Paraoxonase and Arylesterase Activities in Patients with Rheumatoid Arthritis

Romatoid Artrit Hastalarında Paraoxonaz ve Arilesteraz Aktiviteleri

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Abstract

Objective: The aim of the study was to evaluate serum paraoxonase (PON) and arylesterase (ARE) activities as well as lipid hydroperoxide (LOOH) levels in patients with rheumatoid arthritis (RA). We also investigated serum total antioxidant status (TAS) and total oxidative status (TOS) to reveal whether there is an association between the PON/ARE activities and oxidative stress. Our hypothesis is that PON and ARE activities which related to the risk of developing coronary artery disease are low in RA patients.

Patients and Methods: Twenty-five patients with RA and 26 healthy controls were included in the study. Serum PON and ARE activities were measured spectrophotometrically. LOOH levels were measured by ferrous oxidation with xylenol orange assay. TAS, TOS levels were determined by using a novel automated methods.

Results: Paraoxonase and arylesterase activities were significantly lower in patients with RA, LOOH levels were significantly higher ($p<0.001$, $p=0.02$, $p=0.006$, respectively) in patients with RA than in healthy controls. In patients with RA, serum TOS was higher and serum TAS was lower when compared with those of healthy controls ($p < 0.001$). PON was negatively correlated with LOOH ($r = -0.356$, $p = 0.01$), ARE was positively correlated with TAS ($r = 0.429$, $p = 0.002$), LOOH was negatively correlated with TAS ($r = -0.585$, $p = 0.001$).

Conclusion: Our results show that PON and ARE activities, which have antiatherogenic capability, are decreased in patients with RA. PON and ARE activities may be affected by oxidative stress which contribute to the pathogenesis of RA. *(Rheumatism 2007; 22: 132-6)*

Key words: Rheumatoid arthritis, oxidative stress, paraoxonase, atherosclerosis

Özet

Amaç: Bu çalışmanın amacı romatoid artrit (RA) hastalarında serum paraoksonaz (PON), arilesteraz (ARE) aktivitelerini ve lipid hidroperoksid (LOOH) düzeylerini değerlendirmektir. Ayrıca PON/ARE aktivitesi ile oksidatif stress arasında bir ilişki olup olmadığını saptamak için serum total antioksidan durumu (TAS) ve total oksidatif durumu (TOS) da araştırdık. Hipotezimiz RA hastalarında koroner arter hastalığı gelişimi riski ile ilişkili PON ve ARE aktivitelerinin düşük olmasıdır.

Hastalar ve Yöntem: Çalışmaya 25 RA hastası ve 26 sağlıklı kontrol alındı. Serum PON ve ARE aktiviteleri spektrofotometrik olarak ölçüldü. LOOH düzeyleri xylenol orange kiti kullanılarak ferröz oksidasyon tekniği ile ölçüldü. TAS ve TOS düzeyleri yeni geliştirilen otomatik yöntemlerle saptandı.

Bulgular: RA hastalarında paraoksonaz ve arilesteraz aktiviteleri sağlıklı kontrollerden anlamlı olarak düşük, LOOH düzeyleri ise sağlıklı kontrollerden anlamlı olarak yüksekti ($p<0.001$, $p=0.02$, $p=0.006$, sırasıyla). Hastalarda serum TOS sağlıklı kontrollerden yüksek, serum TAS ise sağlıklı kontrollerden düşüktü ($p < 0.001$). PON düzeyleri LOOH düzeyleri ile negatif korelasyon ($r = -0.356$, $p = 0.01$), ARE düzeyleri TAS ile pozitif korelasyon ($r = 0.429$, $p = 0.002$), LOOH düzeyleri TAS ile negatif korelasyon göstermekteydi ($r = -0.585$, $p = 0.001$).

