Evaluation of Oxidative Status in Patients with Brucellosis

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Oxidative stress can be defined as an increase in oxidants and/or a decrease in antioxidant capacity. We aimed to determine total antioxidant capacity (TAC), total peroxide, malondialdehyde and catalase levels in plasma samples, and calculation of oxidative stress index (OSI) in patients with brucellosis to evaluate their oxidative status using a novel automated method. Sixty-nine patients with brucellosis and 69 healthy control subjects were included in the present study. Plasma levels of total peroxide and malondialdehyde were significantly increased in patients as compared with healthy controls ($p<0.001$ and $p<0.001$, respectively). In contrast, TAC level was significantly lower in patients as compared with controls ($p<0.001$). There was no statistically significant difference between the catalase results of the two groups ($p>0.05$). OSI level was significantly increased in patients as compared with healthy controls ($p<0.001$). In conclusion, oxidants were increased and antioxidants were decreased in patients with brucellosis. Oxidative stress was increased in patients with brucellosis.
Key-Words: Brucellosis, oxidants, antioxidants, oxidative stress.

Numerous studies demonstrated that in the many infectious diseases, a variety of inflammatory cells are activated, which lead to production of reactive oxygen and nitrogen species to kill intra-cellular and extra-cellular parasites [1,2]. Reactive oxygen species are one of the crucial molecules that kill bacteria internalized into phagocytic cells, such as polymorphonuclear neutrophils (PMNs) and macrophages [3]. They reside in the phagocytic cells and are released into the phagosome upon engulfment of bacteria [4]. These molecules fight against bacteria by causing DNA strand breaks, degradation of RNA, inhibition of amino acid biosynthesis, and inactivation of membrane transport proteins [5].

Brucella is a Gram-negative, facultative, intracellular pathogen that produces cell toxicity by altering plasma membrane and inducing cell apoptosis [6]. It is stated that the oxidative killing pathways of host macrophages represent a primary mechanism utilized by these host phagocytes to control the intracellular replication of the brucellae [7]. Therefore, it is possible that brucellosis may be related to increased free radical production and antioxidant depletion, and oxidative stress may be implicated in the pathogenesis of brucellosis. Thus, in the present study, we evaluated the oxidative status in patients with brucellosis and healthy controls via measurement of total antioxidant capacity (TAC), oxidants (total peroxide and malondialdehyde–a lipid peroxidation byproduct) and antioxidant (catalase) levels in plasma samples, and calculation of oxidative stress index (OSI).

Material and Methods

The study was conducted at Baskent Univercity, Konya Medical and Research Center in Konya, Turkey. Informed consents were obtained and the study protocol was approved by the Ethical Committee of the Baskent University Hospital. Diagnosis of brucellosis was made by isolation of the bacterium from blood sample and/or serum agglutination test with titers ≥1:160 in conjunction with a compatible clinical presentation [8].

After overnight fasting, peripheral venous blood sample was collected into heparinized tubes for each individual in the study and control groups, and plasma was separated by centrifugation at 1500 x g for 10 min, and the sample was then stored at –80 °C until further analysis of catalase, TAC, total peroxide and malondialdehyde. The blood samples were collected from patients after brucellosis was identified. In the control group, the samples were collected from healty volunteers. At least two blood cultures were also drawn from each patient at diagnosis.

Catalase activity was measured using hydrogen peroxide (H_2O_2) as substrate [9]. The disappearance of H_2O_2 was followed at 240 nm, and enzyme activity was shown as *k*/mg protein (*k*: rate constant of a first-order reaction) at 25 °C. The total antioxidant status of the plasma was measured using a novel automated colorimetric measurement method for TAC developed by Erel [10]. The novel automated method is based on the bleaching of characteristic color of a more stable ABTS [2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation by antioxidants [11]. The assay has excellent precision values, which are lower than 3%. The results were expressed as mmol Trolox equivalent/L. Total peroxide concentrations of plasma were determined by using the FOX2 method with minor modifications [12,13]. The FOX2 test system is based on the oxidation of ferrous iron to ferric iron by the various types of peroxides contained in the plasma samples in the presence of xylenol orange which produces a colored ferric-xylenol orange complex whose absorbance can be measured. The percent ratio of the total peroxide to the TAC gave the OSI, an indicator of the degree of oxidative stress [13]. Collected data were subjected to statistical analysis using

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SPSS statistical package version 13.0. The data obtained from the study and control groups were compared using Student's t-test and chi-square test. Differences were considered statistically significant at $p<0.05$.

Results

Sixty-nine patients with brucellosis and 69 healthy control subjects were included in the present study. Mean age of patients and controls was 45,2±19,03 years and 43,5±16,6 years, respectively ($p>0.05$). Male-to-female ratio of patients and controls was 35/34 and 32/37, respectively ($p>0.05$). In 63 (91%) patients, at least one risk factor for brucellosis was identified: raw milk consumption (90%), close contact with animals (20%), home slaughtering of sheep or cattle (4%), or work in an abattoir (3%). Symptoms of patients were arthralgia (91%), sweating (87%), loss of appetite (86%), fever (83%), headache (36%), chills (26%), and abdominal pain (4%). Elevated erythrocyte sedimentation rate was determined in 94%, leukopenia in 30% and anaemia in 27% of the patients. Blood cultures were positives in 22% of cases.

