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Full Length Research Paper

Evaluation of oxidative stress in patients with tularemia

Sema Koc[1], Erkan Sogut[2], Fazilet Duygu[3], Levent Gurbuzler[1], Ahmet Eyibilen[1] and Ibrahim Aladag[1]

[1]Department of Otorhinolaryngology, Gaziosmanpasa University School of Medicine, Tokat/Turkey.
[2]Department of Biochemistry and Clinical Biochemistry, Gaziosmanpasa University School of Medicine, Tokat/Turkey.
[3]Department of Infectious Disease and Clinic Microbiology, Tokat State Hospital, Tokat/Turkey.

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Tularemia, a zoonotic acute febrile invasive disease with rapid intracellular replication resulting in high bacterial densities, accumulation of phagocytes, and extensive tissue necrosis; may be related to increased free radical production and antioxidant depletion and; oxidative stress. The aim of this study was to investigate serum malondialdehyde (MDA) level and superoxide dismutase (SOD), glutathione peroxidase (GSHPx) activities in patients with tularemia during pre- and post-treatment period and to compare results obtained with data from healthy subjects. A total of 90 subjects (40 patients with tularemia and 50 healthy controls) were enrolled in this study. Peripheral venous blood samples were taken from patients before and 3 months after the treatment. In the control group, blood samples from healthy volunteers were collected only once. Serum MDA level and SOD, GSHPx activities were measured. In the 'before treatment' group, MDA concentration was significantly higher than the control group (p<0.001), but GSH-Px activity was lower than the control group (p = 0.006). The SOD activity was similar in the 'before treatment' group and the control group (p = 0.235). MDA concentration decreased in the 'after treatment' group (p<0.001). In the 'after treatment' group, MDA concentration was significantly higher than the control group (p<0.001). GSH-Px activity in the 'after treatment' group, but the difference was statistically insignificant (p = 0.105). GSH-Px activity was slightly higher in the control group compared to the 'after treatment' group, but the difference was statistically insignificant (p = 0.08). SOD activity has not changed in the 'after treatment' group (p = 0.163). Also, SOD activity was similar in the control and the 'after treatment' group (p = 0.391). In conclusion, the data obtained from the present study showed that patients with tularemia are exposed to oxidative stress, and oxidative stress decreases after the treatment.

Key words: Tularemia, oxidative stres, malondialdehyde, superoxide dismutase, glutathione peroxidase.

INTRODUCTION

Tularemia is a zoonotic disease characterized by different clinical forms. The causative agent of tularemia is a gram-negative coccobacillus, *Francisella tularensis*. *Francisella tularensis* was first isolated from rodents in 1911 in Tulare County, California, USA (Ellis et al., 2002). Tularemia is an acute febrile invasive disease with rapid intracellular replication resulting in high bacterial densities, accumulation of phagocytes, and extensive tissue necrosis (Tärnvik and Chu, 2007). Tularemia is seen in six forms classified according to the route of entry, which are ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal tularemia forms (Hepburn and Simpson, 2008).

All aerobic organisms produce reactive oxygen species (ROS) at physiological concentrations during normal metabolism for various cellular functions. Rates of production and destruction of ROS are in an equilibrium known as the oxidative balance. If the oxidative balance is maintained, ROS have no impact on an organism. However, if the equilibrium is destroyed in favor of free radicals, then oxidative stress develops. Oxidative stress

*Corresponding author. E-mail: drsemakoc@gmail.com. Tel: +905054983511.

is the consequence of an increase in ROS and/or impairment in antioxidant mechanisms (Serafini and Del Rio, 2004; Dogruer et al., 2004).

Lipid peroxidation is an autocatalytic mechanism; fatty acids from the cell membrane become oxidized by a chain reaction. Malondialdehyde (MDA) is an important product of the peroxidation process; its levels correlate with the degree of lipid peroxidation. Therefore, MDA levels are generally used as an indicator of lipid peroxidation (Ogunro and Ologunagba, 2011; Valko et al., 2006).

Antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase (CAT) try to prevent the damaging effects oxidation products have on an organism (Koc et al., 2011; Akanbi et al., 2010). Numerous studies have demonstrated that in various types of infectious diseases, reactive oxygen species are produced by activated inflammatory cells during the inflammatory response in order to kill various intracellular pathogens (Serefhanoglu et al., 2009; Selek et al., 2008). Therefore, it is possible that tularemia may be related to increased free radical production and antioxidant depletion, and oxidative stress may be implicated in the pathogenesis of tularemia.