Sonuç: Sonuçlarımız RA hastalarında antiaterojenik kapasiteye sahip PON ve ARE aktivitesinin düşük olduğunu göstermiştir. PON ve ARE aktiviteleri RA'in patojenezinde rol oynayabilecek oksidatif stresten etkilenebilir. *(Romatizma 2007; 22: 132-6)*

Anahtar kelimeler: Romatoid artrit, oksidatif stress, paraoksonaz, ateroskleroz

Introduction

Rheumatoid arthritis (RA) is characterized by polyarticular synovitis with accompanying degradation of cartilage and bone, which often results in loss of structural integrity (1). This degradation is mediated by several proteolytic enzymes, and current evidence suggests that proinflammatory cytokines are responsible for inducing these catabolic processes (2). Recently, attention has been focused on the role of reactive oxygen species (ROS) produced by activated neutrophils during the inflammatory response (3). It was shown that increased amount of ROS in plasma and synovial fluid may contribute destructive proliferative synovitis in RA (4,5).

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Reactive oxygen species are oxygen-containing molecules that produced during normal metabolism (6). When the production of damaging ROS exceeds the capacity of the body's antioxidant defenses to detoxify them, a condition known as oxidative stress occurs (7).

Reactive oxygen species can cause tissue damage, particularly in the endothelial tissue (8). Lipids and lipoproteins also affected by ROS. The oxidative modification hypothesis of atherosclerosis predicts lipid and protein oxidation in the vascular wall. Further, oxidative stress characterized by oxidized low density lipoprotein contributes to atherogenesis (9). Antioxidants may inhibit atherogenesis and improve vascular function by different mechanisms (10). Enzymatic protection against ROS and the breakdown products of peroxidized lipids and oxidized protein and DNA are provided by many enzyme systems such as superoxide dismutase, catalase, glutathione peroxidase. Apart from these important enzymatic antioxidants, paraoxonase-1 (PON1) appears to have antioxidative properties as well (11). PON1 is enzyme with three activities which are paraoxonase (PON), arylesterase (ARE) and dyazoxonase. PON1 hydrolyses organophosphates, such as paraoxon, aromatic esters, for instance, phenyl acetate, and also lipid peroxidation products, and reduces the accumulation of them. Thus, PON1 prevents the acceleration of atherosclerosis and assumes an antiatherogenic property (12). Recent articles indicated that PON1 reduce oxidative stress in serum and tissues, thus protecting against cardiovascular disease (13).

In this study, we aimed to evaluate serum PON and ARE activities and, lipid hydroperoxide (LOOH) levels in patients with RA. We also investigated serum total antioxidant status (TAS) and total oxidative status (TOS) to reveal whether there is an association between the PON/ARE activities and oxidative stress.

Patients and Methods

Subjects

This study was conducted at the Physical Medicine and Rehabilitation Outpatient Clinic of Harran University, Sanliurfa, Turkey. We treat more than 20 patients a day in our outpatient clinic and have 6 inpatient beds. A consetive sample of out-patients with joint complaints was screened for RA. In a 6-month period, among the 105 cases, referred for the first assessment, 53 did not meet inclusion criteria and 27 subjects refused to participate. The patients satisfied the 1987 revised American College of Rheumatology criteria for classification of RA. Patients with arthritis due to other disease, such as gout, ankylosing spondylitis, Reiter's syndrome, psoriasis, inflammatory bowel disease, systemic lupus erythematosus, Behçet's disease, and adult onset Still disease, neoplastic disease, established deficiency of vitamin B12 or folate and having received any drugs were also excluded. None of the patients were smokers and consumed alcohol. Out of 53 patients, 22 were smoker, 4 had infectious arthritis, 25 were undergoing treatment for RA and 2 were pregnant. Twenty-five RA patients (17 females, 8 males) who never took medical treatment were included in the study. Patients with disease of at least 6 month duration were recruited in this study. Informed consent was obtained from each RA patient.

Control group was consisted of 26 healthy individuals (16 females, 10 males). The controls were recruited from the family of those in the patient group. Controls had no joint complaints and any rheumatological disease. Age and sex distributions in the group of control subjects were similar to those of RA patients. Informed consent was obtained from each control.

Blood samples

Blood samples were obtained following an overnight fasting state. Samples were withdrawn from a cubital vein into blood tubes and immediately stored on ice at 4 °C. The serum was then separated from the cells by centrifugation at 3000 rpm for 10 min and they were analyzed.

Measurement of paraoxonase and arylesterase activities

PON activity was determined using paraoxon as a substrate and measured by increases in the absorbance at 412nm due to the formation of 4-nitrophenol as already described (14). The activity was measured at 25ºC by adding 50µl of serum to 1ml Tris-HCl buffer (100mM at PH 8.0) containing 2mM CaCl2 and 5 mM of paraoxon. The rate of generation of 4-nitrophenol was determined at 412 nm. Enzymatic activity was calculated by using molar extinction coefficient 17 100 M^{-1} cm^{-1}.

