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Full Length Research Paper

Serum paraoxonase and arylesterase activities and oxidant status in patients with brucellosis

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The aim of this study was to the investigate the effect of brucellosis infection on paraoxonase-1 (PON1) activity and oxidative status in patients with brucellosis. Sixty patients with brucellosis (47 acute, 13 subacute) and the 67 healthy controls were used in the study. Serum PON1 and arylesterase activities, total antioxidant capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI), total free sulfhydryl (-SH), and lipid hydroperoxide (LOOH) groups were determined. In addition, HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), total cholesterol (TC) and triglyceride (TG) were measured. Serum PON1 and arylesterase activities, total -SH group levels and TAC were significantly lower in brucellosis patients than controls (P<0.001). TOS, OSI and LOOH levels were significantly higher in brucellosis patients than controls (P<0.001). There were no significant differences between of serum LDL-C, TG and TC levels of patients subjects compared to controls (P > 0.05). Patients with brucellosis are exposed to potent oxidative stress and they have decreased PON1 activity. These predisposal factors might play a role in the pathogenesis for atherosclerosis in patients with brucellosis.

Key words: Brucellosis, PON1 activity, oxidative status, atherosclerosis.

INTRODUCTİON

Brucellosis is a zoonotic diseases caused by *Brucella* spp which are Gram-negative coccobacilli. It remains a significant public health problem in areas of the world where brucella infections are endemic in herbivorous animals (Young, 2005). *Brucella* strains are facultative intracellular bacteria. They have the capacity to survive and replicate for prolonged periods within host macrophages (Pappas et al., 2006). They are internalized into phagocytic cells, and are killed by reactive oxygen and nitrogen species (Franco et al., 2007). In brucellosis, stimulation of macrophages is a significant stage in the progress of the disease in which cells serve as an effector system of cytokines, chemokines and free radicals, to produce highly damaging reactive nitrogen species (RNS) and reactive oxygen species (ROS) (Klebanoff et al., 1980). Thereby, increasing ROS and RNS can cause protein oxidation, lipid peroxidation and DNA damage (Kocyigit et al, 2005). Additionally, it has been demonstrated that serum paraoxonase-1 (PON1) deficiency is related to increased susceptibility to low density lipoprotein oxidation and development of atherosclerosis (Canales and Sanchez-Muniz, 2003). PON1 is a 43- to 45-kDa glycoprotein. It is a calcium-dependent esterase that hydrolyzes a broad spectrum of substrates including organophosphates, arylesters and lactones (Shamir et al., 2005; Rosenblat et al., 2006). In recent years, results of some studies have revealed the presumptive role of several infectious agents in the inflammatory mechanism of atherosclerosis (Kiechl et al.,

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2001; George et al., 2000; Selek et al., 2008).

In the present study, we investigated serum paraoxonase and arylesterase activities along with determination of oxidative status through measurement of total oxidant status (TOS), total antioxidant capacity (TAC), total free sulfhydryl (-SH), lipid hydroperoxide (LOOH) levels and oxidative stress index (OSI). Our aim was to find out if there is any increased susceptibility to atherogenesis, which might be reflected with increased oxidative stress and decreased serum PON1 activity in patients with brucellosis.

MATERİALS AND METHODS

The study was conducted in the Departments of Infectious Diseases and Clinical Microbiology of Medical Faculties of Yuzuncu Yil University and Harran University. The study protocol was carried out in approved by the local research committee for ethics.

Subjects

In the patient group, 60 cases were diagnosed through compatible clinical symptoms and signs compatible and in conjunction with brucella standard agglutination test titer ≥1/160 and/or *Brucella* spp. growth in cultures of blood samples (Buzgan et al., 2010). As a control group, we enrolled 67 healthy volunteers. The patients and healthy volunteers enrolled were aged between 15 and 65 years.

Exclusion criteria

Exclusion criteria included usage of supplemental vitamins, viral hepatitis, smoking habit, chronic diseases such as diabetes mellitus, coronary artery disease, rheumatoid arthritis and malignancy, etc.

