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Serum myeloperoxidase activity and oxidative stress in patients with acute brucellosis

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ABSTRACT

Objectives: The role of infection in the pathogenesis of atherosclerosis has been increasingly discussed. Previous studies have suggested that increased myeloperoxidase activity plays an important role in the pathogenesis of atherosclerosis. The aim of this study was to investigate the serum myeloperoxidase activity and catalase activity along with lipid hydroperoxide (LOOH) levels in patients with acute brucellosis.

Design and methods: Thirty-two patients with brucellosis and 33 healthy controls were enrolled. Serum myeloperoxidase activity, catalase activity and LOOH levels were determined.

Results: Serum myeloperoxidase activity and LOOH levels were significantly higher in patients with brucellosis than controls ($p<0.05$, $p<0.001$), while catalase activity were significantly lower ($p<0.001$). LOOH levels were found to be significantly positively correlated with MPO activity ($r=0.297$, $p=0.016$) in patients.

Conclusions: These results indicate that increased myeloperoxidase activity and decreased catalase activity is associated with increased oxidative stress, which may have a role in atherosclerotic processes in brucellosis patients.

Introduction

Brucellosis is a zoonotic disease widely distributed and remains a significant public health problem mainly in the developing world [1]. The vast majority of cases are attributed to the subtype Brucella melitensis [2]. Brucellosis is a small gram-negative and facultative intracellular pathogenic bacterium, which is able to surviving and replicating within the cells of the mononuclear phagocytic system [3]. Brucellosis is a systemic infection which may involve any organ or system of the body [1].

It has been demonstrated that in the many infectious diseases, a variety of inflammatory cells are activated, which lead to production of reactive oxygen species (ROS) to kill intra-cellular and extra-cellular parasites [4]. ROS are one of the crucial molecules that kill bacteria internalized into phagocytic cells, such as polymorph nuclear neutrophils (PMNs) and macrophages [5]. It has been suggested brucellosis is related to increased oxidative stress and antioxidant depletion. Thus, oxidative stress has been implicated in the pathogenesis of brucellosis [6].

Myeloperoxidase (MPO), which is released during inflammation, is an oxidative enzyme present in phagocytes. MPO may lead to irreversible protein and lipid modification, increasing levels of oxidized low density lipoprotein, and promoting atherogenesis. It is an antimicrobial enzyme found in neutrophils and PMNs [7]. Also, MPO could be a key element for oxidative damage in the human artery wall. MPO is a heme enzyme that uses the oxidizing potential of superoxide and hydrogen peroxide to convert chloride ion to hypochlorous acid (HOCl) and other ROS [8]. MPO and it's the products of its activity have been suggested in atherosclerotic lesions at various stages of severity [9,10]. It has been shown that persons with MPO deficiency had a significantly reduced risk of cardiovascular disease (CVD) [11]. Elevated blood MPO levels have been associated with increased coronary artery disease (CAD) incidence [12,13]. MPO has also been shown to be a strong predict the early risk of myocardial infarction [14].

Oxidative stress and lipid peroxidation products in acute brucella infection has not been fully established. Also, the effect of brucellosis on atherosclerosis is currently well unknown. On the other hand, Apostolou et al. [15] assessed atherogenic lipid profile after treatment of acute infection with brucella. Moreover, effects of oxidant and antioxidant status in patients with acute brucellosis before and after therapy have been evaluated in a human study [16]. To our knowledge, there is limited information in the literature about the oxidative stress

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along with catalase activity in patients with acute brucellosis [6,17]. However, serum MPO activity and catalase activity along with oxidative stress in patients with acute brucellosis have not been reported yet. Therefore, the aim of this study was to investigate serum MPO activity, catalase activity and lipid hydroperoxide (LOOH) levels which is an important biomarker in pathogenesis of atherosclerosis in patients with acute brucellosis.

Methods

Subjects

Thirty-two patients with brucellosis (17 males and 15 females) and 33 healthy controls (17 males and 16 females) were enrolled in the present study.

The acute Brucella infection diagnosis was established by the presence of specific IgM antibodies against Brucella as determined by ELISA. 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 symptoms and signs [2]. The patients with acute brucellosis did not receive any treatment prior to the study.

The control group consisted of 33 healthy subjects without history of chronic and recurrent diseases. The subjects in the control group were asymptomatic with an unremarkable medical history and normal physical examination. None of the control subjects were receiving antioxidant vitamin supplements such as vitamin E or C.

