15th Annual Congress of Iranian Rheumatology Association

The Effect of Zinc Supplementation on Clinical Outcomes of Patients with Spine Brucellosis

Esalatmanesh Kamal[1*] , Soleimani Zahra[2] , Esalatmanesh Roozbeh[1]

1-Autoimmune Diseases Research Center, Kashan University of medical sciences , Kashan, Iran.
2-Infectious Diseases Research Center, Kashan University of medical sciences , Kashan, Iran
***email:** esalat.kamal@yahoo.com **Tel:+989131616775**

INTRODUCTION

Brucellosis is one of the mutual inflammatory diseases between humans and livestock with global transmission [1] which is tied to the chronic disability of humans and reproduction decrease in animals [2]. The diseasecausing Brucella bacterium infects a wide range of domestic and wild mammals [3]. Its annual incidence ranges from 0.3 cases per million people in some developed countries to fewer than 1000 cases per million people in endemic areas [4]. This disease is still one of the most important health issues in the world, especially in parts of the Mediterranean, including Iran, Turkey, the Saudi Arabia Peninsula, and parts of Central and South America [5]. Based on the recent report of WHO, the prevalence of human and animal brucellosis is such that approximately 500,000 cases of human brucellosis are reported annually worldwide [6]. This disease is endemic in Iran, and its prevalence is 225% [7]. Brucellosis has several clinical symptoms including systemic syndrome with fever, sweating, chills and fatigue, insomnia, meningeal syndrome, etc. The severe symptoms of the disease are neurobrucellosis and endocarditis [8]. Brucellosis is transmitted to humans from infected cattle, pigs, sheep, or goats. Moreover, it is transmitted by consuming the unpasteurized animal milk, dairy products, or meat products of infected animals. This disease is also transmitted to another person through human blood and sexual contact [9]. Zinc is the body's second most rare element after iron. the Food and Nutrition Council recommended the required amounts in the diet based on the recognition of the metabolic role of zinc in human nutrition in 1974 [10]. Moreover, zinc involves in the metabolism of liver cells. The amount of zinc in the human body is 2 to 3 grams, which is widely distributed in the human body. The maximum concentration of zinc is found in the liver, pancreas, kidneys, bones, and muscles. Meat, fish, poultry, milk, and dairy products make up 80% of total zinc consumption. Oysters, meat, liver, cheese, whole grains, dried beans, nuts, and soy products are relatively good sources of zinc [11]. Its main role is in regulating gene expression by binding to transcription factors in the gene promoter region [12]. Zinc protects cell membrane lysis against toxins as a cofactor enzyme. This element is not stored in the body. Therefore, it is essential to use supplements and foods rich in zinc. The role of zinc in human health and body function is primarily focused on dietary supplements to promote health and prevent disease. In addition, to use zinc supplementation in diets, zinc is also used for colds, atopic eczema, psoriasis, acne vulgaris, retinal degenerative lesions, age-related macular degeneration, inflammatory bowel disease, and various other disorders. Furthermore, zinc has an important protective performance against the free radicals [14,15,16] as well as being used in the treatment of pneumonia, diarrhea, male fertility, and Alzheimer's disease can be helpful [13]. Brucellosis increases the oxidative stress level and weakens the body's antioxidant defense system [17]. Oxidative stress means excessive production of oxygen free radicals or insufficient levels of the antioxidant system which plays an important role in causing clinical problems such as DNA damage, lipid peroxidation, enzyme inactivation, apoptosis, and cell necrosis [18]. On the other hand, oxidative stress caused by infectious diseases can change the serum levels of rare elements such as zinc [19]. In Zanganeh et al. (2017) research which investigated the effect of brucellosis on rare element levels in 40 patients with brucellosis and 20 healthy individuals, the serum zinc level in the patient and control groups were 76.47 µg/dl and 92.85 µg/dl, respectively. In addition, it was indicated that serum zinc levels in the group of patients with brucellosis (case group) are significantly lower than the group of healthy individuals (control group) [20]. Moreover, serum zinc levels decrease in patients with brucellosis compared with healthy individuals in other studies [21, 22]. Infectious diseases such as brucellosis can influence rare element levels by replacing the hepatic metabolism or increasing urinary excretion. There are other certain inflammatory factors that regulate zinc balance in the body. Some interleukins secreted by leukocytes or active phagocytes can reduce zinc levels by inhibiting the transfer of zinc from plasma to the liver [26,25,24,23]. It was tried in this research to study the effect of zinc supplementation on improving the clinical symptoms caused by brucellosis according to the Significant reduction in serum zinc levels in patients with brucellosis and the antioxidant effects of zinc supplementation considering the increase in oxidative stress in brucellosis and since there has been no research on studying the effect of zinc supplementation on brucellosis.

