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Original Article

Impaired Thiol-Disulfide Balance in Acute Brucellosis

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SUMMARY: The objective of this study was to examine a novel profile: thiol-disulfide homeostasis in acute brucellosis. The study included 90 patients with acute brucellosis, and 27 healthy controls. Thiol-disulfide profile tests were analyzed by a recently developed method, and ceruloplasmin levels were determined. Native thiol levels were 256.72 ± 48.20 μmol/L in the acute brucellosis group and 461.13 ± 45.37 μmol/L in the healthy group, and total thiol levels were 298.58 ± 51.78 μmol/L in the acute brucellosis group and 504.83 ± 51.05 μmol/L in the healthy group ($p < 0.001$, for both). The disulfide/native thiol ratios and disulfide/total thiol ratios were significantly higher, and native thiol/total thiol ratios were significantly lower in patients with acute brucellosis than in the healthy controls ($p < 0.001$, for all ratios). There were either positive or negative relationships between ceruloplasmin levels and thiol-disulfide parameters. The thiol-disulfide homeostasis was impaired in acute brucellosis. The strong associations between thiol-disulfide parameters and a positive acute-phase reactant reflected the disruption of the balance between the antioxidant and oxidant systems. Since thiol groups act as anti-inflammatory mediators, the alteration in the thiol-disulfide homeostasis may be involved in brucellosis.

INTRODUCTION

Brucellosis is the world's most common zoonotic disease, affecting up to half a million people yearly (1). Brucellosis is a serious public health problem, and it disturbs multiple organ systems, such as the gastrointestinal, hematologic, cardiovascular, and nervous systems (2,3). *Brucella* bacteria are Gram-negative, facultative, and intracellular. *Brucella* spp. have the ability to live and reproduce in macrophages (4). When the pathogen bacteria penetrate the host cells, it avoids killing mechanisms and prevents the apoptosis of macrophages and the phagosome-lysosome fusion (5). Thus, *Brucella* spp. inhibit the progression of natural, specific immunity during the initiation of infection, which preserves the pathogen from bactericidal activities of the immune system (6).

There is increasing evidence that activation of inflammatory cells in infection leads to the generation of reactive oxygen and nitrogen species (7). These reactive products are the main cause of tissue damage through inflammation (8). Oxidative killing is one of the significant mechanisms of the intracellular proliferation of *Brucella* pathogens in host macrophages (9). Oxidant-antioxidant molecules, such as superoxide dismutase, catalase, myeloperoxidase, and nitric oxide have a crucial role in the survival and pathogenicity of *Brucella* spp., and they are also associated with the bacterial defense system in brucellosis (7,10,11).

It has been suggested that the presence of inflammatory cytokines and oxidative stress reveal similarities (12). For example, proteins that include sulfhydryl groups and glutathione are accepted as endogenous anti-inflammatory mediators against the reactive oxygen species produced by activation of NADPH oxidase within polymorph nuclear neutrophils in inflammation and tissue damage (13). Thiol antioxidants such as glutathione and N-acetylcysteine inhibit cytokine production (14). Moreover, depletion of reduced glutathione (GSH) levels results in enhanced sensitivity to infection, inflammation, or impaired immunity (15).

Albumin, other proteins, and certain molecules, such as glutathione, homocysteine, and thioredoxin, consist of thiols, which are functional –SH groups (16). Thiols can go through oxidation reactions and turn into their reversible forms called disulfide bonds (–S–S–), and these bonds can then be converted to thiol groups again (17). Thereby, thiol-disulfide homeostasis occurs. Astatic thiol-disulfide balance occupies a crucial point in

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antioxidant defense, inflammation, immune response, apoptosis, and intracellular signaling mechanisms (18).

Ceruloplasmin, an abundant plasma glycoprotein, has multiple functions in the regulation of many biochemical processes, and it also participates in scavenging radicals, angiogenesis, and the inflammatory response (19). Because ceruloplasmin is a positive acute-phase reactant, its plasma concentration is elevated during various processes such as infection, inflammation, tissue injury, and malignancies (20).

The role and importance of thiol-disulfide homeostasis have been reported in several infectious diseases such as Crimean-Congo hemorrhagic fever and acute tonsillopharyngitis (21,22). Altered thiol-disulfide balances were observed in both studies. The aim of this study was to determine a novel profile—the thiol-disulfide balance—in brucellosis and to examine the association between the thiol-disulfide parameters and ceruloplasmin levels. Until now, thiol-disulfide homeostasis has been scarcely investigated in brucellosis, and this study constitutes the first report for ceruloplasmin levels in brucellosis in Turkey.