The aim of this study was to investigate serum MDA level, SOD and GSHPx activities in patients with tularemia during pre- and post-treatment period and to compare results with data from healthy subjects.

MATERIALS AND METHODS

Patients and methods

This is a prospective, controlled study of patients with tularemia. The study was approved by the local ethics committee. Patient's consents were obtained prior to the start of any procedures.

A total of 90 subjects (40 patients with tularemia and 50 healthy controls) were enrolled in this study. All patients underwent a baseline evaluation including a detailed medical history, typical otorhinolaryngologic examination, and blood tests. Tularemia was suspected in individuals living in the epidemic zone who presented with the findings of fever, pharyngitis or tonsillitis and/or cervical lymphadenopathy and who did not respond to penicillin treatment. Exclusion criteria included autoimmune disorders, pregnancy, malignancy, drug or alcohol abuse, human immune deficiency virus infection, other acute infections other than tularemia, chronic respiratory insufficiency and any liver, hematological, cardiovascular, cerebrovascular, metabolic, neurologic, or psychiatric diseases. The patients with tularemia were treated with intramuscular streptomycin (1 g every 12 h) for 14 days.

Diagnosis of tularemia

Blood specimens were obtained from all patients with suspected tularemia. Patient's diagnoses were confirmed by serological tests and polymerase chain reaction (PCR). The microagglutination method was used for the serological diagnosis. Antibody titers of 1:160 and above or positive polymerase chain reaction (PCR) were accepted to be significant for diagnosis (Leblebicioglu et al., 2008). In our patients, a granulomatous inflammation was observed in the histopathological examination of the specimen taken from the cervical lymphadenopathy.

Blood sample collections

Fasting peripheral venous blood samples were taken before and 3 months after the treatment. In the control group, blood samples from healthy volunteers were collected only once. Samples were allowed to clot for 20 min at room temperature before the serum was separated by centrifugation (1500 xg for 10 min at 4℃). The serum samples were separated from the clot within one hour of blood collection and transferred to a clean test tube. Serum samples were stored at –70℃ until the investigation .

Biochemical analysis

The following determinations were made on the samples using commercial chemicals supplied by Sigma (St. Louis, USA). Total (Cu/Zn and Mn) SOD activity was determined according to the method of Sun et al. (1988). The principle of the method is based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine-xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. Serum SOD activity was expressed as units per milliliter serum (U ml^{-1}). Glutathione peroxidase activity was measured by the method of Paglia and Valentine (1967). The enzymatic reaction in the tube, which contains NADPH, reduced glutathione, sodium azide, and glutathione reductase, was initiated by addition of H_2O_2, and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity is expressed as U L^{-1}. The MDA level was determined by a method based on the reaction with thiobarbituric acid (TBA) at 90-100℃ (Esterbauer and Chee seman, 1990). In the TBA test reaction, malondialdehyde (MDA) or MDA-like substances and TBA react together to produce a pink pigment having an absorption maximum at 532 nm. The results were expressed as micromole per liter serum sample (umol L^{-1}).

Statistical analysis

Pearson's chi-square test was used to compare the gender between groups. Gender was presented as count and percentage. The Kolmogorov-Smirnov test was used to evaluate whether the variables were normally distributed. The two independent sample *t* test or Mann Whitney U test were used to compare continuous variables between control and patient groups. Continuous variables were presented as mean (standard deviation (SD) or median (interquartile range[Q1-Q3]). A paired *t* test or Wilcoxon test were used to detect differences between the before- and after-treatment periods. SPSS software 15.0 for Windows (Chicago, IL, USA) was used for all statistical analysis. *P*-values were considered statistically significant when they were <0.05.

RESULTS

The tularemia group consisted of 40 patients, 18 males (45%), between the ages of 17 and 65 years (mean age 48±12 years) and the control group consisted of 50 individuals, 29 males (58%) between the ages of 18 and 65 (mean age 45±14 years). There were no statistical differences in both the age and gender between the patient and control groups.

Table 1. Oxidative stress parameters in the study groups.