METHODOLOGY

This research is a clinical trial that was conducted on brucellosis patients who were definitively diagnosed by a pediatric infectious disease specialist and subspecialist during the requested tests for brucellosis as well as clinical symptoms. The studied population is 40 patients who were referred to the clinic and infectious diseases and pediatrics department of Shahid Beheshti Hospital in Kashan. The mentioned checklist was filled after taking their conscious written consent which includes demographic characteristics and relevant variables to the disease severity. The patients were divided into two intervention groups with zinc supplementation in the form of zinc sulfate capsules 220 mg (containing 50 mg of menthol) and placebo using block randomization method and 4 blocks and were evaluated in terms of clinical response, fever duration, and pain for 6 weeks by a clinical information checklist. A dose of zinc supplementation is used in this research and no serious side effects have been observed following the use of this dose in previous studies [12].

1 Biochemical Evaluation

10 ml of fasting blood samples were taken from patients at first and 6 weeks after zinc supplementation in the laboratory of Shahid Beheshti Hospital in Kashan.

Determination of Inflammatory Markers

A. Inflammatory factor of hs-CRP

A serum with an ELISA device (immunoassay method) is stated in terms of ng/ml to measure hs-CRP [27]. Serum hs-CRP concentration was determined using IBL Kit, Germany with Ref NO: EU 59131. Its mechanism is based on the direct sandwich technic in which two antibody molecules react directly with human CRP.

Determination of Oxidative Stress Biomarkers

Plasma antioxidant capacity was determined by Benzie and Strain methods by a calorimetric method using Cusabio Biotech Co. kit from China [28].
Determination of malondialdehyde was performed by TBARS method and based on the reaction of MDA with thiobarbituric acid at a wavelength of 535 nm [29].

. **Reseach inclusion criterion**

.Being older than 10

.Titrate Wright or Combs Wright at least 1.80 and two mercaptoethanol at least 1.40

.Taking informed consent to participate in research

. **Reseach exclusion criterion**

who suffer from malnutrition

Pregnant women

Having an innate or acquired immunodeficiency

History of zinc sensitivity

affliction to local forms of brucellosis such as endocarditis and meningitis

affliction to the advanced or chronic heart, lung, liver, and kidney disease

In this research, SPSS software (vs. 16) was used to analyze data. Kolmogorov-Smirnov test was used to test the data for normal distribution. The quantitative data was expressed as mean and standard deviation. The independent t-test was used between changes of two groups to compare clinical parameters, inflammatory factor, and oxidative stress biomarkers at the beginning and end of the intervention (6 weeks later) between the two groups of zinc supplementation and placebo.

FINDINGS

Table 1 shows the oxidative stress markers and inflammatory factor of patients in two groups receiving zinc supplementation and placebo. After 6 weeks of intervention, zinc supplementation caused a significant increase in serum levels of total antioxidant capacity than placebo (P <0.001). Zinc consumption had no significant effect on serum levels of malondialdehyde (P = 0.496) and inflammatory factor CRP (P = 0.726) than placebo.

