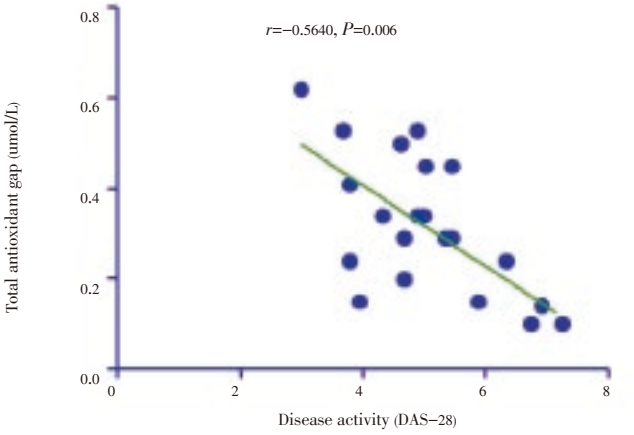

Figure 2. Correlation between antioxidant gap and disease activity of RA calculated as DAS28 score.

4. Discussion

High levels of synovial oxidative stress and mitochondrial mutation burden are strongly associated with low in vivo oxygen tension and synovial inflammation[19].

In the current study serum MDA was found in significantly high levels in RA patients than in controls. Many authors suggested that increased ROS levels in RA may result in a pro-oxidation environment, which in turn could result in increased MDA levels. As a result, LPO may have a role in the pathogenesis of the RA.

It is possible that differences between our results and other investigators results, regarding antioxidant status, is due to differences in the stage of the disease. Chronic joint disease may deplete antioxidant defenses whereas acute inflammation can upgrade them.

We demonstrated significant positive correlation between clinical and laboratory parameters of activity in RA (DAS-28, antioxidant gap and antioxidant status). MDA showed direct correlation with DAS-28, ESR and CRP in RA patients. Our findings agree with Sarban *et al*[20]. and Seven *et al*[21]. WHO demonstrated a significant correlation between oxidative stress and MDA levels in patients with RA, and claimed it would be useful in predicting disease activity. This correlation between MDA and antioxidants versus parameters of activity in RA makes it possible to use them as a surrogate measure of disease activity. We demonstrated a significant positive correlation between serum MDA and erythrocyte GST activity and plasma Cp concentration, another direct correlation was detected between SOD and CAT. This correlation clarifies that oxidative stress leads to increased antioxidant enzyme activities to restore the oxidant/antioxidant system balance which is shifted in favor of LPO which could lead to the tissue damage observed in RA, as confirmed by Jaswal *et al*[22].

In conclusion, the light of previous findings, it is possible to conclude that increased oxidative stress in RA patients evidenced by increased serum MDA, have led to compensatory changes in total antioxidant capacity. These findings confirm the role of oxidative stress in the pathogenesis of RA and those LPO markers such MDA and antioxidants can serve as surrogate markers for disease activity in RA.

Conflict of interest statement

We declare that we have no conflict of interest.

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