	GSHPx*	MDA†	SOD*
Before treatment (n = 40)	581±179 [b]	3.40 (2.79-4.02) [a]	4.33±1.23
After treatment (n = 40)	633±150 [c]	2.00 (1.74-2.70) [a,d]	3.83±1.43
Control (n = 50)	709±251	1.36 (1.30-1.52)	4.08±0.86

*, Values are presented as means ± SD; †, Values are presented as median and interquartile range (Q1-Q3); [a], p <0.001 *vs.* Control; [b], p =0.006 *vs.* Control; [c], p =0.08 *vs.* Control; [d], p <0.001 *vs.* before treatment.

In the 'before treatment' group, MDA concentration was significantly higher than the control group (3.40 [2.79-4.02] vs. 1.36 [1.30-1.52], p<0.001), but GSH-Px activity was lower than the control group (581±179 vs. 709±251, p=0.006). The SOD activity was similar in the 'before treatment' group and the control group (4.08±0.86 vs. 4.33±1.23, ps = 0.235). MDA concentration decreased in the 'after treatment' group (3.40 [2.79-4.02] vs. 2.00 [1.74-2.70], p<0.001). In the 'after treatment' group, MDA concentration was significantly higher than the control group (2.00 [1.74-2.70] vs. 1.36 [1.30-1.52], p<0.001). GSH-Px activity increased in the 'after treatment' group, but the difference was statistically insignificant (581±179 vs. 633±150, p = 0.105). GSH-Px activity was slightly higher in the control group compared to the 'after treatment' group, but the difference was statistically insignificant (709±251 vs. 633±150, p = 0.08). SOD activity has not changed in the 'after treatment' group (4.33±1.23 vs. 3.83±1.43, p=0.163). Also, SOD activity was similar in the control and the 'after treatment' group (4.08±0.86 vs. 3.83±1.43, p = 0.391). Table 1 summarizes the statistical results.

DISCUSSION

In order to investigate the effect of tularemia on oxidative status, we measured the oxidants (malondialdehyde) and the antioxidants (SOD, GSHPx). To the best of our knowledge, there is no prior study investigating these biomarkers of oxidation and antioxidant defense in patients with tularemia.

Reactive oxygen species (ROS) such as hydroxyl radicals (HO•), peroxyl radicals (ROO•), the superoxide anion (O2•−), hydrogen peroxide (H_2O_2), nitrogen oxide (NO•), and hypochlorous acid (HOCl) are produced for crucial cellular reactions in physiological concentrations (Koc et al., 2011; Ogunro et al., 2010). The generation of reactive oxygen species (ROS) by activated immune cells is essential for innate immune defense during inflammatory response and they are involved in damaging cellular structures and disrupting crucial cellular functions. Therefore, ROS have been reported to play a role in the pathogenesis of many inflammatory diseases such as rheumatoid arthritis, ankylosing spondylitis, brucellosis, and tuberculosis (Serefhanoglu et al., 2009; Selek et al., 2008; Erdem et al., 2010; Desai et al., 2010). Reactive oxygen species (ROS) are also implicated in otolaryngological diseases including otitis media, chronic adenotonsillitis, chronic sinusitis, tinnitus, hearing loss, and nasal polyps (Kiroglu et al., 2006; Westerveld et al., 1997; Ozcan et al., 2010; Capaccio et al., 2011; Parks et al., 1996).

Reactive oxygen species (ROS) can cause oxidative damage to DNA, RNA, lipid peroxidation pathways, proteins contributing to barrier integrity modification, and other types of molecular oxidation reactions (Kiroglu et al., 2006). Main targets of ROS are the polyunsaturated fatty acids in cell membranes causing lipid peroxidation and malondialdehyde (MDA) formation, which may lead to damage of cellular structures and function. Therefore, the assay for MDA is often used as a marker for oxidative stress in several inflammatory diseases (Kaygusuz et al., 2003). Defense systems protecting from free radical damage consist of a network of both enzymatic and non-enzymatic antioxidants. Non-enzymatic systems include glutathione, *β*-carotene, ascorbic acid, and tocopherols. Enzymatic systems include superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase (CAT) (Serefhanoglu et al., 2009). By detoxifying reactive oxygen species, antioxidant enzymes constitute the main protective mechanism of infected tissue against free radical mediated injury. Detoxification of the ROS begins with the conversion of extremely toxic and reactive superoxide (O2_) radicals into molecular oxygen (O2) and H_2O_2 through the activation of superoxide dismutases. Catalase, GSH-Px, and glutathione reductase then convert the H_2O_2 to H_2O by attaching the hydroxyl groups to various organic molecules (Guina et al., 2007).