Table 1. Changes in Biomarkers of Oxidative Stress and Inflammatory Factors in Two Groups of Patients with Spine Brucellosis

	Placebo Group (n=20)			Zinc group (n=20)			1 P
	The research beginning	Sixth week	Changes	The research beginning	Sixth week	Changes	
TAC	318.66±9.9	751.96±8.4	432.62±8.5	333.89±7.0	954.136±5.7	620.156±7.6	<0.001
(mmol/L) MDA	7.0±4.7	4.0±0.5	-3.0±4.6	7.0±3.8	3.1±7.1	-3.1±5.3	0.496
(µmol/L) hs-CRP (mg/L)	20.9±3.0	8.3±4.6	-11.6±9.4	19.7±0.6	7.2±6.7	-11.6±3.1	0.726

hs-CRP, high-sensitivity C-reactive protein; MDA, malondialdehyde; TAC, total antioxidant capacity

Data are presented as mean ± standard deviation or number (%).

1 is the calculation based on the changes between the end and beginning of the intervention of two groups using independent t-test

RESULTS AND DISCUSSION

This research indicates that consumption of zinc supplementation in patients with brucellosis has significantly reduced the duration of fever and pain and significantly increased serum levels of total antioxidant capacity in comparison to the placebo, while it could not have a significant effect on inflammatory factor CRP and MDA serum levels.

Brucellosis is an intracellular gram-negative bacterium that can survive and multiply inside phagocytes for a long time and so it can lead to chronic infection [30, 31]. It was shown in the literature that various immune cells are activated in many infectious inflammatory diseases which produce oxygen free radicals destroying intracellular and extracellular microbes [34-32]. Actually, these free radicals are one of the vital molecules in killing bacteria inside phagocytes such as neutrophils and macrophages, which act through the peroxidation of fatty acids, proteins, and DNA [37-34]. Brucella invades macrophages in the early stages of infection and adapts to the acidic environment and multiplies in them, where it produces oxygen free radicals such as and H2O2 by anaerobic metabolism [38, 39]. It was observed that the free radical pathway is one of the main ways to prevent the proliferation of Brucella bacteria inside phagocyte cells. This mechanism ultimately damages DNA to the extent to be in contradiction with life by disrupting DNA replication and DNA polymerase and leads to cell apoptosis [34, 40, and 41]. Serefhanoglu et al. (2009) research showed this fact that the overall antioxidant capacity of the body decreases in patients with brucellosis, but oxidative stress increases, and in fact oxidative stress is one of the effective factors in the pathogenesis of brucellosis [17, 42]. Moreover, it was indicated in another research that serum malondialdehyde levels in patients with brucellosis were significantly higher than in the control group, while the total antioxidant capacity of plasma decreased significantly [43]. In addition, zinc has an important protective function against free radicals as an antioxidant [14-14]. Moreover, it was observed that the caused oxidative stress by infectious diseases can change the serum levels of rare elements such as zinc [19]. In a conducted research by Zanganeh et al. (2017) to investigate the effect of Brucellosis on rare element levels in 40 patients with brucellosis and 20 healthy individuals that the serum level in the patients and control groups was 76.47 µg/dl and 92.9292 µg/dl, respectively. it was indicated that serum zinc levels in the group of patients with brucellosis (case group) are significantly lower than the group of healthy individuals (control group) [20]. Furthermore, in another research by Cesur et al. (2005), the serum level in the patients and control groups was 79.5 µg/dl and 83 µg/dl, respectively, and showed that serum zinc levels in patients with brucellosis are significantly lower than in healthy individuals [21]. In Suat Skin et al. (2004) research that the serum level of some rare elements in sheeps with brucellosis was studied, it was reported that serum zinc levels in sheep with brucellosis were significantly lower than in healthy sheep, but no significant change was observed in other rare elements (44).