Blood sample collection

Blood samples were obtained following an overnight fasting state and collected into empty tubes and immediately stored in ice at 4°C. The serum samples were then separated from the cells by centrifugation at 3000 rpm for 10 min. Serum samples were stored at –81°C until analysis.

Measurement of paraoxonase and arylesterase activities

Paraoxonase activities measurements were performed in the absence (basal activity) and presence of NaCl (salt-stimulated activity) (Seres et al., 2004). Briefly, the rate of paraoxon hydrolysis was measured by the increase of absorbance at 412 nm at 25°C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 17,100 M^{-1} cm^{-1}. Paraoxonase activity was expressed as U/L serum. Phenylacetate was used as a substrate to measure the arylesterase activity. The reaction was started by the addition of the serum and the increase in absorbance was read at 270 nm. Blanks were included to correct the spontaneous hydrolysis of phenylacetate. Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol, 1310 M^{-1} cm^{-1}. One unit of arylesterase activity was defined as 1 µmol phenol generated/min under the above conditions and expressed as U/L serum. The phenotype distribution of PON1 was determined in the presence of 1 mol/L NaCl (salt-stimulated paraoxonase). The ratio of the salt-stimulated paraoxonase activity to the arylesterase activity was used to assign individuals to one of the three possible phenotypes (Eckerson et al., 1983). Measurement of total oxidant status (TOS) of serum was determined using a novel automated measurement method (Erel, 2005). 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). The assay has excellent precision values lower than 2%.

Measurement of LOOH levels

Serum LOOH levels were measured with the ferrous ion oxidation–xylenol orange assay. The principle of the assay depends on the oxidation of ferrous ion to ferric ion through various oxidants and the produced ferric ion is measured with xylenol orange. LOOH's are reduced by triphenyl phosphine (TPP), which is a specific reductant for lipids. The difference between with and without TPP pretreatment gives LOOH levels (Arap and Steghnes, 2004).

Measurement of total free sulfhydryl groups (-SH)

Measurement of total free sulfhydryl groups briefly, 1 ml of buffer containing 0.1 MTris, 10 mMEDTA, pH 8.2, and 50 µl serum was added to cuvettes followed by 50 µl 10 mMDTNB in methanol. Blanks were run for each sample as a test, but there was no DTNB in the methanol. Following incubation for 15 min at room temperature, sample absorbance was read at 412 nm on a Cecil 3000 spectrophotometer. Sample and reagent blanks were subtracted. The concentration of sulfhydryl groups was calculated using reduced glutathione as free sulfhydryl group standard and the result was expressed as millimolars (Erel, 2004).

Measurement of the total antioxidant capacity

TAC of serum was determined using a novel automated measurement method, developed by Erel (2004); Kosecik et al. (2005). In this method, hydroxyl radical, which is the most potent biological radical, is produced. 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. Using this method, 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 lower than 3%. The results are expressed as mmol Trolox Equiv./L.

Measurement of total oxidant status

TOS of serum was determined using a novel automated measurement method, developed by Erel (2005). 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

Table 1. Demographic and lipids parameters of the patients and the control group.

Parameter	Patients (n=60)	Control (n=67)	P value
Women/man	31/29	35/32	Ns
Age (mean±SD)	39.23±16.10	37.2±14.30	Ns
BMI (kg/m^2) (mean±SD)	21.12±3.24	22.18±2.26	Ns
TG (mg/dL)	201.85±111.38	156.15±115.11	Ns
TC (mg/dL)	165.13±43,66	176.56±38.36	Ns
HDL-C (mg/dL)	22.92±12.96	30.04±12.93	<0.05
LDL-C (mg/dL)	102.40±34.287	103.61±34.39	Ns

BMI: body mass index, TG: triglyceride, TC: total cholesterol, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol. Ns = non-significant.

Table 2. Paraoxonase activity, oxidant and antioxidant parameters in patients and the control group.