Kaygusuz et al. (2003) reported that in patients with chronic tonsillitis, oxidative stress is increased and SOD activity is reduced concomitantly with an increase in MDA levels. Serefhanoglu et al. (2009) evaluated 69 brucellosis patients and 69 healthy controls for oxidative stress. They reported that plasma levels of total peroxide and malondialdehyde were significantly increased in experimental group patients compared with healthy controls, and total antioxidant capacity level was significantly lower in experimental patients as compared with controls. However, there was no statistically significant difference between the catalase results of the

two groups. Prasad et al. (2008) evaluated 100 patients with leprosy and 50 healthy controls for oxidative stress. They determined that blood glutathione content and erythrocyte antioxidant enzyme activities of GSH-Px and glutathione reductase were lower in leprosy patients with chronic granulomatous infection than those in the control group. Moreover, they reported that oxidative stress was associated with insufficient antioxidant defense potential in subjects with leprosy. Melek et al. (2006) inoculated *Brucella melitensis*, an intracellular pathogen leading to chronic infection, into rats in their experimental study. They reported the formation of oxidative stress, as well as decreased activities of antioxidant enzymes like glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) which indicated that oxidative stress may be important in the pathogenesis of brucellosis. Naderi et al. (2011) found that antioxidant activities were decreased in patients with pulmonary tuberculosis as compared to control group. They reported that oxidant/antioxidant imbalance induced by inflammation may have an impact on antioxidant activities.

Conclusion

In our study, in the ‘before treatment’ group, MDA concentration was significantly higher than the control group, but GSH-Px activity was lower than the control group. The SOD activity was similar in the ‘before treatment’ group and the control group. MDA concentration decreased in the ‘after treatment’ group. In the ‘after treatment’ group, MDA concentration was significantly higher than the control group. GSH-Px activity increased in the ‘after treatment’ group, but the difference was statistically insignificant. GSH-Px activity was slightly higher in the control group compared to the ‘after treatment’ group, but the difference was statistically insignificant. SOD activity has not changed in the ‘after treatment’ group. Also, SOD activity was similar in the control and the ‘after treatment’ group. In conclusion, the data obtained from the present study showed that patients with tularemia are exposed to potent oxidative stress. However, further studies with larger sample sizes are needed to define the exact role of oxidative stress in the pathogenesis and treatment of the disease.

REFERENCES

Akanbi OM, Odaibo AB, Ademowo OG (2010). Effect of antimalarial drugs and malaria infection on oxidative stress in pregnant women. Afr. J. Reprod. Health, 14(3): 209-212.

Capaccio P, Pignataro L, Gaini LM, Sigismund PE, Novembrino C, De Giuseppe R, Uva V, Tripodi A, Bamonti F (2011). Unbalanced oxidative status in idiopathic sudden sensorineural hearing loss. Eur. Arch. Otorhinolaryngol. Jun 26. [Epub ahead of print]

Desai PB, Manjunath S, Kadi S, Chetana K, Vanishree J (2010). Oxidative stress and enzymatic antioxidant status in rheumatoid arthritis: a case control study. Eur. Rev. Med. Pharmacol. Sci., 14(11): 959-967.

Ellis J, Oyston PC, Green M, Titball RW (2002). Tularemia. Clin. Microbiol., 15(4): 631-646.

Dogruer ZN, Unal M, Eskandari G, Pata YS, Akbaş Y, Cevik T, Cimen MY (2004). Malondialdehyde and antioxidant enzymes in children with obstructive adenotonsillar hypertrophy. Clin. Biochem., 37(8): 718-21.

Erdem FH, Karatay S, Yildirim K, Kiziltunc A (2010). Evaluation of serum paraoxonase and arylesterase activities in ankylosing spondylitis patients. Clinics, 65(2): 175-179.

Esterbauer H, Cheeseman KH (1990). Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol., 186: 407-421.

Guina T, Radulovic D, Bahrami AJ, Bolton DL, Rohmer L, Jones-Isaac KA, Chen J, Gallagher LA, Gallis B, Ryu S, Taylor GK, Brittnacher MJ, Manoil C, Goodlett DR (2007). MglA regulates Francisella tularensis subsp. novicida (Francisella novicida) response to starvation and oxidative stress. J. Bacteriol.,189(18): 6580-6486.