MATERIALS AND METHODS

Study design: A total of 90 patients (51 men, 39 women) with acute brucellosis and 27 healthy controls (12 men, 15 women) were included in the study. Diagnosis of acute brucellosis was based on laboratory and clinical findings and was determined in the following ways: by a 1/160 or higher titer in a standard tube agglutination (STA) test; by a 4-fold increase in titers between 2 STA tests performed 2 weeks apart; by one of the blood tests in addition to the presence of clinical symptoms (arthralgia, fever, sweating, chills, headache, and malaise) within the last 8 weeks; and/or by the growth of *Brucella* spp. in appropriately prepared culture media (23).

Patients who had a previous diagnosis of brucellosis, were undergoing treatment for brucellosis, had pre-existing systemic, infectious, or inflammatory diseases (such as diabetes mellitus, anemia, hypertension, liver or renal disease, coronary artery disease, rheumatoid arthritis, or pulmonary disease), current smokers, or who were abusing alcohol, were excluded. The control group consisted of healthy subjects who had normal physical examinations and routine clinical laboratory tests. No patients or controls were taking vitamin supplements.

The study protocol was approved by the local ethics committee (Clinical Research Ethics Committee of Yildirim Beyazit University Faculty of Medicine, approval date and No. was 21/10/2015-211), and written informed consent was received from all subjects involved in the study.

Venous blood samples were obtained from all participants following an overnight fasting period. The sera were separated from the cells by centrifugation at 1,800 × *g* for 10 min and stored at −80°C until analyses were performed.

Serum thiol-disulfide profile tests were performed using a novel automated method with an automated analyzer (Cobas 501, Roche, Mannheim, Germany) (24). The native and total thiol amounts were measured as a paired test. The half value of the difference between total and native thiol concentrations gave the disulfide amounts. The disulfide/native thiol ([–S–S–] / [–SH]), disulfide/total thiol ([–S–S–] / [–SH + –S–S–]), and native thiol/total thiol ([–SH] / [–SH + –S–S–]) ratios were also calculated.

Ceruloplasmin levels were measured by the method described by Erel (25). Total protein and albumin levels were determined with commercially available assay kits (Roche) with an auto-analyzer (Cobas 501).

Statistical analysis: Distributions of data were evaluated with the Kolmogorov-Smirnov test and the Shapiro-Wilk test. Values were presented using mean and standard deviation for the normally distributed variables. Independent sample *t*-tests were used for comparison between groups. Pearson's correlation coefficients were calculated to examine the relationships between parameters, and a scatter plot graph was created for visual representation. In all analyses, a p-value < 0.05 was considered statistically significant. SPSS software ver. 22.0 was used for statistical calculations (Chicago, IL, USA).

RESULTS

Ninety patients with acute brucellosis and 27 healthy controls were enrolled in the study. The demographic and clinical characteristics of subjects are shown in Table 1. No statistically significant differences were observed in age and sex between groups. Serum albumin

Table 1. Clinical characteristics of the study groups

	Brucellosis (n = 90)	Control group (n = 27)	p-value[1)]
Age (yr)	44.14 ± 13.63[2)]	39.18 ± 12.02	NS
Sex (man/woman)	51/39	12/15	NS
Albumin (g/dL)	4.45 ± 0.35	4.54 ± 0.29	NS
Total protein (g/dL)	7.49 ± 0.44	7.31 ± 0.40	NS
Ceruloplasmin (U/L)	197.75 ± 34.47	146.70 ± 21.47	p < 0.001

1): p < 0.05 was accepted as statistically significant.
2): Values are mean ± SD.
NS, not significant.

Table 2. Thiol-disulfide parameters of subjects

Parameter	Brucellosis (n = 90)	Control group (n = 27)	p-value[1)]
Native thiol, μmol/L	256.72 ± 48.20[2)]	461.13 ± 45.37	< 0.001
Total thiol, μmol/L	298.58 ± 51.78	504.83 ± 51.05	< 0.001
Disulfide, μmol/L	20.93 ± 5.34	21.87 ± 5.96	≥ 0.05
Disulfide/Native thiol, %	8.37 ± 2.42	4.73 ± 1.24	< 0.001
Disulfide/Total thiol, %	7.10 ± 1.74	4.30 ± 1.03	< 0.001
Native thiol/Total thiol, %	85.79 ± 3.48	91.38 ± 2.07	< 0.001

1): p-value < 0.05 was accepted as statistically significant.
2): Values are mean ± SD.