It has been shown in various studies that the use of zinc supplementation can be effective on the serum levels of inflammatory factors and biomarkers of oxidative stress. Based on Kelishadi et al. research, daily consumption of 20mg of zinc has significantly reduced the inflammatory factor CRP and malondialdehyde (reduced fatty acid peroxidation) in children with metabolic syndrome [45]. Moreover, Coles et al. research showed that daily prescription of 20 mg of zinc has significantly reduced serum CRP levels and length of hospital stay in children with severe pneumonia [46]. It was shown in Kim et al. (2014) research that daily intake of 30 mg of zinc gluconate has significantly reduced serum CRP and IL-6 levels in obese women [47]. Moreover, it has been shown in the human studies that Zinc prescription to people who are deficient in this element may prevent inflammatory and infectious diseases such as severe pneumonia, which shows a strengthening of the immune system after zinc prescription [48, 49]. Another research was conducted about Zinc prescription for patients with HIV-1 by Mburu et al. (2009) that finally observed that the amount of Zinc in patients with high inflammatory and CRP is lower than other patients. In addition, it was observed that the reverse process, inflammatory decrease, will increase the plasma zinc. It can be concluded that the inflammatory process can reduce the amount of zinc in the body and so the zinc supplementation prescription can be useful in these patients [50]. The limitations of this research include the lack of measuring the other inflammatory factors and markers of oxidative stress such as TNF-α, TGF-β, IL-6, superoxide dismutase, glutathione peroxidase, and so on because of the research budget limitation.

CONCLUSION

It can be concluded based on all the observed results in this research that zinc can improve brucellosis based on its anti-inflammatory and antioxidant effects. Therefore, it seems that prescribing zinc supplementation can play an important role in the recovery of patients with brucellosis as adjunctive therapy along with antibiotic treatments.

Acknowledgment

This research is adopted from the thesis from Kashan University of Medical Sciences. Thereby, we appreciate the Vice-Chancellor for Research and Technology and the Student Research Committee of Kashan Medical School for completing this article.

Ethical Considerations

The protocol of this research was based on international law and was approved by the University Ethics Committee.
Before people participated in this research, the methodology was explained to them and all interview and intervention steps were conducted after taking the written consent of the individuals and the supervision of the infectious disease specialist.
All the conducted services and tests for patients were free using the specialized budget.
In addition, the request of those who wanted to exit the research was accepted.
All patients received standard brucellosis treatment (doxycycline and rifampin) for 6 weeks.

