THE EFFECT OF ZINC SUPPLEMENTATION ON CLINICAL SYMPTOMS OF BRUCELLOSIS PATIENTS (QUASI-EXPERIMENTAL STUDY)

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ABSTRACT

Background and Objective: Increased oxidative stress and inflammatory factors may play pivotal roles in the pathogenesis of brucellosis. This study was carried out to evaluate the effects of zinc supplementation in patients with brucellosis.

Methods: This randomized, double-blind, placebo-controlled trial was conducted among 82 patients with brucellosis. Subjects were randomly allocated into two groups to receive either 220 mg zinc sulfate (n=41) or placebo (n=41) for 6 weeks. Blood samples were obtained at baseline and after the 6-week intervention to determine outcomes.

Results: The difference between the zinc group and placebo group for fever duration (5.4±2.5 vs. 7.2±2.4 days, P=0.003), and length of musculoskeletal pain (13.2±3.1 vs. 17±3.5 days, P<0.001) was statistically significant. After the 6-week intervention, compared with the placebo, zinc supplementation resulted in significant increases in total antioxidant capacity (TAC) (620.7±156.6 vs. 432.8±62.5 mmol/lit, P<0.001). Supplementation with zinc had no significant impact on other outcomes.

Conclusion: Zinc supplementation among patients with brucellosis had beneficial effects on clinical outcomes and body anti-oxidative defense system.

Keywords: Zinc Supplementation, Clinical Outcome, Brucellosis.

I. 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 disease-causing 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.

II. 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 82 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
A. Plasma antioxidant capacity was determined by Benzie and Strain methods by a calorimetric method using Cusabio Biotech Co. kit from China [28].

B. 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].

- Research 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

- Research 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.

### III. FINDINGS

Table 1 shows the general differences of the brucellosis disease at the beginning of intervention in two groups of taking zinc supplementation and placebo. There is no significant difference between the two groups of taking zinc supplementation and placebo based on means of age, frequency percentage, gender, habitat, and nationality.

| Table 1. General Specification of Research Participants |
|---------------------------------|----------------|----------------|----------|
| Age (year)                      | Zinc group (n=37) | Placebo Group (n=36) | P 1      |
| gender                          | 35.13±7.9        | 39.14±8.3        | 0.22†    |
| Female                          | 15 (40.5)        | 13 (36.1)        | 0.81     |
| Male                            | 22 (59.5)        | 23 (63.9)        | 0.46     |
| Habitat                         |                |                |          |
| village                         | 26 (70.3)        | 22 (61.1)        |          |
| City                            | 11 (29.7)        | 14 (38.9)        | 0.78     |
| Nationality                     |                |                |          |
| Iranian                         | 28 (75.7)        | 29 (80.6)        |          |
| Foreigner citizens              | 9(24.3)          | 7 (19.4)         |          |
| CRP serum levels at the beginning of the intervention | 19.7±0.6 | 20.9±3.0 | 0.49† |
| Serum levels of malondialdehyde at the beginning of the intervention | 7.0±3.8 | 7.0±4.7 | 0.67† |
| Serum levels of total antioxidant capacity at the beginning of the intervention | 333.89±7.0 | 318.66±9.9 | 0.42† |
Data are presented as mean ± standard deviation or number (%).

1 is the result of Pearson Chi-squared

† is independent t-test result

Table 2 shows the fever duration and pain of patients in two groups of taking zinc supplementation and placebo. The duration of fever and the duration of pain in patients receiving zinc supplementation were significantly shorter than in patients receiving placebo.

**Table 2. Clinical Implications for Patients with Brucellosis**

<table>
<thead>
<tr>
<th></th>
<th>Placebo Group (n=36)</th>
<th>Zinc group (n=37)</th>
<th>P 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain duration (days)</td>
<td>17.3±0.5</td>
<td>13.3±2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fever Pain duration (days)</td>
<td>7.2±2.4</td>
<td>5.2±4.5</td>
<td>0.003</td>
</tr>
</tbody>
</table>

1 is calculated by independent t-test.

Table 3 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 3. Changes in Biomarkers of Oxidative Stress and Inflammatory Factors in Two Groups of Patients with Brucellosis**

<table>
<thead>
<tr>
<th></th>
<th>Placebo Group (n=36)</th>
<th>Zinc group (n=37)</th>
<th>P 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>The research beginning</td>
<td>318.66±9.9</td>
<td>751.96±8.4</td>
<td>432.62±8.5</td>
</tr>
<tr>
<td>Sixth week</td>
<td>7.0±4.7</td>
<td>4.0±0.5</td>
<td>-3.0±4.6</td>
</tr>
<tr>
<td>Changes</td>
<td>333.89±7.0</td>
<td>7.0±3.8</td>
<td>3.1±7.1</td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>20.9±3.0</td>
<td>8.3±4.6</td>
<td>19.7±0.6</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>20.9±3.0</td>
<td>8.3±4.6</td>
<td>19.7±0.6</td>
</tr>
</tbody>
</table>

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

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

**IV. 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 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.

V. 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

1 The protocol of this research was based on international law and was approved by the University Ethics Committee.

2 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.

3 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.

REFERENCES


