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Original Article

Serum paraoxonase, TAS, TOS and ceruloplasmin in brucellosis

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Abstract: It is possible that brucellosis may be related to increase free radical production and antioxidant depletion. Thus, in the present study we aimed to evaluate the oxidative status in patient with brucellosis and healthy controls. Methods: This study includes the patients with brucellosis diagnosed by clinical findings and positive agglutination titer. The paraoxonase, ceruloplasmin, total antioxidant capacity and total oxidant status values were measured from the samples taken. The oxidative stress index value was calculated through the total antioxidant capacity and total oxidant status values. Results: A total number of 93 people, 40 women (43%) and 53 men (57%) were included to the study. The levels of ceruloplasmin were found higher in patients when compared to the control group ($p < 0.001$). The total antioxidant capacity level was found significantly higher in the patients group when compared to the control group ($p < 0.001$). The oxidative stress index value was significantly lower in the patients group when compared to the control group ($p < 0.001$). The paraoxonase-1 level was not different in control and patient groups ($p = 0.077$). Conclusions: Brucellosis is an infection that is frequently seen in Mediterranean countries. This infection breaks the oxidant and antioxidant balance. In this disease, oxidant-antioxidant system indicators such as ceruloplasmin, total antioxidant capacity, total oxidant status and oxidative stress index can be used for showing the role of the brucella infection and for the monitoring of the treatment results.

Keywords: Brucella, total antioxidant capacity, total oxidant status, paraoxonase, ceruloplasmin

Introduction

Brucellosis is a systemic zoonotic disease caused by brucella type bacteria. Even though it is primarily an animal disease, over 500,000 cases in a year are notified in human [1]. Although brucellosis is an endemic disease in many developing countries such as Middle East, Mediterranean region, Asia, Africa, and South America, it remains underestimated because of underreporting and under-diagnosing [2].

Brucella types are facultative intracellular bacteria. They can live and reproduce in macrophages. How exactly they can maintain intracellular life is not known, the facts that they can live in acidic environment, they can prevent the apoptosis of macrophages and the phagosome-lysosome fusion and they can depress the cellular immune response. Brucella achieves to survive in intracellular environment by inhibiting the bactericidal response with these protection mechanisms. Reactive oxygen products play an important role in the removal of the phagocytosed bacteria. Reactive oxygen products also cause protein oxidation; lipid peroxidation and DNA damage [3]. The host macrophage oxidative burst shows a main mechanism that controls the intracellular replication of brucella, so oxidative stress and antioxidant defense mechanism are important items in brucellosis [4].

Ceruloplasmin (CP) is a copper-dependent ferroxidase involved in the acute-phase reaction with both anti- and pro-oxidant activities. It plays a role in iron (Fe) homeostasis and protec-

tion against free radical-initiated cell injury [5]. In addition to ferroxidase activity, CP contributes to anti-oxidant defense by scavenging of H_20_2 and inhibits superoxide-induced lipid peroxidation [6].

Paraoxonase-1 (PON1) plays an important role in the protection of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) from oxidation by hydrolyzing lipid peroxides accumulation and its activity has been shown to be inversely associated with oxidative stress in human serum. PON1 is a HDL-associated antioxidant enzyme. In addition, human PON1 contributes to the antiatherogenic effect of HDL [7, 8].

It is possible that brucellosis may be related to increase free radical production and antioxidant depletion. Thus, in the present study, we aimed to evaluate the oxidative status in patient with brucellosis and healthy controls by measurement of serum CP, PON1, total antioxidant capacity (TAC) and total oxidant status (TOS), and calculation of oxidative stress index (OSI).

Material and methods

Patient's selection

This study includes 59 patients with brucellosis diagnosed by clinical findings and positive agglutination titer and these patients were followed up at the Department of Infectious Diseases, Ministry of Health Batman Regional Government Hospital. As a control group, we enrolled 34 healthy volunteers. Patients with severe renal dysfunction and infectious diseases such as viral hepatitis and chronic diseases such as diabetes mellitus and patients who use supplemental vitamins, smoking, and pregnancy were excluded. The study protocol was approved by the local Ethical Committee.

Blood sample collection

The blood samples were collected from the patients who were first identified. These patients didn't have any treatment. Blood samples were obtained following an overnight fasting state and collected into empty tubes (BD diagnostics-preanalytic systems, UK) at 08:00-09:00 A.M. The serum samples were separated from the cells by centrifugation at 3000 rpm for 10 min. Serum samples were stored at -40°C until analysis. Calcium EDTA containing tubes were not used for collecting serum samples because calcium EDTA inhibited PON1 activity.