1. Bennett JE, Dolin R, Blaser MJ. (2014). Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases: 2-Volume Set. Elsevier Health Sciences.
2.Kasper D, Fauci A, Hauser S, et al. (2015). Harrison's principles of internal medicine, 19e. Mcgraw-hill.
3.Health WOfA. (2012). Manual of diagnostic tests and vaccines for terrestrial animals: mammals, birds and bees. Biological Standards Commission, World Organization for Animal Health Paris, France.
4.Skalsky K, Yahav D, Bishara J, et al. (2008). Treatment of human brucellosis: systematic review and meta-analysis of randomised controlled trials. Bmj, 336: 701-4.
5.Young EJ, Corbel MJ. (1989). Brucellosis: clinical and laboratory aspects. CRC press.
6.Pappas G, Papadimitriou P, Akritidis N, et al. (2006). The new global map of human brucellosis. The Lancet infectious diseases, 6: 91-9.
7.Zamani A, Daneshjoo K.(2005). Brucella antibody titer (Wright's test) in healthy primary school children in Tehran. Iranian Journal Of Pediatrics, 15: 249-54.
8.Dean AS, Crump L, Greter H, et al. (2012). Clinical manifestations of human brucellosis: a systematic review and meta-analysis. PLoS Negl Trop Dis., 6: e1929.
9.Mesner O, Riesenberg K, Biliar N, et al. (2007). The many faces of human-to-human transmission of brucellosis: congenital infection and outbreak of nosocomial disease related to an unrecognized clinical case. Clinical Infectious Diseases, 45: e135-e40.
10.Shils ME, Shike M. (2006). Modern nutrition in health and disease. Lippincott Williams & Wilkins.
11.Mahan LK, Escott-Stump S. (2004). Krause's food, nutrition, & diet therapy. Saunders Philadelphia.
12.Mohammad MK, Zhou Z, Cave M, et al. (2012). Zinc and liver disease. Nutrition in Clinical Practice, 27: 8-20.
13.Gaetke LM, McClain CJ, Talwalkar RT, et al. (1997). Effects of endotoxin on zinc metabolism in human volunteers. Am J Physiol., 272: E952-6.
14.Ghasemi H, Karimi J, Goodarzi MT, et al. (2016). Seminal plasma zinc and selenium levels and their relation to spermatozoa parameters in semen of diabetic men. International Journal of Diabetes in Developing Countries, 36: 34-9.
15.Dresler S, Illek J, Zeman L. (2016). Effects of organic selenium supplementation in weaned calves. Acta Veterinaria Brno.; 85: 48-53.
16.Song Y, Leonard SW, Traber MG, et al. (2009). selenium deficiency affects DNA damage, oxidative stress, antioxidant defenses, and DNA repair in rats. J Nutr., 139: 1626-31.
17.Serefhanoglu K, Taskin A, Turan H, et al.(2009). Evaluation of oxidative status in patients with brucellosis. Braz J Infect Dis., 13: 249-51.
18.Birben E, Sahiner UM, Sackesen C, et al. (2012). Oxidative stress and antioxidant defense. World Allergy Organ J., 5: 9-19.
19.Vicari E, La Vignera S, Calogero AE. (2012). Oxidative stress and infection. Studies on Men's Health and Fertility: Springer;. p. 551-70.
20.Zanganeh N, Siahpoushi E, Kheiripour N, et al. (2017). Brucellosis Causes Alteration in Trace Elements and Oxidative Stress Factors. Biol Trace Elem Res., 1-5.
21.Cesur S, Kocaturk PA, Kavas GO, et al. (2005). Serum copper and zinc concentrations in patients with brucellosis. J Infect., 50: 31-3.
22.Ekin S, Kozat S, Gunduz H, et al. (2004). Levels of some trace elements and rheumatoid factor in sheep with brucellosis. Biological Trace Element Research., 99: 123-8.
23.Kalkan A, Bingol N, Bulut V, et al. (2000). Serum cobalt, zinc and selenium concentrations in brucellosis. İnfeksiyon Dergisi= Turkish Journal of Infection., 14: 205-8.
24.Milanino R, Marrella M, Gasperini R, et al. (1993). Cobalt and zinc body levels in inflammation: an overview of the data obtained from animal and human studies. Agents and actions., 39: 195-209.
25.Arai K-i, Lee F, Miyajima A, et al. (1990). Cytokines: coordinators of immune and inflammatory responses. Annual review of biochemistry., 59: 783836.
26.Milanino R, Cassini A, Conforti A, et al. (1986). Cobalt and zinc status during acute inflammation: studies on blood, liver and kidneys metal levels in normal and inflamed rats. Agents and actions, 19, 215-23.