As seen in Table 1, plasma levels of total peroxide and malondialdehyde were significantly increased in patients as compared with healthy controls ($p<0.001$ and $p<0.001$, respectively). In contrast, TAC level was significantly lower in patients as compared with controls ($p < 0.001$). There was no statistically significant difference between the catalase results of the two groups ($p>0.05$). OSI level was significantly increased in patients as compared with healty controls ($p<0.001$).

Discussion

In order to investigate the effect of brucellosis on oxidative status, we measured total antioxidant capacity (TAC), oxidative stress index, the oxidants (malondialdehyde and total peroxide) and the antioxidant (catalase). To the best of our knowledge, there is no prior study investigating these biomarkers of oxidation and antioxidant defense in patients with brucellosis. Reactive oxygen species (ROS) are produced during many metabolic and physiological processes. Organisms have several enzymatic and non-enzymatic antioxidant systems that overwhelm harmful effects of these ROS. Under certain conditions, antioxidants mechanisms are impaired and/or ROS are increased and antioxidant mechanisms may become insufficient to prevent oxidative damage completely. Consequently, oxidative stress develops [14,15].

Table 1. Plasma oxidative and antioxidative parameters of patients with brucellosis and healty controls.

Patients (n=69)	Healty	Controls (n=69)	p
Catalase (k/mg prot)	25.41 ±35.77	26.52 ±47.52	0.877
TAC (mmol Trolox Equiv/L)	0.62 ±0.30	1,08 ±0,29	<0.001
Total peroxide (μmol H_2O_2/L)	38.21 ±11.61	21.64 ±7.75	<0.001
OSI (arbitrary unit)	85.54 ±69.03	21.38 ±9.36	<0.001
Malondialdeyde (mmol/L)	12.36 ±4.75	5.91 ±4.08	<0.001

Values are mean ±SD, TAC: total antioxidant capacity; OSI: oxidative stress index.

Although the level of plasma antioxidant components can be measured separately, these measurements are time consuming, costly, unpractical and labour intensive [11]. It is known that, various antioxidants in plasma have additive effects on oxidative status, and the cooperation of the antioxidants prevents adverse effects of free radicals [16]. Measurement of individual antioxidant components may not accurately reflect the total antioxidant status [11], and measurement of TAC practically represents all of them and therefore can reflect the oxidative status of the organism [10,17]. Various measurement methods have been developed to measure total antioxidant status, but there is not yet an accepted reference method. We used a novel automated colorimetric measurement method for TAC developed by Erel [10]. The measurement of TAC by this assay is sensitive, reliable and specific [10]. In our study, we found that TAC level was significantly decreased in the patients with brucellosis compared to the healthy controls, indicating that the anti-oxidative defense of these patients was impaired. A possible mechanism for the decreased level of TAC could be that the radical-scavenging antioxidants were consumed by the increased free radical activity associated with *Brucella* infection. This opinion was supported by the fact that in the current study, in the patients with brucellosis, significant increases in the individual oxidants, total peroxide and malondialdehde, were detected compared to control subjects. Malondialdehde is produced from lipid peroxidation, and is one of the oxidative stress markers in plasma [18].

In the present study OSI was significantly higher in the patients than controls. OSI is the ratio of the total plasma peroxide level to TAC, and is an indicator of oxidative stress. It has been suggested that OSI may reflect the state of oxidative status more accurately than TAC [13,19].

In the current study, catalase level showed no difference between the patients and controls. Catalase serves as an intracellular antioxidant enzyme, and is a member of free radical and ROS scavenging system [20,21]. Melek et al. [22] using a rat model investigated the activity of catalase and malondialdehde in *Brucella* infection. Similar to our findins, in these mice, while *Brucella* infection significantly increased malondialdehde concentration in the plasma, brain, liver, and spleen, the pathogen did not affect catalase activities in any of specimens apart from in liver. In this study, catalase activity in the liver was increased at 30th day of post-infection and then declined to basal level at 45th day.

The oxidative killing by polymorphonuclear leukocytes and macrophages plays a primary role in the elimination of intracellular brucellae. The organism has ability to resist the

oxidative killing by several incompletely understood mechanisms and thereby can survive and multiply in the these phagocytic cells of the host [23]. Consequently, the ongoing inflammation characterized by activated neutrophils and macrophages is possibly associated to the high production of reactive oxygen species, as we have observed in the present study.

While there is no any report in the literature about TAC, OSI, total peroxide, malondialdehde and catalase in patients with brucellosis, in the several studies, similar to our findings increase in OSI, total peroxide and MDA and/or decrease in TAC have been reported in subjects with several conditions, including chronic hepatitis B infection [24], preeclampsia [25], exposure to passive smoking [26], cutaneous leishmaniasis [27], and chronic renal failure [21]. Ece et al. [21] found significantly lower catalase level in the children with chronic renal failure compared with the control group.

In conclusion, according to the data obtained from the present study, decreased TAC levels, and increased malondialdehde and total peroxide levels together with increased OSÝ showed that the patients with brucellosis infection were exposed to potent oxidative stress.

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