Measurement of CP and PON1 activities

CP levels were measured by nephelometric method (Image 800, Beckman Coulter, Fullerton, CA). Serum PON1 levels were measured spectrophotometrically by modified Eckerson method. Initial rates of hydrolysis of paraoxon (diethyl-p-nitrophenylphosphate; Sigma Chemical Co. London, UK) were determined by measuring liberated- p -nitrophenol at 412 nm at 37°C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 17,000 M^{-1} cm^{-1}. PON1 activity was expressed as U/L serum.

Measurement of the TAC

TAC of serum was determined using a novel automated measurement method, developed by Erel [9]. In this method, hydroxyl radical, which is the strongest 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 dianisidine radical cation, produced by the hydroxyl radical, they 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 TOS

Total oxidant status was measured by Erel's methods [10]. Oxidants which are 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

Table 1. Patient characteristics

	Patient Mean ± SD (Range)	Control Mean ± SD (Range)	*P* value
Age (year)	31.2 ± 12.9 (15-65)	34.1 ± 9 (21-50)	0.11
AST (U/L)	36.5 ± 19.9 (13-95)		
ALT (U/L)	27.9 ± 16.1 (6-83)		
Triglyceride (mg/dL)	144.2 ± 29 (77-218)	141.1 ± 28.5 (84-194)	0.374
Cholesterol (mg/dL)	176 ± 23.6 (117-215)	170.6 ± 27.9 (113-215)	0.505
HDL (mg/dL)	38.4 ± 7.9 (25-66)	42 ± 10.1 (27-68)	0.101
Htc (%)	38.1 ± 4.2 (27.9 ± 49.5)		
Plt (10^9/L)	244.7 ± 73.9 (123-518)		
CRP (mg/L)	2.28 ± 3.46 (0.1-14.5)		
Sedimentation (mm/h)	25.1 ± 18 (1-67)		
Ceruloplasmin (mg/dL)	38.08 ± 13.12 (14-90)	29 ± 12.1 (14-89)	< 0.001*
PON1 (U/L)	50.5 ± 29.4 (13-131)	64.5 ± 38 (21-171)	0.077
TAS (mmol Trolox Equiv/L)	1.06 ± 0.33 (0.32-1.83)	0.87 ± 0.57 (0.21-3.83)	< 0.001*
TOS (μmol H_2O_2 Equiv/L)	20.23 ± 6.31 (6.82-44)	28.87 ± 6.39 (18.7-51.04)	< 0.001*
OSI (Arbitrary Unit)	2.09 ± 0.92 (0.6-5.19)	4.41 ± 3.89 (0.67-24.3)	< 0.001*

*P-value of less than 0.05 was accepted as statistically significant.

peroxide equivalent per liter (μmol H_2O_2 Equiv/L).

Oxidative stress index

The ratio of TOS level to TAC level was accepted as OSI. The OSI value was calculated according to the following Formula [11]. OSI (Arbitrary Unit) = TOS (_mol 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 by using an autoanalyzer (Architect c4000, Abbott, US) with spectrophotometrically. Serum TC, TG, HDL-C and LDL-C levels were measured at the same day after the blood was collected.

Statistical analyses

Statistical analyses were performed using the SPSS software version 15. Whether there was a gender difference between the patients and the control group was investigated through Chi-squared test. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov test) to determine whether or not they were normality distributed. The cases in which the *p* value was above 0.05 in the Kolmogorov-Smirnov test were accepted as the normal distribution. Since the age, TG, TC, HDL, CP, PON1, TOS, TAC and OSI were not normally distributed; the differences between the patients and the control group were investigated through the non-parametrical Mann-Whitney U test.

While investigating the associations between non-normally distributed, the correlation coefficients and their significance were calculated using the spearman test. A *p*-value of less than 0.05 was accepted as statistically significant. Statistical analyses were performed using the SPSS 15.0 statistical package (SPSS, Inc., Chicago, IL).

Results

A total number of 93 people, 40 women (43%) and 53 men (57%), participated to the study. 59 people (63.4%) participating to the study were in the patients group and 34 people (36.6%) were in the healthy control group. No difference in terms of gender was observed between the patient and the control group (p: 0.786). In the patient group, there were 26 women (44.1%) and 33 men (55.9%). In the control group there were 14 women (41.2%) and 20 men (58.8%). No difference in terms of age was observed between the two groups (p = 0.11). The average ages of the patients were 31.2 ± 12.9 (Range 15-60) (**Table 1**).

The levels of CP were higher in the patients group when compared to the control group (p <

0.001). In the patient group, the average level of CP was 38.08 ± 13.12 (Range 14-90) and it was 29 ± 12.1 (Range 14-89) in the control group.

