

LEVELS OF PLASMA LIPID PEROXIDE PRODUCTS AND ANTIOXIDANT STATUS IN RHEUMATOID ARTHRITIS

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Abstract. Oxygen free radicals have been implicated as mediators of tissue damage in patients with rheumatoid arthritis. The aim of our study was to assess the lipid peroxide products and antioxidant status in rheumatoid arthritis patients (RA). The study involved determination of two plasma lipid peroxide products, malondialdehyde (MDA) and conjugated dienes (CD), two plasma antioxidant vitamins (C and E) in 91 RA patients and 26 healthy subjects. The results showed that rheumatoid patients had increased plasma CD but not MDA and decreased plasma vitamin E, when properly expressed per unit cholesterol and triglyceride. This finding suggested that RA patients had increased oxidant stress that might play a role in the tissue damage and inflammation process of this disease.

INTRODUCTION

Rheumatoid arthritis (RA) is characterized by a chronic hypertrophic synovitis leading to destruction of connective tissues and functional damage of cartilage and bony structure. Reactive oxygen species (ROS) play an important role in tissue injury in this disease (Blake *et al*, 1989). Several mechanisms exist whereby oxygen radicals might be generated within the joint in RA. These include the release of such species from activated synovial macrophages and polymorphs (Babior, 1978; Halliwell, 1982), the prostaglandin pathway (Egan *et al*, 1976) and xanthine oxidase mediated synovial ischemic reperfusion injury. The prime targets of ROS attack are the polyunsaturated fatty acids (PUFA) in the membrane lipids causing lipid peroxidation which may lead to disorganization of cell structure and function (Floyd, 1990). Hence, lipid peroxides (LPO) produced from PUFA in the presence of free radicals may reflect the level of free radicals in the body.

To circumvent the damage caused by the ROS, multiple defense systems collectively called "antioxidants" are present in human serum, as well as in erythrocytes.

Patients with rheumatoid arthritis have been reported to have elevated levels of lipid peroxides, which include malondialdehyde in the serum and synovial fluid (Lunec *et al*, 1981; Ozgunes *et al*, 1995; Gambhir *et al*, 1997), and lower levels of antioxidant vitamins A and E in serum in some, but not all studies (Hankanen *et al*, 1989; Bendich and Cohen, 1996). Overwhelming production of free radicals or insufficient antioxidant may play a signifi-

cant role in the tissue-damaging and inflammation perpetuating process in RA (Merry *et al*, 1989). Study of the formation of LPO products and antioxidant status in patients suffering from RA would be an initial step towards elaboration of the oxidant-antioxidant equilibrium among these patients, and for subsequent evaluation of LPO products in relation to the clinical course of the disease with implications for treatment with antioxidants.

MATERIALS AND METHODS

Ninety-one patients, all females aged 21-83 years, were followed at the outpatient Rheumatology clinic of Ramathibodi Hospital. All cases met the revised criteria for rheumatoid arthritis of the American College of Rheumatology. Duration of illness was between 10 months to 33 years. Six of them had inflamed and swollen joints on the day of taking blood. Seropositivity for antinuclear antibodies (titer \geq 1:16) and anti-double stranded-DNA antibodies was found in 45 patients and 1 case respectively. Twenty-six healthy subjects, all females aged 18-68 years, served as controls. Blood samples were drawn from the patients and control subjects with their informed consent.

Hemoglobin (Hb), hematocrit (Hct), white blood cell counts (WBC) and erythrocyte sedimentation rate (ESR) were performed in all patients, except that ESR data were not obtained from 7 patients.

The plasma from ethylene diamine tetraacetic acid anticoagulated blood was separated, immediately stored at -80°C and used for determination of

malondialdehyde (MDA), conjugated dienes (CD), vitamin E (Vit E), vitamin C (Vit C), cholesterol and triglyceride. MDA was determined by its reaction with thiobarbituric acid (TBA) to form a red MDA-TBA complex (Das *et al*, 1990) which was quantitated by fluorometry (Richard *et al*, 1991). CD was measured as relative absorbance at 233 nm wavelength (Beuge and Aust, 1978; Hunter and Mohamed, 1986). Plasma vitamins E and C were determined by a fluorometric method (Taylor *et al*, 1976) and a spectrophotometric method (Omaye *et al*, 1979) respectively while plasma cholesterol and triglyceride were quantitated with a commercial enzymatic kit.

Statistical analysis

All data were expressed as mean±SD. Unpaired student's *t*-test was used for between-group comparisons. Differences were considered of statistical significance when the *p*-value was less than 0.05.

RESULTS

The age, biochemical and hematological data of controls and RA patients are shown in Table 1. The mean ages of RA patients were higher than the controls (RA = 48.8±12.8 years *vs* controls =

40.8±14.5 years). The patient group had significantly lower values of plasma cholesterol, hemoglobin and hematocrit than the control subjects. The WBC counts and ESR values were presented only in RA patients and their ranges were 2.59-13.4 and 1-130 respectively.

Table 2 demonstrates that the RA patients tended to have increased plasma MDA levels compared with controls but it did not reach the statistical significance. The concentrations of CD were markedly increased in the patients. Plasma vitamin E values in RA patients had a tendency to be decreased but they were significantly lower when vitamin E was expressed per millimole of plasma triglyceride plus plasma cholesterol. The concentrations of plasma vitamin C did not differ significantly between the two groups.

