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Evaluation of the oxidant/antioxidant status in patients with brucellosis treated with a combination of two types of antibiotics

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Abstract

Aim: This study aimed to evaluate the oxidant/antioxidant status of patients treated for brucellosis and to determine the effects of a combination of antimicrobial agents (doxycycline, streptomycin and rifampicin) that could be used in the treatment of brucellosis.

Material and Methods: Sixty (60) acute patients with brucellosis and thirty (30) healthy volunteers were enrolled in the study. Doxycycline-streptomycin (DOX-STR) and doxycycline-rifampicin (DOX-RIF) were used in the treatment of brucellosis. MDA levels, total oxidant status (TOS), and superoxide dismutase, catalase levels and total antioxidant capacity (TAC) were determined in patients treated with brucellosis. Also, TOS/TAC index (OSI) was calculated.

Results: Before therapy, MDA, TOS, SOD, catalase levels and OSI value were significantly higher, and TAC was significantly lower in brucella patients compared with healthy subjects ($p < 0.001$). After therapy, MDA, TOS, SOD, catalase levels, and OSI value significantly decreased, and plasma TAC levels significantly increased compared to the pre-treatment group, especially in the DOX-STR regimen ($p < 0.001$).

Discussion: Over-production of oxidants and depletion of TAC in patients with brucellosis are associated with inflammatory nature of brucellosis. Measurement of oxidant/antioxidant status may be useful for monitoring patients who are recovering, and the treatment of patients with antibiotic combination, especially (DOX-STR) will ameliorate their antioxidant system.

Keywords

Brucellosis, Total Oxidant Status (TOS), Total Antioxidant Capacity (TAC), Oxidative Stress Index (OSI), Treatment, Antibiotic Combination

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Introduction

Human brucellosis, also known as "Mediterranean" or "Malta fever", is a zoonosis and transmitted to humans through direct contact with infected animals or by eating their products. It causes loss of appetite and body weight, undulant fever, arthritis, spondylitis, endocarditis, osteomyelitis. It is an important public health and affects people in Turkey or developing countries such as the Middle East, Asia, Africa, Mediterranean countries of Europe and South America (WHO. Brucellosis fact sheet N173. WHO, Geneva, Switzerland; 1997.p.23-37).

Brucella organisms are gram-negative coccobacillus, facultative intracellular bacteria that reside and replicate in a vacuolar compartment within macrophages of the infected human. Numerous studies reported that lipopolysaccharides found in the outer membranes of brucella bacteria increase nitric oxide (NO) production in macrophages through IFN-γ; and thus superoxide and hydrogen peroxide have an important effect in protecting against brucella [1,2]. Experimental studies has identified the production of reactive oxygen species (ROS) by induced macrophage activation with IFN-γ [1-4].

Previous studies have found an increment in the expression of antioxidant enzymes such as NO reductase, superoxide dismutase (SOD), catalase (CAT) in brucella patients [5,6]. This outcome was attributed to increased free radical production and antioxidant depletion. Thus, the resulting oxidative stress plays an important role in the pathogenesis of brucellosis [6]. Generally, this situation was monitored with determining the total oxidant/antioxidant status. The therapeutic power of antibiotic combinations used in patients within the scope of this study will also be determined by oxidative stress and antioxidant agents that will be measured before and after treatment in patients, and will further contribute to the determination of effective treatment protocols.

Material and Methods

Study population

Brucella-infected individuals attending the outpatient Clinic of Infectious Diseases at the Cankiri State Hospital were enrolled in the study. The study was approved by the Research Ethics Committee of the University of Zonguldak Karaelmas/Turkey (2011/06). Sixty brucella-infected patients aged 18 – 50 years (32 men and 28 women), and 30 healthy controls aged 19 – 45 years (15 men and 15 women) were included in the study. Table 1 shows demographic features of individuals. No statistically significant difference was found between the groups (age, gender, body mass index (BMI), BMI score, and waist circumference).

Patients were assigned to the Brucella-infected group if they had an epidemiological history (contact with animals and animal products), clinical symptoms (hyperhidrosis, undulant fever and joint pain, etc.), and confirmed laboratory findings (determination with blood culture, serum agglutination test [SAT] with titres ≥ 1/160). Exclusion criteria for the patient and control groups were pregnancy, drug use, alcohol or tobacco use , obesity (BMI ≥ 30), gastro/metabolic/inflammatory diseases, abdominal surgery.