The TAC level was significantly higher in the patient group when compared to the control group ($p < 0.001$). In the patients group, the average value of TAC was 1.06 ± 0.33 (Range 0.32-1.83) and it was 0.87 ± 0.57 (Range 0.21-3.83) in the control group. The TOS level was significantly higher in the control group when compared to the patients group ($p < 0.001$). The average value of TOS was 28.9 ± 6.4 (Range 18.7-51) in the control group and it was 20.2 ± 6.3 (6.82-44) in the patients group. The OSI value was significantly lower in the patients group when compared to the control group ($p < 0.001$).

The PON1 level was not different between the control group and the patients groups ($p = 0.077$). The PON1 levels and the TC levels were negatively correlated ($p = 0.013$, $r = -0.256$). However, a significant positive correlation was found with HDL ($p < 0.001$, $r = 0.389$).

The CP values were not correlated with TAC, but correlated with TOS ($p = 0.53$, $p = 0.001$). The correlation coefficient between CP and TOS was determined as 0.334. No correlation was determined between TAC and TOS ($p = 0.101$).

There was a negative correlation between TAC and age ($p = 0.013$, $r = -0.257$). Age, TOS and PON1 levels were unrelated ($p = 0.194$, $p = 0.98$).

Discussion

In this study, while the CP, TOS and OSI values were found to be statistically significantly different between patients with Brucellosis and the control group, the PON1 values were found to be indifferent. PON1 is a multifunctional antioxidant enzyme. PON1 protects LDL and HDL from oxidation. In their study in which they researched the factors playing role in the atherogenesis of patients with brucellosis, Apostolu et al had shown that the PON1 level increased when compared to the control group [12]. However in our study, the PON1 level wasn't different between the patient group and the control group. We are thinking that it is necessary to study more on the research of the PON1 level change in patients with brucellosis.

CP is an anti-oxidant molecule having an important role in iron and copper homeostasis and it also has an important role in the protection against oxidative stress. CP plays a part as a positive acute phase reactant in the tissue repair processes by means of the host immune system [13]. As a radical scavenger CP, keeps the free oxygen in the circulation and it undertakes a protective function against tissue injuries which are created by free oxygen radicals [14]. The CP levels increase in physical exercise, pregnancy, ovarian hyperfunction, arteriosclerosis, epilepsy, cholestasis, alcoholic liver injury, diabetes mellitus, chronic inflammatory processes, malignant tumors and chronic infections [15-17]. It has been shown that CP level increases in aspergillosis and pulmonary cystic echinococcosis [18, 19]. The level of acute phase reactants increases in brucellosis [20]. It has been shown that the level of CP, which is an acute phase reactant increases in our study. The CP level was found to be positively correlated with the TOS in our study. Oxidative burst plays an important role in the antibacterial processes of phagocytic cells against the brucellosis. The oxidative stress, which appears during the removal of the brucella within the cell through oxidative burst, may be the reason why the level of CP increases in patients with brucellosis.

The separate measurement of the concentration of different oxidants and antioxidants in the laboratory environment takes a long time, it is also expensive and it requires complex techniques. Besides, the separate measurement of the different oxidant-antioxidant molecules and assembling and evaluating the result are not practical. TAC and TOS are the additive measurement parameters of antioxidant effects that enable the measurement of all the antioxidant and oxidant parameters by being evaluated together. The TOS/TAC ratio, in other words the OSI, is an indicator of the oxidative stress degree and shows the anti-oxidation and oxidation redox balance [21]. Usta et al have found significantly higher TAC, TOS and OSI values in patients with Brucella Canis [22]. The measurement values in this study are lower than the values we found in our study. Patients infected with straight Brucella types had been taken in our study. In their study in which oxidative stress is evaluated in patients with straight brucellosis, Serefhanoğlu et al have had the similar results with our study [23].

The TAC, TOS and OSI measurement do not only show the oxidative/anti-oxidative condition during the diagnosis, but they also play a role in the monitoring of the treatment. Karaaağaç et al had shown that the TOS and OSI values increased and the TAC value decreased during the treatment in patients with brucellosis [24].

Brucellosis is an infection that is frequently seen in Mediterranean countries and that may become chronic. This infection damages the oxidant and antioxidant balance. The oxidant - antioxidant balance plays a role in the etiology of many chronic degenerative diseases such as cancer and atherosclerosis. Oxidant - antioxidant system indicators such as CP, TAC, TOS and OSI can be used for the determination of the role of the brucella infection in these diseases and for the monitoring of the treatment results.

Disclosure of conflict of interest

None.

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