Parameter	Patients (n=60) Mean±SD	Control (n=67) Mean±SD	P value
Paraoxonase (U/L)	117.85±43.42	162.57±40.06	<0.001
Arylesterase (kU/L)	125.39±64.42	169.49±72.2	<0.001
-SH (mmol/L)	0.32±0.01	0.54±0.0.06	<0.001
TAC (mmol Trolox Equiv./L)	1.27±0.21	1.38±0.16	<0.001
LOOH (µmol H2O2 equiv./L)	33.72±42.82	6.93±3.62	<0.001
TOS (µmol H2O2 Equiv./L)	50.32±45.81	15.05±10.42	<0.001
OSI (arbitrary unit)	4.41±4.60	1.10±0.79	<0.001

TAC: total antioxidant capacity, TOS: total oxidant status, OSI: oxidative stress index, -SH: total free sulfhydryl, LOOH: lipid hydroperoxide.

results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 Equiv./L).

Oxidative stress index

Percentage ratio of TOS level to TAC level was accepted as OSI. For calculation, the resulting unit of TAC was changed to mmol/L, and the OSI value was calculated according to the following Formula (Karaagac et al., 2010). OSI (Arbitrary Unit) = TOS (µmol H_2O_2 Equiv./L)/TAC (mmol Trolox Equiv./L).

Other parameters

The levels of HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), total cholesterol (TC) and triglyceride (TG) were measured using commercially available assay kits (Abbott®, Illinois, USA) with an autoanalyzer (Aeroset®, Abbott®, Illinois, USA).

Statistical analysis

All analyses were conducted using SPSS 11.5 (SPSS for Windows 11.5, Chicago, IL). Continuous variables were expressed as mean ± standard deviation (S.D). Parameters comparisons were performed using the Sutent's t-test. All statistical tests were two-sided. A P-value < 0.05 was considered as significant.

RESULTS

The demographic characteristics and lipids values of the 60 patients with brucellosis (47 acute and 13 subacute) and 67 healthy controls are shown in Table 1. No statistically significant difference was observed when the study groups were compared in terms of age, gender or body mass index (BMI) (P> 0.05). There were no significant differences between serum LDL-C, TG and TC levels of patients group compared to controls (P > 0.05) (Table 1). Serum paraoxonase, arylesterase activities, total -SH group levels and TAC were significantly lower in brucellosis patients than controls (P<0.001), while TOS, OSI and LOOH levels were significantly higher (P<0.001) (Table 2).

DİSCUSSİON

Bucella are internalized in to phagocytic cells and are killed by reactive oxygen and nitrogen species. Therefore brucellosis related to increased free radical production and antioxidant depletion, and oxidative burst represents plays rol in the pathogenesis of brucellosis. A decrease in total antioxidative capacity and an increase in total oxidative status and OSI showed that the patients with brucellosis infection were exposed to potent oxidative stress (Serefhanoglu et al., 2009; Serdar et al., 2006). In our study, a novel automated colorimetric measurement method for oxidative status (TAC, TOS and OSI) was developed by Erel, in our university. This method has