Table 3. The relationship between thiol-disulfide profiles and ceruloplasmin levels

Ceruloplasmin (n = 117)	Native thiol	Total thiol	Disulfide	Disulfide /Native thiol	Disulfide /Total thiol	Native thiol /Total thiol
r-value[1)]	–0.590	–0.572	0.020	0.496	0.503	–0.503
p-value[2)]	< 0.001	< 0.001	≥ 0.05	< 0.001	< 0.001	< 0.001

1): The r-value is the Pearson's correlation coefficient.
2): The p-value is the probability using a given statistical model.

levels were lower in the acute brucellosis group than the control group, but this was not statistically significant ($p \geq 0.05$); the total protein levels were higher in the patient group than the healthy group ($p \geq 0.05$). Serum ceruloplasmin levels of the acute brucellosis group were higher than those of the control group ($p < 0.001$).

Table 2 describes the thiol-disulfide parameters for all participants. In the acute brucellosis study group, native thiol levels were significantly lower than that of the healthy controls ($p < 0.001$). When the 2 groups were compared based upon the disulfide levels, there was no statistically significant difference between the groups ($p \geq 0.05$). Serum disulfide levels were similar in both the acute brucellosis and the control groups. The acute brucellosis group had significantly lower the total thiol levels than the control groups ($p < 0.001$). In addition, the disulfide/native thiol ratios and disulfide/total thiol ratios were significantly higher, and native/total thiol ratios were significantly lower in patients with acute brucellosis than in the healthy controls ($p < 0.001$, for all ratios).

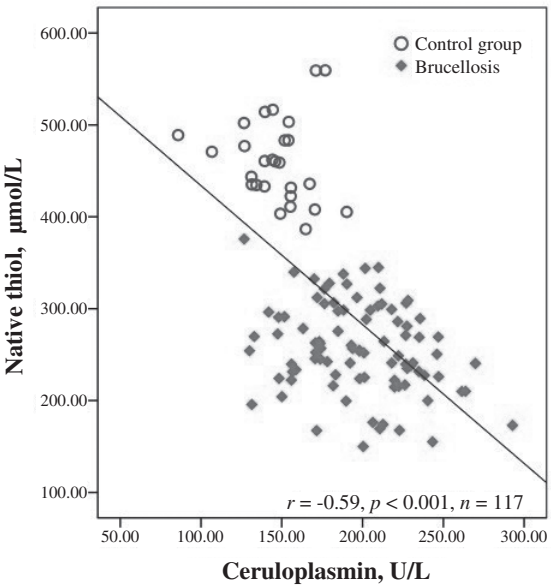

Fig. 1. The association of native thiol and ceruloplasmin levels.

As seen in Table 3 and Fig. 1, the relationships between ceruloplasmin and thiol-disulfide parameters were evaluated. Serum native and total thiol levels inversely correlated with ceruloplasmin levels (r = –0.590, $p < 0.001$; r = –0.572, $p < 0.001$, respectively). However, no correlation was found between disulfide and ceruloplasmin levels ($p \geq 0.05$). There were positive correlations between serum ceruloplasmin levels and disulfide/native thiol ratios and disulfide/total thiol ratios (r = 0.496, $p < 0.001$; r = 0.503, $p < 0.001$). In addition, a significant negative correlation was found between native/total thiol ratios and ceruloplasmin levels (r = –0.503, $p < 0.001$).

DISCUSSION

Thiol groups of proteins are not just antioxidant buffers; they also regulate the redox system (17). The thiol-disulfide balance is a significant member of various processes consisting of antioxidant defense, immune response, modulation of enzyme activity, and apoptosis (26). Dynamic thiol-disulfide homeostasis is implicated in several disorders (27,28). For example, it has been demonstrated that impaired thiol-disulfide balance has been involved in a variety of diseases such as myocardial infarction, preeclampsia, polycystic ovary syndrome, diabetes mellitus, and cancer (29).

Thiol-disulfide homeostasis has also been evaluated in various infectious diseases such as Crimean-Congo hemorrhagic fever and acute tonsillopharyngitis (21,22). Thiol-disulfide balances were found to be shifted to the oxidative side in both studies (21,22). There were positive or negative relationships between thiol-disulfide

tests and some other well-known oxidant, and antioxidant parameters, including total antioxidant status, total oxidant status, paraoxonase, arylesterase, and ceruloplasmin in Crimean-Congo hemorrhagic fever (21). In acute tonsillopharyngitis, C-reactive protein values and white blood cell counts were negatively correlated with the native and total thiol levels (22).