ARE activity was measured using phenylacetate as a substrate. Serum was diluted 400 times in 100mM Tris-HCl buffer, pH = 8.0. The reaction mixture contained 2.0 mM phenylacetate (Sigma Chemical Co) and 2.0 mM $CaCl_2$ in 100mM Tris-HCl buffer, pH = 8.0. Initial rates of hydrolysis were determined by following the increase of phenol concentration at 270 nm at 37 °C on a CE 7250 spectrophotometer (Cecil Instruments Limited, UK) (15). Enzyme activities were expressed in international units (U) or kilounits (kU) per 1 litre of sera.

Measurement of total antioxidant status

Plasma TAS levels were determined using a novel automated measurement method, developed by Erel (16). In this method, hydroxyl radical, which is the most potent radical, is produced via Fenton Reaction. In the classical Fenton reaction, the hydroxyl radical is produced by mixing of ferrous ion solution and hydrogen peroxide solution. In the most recently developed assay by Erel, same reaction is used. In the assay, ferrous ion solution, which is present in the Reagent 1, is mixed by hydrogen peroxide, which is present in the Reagent 2. The sequential produced radicals such as brown colored dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. In this assay, antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay has got excellent precision values, which are lower than 3%. The results are expressed as mmol Trolox equiv / l.

Measurement of total oxidant status

Plasma TOS levels were determined using a novel automated measurement method, developed by Erel (17). In this method, oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equiv. / L).

Measurement of LOOH levels

Lipid hydroperoxide amount was measured by a new automated method using xylenol orange. In this method, lipid hydroperoxide oxidizes ferrous ions to ferric ions. The produced ferric ions make a colored mega complex with xylenol orange. The absorbance is measured 570 nm (18).

Statistical analysis

Student's t test and Pearson's correlation analyses were performed by using SPSS for Windows, Release 11.5 computer program (SPSS Inc, Chicago, IL) and $p \leq 0.05$ was considered statistically significant.

Results

The RA subjects were 25 individuals (17 females, 8 males) aged 26 to 45 years (mean age: 37.9±5.4). The control group consisted of 26 healthy individuals (16 females, 10 males) aged 27 to 42 years (mean age: 36.8±4.9).

Demographical characteristics of the subjects are shown in table 1. There were no significant differences between RA subjects and controls with respect to age, gender, and body mass index (BMI).

Laboratory findings of the patients and controls are presented in Table 2. PON and ARE activities were significantly lower (p=0.024), and LOOH levels were significantly higher (p=0.006) in patients with RA compared to controls. Serum TOS was higher in patients than in healthy controls (p<0.001). Serum TAS was lower in patients than in healthy controls (p<0.001). In addition, PON was negatively correlated with LOOH (r=-0.356, p=0.01), ARE was positively correlated with TAS (r=0.429, p=0.001), LOOH was negatively correlated with TAS (r=-0.585, p=0.002).

Discussion

RA is a systemic, chronic inflammatory disease that primarily affects joints and leads to pain, deformity, joint destruction, and disability (19). Epidemiological studies have shown an increased premature mortality in patients with RA compared with the general population (20).

Several investigators reported an excess of cardiovascular morbidity and mortality among RA patients (21,22). Inactivity, side effects associated with drug use, dyslipidemia, increase in homocystenia, and increase in trombotic factors were put forward as causes of the accelerated atherosclerosis in RA (23). However, persons with RA have an increased incidence of myocardial infarction and death from coronary artery disease not explained by conventional coronary risk factors (24).

Systemic inflammation occurs as evidenced by increased levels of inflammatory marker in blood, and by the presence of extraarticular manifestations of the disease in RA (25, 26). Kumon et al. (27) reported that serum paraoxonase activity was down-regulated by IL-1 and TNF-α (28). In similar, Sattar and coworkers summarized the implications of the systemic inflammatory response in the development of accelerated atherosclerosis in RA (21). According to this study, proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 1Beta (IL1β), and interleukin 6 (IL-6), generated in the synovial tissue, can be released into the systemic circulation. These circulating cytokines are in a position to alter the function of distant organs, including adipose, skeletal muscle, liver, and vascular endothelium, to generate a spectrum of proatherogenic changes that include endothelial dysfunction, insulin resistance, a characteristic dyslipidemia, prothrombotic effects, and pro-oxidative stress.