Brucellosis is usually treated with antibiotic combinations such as doxycycline-streptomycin (DOX-STR) and doxycycline-rifampicin (DOX-RIF) (Joint FAO/WHO Expert Committee on Brucellosis. Joint FAO/WHO Expert Committee on Brucellosis: sixth report. WHO Technical Report Series No. 740. Geneva; 1986.p.56-7). Treatment regimens of the patients with brucellosis in this study were 200 mg/day orally doxycycline plus 1 g/day intramuscularly streptomycin for 45 days or 200 mg/day orally doxycycline plus 600 mg/day orally rifampicin for 45 days.

Sample Collection and Processing

Blood specimens (5mL) before and after treatment were collected by venipuncture from participants and inoculated in bottles including anticoagulant (EDTA). The samples of 2 mL were stored at –70 °C until further analysis of MDA, total oxidant status (TOS), SOD, CAT, and total antioxidant capacity (TAC).

Analytical Procedures and Instruments

Firstly, oxidative stress parameters MDA and TOS were analyzed in plasma samples. MDA levels were analyzed using a modified method of Trichloroacetic Acid– Thiobarbituric Acid (TCA – TBA), at 532 nm spectrophotometrically. MDA values were expressed as mmol/L [7]. An automated measurement method developed by Erel was used in the determination of TOS [8]. In this method, oxidants present in the sample oxidize the ferrous ion complex to ferric ion. The color intensity is then measured at 660 nm spectrophotometrically, and this indicates the total amount of oxidants present in the sample. The results were expressed in μmol H2O2 equiv./L.

Secondly, the antioxidant enzyme (SOD, CAT) levels and TAC were analyzed. Plasma SOD activity was measured using Nitro Blue Tetrazolium (NBT) technique [9]. The volume of 3 ml of SOD assay mixture consisted of 0.1 ml ethanol phase (ethanol–chloroform 5/3 volume and plasma 1 volume), 0,05 mL xanthine oxidase 2.85 mL of solution containing 0.12 mM xanthine, 0.12 mM NaEDTA, 30.6 μM nitro blue tetrazolium, and 0.06 g/L bovine serum albumin (BSA). The reaction was stopped with 1 mL of 0.02 mM CuCl2, and the absorbance was recorded at 560 nm. SOD activity was expressed in terms of IU/g Hb. Plasma CAT activity was measured by the Aebi method [10]. In the assay, 0.05 mL of plasma, 0,5 mL hydrogen peroxide, 1,5 mL 100 mM phosphate buffer (pH 7), 0,95 mL were mixed and kinetic measurement was recorded using destruction of hydrogen peroxide with CAT at 240 nm. The activity was expressed as k/mg protein. An automated measurement method developed by Erel was used in the determination of TAC levels [11]. In this assay, the hydroxyl radical is produced by the Fenton reaction and when the sample is added to the mixture, the antioxidants in the sample suppressed the oxidative reactions, preventing color change. Thus, we can measure the total antioxidant capacity of the sample. Briefly, 0.03 mL of plasma sample, and 0,5 mL of reagent 1 were mixed, and then 0.067 mL of reagent 2 was added. Differences in the absorbance of the solution at 660 nm were measured after 30 s and 5 min. TAC levels were expressed in mmol Trolox equiv./L.

Finally, the percentage of TOS to the TAC was calculated to obtain the oxidative stress index (OSI), an indicator of the degree of oxidative stress.

Statistical Analysis

While evaluating the findings obtained in the study, the SPSS

program (Statistical Package for Social Sciences 13.0, USA) was used for statistical analysis. The Mann-Whitney-U test and Spearman's correlation analysis were used to compare differences between groups. The level of significance, p-value was 0.001 and all data in the tables were expressed as mean ± SD.

Results

Hematological and biochemical data at the initiation and after 45 days of combined antibiotic therapy are shown in Table 2. As can be seen in Table 2, increased white blood cell, glucose, aspartate amino-transferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), C-reactive protein (CRP), very low-density lipoprotein (LDL), total cholesterol levels were found in patients with brucellosis compared to healthy controls. Decreased total bilirubin, creatinine, lactate dehydrogenase (LDH), high-density lipoprotein (HDL) levels were found in the same group compared to controls.