Brucellosis is a systematic inflammatory illness that can act upon any system in the organism (2). Until now, there has been no report evaluating thiol-disulfide homeostasis in acute brucellosis. As can be seen in Table 2, native and total thiol levels were lower in the brucellosis patients than in the healthy controls, while disulfide amounts were similar in both groups. Research in oxidative stress has shown that the measured thiol and disulfide values are not wholly consistent with the expected values for thiol oxidation to disulfide (30). For example, a decrease in thiol without a corresponding increase in disulfide, or an increase in disulfide with no decrease in thiol can be observed. In this situation, evaluating the thiol/disulfide ratios instead of the thiol and disulfide concentrations led to conclusions implicating oxidative stress (30).

Despite the fact that the disulfide levels were not different between the 2 groups, the disulfide/native thiol ratios, and the disulfide/total thiol ratios were higher, and native/total thiol ratios were lower in the brucellosis group compared to the control group (Table 2). These results indicated that thiol-disulfide homeostasis was impaired in acute brucellosis. Several experimental studies have reported that alteration of the thiol/disulfide ratios leads to modifications in cellular status (18). Additionally, it has been demonstrated that an increase in the GSH/oxidized glutathione (GSSG) redox status causes proliferation, while a decrease in the GSH/GSSG redox status leads to apoptosis (31). Therefore, prevention of apoptosis of macrophages by *Brucella* pathogens supports the theory that *Brucella* pathogens lead to depletion in the cellular immune response.

Ceruloplasmin, a positive acute-phase reactant, takes part in the immune system response (20). Additionally, it plays a protective role against oxygen radicals (19). In this study there were strong inverse relationships between native and total thiols and ceruloplasmin levels (Table 3 and Fig. 1). Statistically significant positive correlations were also observed between disulfide/native thiol ratios and disulfide/total thiol ratios, and ceruloplasmin levels (Table 3). Similarly, Demirpence et al. found a relationship between ceruloplasmin levels and total oxidant status (32). In line with these outcomes, it is exciting and encouraging to consider thiols as a negative acute-phase reactant. The positive and negative relationships between thiol-disulfide profiles and ceruloplasmin levels indicate the disruption of the balance between the antioxidant and oxidant systems.

Serefhanoglu et al., Karahocagil et al., and Esen et al. previously demonstrated an oxidant-antioxidant imbalance in *Brucella* infection (33–35). These researchers found diminished antioxidant status and an elevated oxidative state in brucellosis. Karaagac et al. evaluated oxidative stress before and after therapy in *Brucella* infection (36). They also found increased oxidant capacity and decreased antioxidant parameters before therapy, and they obtained increased antioxidant levels after treatment (36). Therefore, it is well known that brucellosis is involved in enhanced free radical production and a depleted antioxidant system (10, 33–36). Oxidative burst and oxidative killing in macrophages have a significant and primary role in the control of *Brucella* pathogens (9). Consequently, inflammation is associated with an imbalance between antioxidant and oxidant systems in *Brucella* infection.

The thiol side-chain of cysteine is easily oxidized to form disulfide bonds (17). According to the capacity of thiols to undergo redox reactions, cysteine has antioxidant characteristics (37). Furthermore, cysteine is associated with the synthesis of glutathione and taurine, which have a significant role in the defense system against an oxidative state (38). Cysteine has been reported to show anti-inflammatory effects (14). Drugs that contain cysteine have been used to cure intestinal inflammation and have created promising outcomes (13). In a recent study, the free amino acid form, L-cysteine, was deployed against inflammatory bowel disease (37).

In conclusion, the balance between thiol-disulfide pairs is impaired in acute brucellosis. Depleted native and total thiol serum levels may reflect their decreasing effects against inflammation and oxidative stress. The elevated disulfide/native thiol ratios and disulfide/total thiol ratios, and diminished native/total thiol ratios exhibit enhanced oxidation in brucellosis. Moreover, there is a strong association between thiol-disulfide parameters and ceruloplasmin, which is a positive acute-phase reactant. Supplementation with thiol-containing compounds can be discussed. Restoration of the thiol-disulfide homeostasis may have potential benefits in the inflammatory response and may provide new approaches to therapy in *Brucella* infection.

Conflict of interest None to declare.

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