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BRIEF REPORT

Serum zinc and copper concentrations in brucellosis patient

Ahmadreza Mobaien[1*], Mahboubeh Hajiabdolbaghi[2], Siroos Jafari[2], Ali Alipouran[2], Mehdi Ahmadi[1], Peyman Eini[3], Henk L. Smits[4]

[1]*Department of Infectious Diseases and Tropical Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*
[2] *Iranian Research Center for HIV/AIDS (IRCHA), Tehran University of Medical Sciences, Tehran, Iran*
[3]*Department of Infectious Diseases and Tropical Medicine, Hamedan University of Medical Sciences, Hamedan, Iran*
[4]*KIT Biomedical Research, Royal Tropical Institute/Koninklijk Instituut voor de Tropen (KIT), Amsterdam, Netherlands*

ABSTRACT

Background: Micronutrients such as zinc (Zn) and copper (Cu) have a modulatory effect on immune system. Altered serum concentrations of these nutrients have been described in patients with specific disease conditions. The aim of this study was to evaluate serum Zn and Cu level alterations in patients with brucellosis in comparison with healthy individuals.

Patients and methods: Serum Zn and Cu level of 43 patients with brucellosis (34 men and 9 women) were compared with 43 matched healthy controls. Serum micronutrient concentrations were measured by automatic absorptive spectrophotometry.

Results: Mean serum Cu concentration was significantly higher in subjects with brucellosis when compared with age-matched healthy controls ($p<0.05$). Mean serum Zn level was decreased in female patients compared with controls ($p<0.05$), however, there was no significant difference between male patients and controls.

Conclusion: Serum Zn and Cu concentrations may alter in patients with brucellosis during the period of infection. Further studies are needed to determine whether these micronutrients have an effect on disease severity and outcome. Measuring serum Cu level may be suggested as a complementary screening tool for brucellosis.

Keywords: *Brucellosis, Zinc, Copper, Micronutrient*.
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INTRODUCTION

Brucellosis is still endemic in Iran and according to the reports of Center for Diseases Control of the Ministry of Health (Iran), the prevalence has increased from 17 cases per 100,000 people in 2000 to 39 cases per 100,000 people in 2006 (1). Human brucellosis is a non-specific febrile illness without distinctive signs or characteristic symptoms (2). Although its mortality is low, brucellosis is a serious disease that may affect multiple organ systems and cause various debilitating complications. Brucellosis is treated with a combination of antibiotics, most commonly doxycycline in combination with an aminoglycoside, rifampicin, or both (3). The disease may be prevented through the vaccination of livestock and the pasteurization of milk. However, most of the livestock in Iran are not vaccinated and, especially in rural areas, much of

Received: 19 January 2010 *Accepted*: 2 April 2010
Reprint or Correspondence: Ahmadreza Mobaien, MD. Department of Infectious Diseases and Tropical Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
E-mail: amobaien@yahoo.com

the dairy products are not pasteurized, while unpasteurized dairy products may be sold at markets and shops in cities. Brucellosis affects both men and women, but in occupationally exposed populations, such as farmers and butchers, more men than women become infected (4).

Brucella is a facultative intracellular, gram-negative bacterium. Following infection through penetration of the mucosal epithelium, the bacteria migrate either free or within phagocytic cells to lymph nodes from where they may spread to any organ system via infected macrophages. Several bacterial factors are involved in the virulence and pathogenicity of *Brucella* including type IV secretion system, lipopolysaccharide, and BvrR/BvrS component signaling system. These factors are thought to determine cell adherence and entry, enhance intracellular survival and replication and modulate immune resistance and response to infection (5). The pathogenicity and immunity to *Brucella* infection may be modulated by several predisposing human factors, such as polymorphism of the TGF-beta1 gene and polymorphism of the transmembrane region of the major histocompatibility complex class I chain-related gene A (6,7). Shortly after infection, the host responses, through the innate immune system, will start. Later on, during the infection, signals produced during the innate response and cytokines released by infected macrophages trigger humeral and cellular immune cells, and a specific immune response is developed. Cellular immunity to *Brucella* depends on the balance in the production of Th1 and Th2 cytokines. Th1 cytokines might induce resistance whereas Th2 cytokines seem to predispose to brucellosis (8).

Immunity and resistance to infection is affected by the nutritional status. Micronutrients such as Zn and Cu may affect and modulate immune responses and thereby contribute to resistance to infectious diseases (9). A study by Cesur and coworkers has shown that, in brucellosis, serum Zn levels are reduced whereas serum Cu levels are increased (10). A similar observation was made by Ciftci and coworkers in patients with pulmonary tuberculosis (11). Infection may also affect serum trace element concentration due to altered or impaired liver physiology or increased loss in the urine. The status of the serum trace element levels could have direct consequences for susceptibility to infection with *Brucella* and prospects for recovery of infected patients. Therefore, use of some trace elements (like Zn) or avoiding some others (like Cu) might be helpful in treatment of brucellosis (for resistance or relapse). Meanwhile, whether trace elements change during therapy and zinc and copper serve as new markers in brucellosis patients are questions that require further studies. In the present study we investigated serum level of Cu and Zn in Iranian brucellosis patients to compare the level of these micronutrients in infectious versus healthy subjects.