DISCUSSION

From all rheumatoid arthritis patients, 33/91 (33%) had low levels of Hb (Hb ≤ 11 g%), 41/91 (45%) had low levels of Hct (Hct ≤ 36%), 9/91 (10%) had low WBC counts (WBC ≤ 4,500 cells/μl) and 79 of 84 patients (94%) had high ESR (ESR > 20).

Table 1
The age, biochemical and hematological data of controls and patients.

Parameter	Control (n=26)	RA patient (n = 91)
Age (years)	40.8±14.5 (18 - 68)	48.8±12.8 ^a (21 - 83)
Cholesterol (mg/dl)	213.4±32.6 (158 - 290)	192.5±39.5 ^b (117 - 304)
Triglyceride (mg/dl)	102.2±47.8 (45 - 196)	128.4±81.9 (37 - 495)
Uric acid (mg/dl)	-	5.84±2.34 (1.3 - 15.5)
Hemoglobin (g/dl)	12.92±1.21 (10.5 - 15.2)	11.53±1.48 ^c (6.9 - 15.6)
Hematocrit (%)	40.4±3.42 (30 - 45)	35.6±4.36 ^d (22.6 - 47.7)
WBC* (cells x 10 ³ /μl)	-	7.54±2.66 (2.59 - 13.4)
ESR* (mm/hr)	-	60.6±27.9 (1 - 130)

a = significant difference from the controls at *p* < 0.007; b = significant difference from the controls at *p* < 0.027; c = significant difference from the controls at *p* < 0.00001; d = significant difference from the controls at *p* < 0.00001.
* WBC = white blood cell, ESR = erythrocyte sedimentation rate; data in parentheses are ranges

Table 2
Plasma lipid peroxide and antioxidant levels in the controls and patients.

Parameter	Control (n = 26)	RA patient (n = 91)
Plasma MDA ($\mu\text{mol/l}$)	1.13 \pm 0.26	1.22 \pm 0.76
Plasma CD (OD/ml)	0.45 \pm 0.09	0.60 \pm 0.25 ^a
Plasma VitE (mg/dl)	1.33 \pm 0.27	1.23 \pm 0.41
Plasma VitE (mg/g cholesterol)	6.60 \pm 0.93	6.38 \pm 1.41
Plasma VitE: chol+ trig ($\mu\text{mol}/\text{mmol}$)	5.16 \pm 0.60	4.75 \pm 1.10 ^b
Plasma VitC (mg/dl)	1.18 \pm 0.37	1.06 \pm 0.39

MDA = malondialdehyde, CD = conjugated dienes

^a = significant difference from the controls at $p < 0.00001$

^b = significant difference from the controls at $p < 0.021$

The lipid peroxide (LPO) products, especially MDA, have been established as indicators of lipid peroxidation (Kwon and Watts, 1964). Since MDA is much easier to measure with the thiobarbituric acid method, it is more commonly used in clinical trials, although there is criticism of the specificity of this method. Conjugated dienes, another marker which is an intermediate of lipid peroxidation, were included in this study. The determination of MDA and CD values was used as indicators of lipid peroxidation and also to study its consequence on antioxidant status.

Our results showed that rheumatoid arthritis patients had a tendency to have increased plasma MDA as compared to controls but it did not reach statistical significance. This finding differed from other studies, which found that RA patients with active disease had an increase in the levels of MDA (Ozgunes *et al*, 1995; Gambhir *et al*, 1997). Most of our patients were not in the active stage of disease on the day the blood was drawn. However, the concentrations of CD were significantly increased in the patients. Significantly elevated levels of CD in RA patients were accepted as indirect evidence of increase lipid peroxidation.

Vitamin E, which is an important lipid soluble antioxidant, is transported in serum with serum lipid. Thus the level of plasma vitamin E was expressed per unit plasma lipids. When vitamin E was appropriately expressed per unit cholesterol and triglyceride as suggested by Regnstrom *et al* (1996), its levels in the plasma of RA patients were significantly lower than the controls. This report was confirmed by other studies (Situnayake *et al*, 1991; Comstock *et al*, 1997). The low concentrations of this antioxidant may in some way relate to the development of RA disease, either directly or as an associations with etiologic factors. Heliovaara *et al* (1994) have demonstrated that a low antioxidant

level was a risk factor for development of RA disease. It is possible that vitamin E was consumed in the process of counter-balancing the free radicals. A decrease in plasma vitamin E was also observed in juvenile rheumatoid arthritis (Araujo *et al*, 1998). Edmonds *et al* (1997) have reported that treatment with vitamin E in RA patients can not reduce clinical indices of inflammation, but increases analgesic action.

Vitamin C is one of the most efficient water soluble antioxidants. Our finding revealed that the levels of vitamin C in RA patients did not differ from the controls. Situnayake *et al* (1991) have reported a significant decrease in plasma vitamin C levels in RA patients with moderately active inflammatory activity, and 70% of these patients were seropositive for rheumatoid factor. In our study we did not measure the disease activity of the subjects on the day of taking blood.

We concluded that patients with RA presented an imbalance in the oxidant-antioxidant system that markedly increased lipid peroxide products, particularly CD, and significantly decreased lipid soluble antioxidant vitamin E. These changes might play a role in the tissue damage and inflammation process in this disease.

In summary, our data provided preliminary evidence that patients suffering from RA had a significant increase in lipid peroxide products, especially CD, and a significant decrease in antioxidant vitamin E. In the future, mechanisms to decrease the generation of lipid peroxide and/or antioxidant therapy may develop into new avenue of therapeutic intervention.

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