In the present study, we found that the levels of LOOH and TOS were increased, and PON1 activities and TAS were

Table 1. Demographic and clinical data of patients with rheumatoid arthritis

	Patients (n = 25)	Controls (n = 26)	p
Age	37.9 ± 5.4	36.8 ± 4.9	> 0.05
Gender (Male/Female)	17 / 8	16 /10	> 0.05
BMI	29.9 ± 3.3	27.6 ± 3.8	> 0.05

RA- rheumatoid arthritis, BMI- body mass index

Table 2. Laboratory findings of the patients and controls

	Patients (n = 25)	Controls (n = 26)	p
ESR (mm/h)	49.5±14.3	11.3±3.3	<0.001
CRP (mg/L)	2.1±1.8	1.7±0.8	0.3
LOOH (μmolH_2O_2)	5.5±1.9	1.9±0.9	0.006
PON (U/L)	73.8±17.4	100.6±44.4	<0.001
ARE activity (U/L)	64.4±6.5	68.2±5.2	0.024
TOS (mol H2O2/L)	10.022.6	7.41.4	<0.001
TAS (meq Troloks/L)	0.9±0.7	1.01±0.7	<0.001

ESR- erytrocyte sedimentation rate. LOOH- lipid hydroperoxide, TAS- total antioxidant status, TOS- total oxidative status, CRP- C-reactive protein, PON- paraoxonase, ARE- arylesterase

Table 3. Significant Correlations among parameters in the patients group

	r	p
PON - LOOH	-0.356	0.01
ARE - TAS	0.429	0.001
LOOH - TAS	-0.585	0.002

LOOH- lipid hydroperoxide, TAS- total antioxidant status, TOS- total oxidative status, PON- paraoxonase, ARE: arylesterase decreased in the patient group. Further, PON was negatively correlated with LOOH, and ARE was positively correlated with TAS. These results might suggest that changes in oxidative/antioxidative status might be responsible for the decrease in the activity of PON1 observed in RA. Baskol et al. (29) reported that increased ROS levels in RA might result in a pro-oxidation environment, which in turn could result in decreased antioxidant PON1 activity and increased malondialdehyde levels.

It has been previously shown that PON1 activity was decreased in some diseases due to ROS pathogenesis under oxidative stress and inflammation condition such as ulcerative colitis and Behcet's disease (3,30). The excessive production of ROS can damage protein, lipids, nucleic acids, and matrix components. ROS can attack double bonds in polyunsaturated fatty acids, and thus induce lipid peroxidation; this in turn results in more oxidative damage. Increased plasma oxLDL concentrations have been reported in patients with hypercholesterolemia, end-stage renal disease, transplant coronary atherosclerosis, diabetes, coronary artery disease and the metabolic syndrome (31-34). Aviram et al. (35,36) clearly demonstrated that PON1 inactivation by oxLDL resulted in both the reduction of paraoxonase and arylesterase activities and serum paraoxonase activity and HDL susceptibility to oxidation to be inversely correlated. Navab et al. (37) suggested that paraoxonase activity also protects the antiatherogenic activity of HDL.

It was known that oxidative stress can cause atherogenic, vasculotoxic, and tissue injury, however, the mechanism of this association, and whether it is direct or indirect, remains to be explored. Increased inactivation of PON1 which is related to increased generation of ROS may explain the susceptibility of RA patients to atherosclerosis. There are some limitations in our study. The patients were not evaluated according to serum lipids and lipoproteins, and echocardiographic findings. Another limitation is the relatively small sample size that could limit our ability to generalize the results to RA patients in general. The decrease in the activities of PON and ARE enzymes, which have antioxidant and antiatherogenic properties, might be influencing both progression of the disease and the development of atherosclerosis in RA. These findings might provide evidence that early treatment inflammatory process may reduce the risk of atherosclerosis and cardiovascular events in RA. Further studies are necessary to determine the role of PON activity on the development of cardiovascular diseases in RA.

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