Thiol/disulphide balance and ischemia modified albumin levels in relapsed brucellosis patients

Mehtap Hülya Aslan1, Ömer Karaşahin2, Sibel İba Yılmaz2, Ali Kurt1, Pervin Baran4, Cemile Koca Biçer4

1Department of Clinical Microbiology, Erzurum Regional Training and Research Hospital, Erzurum, Turkey
2Department of Infectious Diseases and Clinical Microbiology, Erzurum Regional Training and Research Hospital, Erzurum, Turkey
3Department of Clinical Pathology, Erzurum Regional Training and Research Hospital, Erzurum, Turkey
4Department of Clinical Biochemistry, Yıldırım Beyazit University Faculty of Medicine, Ankara, Turkey

ABSTRACT

Objectives: In the study, it was aimed to determine the Thiol/Disulphide profile in patients with relapsed brucellosis and the relationship between Ischemia Modified Albumin (IMA) Levels and Thiol/Disulphide profile.

Methods: Native thiol, Total thiol, Disulphide, Disulphide/Native thiol, Disulphide/Total thiol, IMA levels were measured in forty patients with relapsed brucellosis and healthy control group by using the newly developed method in this cross-sectional study.

Results: There was no statistically significant difference in patients with relapse brucellosis despite the fact that it was lower than total thiol control group (p > 0.05). Disulphide was detected high in patients with brucellosis but no statistically significant difference was found (p > 0.05). Native thiol and total thiol ratios of disulphide were found to be statistically higher in patients with relapsed brucellosis (p < 0.05). In addition, the ratio of native thiol and native thiol to total thiol was statistically lower than the control group (p < 0.05). The levels of IMA were statistically significant in patients with relapsed brucellosis compared to the control group (p < 0.05). There was a statistically significant positive correlation between IMA values and Disulphide and Disulphide/Native thiol, Disulphide/Total thiol, Native thiol/Total thiol ratios (r = 0.514, r = 0.527, r = 0.527, r = 0.527; respectively).

Conclusions: It is known that the response of brucellosis treatment can be followed up with oxidative stress markers and it can also be used as a relapse indicator in our study.

Keywords: Relapse brucellosis, thiol/disulphide, ischemia modified albumin

Brucellosis is a zoonotic infectious disease that affects half a million people annually and is considered one of the most important problems for public health [1]. Brucella species can grow and survive in macrophages [2]. Oxidative explosion in host macrophages is the primary mechanism controlling intracellular replication of brucella. In addition, inhibitors of reactive oxygen species have been reported to reduce anti-Brucella activity of macrophages [3]. Therefore, oxidant-antioxidant molecules have been reported to have a very significant role in Brucella endurance and pathogenesis. It is reported that brucellosis may be associated with production of oxygen radicals and the emergence of antioxidant depletion,
and that oxidative stress plays a role in the patogenesis of brucellosis [4].

Thiols are mercaptans based sulfhydryl residues that play a significant role in the coordination defense network [4]. In the case of oxidative stress, with the oxidation reaction of cysteine residues, thiols oxides and reversible disulfide bonds are formed. Disulphide bond can be reduced again to thiol group. In this way, thiol-disulphide homeostasis occurs [4]. In various diseases such as cerebral ischemia, pulmonary thromboembolism, coronary artery disease, preeclampsia, polycystic over syndrome, celiac disease, impaired thiol disulfide balance has been demonstrated [5-8]. Inflammatory cells which get activated through the production of reactive oxygen and nitrogen compounds fight against microbial pathogens that are considered to be causative agents a variety of infectious diseases [9]. For this reason, Thiol-Disulphide homeostasis was evaluated in infectious diseases such as Crimean Kongo Hemorrhagic Fever and tonsillopharyngitis, and a shift to oxidative side of balance was indentified [10]. The ischemia modified albumin (IMA) results from the modification of cobalt binding sites from the N-terminal end of albumin with the emergence of free radicals in ischemic tissue [11]. This new albumin molecule, which has lost its ability to bind cobalt, has been reported to be an early marker of ischemia. Furthermore, the use of IMA as a marker for sepsis and community-acquired pneumonia associated with oxidative stress and ischemic damage has been reported in the literature [12, 13].

In our study, it was aimed to determine the relationship between thiol-disulphide profile and IMA use as an oxidative stress indicator in patients with relapsed brucellosis.

METHODS

Study Population

This cross-sectional coverage consisted of 40 patients with relapsing disease and 40 healthy individuals who had been diagnosed with brucellosis within the last 6 months who applied to infectious diseases polyclinic. The age and gender of the control group were in agreement with the patient group. Those with known infections, inflammatory or chronic diseases and those who smoke or drink alcohol were excluded.

RESULTS

Forty brucellosis patients and 40 healthy individuals were included in the study. There was no statistically significant difference between the age of the patients included in patient and control group. Demo-
graphic and laboratory data between the two groups were presented in Table 1. Relapsing brucellosis patients were found to have a significantly higher sedimentation value than healthy control group. No statistically significant difference was found in patients with brucellosis despite the low detection rate compared to the total thiol control group ($p > 0.05$). Disulphide was found high in patients with brucellosis.