The study protocol was carried out in accordance with the Helsinki Declaration as revised in 2000 and approved by the local ethics committee. All subjects were informed about the study, and written consent was obtained from each subject.

Exclusion criteria

The exclusion criteria included a history of alcohol abuse, habitual smoking, intravenous drug abuse, pregnancy, the use of antioxidant supplements, hypertension, diabetes mellitus, liver or renal disease, rheumatoid arthritis and pulmonary disease. None of acute brucellosis patients was receiving a special diet.

Blood samples

Blood samples were obtained following an overnight fasting period before treatment. Blood samples were collected into empty tubes and immediately stored on ice at 4 °C. The serum samples were then separated from the cells by centrifugation at 3000 rpm for 10 min. The serum were stored in plastic tubes at −80 °C and were used for analyzing MPO activity, catalase activity and LOOH levels.

Measurement of lipid hydroperoxide levels

Serum LOOH levels were measured with the ferrous ion oxidation–xylenol orange assay [18]. 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.

Measurement of catalase activity

Catalase activity was measured using hydrogen peroxide (H_2O_2) as substrate [19]. The disappearance of H_2O_2 was followed at 240 nm, and enzyme activity was expressed in units per liter of serum (U/L) at 25 °C.

Measurement of myeloperoxidase activity

Serum MPO activity was determined by the method of Klebanoff and Clark [20] and was based on kinetic measurement of the formation rate of the yellowish-orange product of the oxidation of o-dianisidne with myeloperoxidase in the presence of hydrogen peroxide (H_2O_2) at 460 nm. One unit of myeloperoxidase was defined as that degrading 1 μmol of H2O2 per minute at 25 °C. A molar extinction coefficient of $1.3\times104\ M^{-1}\ cm^{-1}$ of oxidized 0-dianisidine was used for the calculation. Myeloperoxidase activity was expressed in units per liter of serum (U/L).

Statistical analysis

The results are expressed as the mean ± standard deviation. The comparisons of parameters of acute brucellosis and healthy controls were performed using Student's *t*-test. The correlation analyses were performed using Pearson's correlation test. The results were considered statistically significant when p value was less than 0.05. The data were analyzed using the SPSS® for Windows (Version 11,0).

Results

The demographic characteristics of the acute brucellosis and control subjects are presented in Table 1. There were no significant differences between the groups in age, gender and body mass index ($p>0.05$) (Table 1).

Serum MPO activity and LOOH levels were significantly higher in the acute brucellosis than controls ($p<0.05$, $p<0.001$), while catalase levels were significantly lower ($p<0.001$) (Table 2).

Catalase activity was found to be significantly negatively correlated with MPO activity ($r=-0.330$, $p=0.007$) and LOOH levels ($r=-0.421$, $p=0.002$). Serum LOOH levels were also found to be significantly positively correlated with MPO activity ($r=0.297$, $p=0.016$).

No significant correlation was found between serum agglutination test with titers, and MPO activity, catalase activity and LOOH levels ($p>0.05$) in patients with acute brucellosis.

The number of white blood cells were higher in patients with acute brucellosis than controls (8087 ± 4992 in patients, 6992 ± 2619 in controls; respectively), but this was not statistically significant ($p>0.05$).

Linear regression analysis was performed to identify factors that appeared to exert some independent influence on MPO levels. As independent variables, catalase activity, LOOH levels, the number of white blood cells and age were included. However, no effect of the variables on MPO activity was observed.

Discussion

In the present study, we investigated serum MPO activity, catalase activity and LOOH levels in patients with acute brucellosis. We found that brucellosis patients had increased MPO activity and LOOH levels compared with healthy subjects. Additionally, we observed that serum catalase activity was significantly lower in patients with acute brucellosis than controls. To our knowledge, this study shows for the first time that acute brucellosis is associated with increased

Table 1
Demographic characteristics in patients with acute brucellosis and healthy controls.

Parameters	Patients (n = 32)	Controls (n = 33)	p
Age (years)	38.2 ± 4.1	37.1 ± 2.5	ns
Sex (female/ male)	17/15	17/16	ns
Body mass index (kg/m^2)	22.1 ± 0.3	21.2 ± 1.1	ns

Values are mean ± SD.
ns = non significant.