27.Michalowicz BS, Novak MJ, Hodges JS, et al. (2009). Serum inflammatory mediators in pregnancy: changes after periodontal treatment and association with pregnancy outcomes. J Periodontol., 80: 1731-41.
28.Erel O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem., 37: 277-85.
29.Bandeira Sde M, Guedes Gda S, da Fonseca LJ, et al. (2012). Characterization of blood oxidative stress in type 2 diabetes mellitus patients: increase in lipid peroxidation and SOD activity. Oxid Med Cell Longev., 2012: 819310.
30.Franco MP, Mulder M, Gilman RH, et al. (2007). Human brucellosis. Lancet Infect Dis., 7: 775-86.
31.Colmenero JD, Reguera JM, Martos F, et al. (1996). Complications associated with Brucella melitensis infection: a study of 530 cases. Medicine (Baltimore), 75: 195-211.
32.Gantt KR, Goldman TL, McCormick ML, et al. (2001). Oxidative responses of human and murine macrophages during phagocytosis of Leishmania chagasi. J Immunol., 167: 893-901.
33.Orem A, Efe H, Deger O, et al. (1997). Relationship between lipid peroxidation and disease activity in patients with Behcet's disease. J Dermatol Sci.; 16: 11-6.
34.Halliwell B, Gutteridge JM. (2015). Free radicals in biology and medicine. Oxford University Press, USA.
35.Dieffenbach CW, Tramont EC. (2015). Innate (general or nonspecific) host defense mechanisms. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases: Elsevier;. p. 26-33. e2.
36.Young E, Mandell G, Bennett J, et al. (2009). Brucella species Principles and practice of infectious diseases, 226.
37.Chambers JC, Haskard DO, Kooner JS. (2001). Vascular endothelial function and oxidative stress mechanisms in patients with Behcet's syndrome. J Am Coll Cardiol., 37: 517-20.
38.Melek IM, Erdogan S, Celik S, et al. (2006). Evaluation of oxidative stress and inflammation in long term Brucella melitensis infection. Mol Cell Biochem., 293: 203-9.
39.Rest RF, Robertson DC. (1975). Characterization of the electron transport system in Brucella abortus. J Bacteriol., 122: 139-44.
40.Jiang X, Leonard B, Benson R, et al. (1993). Macrophage control of Brucella abortus: role of reactive oxygen intermediates and nitric oxide. Cell Immunol., 151: 309-19.
41.Erdogan S, Tosyali E, Duzguner V, et al. (1993). Cisplatin reduces Brucella melitensis-infected cell number by inducing apoptosis, oxidant and proinflammatory cytokine production. Res Vet.
42.Esen R, Aslan M, Kucukoglu ME, et al. (2015). Serum paraoxonase activity, total thiols levels, and oxidative status in patients with acute brucellosis. Wien Klin Wochenschr., 127: 427-33.
43.Zanganeh N, Siahpoushi E, Kheiripour N, et al. (2018). Brucellosis causes alteration in trace elements and oxidative stress factors. Biological Trace Element Research, 182: 204-8.
44.Ekin S, Kozat S, Gunduz H, et al.(2004). Levels of some trace elements and rheumatoid factor in sheep with Brucellosis. Biological Trace Element Research, 99: 123-8.
45.Kelishadi R, Hashemipour M, Adeli K, et al. (2010). Effect of zinc supplementation on markers of insulin resistance, oxidative stress, and inflammation among prepubescent children with metabolic syndrome. Metabolic syndrome and related disorders, 8: 505-10.
46.Coles CL, Bose A, Moses PD, et al. (2007). Infectious etiology modifies the treatment effect of zinc in severe pneumonia. The American journal of clinical nutrition, 86: 397-403.
47.Kim J, Ahn J.(2014). Effect of zinc supplementation on inflammatory markers and adipokines in young obese women. Biological Trace Element Research, 157: 101-6.
48.Brooks WA, Santosham M, Naheed A, et al. (2005). Effect of weekly zinc supplements on incidence of pneumonia and diarrhoea in children younger than 2 years in an urban, low-income population in Bangladesh: randomised controlled trial. Lancet, 366, 999-1004.
49.Bhandari N, Bahl R, Taneja S, et al. (2002). Effect of routine zinc supplementation on pneumonia in children aged 6 months to 3 years: randomised controlled trial in an urban slum. Bmj, 324: 1358.
50.Mburu AS, Thurnham DI, Mwaniki DL, et al. (2010). The influence of inflammation on plasma zinc concentration in apparently healthy, HIV+ Kenyan adults and zinc responses after a multi-micronutrient supplement. Eur J Clin Nutr., 64: 510-7.