PATIENTS and METHODS

Forty-three patients with active brucellosis diagnosed in 2006 at Infectious Diseases Unit of Imam Khomeini Hospital in Tehran and Farshchian Hospital affiliated to Hamadan University of Medical Sciences in Hamadan, were included. All patients were in acute phase and clinical signs and symptoms had been commenced during the recent 3 months prior to their admission, while none had been received antibrucella antibiotics. All patients had clinical signs and symptoms relevant to brucellosis and a Wright serum agglutination test titer $\geq$1:160. Thirty-four patients were males and nine were females. The mean age of brucellosis patients was 40.1 years (range, 25 to 55 years). As a control group, 43 age- and sex-matched healthy volunteers with no underlying disease were enrolled.

The study protocol was approved by Tehran Medical University Ethics Committee, however, all patients and controls were requested to sign an informed consent.

For measurement of Zn and Cu levels, a 10 ml fasting venous blood was drawn from subjects. Blood samples were collected without addition of an anticoagulant. Freshly collected blood samples were spun at 250 rpm for 15 minutes to obtain the serum. Having diluted with deionised water, Zn and Cu serum concentrations were measured by atomic absorption spectrophotometer using Randox Zn and Cu serum level kits (Antrim, UK).

Finally, all data analysis was performed using SPSS software (version 14.0, SPSS Inc., Chicago, USA). T-test was used for data analysis. All data were presented as mean ± standard deviation (SD). Statistical significance was defined as $p<0.05$.

RESULTS

The most common clinical findings were arthralgia (86%), fever (79%), back pain (77%), and diaphoresis (72%) as shown in table 1. Most male patients presented with two or more symptoms and signs whereas females presented with just one sign or symptom. All (100%) patients recalled the consumption of unpasteurized dairy products, and 27 (79%) male patients reported contact with livestock or livestock products. Among male patients, 20 (58%) were farmers, 7 (20%) were domestic animal husbandmen, 4 (11%) were students, and 3 were laborers. All nine women were housewives.

Table 1. Clinical signs and symptoms of patients with brucellosis

Sign or symptom	Total (%)	Men(%)	Women(%)
Arthralgia	37(86)	28(82)	9(100)
Fever	34(79)	28(82)	6(66)
Back pain	33(76)	24(70)	9(100)
Diaphoresis	31(72)	28(82)	3(33)
Weakness	19(44)	17(50)	2(22)
Weight loss	18(41)	14(41)	4(44)
Headache	15(34)	10(29)	5(55)
Chill	6(13)	6(17)	0(0)
Cough	2(4)	2(5)	0(0)
Splenomegaly	2(4)	2(5)	0(0)
Hepatomegaly	0(0)	0(0)	0(0)
Lymphadenopathy	0(0)	0(0)	0(0)
Rash	0(0)	0(0)	0(0)

Compared with healthy controls, the brucellosis patients were slightly more often exposed to both non-pasteurized dairy products and livestock ($p<0.05$). Significantly higher serum Cu concentrations ($p<0.01$) were found in brucellosis patients compared with healthy individuals (table 2). Serum Cu levels were increased about 1.5 fold in men and 1.8 fold in women. Although male patients generally had higher serum Cu levels than females, this difference did not reach a statistically significant level. No effect of gender on the serum Cu level was observed for the controls. Serum Zn level was 0.85 fold reduced in males and 0.67 fold in females when compared with controls, but this difference was statistically significant ($p<0.05$) for female patients only (table 2). Among controls, serum Zn level was about 1.14 higher in females.

Table 2. Serum Cu (µg/dl) and Zn level (µg/dl) in patients and controls with respect to gender

	Patients	Controls
Cu level		
All*	160.84±54.61	101.74±27.37
Men*	154.68±49.89	100.91±23.15
Women**	184.11±67.97	101.22±18.97
Zn level		
All	91.47±46.91	107.19±35.18
Men	94.41±47.80	104.03±36.52
Women**	80.33±44.20	119.11±28.17

*$p<0.01$, **$p<0.05$

DISCUSSION

Micronutrients play an important role in immunity (12,13). Consistent with an earlier report, we found higher serum Cu levels in patients with brucellosis (10). In a study conducted by Cesur and coworkers the Cu levels were increased 1.34 fold, however, we found an almost 1.6 fold increment in brucellosis patients. Cesur and coworkers also found a significant 0.96 fold reduced Zn level in brucellosis patients compared with controls. In our study Zn levels were 0.85 fold lower in brucellosis patients compared with controls, but the difference was significant for female patients only.

Cu and Zn levels are known to present a wide variation between different groups (14). In our study, the variation in Zn levels within the patients and control groups was larger than what reported by Cesur et al in Turkey, a finding that might have masked an effect of infection with *Brucella*.

Many different factors may influence micronutrient levels, including nutritional status and gender. We did not find the effect of gender on Cu levels in the healthy control group. However, higher Zn levels were observed in health females compared with males. In contrast, in studies investigating the normal population, higher Cu levels of females compared with males were observed (for instance, in a study performed on the Canari Islands). Nevertheless, no difference in Zn level was noted with respect to gender (15). In a study conducted in Tehran, Farzi et al reported a similar effect of gender on Cu and Se levels but not on Zn levels (16).