Table 3 described the oxidative and anti-oxidative parameters for all participants. Significantly increased MDA, TOS, SOD and CAT levels were found in patients with acute brucellosis compared to healthy controls ($p < 0.001$). On the other hand, significantly decreased TAC levels were found in patients with acute brucellosis compared to the controls ($p < 0.001$). Also, the TOS/TAS ratio (OSI index) was significantly higher, in patients with acute brucellosis than in the healthy controls ($p < 0.001$). When comparing two treatments with the patient group, the oxidant/antioxidant status of the patients normalized after the treatment period.

Table 3 also shows outcome data for patients treated with the antibiotic combination DOX-STR or DOX-RIF. When comparing the two treatment regimens, there was no significant difference ($p > 0.05$). Comparison of different antibiotic combinations showed the benefit of using DOX-STR. Thus, a non-statically significant normalization of the oxidant/antioxidant status of patients was due to DOX-STR treatment in patients with acute brucellosis.

Table 1. Demographic features control and patient groups

	Control	Patients
N	30	60
Gender (M/F)	15/15	32/28
Age* (year)	33.2 ± 8.1	34.3 ± 8.7
BMI* (kg/m2)	23.5 ± 4.2	22.8 ± 2.7
BMI score*	2.03 ± 0.35	1.97 ± 0.41
Waist circumference*	89.42 ± 8.22	87.32 ± 7.15

*Mean ± standard deviation; P-value: p>0,05

Table 2. Hematological and biochemical findings of the control and patient groups

	After 45 days of treatment with			
Variables	**Control**	**Patients**	**(DOX-STR)**	**(DOX-RIF)**
Red blood cells ($\times 10^6$/µL)	4.31 ± 0.84	4.37 ± 0.61	4.41 ± 0.47	4.33 ± 0.52
White blood cell ($\times 10^3$/µL)	6.92 ± 2.9	8.80 ± 3.2*	8.25 ± 5.3*	7.36 ± 4.8*
Total bilirubin (mg/dL)	0.71 ± 0.18	0.63 ± 0.13*	0.65 ± 0.22*	0.68 ± 0.35*
Albumin (g/dL)	4.04 ± 0.37	3.70 ± 1.9	4.11 ± 0.8**	3.92 ± 1.3**
Glucose (mg/dL)	87.35 ± 11.75	111.19 ± 31.16*	105.22 ± 32.41*	106.34 ± 24.46*
Creatinine (mg/dL)	0.94 ± 0.21	0.70 ± 0.17*	0.84 ± 0.22**	0.81 ± 0.25**
C-reactive protein (mg/L)	5.2 ± 1.4	30 ± 3.85*	9 ± 1.67**	10 ± 1.95**
Aspartate amino transferase (IU/L)	25.2 ± 7.4	48.0 ± 9.34*	28.2 ± 8.42**	32.7 ± 7.26**
Alanine aminotransferase (IU/L)	27.7 ± 8.3	42.0 ± 7.2*	31.3 ± 6.31**	36.4 ± 5.98**
Lactate dehydrogenase (IU/L)	583 ± 124.6	540 ± 142.7*	564 ± 131.8	572 ± 99.1**
Gamma glutamyl transferase (IU/L)	65.32 ± 24.1	70 ± 17.3*	64.27 ± 31.8**	67.14 ± 19.7
Alkaline phosphatase (IU/L)	115.45 ± 14.5	310 ± 16.4*	195.2 ± 27.8*,**	228 ± 13.9*,**
Triglycerides (mg/dL)	125.81 ± 74.3	134.65 ± 62.8	127.38 ± 21	132.43 ± 35.6
HDL (mg/dL)	58 ± 5.6	42 ± 4.9*	51 ± 3.8*,**	49 ± 2.7*,**
LDL (mg/dL)	125.3 ± 25.3	155.4 ± 14.5*	133.2 ± 35.6*,**	145.2 ± 19.1*,**
Total cholesterol (mg/dL)	178.24 ± 19.43	207.32 ± 22.7*	171.12 ± 15.76**	176.19 ± 13.5²**

Results are represented as mean ± SD, *P < 0.001, significant compared with controls; **P < 0.001, significant compared with patients

Table 3. Comparison of TOS, TAC, and OSI levels between controls and brucellosis patients before and after treatment.