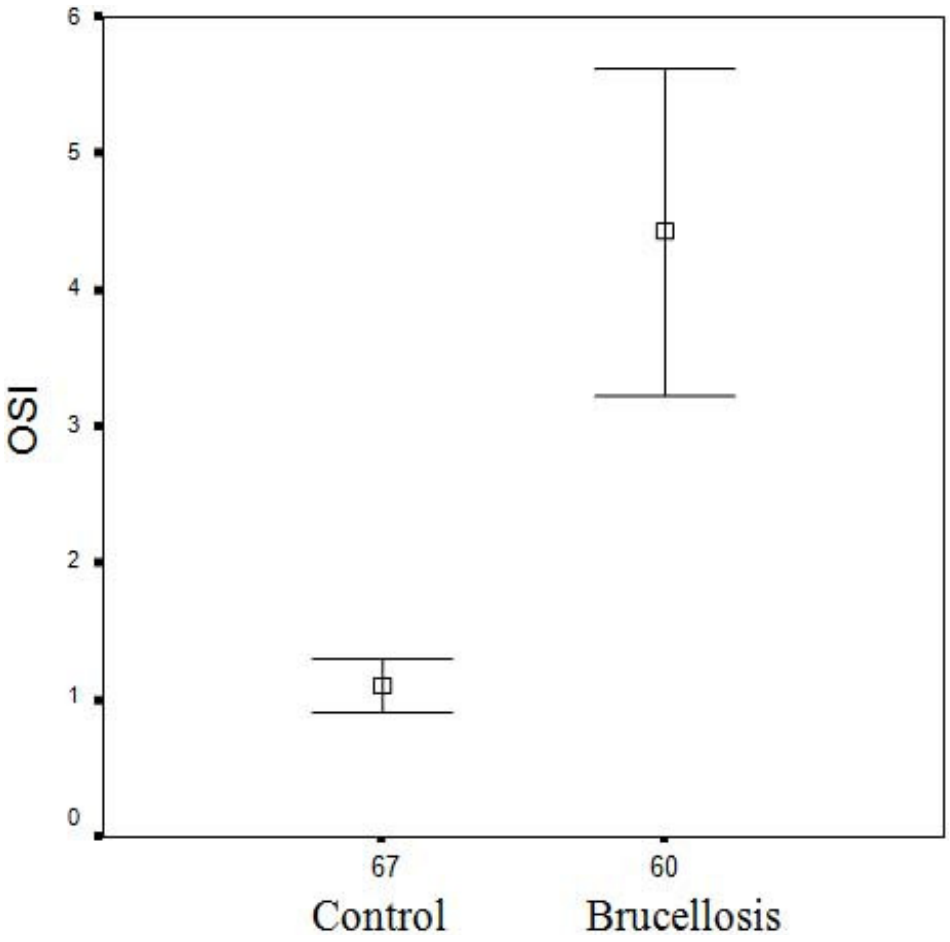

Figure 1. The OSI level of patients and control groups.

advantages in comparison with other methods. It is simple, cost-effective, reliable and sensitive. It does not interact with normally available serum components such as serum lipids, bilirubin or anticoagulants (Erel, 2004, 2005).

Oxidative stress, owing to increased lipid and protein oxidation products and decreased antioxidant enzymes is associated with cardiovascular diseases and affects PON1 activities (Aviram et al., 1999; Mc Elven et al., 1986). In humans, epidemiologic studies have represented that PON1 has an independent risk factor for coronary artery disease (Rozenberg et al., 2003; Aviram and Rosenblat, 2004). Serum PON1 expression is down-regulated by oxidative stress (Mackness et al., 2003). Furthermore, Mackness et al. (2003) have reported that PON1 is inactivated under oxidative conditions. Also PON1 activity is low in serum of atherosclerotic patients (Srinivasan et al., 2004). In the present study, we showed that serum paraoxonase, arylesterase activities and total -SH group levels were significantly lower in patients with brucellosis than controls, while LOOH levels TOS and OSI were significantly higher. Therefore, it has been suggested that OSI may reflect the state of oxidative status in brucellosis. The patients and control group levels of OSI are shown in Figure 1 and the both groups PON1 activities are shown in Figure 2. To the best of our knowledge, there is no prior study investigating PON1, arylesterase, oxidative status, lipid hydroperoxide and total free sulfydryl in patients with brucellosis. We have demonstrated that oxidative stress markers were elevated in patients with brucellosis, which provides evidence for enhanced free-radical mediated processes. In addition, we can suggest that increased oxidative status and decreased serum PON1 activity may prone patients with brucellosis to the development of atherosclerosis through increased susceptibility to lipid peroxidation. The association between PON1 and carotid artery intima-media thickness and autopsy verified atherosclerosis of celiac, superior and inferior mesenteric and coronary arteries have been reported (Leus et al., 2000; Malin et al., 2001; Rontu et al., 2003). Several reports have revealed relationship between serum con-centration and activity of PON1 and presence and extent of atherosclerotic diseases (Rontu et al., 2003; Mackness et al., 2001; Gran'er et al., 2006). Decrease in PON1 activity under oxidative stress was mostly attributed to