In brucellosis, different routes of exposure may lead to infection and disease. In the current study group, all patients reported consumption of fresh dairy products. In addition, a large proportion of the male patients reported professional contact with livestock which is considered a major risk factor for brucellosis. Professional contact with livestock could result in a more frequent exposure to the pathogen of the male patients and this could influence their immune status and, hence, have an effect on their serum micronutrient levels.

The difference in the number of disease symptoms and signs between male and female patients may point to a difference in the disease status between the two groups, and this in turn could be reflected by the difference in Zn levels.

Changes in serum level of trace elements have also been observed in patients with other infectious diseases. Low plasma Zn levels predicted the subsequent development of lower respiratory tract infections and diarrhea among Indian infants (17). Increased Cu levels along with reduced Zn levels were measured in the serum of patients diagnosed with vivax malaria (18). Patients with pulmonary tuberculosis showed an increase in serum Cu levels (11). The meaning of these changes is not yet clear. Such changes could be the direct or indirect effect of defense strategies to prevent growth or adhesion of the pathogen, or to improve the host immune defense mechanism.

In conclusion, Cu levels are clearly increased in patients with brucellosis whereas Zn levels showed a moderate reduction in female brucellosis patients. The significance of these changes is unclear. Perhaps increased Cu levels point to an increased activity of the host defense system. The difference between the two genders could be the result of the difference in the nutritional status, the effect of hormonal difference, the difference in route or frequency of exposure, or the immune status.

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REFERENCES

1. Center for Diseases Control of the Ministry of Health, Treatment and Medical Education of Iran. Information and statistics center of Iran. 2006.

2. Pappas G, Akritidis N, Bosilkovski M, Tsianos E. Brucellosis. N Engl J Med. 2005;352:2325-36.

3. Ariza J, Gudiol F, Pallares R. Treatment of human brucellosis with doxycycline plus rifampin or doxycyclin plus streptomycin. A randomized, double-blind study. Ann Intern Med. 1992;117:25-30.

4. Karimi A, Alborzi A, Rasooli M, Kadivar MR, Nateghian AR. Prevalence of antibody to *Brucella* species in butchers, slaughterers and others. East Mediterr Health J. 2003;9(1-2):178-84.

5. Ko J, Splitter GA. Molecular host-pathogen interaction in brucellosis: current understanding and future approaches to vaccine development for mice and humans. Clin Microbiol Rev. 2003;16:65-78.

6. Bravo MJ, Colmenero JD, Martin J, Alonso A, Caballero A. Polymorphism of the transmembrane

region of the MICA gene and human brucellosis. Tissue Antigens. 2007;69:358-60.

7. Bravo MJ, Colmenero JD, Queipo-Ortuño MI, Alonso A, Caballero A. TGF-b1 and IL-6 gene polymorphism in Spanish brucellosis patients. Cytokine 2008;44:18–21.

8. Galanakis E, Makis A, Bourantas KL, Papadopoulou ZL. Interleukin-3 and interleukin-4 in childhood brucellosis. Infection. 2002;30:33–4.

9. Munoz C, Rios E, Olivos J, Brunser O, Olivares M. Iron, copper and immunocompetence. Br J Nutr. 2007; 98: S24-S28.

10. Cesur S, Kocaturk PA, Kavas GO, Aksaray S, Tezeren D, Ciftci U. Serum copper and zinc concentration in patients with brucellosis. J Infect. 2005; 50:31–33.

11. Ciftci UV, Ciftci B, Yis Ö, Guney Y, Bilgihan A, Ogretensoy M. Changes in serum selenium, copper, zinc levels and Cu/Zn ratio in patients with pulmonary tuberculosis during therapy. Biol Trace Elem Res. 2003; 95:65-71.

12. Dardenne M. Zinc and immune function. Eur J Clin Nutr 2002;56(3):S20–S23.

13. Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 1998;68:447S–463S.

14. Calder PC, Kew S. The immune system: a target for functional foods? Br J Nutr 2002;88:Suppl 2:S165-77.

15. Romero CD, Sanchez PH, Blanco FL, Rodriguez ER, Majem LS. Serum copper and zinc concentrations in a representative sample of the Canarian population. J Trace Elem Med Biol. 2002;16:75-81.

16. Farzin L, Moassesi ME, Sajadi F, Amiri M, Shams H. Serum levels of antioxidants (Zn, Cu, Se) in healthy volunteers living in Tehran. Biol Trace Elem Res. 2009;129(1-3):36-45.

17. Bahl R, Bhandari N, Hambridge KM, Bhan MK. Plasma zinc as a predictor of diarrhoeal and respiratory morbidity in children in an urban slum setting. Am J Clin Nutr 1988;68:414S–417S.

18. Seyrek A, Kocyigit A, Erel O. Essential trace elements selenium, zinc, copper, and iron concentrations and their related acute-phase proteins in patients with vivax malaria. Biol Trace Elem Res. 2005;106(2):107-15.