	After 45 days of treatment with			
Variables	**Control**	**Patients**	**(DOX-STR)**	**(DOX-RIF)**
MDA (mmol/L)	5.63 ± 2.7	12.73 ± 0.6*	7.58 ± 3.4**	8.45 ± 2.1**
TOS (µmol H_2O_2 equiv./L)	20.32 ± 5.7	42.38 ± 8.5*	30.72 ± 9.2**	34.63 ± 10.8**
SOD (IU/g Hb)	1008.5 ± 91.3	1312.4 ± 100.8*	1156.2 ± 89.4**	1247.5 ± 115.9**
Catalase (k/mg protein)	23.26 ± 20.3	28.61 ± 10.5*	26.14 ± 16.4**	27.24 ± 13.5**
TAC (mmol Trolox equiv./L)	1.15 ± 0.3	0.82 ± 0.4*	1.05 ± 0.5**	0.97 ± 0.3**
OSI (arbitrary unit)	17.67 ± 19.23	51.68 ± 21.25*	35.70 ± 36**	25.85 ± 13**

Discussion

With regard to disease progression, some biochemical parameters, such as AST, ALT, GGT and CRP levels were significantly higher in patients with acute brucellosis compared to healthy controls. When comparing those parameters of the brucella group before and after treatment, a significant decrease was observed, indicating the effectiveness of the treatment. These results were consistent with other studies [12,13].

When an infectious bacterium enters the body, macrophages and dendritic cells are activated for antigen processing in the normal immune response, leading to an overproduction of reactive oxygen species in inflammatory diseases such as brucellosis [1,2]. Antioxidant enzymes such as superoxide dismutase, catalase, myeloperoxidase, and NO reductase play a very important role in the elimination of ROS species and are associated with the bacterial defense system in Brucella [14]. In the present study, these changes were analyzed by monitoring the oxidants such as MDA, TOS and antioxidants such as SOD, CAT, TAC and OSI. In this study, patients with brucellosis had significantly increased MDA, TOS, SOD, CAT levels and OSI value compared with healthy controls. Additionally, significantly decreased TAC levels were found in patients with acute brucellosis than in controls. In a numerous studies, these biomarkers of oxidative/antioxidative have been investigated in acute brucellosis. In a rat model, found an increase in MDA levels in plasma and tissues such as the brain, liver, and spleen, and CAT activity in the liver. Esen et al. [15] observed significantly decreased serum paraoxonase–arylesterase (PON) activities, TAC and thiols levels, while increased lipid hydroperoxide, TOS and OSI values in acute brucellosis patients than in controls. Otherwise, Karahocagil et al. [16] found significantly decreased CAT activity in the brucella patients than in controls. Karaagac et al. [17] measured the TAC and TOS levels in patients before and after therapy, and calculated OSI. They determined significantly higher TOS and OSI levels and lower TAC values in brucella patients when compared to those before and after therapy. After treatment, they reported decreased TOS and OSI levels, whereas TAC levels increased. It is thought that the elevation of free oxygen radicals and a decrease in antioxidant capacity may have an importance in the pathogenesis of brucellosis.

Antibiotic combinations such as doxycycline - streptomycin (DOX-STR) and doxycycline - rifampicin (DOX-RIF) are commonly used in the treatment of brucellosis. In two different meta-analyses evaluating antibiotic combinations in brucellosis, the usage of DOX-STR is reported to be superior to that of DOX-RIF [18,19]. According to our data, DOX-STR regimen was superior in the normalization of aminotransferases, and another probable reason may be related to the hepatotoxicity of rifampicin [20]. In the present study, these effects of antibiotic regimens were analyzed by monitoring the oxidant and antioxidant status of the post-treatment groups compared to controls. DOX-STR treatment of patients with brucellosis further reduced MDA, TOS, SOD, CAT levels, and OSI value compared with healthy controls. Additionally, it is also observed that the same regimen further increased plasma TAC levels after therapy. These results indicate that the use of DOX-STR regimen particularly improves the antioxidant status of the brucella patients, and this should be taken into consideration in designing treatment.

Conclusion

It is thought that the rise of free oxygen radicals and the decrease of antioxidant capacity may play a role in the pathogenesis of brucellosis. The antioxidant status of the blood can be determined by measuring the total oxidant/antioxidant status. In this study, the therapeutic power of antibiotic combinations used in patients was determined by the oxidant and antioxidant agents measured before and after treatment in patients, and the addition of antioxidants to effective treatment protocols for brucella will make an extra contribution.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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