### Table 1. Demographic characteristics and laboratory parameters of the study groups

<table>
<thead>
<tr>
<th></th>
<th>Brucellosis (n = 40)</th>
<th>Control group (n = 40)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year), median (min-max)</td>
<td>43 (16-75)</td>
<td>39.5 (18-74)</td>
<td>0.707</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>19/21</td>
<td>15/25</td>
<td>0.131</td>
</tr>
<tr>
<td>CRP (mg/dL), median (min-max)</td>
<td>0.55 (0.20-16.00)</td>
<td>0.32 (0.32-1.98)</td>
<td>0.107</td>
</tr>
<tr>
<td>Sedimentation (mm/h), median (min-max)</td>
<td>14.0 (2.0-41.0)</td>
<td>5.5 (2.0-38.0)</td>
<td><strong>&lt; 0.001</strong></td>
</tr>
<tr>
<td>Leukocyte count ($\times 10^3$/L), median (min-max)</td>
<td>6.65 (3.08-22.40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet count ($\times 10^3$/L), median (min-max)</td>
<td>259 (95-537)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (U/L), median (min-max)</td>
<td>27.5 (10.0-133.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (U/L), median (min-max)</td>
<td>24.5 (12.0-85.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL), median (min-max)</td>
<td>0.73 (0.50-1.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL), median (min-max)</td>
<td>14.00 (9.40-18.10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous treatment, n (%)</td>
<td>Rifampicin+doxycycline 37 (92.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rifampicin+doxycycline+ aminoglycoside 1 (2.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Doxycycline+ quinolones 2 (5.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CRP = C-reactive protein, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase

### Table 2. Thiol/disulfide profiles and ischemia-modified albumin levels of the study group

<table>
<thead>
<tr>
<th></th>
<th>Brucellosis (n = 40)</th>
<th>Control group (n = 40)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMA</td>
<td>78.05 (71.80-90.50)</td>
<td>75.90 (68.50-83.30)</td>
<td><strong>0.003</strong></td>
</tr>
<tr>
<td>Native thiol ($\mu$mol/L)</td>
<td>280.95 (126.70-414.00)</td>
<td>322.05 (211.50-390.90)</td>
<td><strong>0.028</strong></td>
</tr>
<tr>
<td>Total thiol ($\mu$mol/L)</td>
<td>332.35 (152.70-496.10)</td>
<td>347.45 (232.10-437.50)</td>
<td>0.214</td>
</tr>
<tr>
<td>Disulphide ($\mu$mol/L)</td>
<td>20.75 (0.80-43.60)</td>
<td>13.50 (0.35-30.85)</td>
<td>0.066</td>
</tr>
<tr>
<td>Disulphide/Nativethiol (%)</td>
<td>7.26 (0.22-15.96)</td>
<td>4.85 (0.14-10.58)</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td>Disulphide/Total thiol (%)</td>
<td>6.34 (0.22-12.10)</td>
<td>4.42 (0.14-8.74)</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td>Native thiol/Total thiol (%)</td>
<td>87.30 (75.80-99.56)</td>
<td>91.15 (82.53-99.72)</td>
<td><strong>0.009</strong></td>
</tr>
</tbody>
</table>

Data were expressed as median (minimum – maximum), IMA = Ischemia modified albumin,
cellosis but no statistically significant difference was detected \((p > 0.05)\). The native thiol and total thiol ratios of disulphide were statistically higher in patients with relapsed brucellosis \((p < 0.05)\). In addition, the ratio of total thiole to native thiol and native thiol was statistically lower than the control group \((p < 0.05)\).

IMA levels were found statistically higher in patients with brucellosis than control group \((p < 0.05)\) (Table 2). There was a statistically significant positive correlation between IMA value and Disulphide and Disulphide/Native Thiol, Disulphide / Total thiol, Native thiol/ Total thiol ratios \((p < 0.05)\) (Table 3).

### DISCUSSION

Relaps of brucellosis occurs as an important problem during the treatment. Cellular immunity and treatments provided bacterial elimination; however, chronic and relapsed infections were seen due to the presence of intracellular microorganisms. Previously, *Brucella* –PCR was used for the detection of *Brucella* relapse [16]. In our study, it was aimed to determine the thiol/ disulphide profile in patients with relapsed brucellosis and, at the same time to determine the relationship between IMA levels and thiol/ disulfide profile. The mechanism by which *Brucella* saved itself without being killed in an intercellular fashion was not fully understood. However, it was reported that the main mechanism that controls intracellular replication was the oxidative explosion in host macrophages [3]. The endogenous reactive oxygen compounds of aerobic respiratory metabolism against *Brucella* strains have been shown to be exogenously produced for both endogenous and anti-*Brucella* activities of macrophages [3]. Reactive oxygen compounds responsible for oxidative stress was elevated in inflammation leading to cell damage via peroxidation of double –chain fatty, protein and DNA[17]. It was reported that this condition is responsible for the pathogenesis of diseases through accompanying the primary disease [18]. There were studies showing that oxidative stress plays a role in the pathogenesis of various bacterial infectious diseases such as Hepatitis C, influenza, HIV, Sepsis, Crimean Congo Hemorrhagic fever, as well as non-infectious diseases [19-23]. In these cases, oxidant capacity increased and deterioration was observed in antioxidants [19]. In support of this situation, serum antioxidant activity indicators such as total peroxide malondialdehyde, paroxonase and arylesterase in osteomyelitis, tuberculosis, brucellosis and Hepatitis B infections were significantly lower than those in healthy individuals, as shown in literature [4]. Also in previous studies, reduced total antioxidant capacity in *Brucella* infection has been explained by the depletion of antioxidants as an elevated free radical scavenger [4].