Hepburn MJ, Simpson AJ (2008). Tularemia: current diagnosis and treatment options. Expert Rev. Anti. Infect. Ther., 6(2): 231-240.

Kaygusuz I, Ilhan N, Karlıdag T, Keles E, Yalcin S, Cetiner H (2003). Free radicals and scavenging enzymes in chronic tonsillitis. Otolaryngol. Head Neck Surg., 129(3): 265-268.

Kiroglu AF, Noyan T, Oger M, Kara T (2006). Oxidants and antioxidants in tonsillar and adenoidal tissue in chronic adenotonsillitis and adenotonsillar hypertrophy in children. Int. J. Pediatr. Otorhinolaryngol., 70(1): 35-38.

Koc F, Kalay N, Ardic I, Ozbek K, Celik A, Ceyhan K, Kadi H, Karayakali M, Sahin S, Altunkas F, Onalan O, Kaya MG (2011). Antioxidant status and levels of antioxidant vitamins in coronary artery ectasia. Coron. Artery Dis., 22(5): 306-310.

Leblebicioglu H, Esen S, Turan D, Tanyeri Y, Karadenizli A, Ziyagil F, Goral G (2008). Outbreak of tularemia: a case-control study and environmental investigation in Turkey. Int. J. Infect. Dis., 12(3): 265-269.

Melek IM, Erdogan S, Celik S, Aslantas O, Duman T (2006). Evaluation of oxidative stress and inflammation in long term Brucella melitensis infection. Mol. Cell Biochem., 293(1-2): 203-209.

Naderi M, Hashemi M, Komijani-Bozchaloei F, Moazeni-Roodi A, Momenimoghaddam M (2011). Serum paraoxonase and arylesterase activities in patients with pulmonary tuberculosis. Pathophysiology, 18(2): 117-20.

Ogunro PS, Ogungbamigbe TO, Muhibi MA (2010). The influence of storage period on the antioxidants level of red blood cells and the plasma before transfusion. Afr. J. Med. Med. Sci., 39(2): 99-104.

Ogunro PS, Ologunagba PO (2011). The Effect of Palm Wine on Lipid Peroxidation and Antioxidant status of Rural dwellers in South West Nigeria. Niger Postgrad. Med. J., 18(3): 186-90.

Ozcan C, Tamer L, Ates NA, Görür K (2010). The glutathione-S-transferase gene polymorphisms (Gstt1, Gstm1, and Gstp1) in patients with non-allergic nasal polyposis. Eur. Arch. Otorhinolaryngol., 267(2): 227-232.

Paglia DE, Valentine WN (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70: 158-169.

Parks RR, Huang CC, Haddad J Jr (1996). Middle ear catalase distribution in an animal model of otitis media. Eur. Arch. Otorhinolaryngol., 253(8): 445-449.

Prasad CV, Kodliwadmath MV, Kodliwadmath GB (2008). Erythrocyte glutathione peroxidase, glutathione reductase activities and blood glutathione content in leprosy. J. Infect., 56(6): 469-473.

Selek S, Cosar N, Kocyigit A, Erel O, Aksoy N, Gencer M, Gunak F, Aslan M (2008). PON1 activity and total oxidant status in patients with active pulmonary tuberculosis. Clin. Biochem., 41(3): 140-144.

Serafini M, Del Rio D (2004). Understanding the association between dietary antioxidants, redox status and disease: is the total antioxidant capacity the right tool? Redox Rep., 9(3): 145-152.

Serefhanoglu K, Taskin A, Turan H, Timurkaynak FE, Arslan H, Erel O (2009). Evaluation of oxidative status in patients with brucellosis. Braz. J. Infect. Dis., 13(4): 249-251.

Sun Y, Oberley LW, Li Y (1988). A simple method for clinical assay of superoxide dismutase. Clin. Chem., 34(3): 497-500.

Tärnvik A, Chu MC (2007). New approaches to diagnosis and therapy

of tularemia. Ann N Y Acad. Sci., 1105: 378-404.
Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006). Free radicals, metals and antioxydants in oxidative stress-induced cancer. Chem. Biol. Interact., 160(1): 1-40.
Westerveld GJ, Dekker I, Voss HP, Bast A, Scheeren RA (1997). Antioxidant levels in the nasal mucosa of patients with chronic sinusitis and healthy controls. Arch. Otolaryngol. Head Neck Surg., 123(2): 201-204.