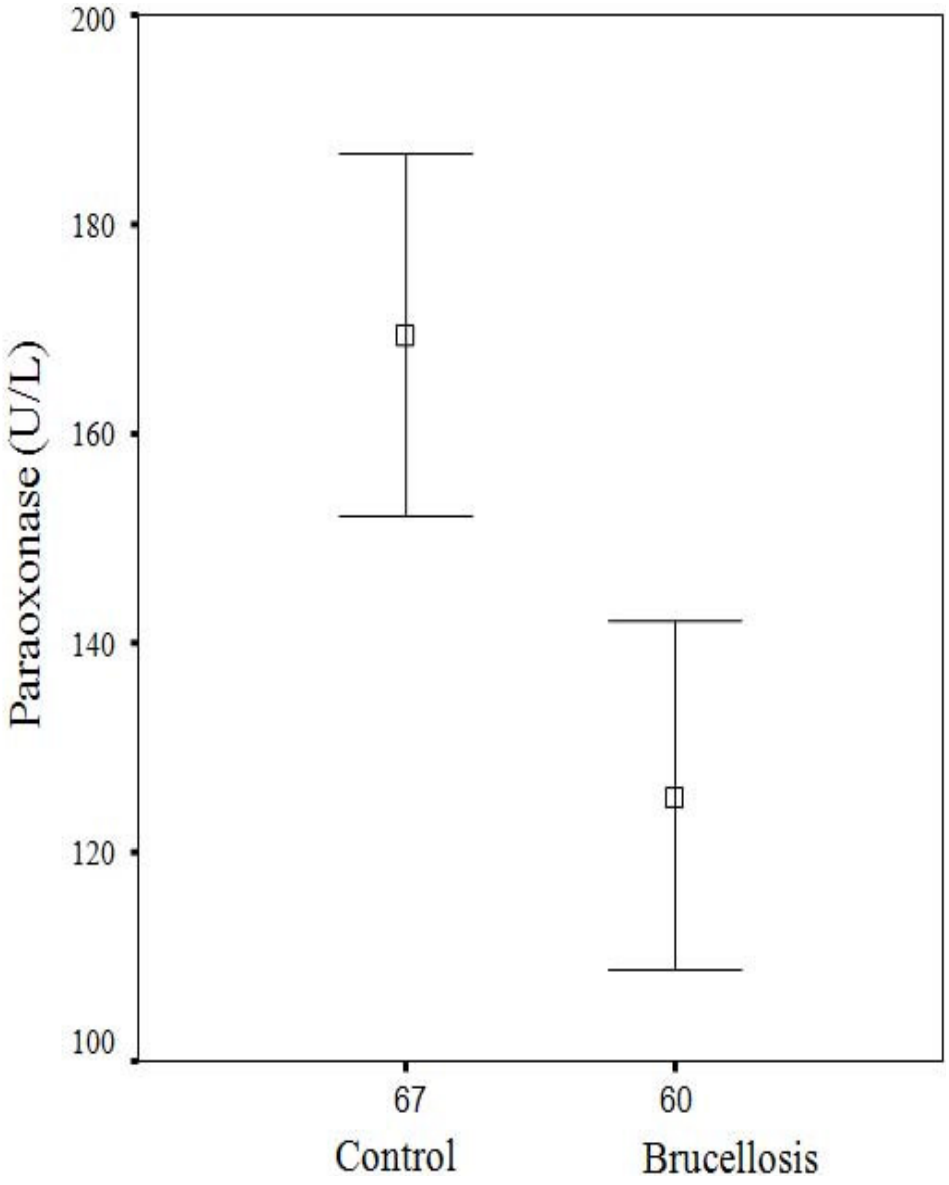

Figure 2. The Paraoxonase activity of patients and control groups.

changes in the redox status of the protein's free sulfhydryl groups since sulfhydryl compounds prevented the inhibition of PON1 activity caused by ROS. Free sulfhydryl groups of proteins constitute the main antioxidant component of serum (Rozenberg and Aviram, 2004). It has been shown that total -SH group levels are associated with the extent of coronary heart disease (Gur et al., 2006). In the present study, both decreased total -SH group levels and decreased PON1 activity may be attributed to development of atherosclerosis in patients with brucellosis.

In conclusion, findings of the present study have shown that serum paraoxonase and arylesterase activities are significantly reduced while oxidative status is increased. Decreased serum paraoxonase and arylesterase activities might play a role in the pathogenesis for atherosclerosis in patients with brucellosis.

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