Thiol groups were the main antioxidant components of serum and prevented tissue damage by reacting and neutralizing these molecules with free oxygen radicals and lipid peroxides [10]. Thiols can enter oxidation reactions, convert to the inverse forms called disulfide bonds (\(-S-S\)), which can then be converted to thiol groups. Thus thiol-disulfide homeostasis occurs [24]. In a study of thiol-disulfide balance in acute brucellosis, it was reported that an increase in disulfide could be observed without a corresponding decrease in thiol or a decrease in thiol without a corresponding increase in disulfide. In this case, when the ratio of disulfide / thiol was evaluated, it was stated that the healthiest results regarding oxidative stress can be achieved [25]. In our study, the disulphide-total thiol and disulphide/native thiol ratios were found to be statistically higher than those of healthy individuals as Kolgelier et al. [25] found in patients with acute brucellosis. As a result, increased oxidative stress can also be found in relapsed brucellosis patients.

Karaağaç et al. [26] found that after *Brucella* in-

<p>| Table 3. Relations between thiol-disulphide profiles and ischemia-modified albumin levels |
|----------------------------------|---------------------------------|----------------|----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Native thiol</th>
<th>Total Thiol</th>
<th>Disulphide</th>
<th>Disulphide/Native thiol</th>
<th>Disulphide/Total thiol</th>
<th>Native thiol/Total thiol</th>
<th>Sedimentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMA</td>
<td>0.189</td>
<td>-0.65</td>
<td>0.514</td>
<td>0.527</td>
<td>0.527</td>
<td>0.527</td>
</tr>
<tr>
<td>(n = 80)</td>
<td>0.012</td>
<td>0.692</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.123</td>
</tr>
</tbody>
</table>

IMA = Ischemia modified albumin
fection treatment, oxidant capacity and antioxidant parameters reduced, and antioxidant levels increased. In this study, it has been reported that oxidant-antioxidant balance, which can be achieved by treatment with oxidative stress, may contribute to treatment outcome [26]. In our study, the high ratio of disulfide/thiol suggests that it may contribute to follow-up treatment in terms of relapse development undertreatment.

Oxidative stress-induced conformational change in the N-terminal end of albumin, metabolic ions such as cobalt and copper which tie with the N-terminal end fail due to conformational change and was called altered albumin-shaped ischemic modified [12]. IMA was a newly developed marker that increases in ischemic and oxidative stress-related pathological conditions such as myocardial infarction, pulmonary embolism, COPD and cerebrovascular event [27]. In inflammatory diseases, high IMA levels have been shown to be indicative of oxidative stress, together with inflammation and oxidative stress associated with the pathogenesis of these diseases [28]. Furthermore, the use of IMA as a marker in infectious diseases such as sepsis and community-acquired pneumonia associated with oxidative stress and ischemic damage has been reported in the literature. In these diseases, it has been recognized as an important marker of ischemic damage in tissues and organs, helping to identify and quantify oxidative stress [13]. In our study, IMA value was statistically found to be significantly higher in patients with relapsed brucellosis than in healthy subjects. In addition, a moderately strong positive correlation was detected between disulfide/thiol ratios. This moderately strong relationship was used control the accuracy of parameters used as oxidative stress indicators in cases of relapsing brucellosis.

Limitations

Limitation in our study was firstly not compared with other oxidative stress markers used as an indicator of oxidative stress. Another limitation is that it was performed with a small number of patients.

CONCLUSION

Thiol/disulphide balance, previously balance, previously studied in acute brucellosis, has also been shown to be oxidative in our study of relapsing Brucella cases. IMA was higher in the study group than in the control group and correlation with disulphide/thiol ratios was also considered as a sign of increased oxidative stress in relapsed brucellosis cases. Previous studies have shown that brucellosis treatment response can be followed up with oxidative stress markers and in our study; it has been shown that it can also be used as a relapse indicator.

Authors’ Contribution

Study Conception: MHA, CKB, AK; Study Design: ÖK, SIY, PB; Supervision: MHA, ÖK, AK; Funding: CKB, PB, MHA; Materials: ÖK, SIY, MHA; Data Collection and/or Processing: CKB, ÖK, PB; Statistical Analysis and/or Data Interpretation: ÖK, CKB, SIY; Literature Review: AK, ÖK, CKB; Manuscript Preparation: ÖK, MHA, SIY and Critical Review: PB, AK, MHA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

REFERENCES