The effects of zinc supplementation on serum zinc, alkaline phosphatase activity and fracture healing of bones

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ABSTRACT

الأهداف: لتحديد أثر الزنك التكميلي على تكوين الجسم الثفني، ونشاط مصل الزنك وفوسفات الكالين لدى البشر.

الطريقة: أجريت هذه التجربة السريرية العشوائية العمياء والمزدوجة على 60 مريضاً تعرضوا لكسر في العظام، وتمت إحالتهم إلى مستشفى الشهداء – تابريز – إيران، خلال الفترة من أغسطس وحتى ديسمبر 2007م. تم تقسيم المرضى إلى مجموعتين: مجموعة الحالة (عدد=30) وتلقت كبسولة واحدة من سولفيت الزنك المتكون من 50mg من الزنك يومياً، وتلقت مجموعة التحكم (عدد=30) بلاسيبو لمدة 60 يوم. تم تحديد المعلومات الفردية والسريرية بواسطة الاستبيانات، ومقدار التناول الغذائي بواسطة سجلات الطعام لكل ثلاثة أيام في بداية ونهاية التجربة. تم قياس مصل الزنك وفوسفات الكالين بواسطة (AAS)، وبواسطة الطريقة الأنزيمية على التوالي. تم تقييم تشكل الجسم الثفني خلال التئام الكسر بواسطة تصوير العظام إشعاعياً.

النتائج: لم يكن هنالك فرقاً ملحوظاً في النشاط البدني، الجنس، العمر، نوع الكسور، والتغذية بين المجموعتين. سبب أعطاء الزنك ارتفاع ملحوظ في مصل الزنك ونشاط فوسفات الكالين. أظهر تقييم العظام بالأشعة السينية تقدم ملحوظ في تشكل الجسم الثفني في مجموعة الحالة بالمقارنة مع مجموعة التحكم.

خاتمة: أظهرت هذه الدراسة إمكانية تحفيز شفاء الكسور باستخدام الزنك التكميلي، ويحتاج إلى المزيد من الدراسة.

Objectives: To determine the effect of zinc supplementation on callus formation, serum zinc and alkaline phosphatase activity in humans.

Methods: This randomized, double-blind, placebo controlled clinical trial was conducted on 60 patients with traumatic bone fracture referred to Shohada Hospital of Tabriz, Iran from August to December 2007. Subjects were randomly divided into 2 groups: cases (n=30), receiving one capsule of zinc sulfate consists of 50 mg zinc each day and the controls (n=30), receiving placebo for 60 days. Individual and clinical information was determined by a questionnaire: nutritional intake by 3 days food records at the beginning and the end of trial. Serum zinc and alkaline phosphatase was measured by atomic absorption spectroscopy, and by enzymatic method. Callus formation during fracture healing was evaluated by radiography of the bone.

Results: There was no significant difference in physical activity, gender, age, type of fractures, and nutrient intake, between the 2 groups. The administration of zinc caused a significant elevation of serum zinc and alkaline phosphatase activity. Assessment of bone x-rays showed a significant progress in callus formation in cases compared to the controls.

Conclusion: This study shows that zinc supplementation can stimulate fracture healing, however, it needs further study.

Saudi Med J 2008; Vol. 29 (9): 1276-1279

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Received 10th May 2008. Accepted 27th 2008.

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Zinc was known as essential mineral in 1970.[1] It has a wide variety of physiologic roles in biochemical processes of human and many animals, such as growth process.[1,2] Zinc deficiency induces retardation of bone growth,[3,4] suggesting that the metal is required for the growth, development and maintenance of healthy bones. Several studies demonstrated that zinc plays a role in the stimulation of bone formation and in the inhibition

of bone resorption.[5,6] Several zinc dependent enzymes and hormones are involved in bone metabolism.[7] For example, zinc has been shown to stimulate the activity of alkaline phosphatase, which is involved in bone mineral deposition.[8] Alkaline phosphatase was the first zinc enzyme to be discovered in which 3 closely spaced metal ions (2 zinc ions and one magnesium) are present at the active center. Zinc ions at all 3 sites also produce a maximally active enzyme.[9] Rossi et al[10] have shown that zinc deficiency in growing rats results reduced bone growth and force is required before breaking the bone. The cellular mechanism of zinc action has been demonstrated to stimulate proliferation and differentiation in osteoblastic cells.[11] Chemical factors, which can stimulate the healing of bone fracture in humans have not been fully developed and the most of finding on the effect of zinc on fracture healing are limited to the animal study. However, zinc nutritional deficiency is a global health problem. It has been estimated that almost half of world population does not get enough zinc from their food programs especially in developing countries.[12,13] The present study was undertaken to determine the effect of zinc supplementation on fracture healing and relation between callus formation with zinc and alkaline phosphatase activity in serum in patient with bone fracture.

Methods. This study was a randomized, double-blind, placebo controlled clinical trial. The study population was 60 men and women, aged 20-50 years old with traumatic long bone fracture referred to Shohada Hospital of Tabriz, Iran from August to December 2007. These patients had no history of osteoporosis, osteoarthritis, kidney stones, diabetes, and other endocrine disorders. Also, they were not taking any medication or supplementation known to influence bone metabolism or zinc status, although these diseases were the exclusion criteria for this study. This study was approved by the Regional Medical Research Ethic Committee of Tabriz Medical Sciences University. All patients signed the informed consent for participation in this study.

Patients were randomly divided into 2 groups, supplement group (n=30) received one capsule of 220 mg zinc sulfate contain 50 mg zinc, and control group (n=30) receiving placebo contain starch, each day for 60 days. The participants completed a health questionnaire covering the physical activity at work, previous fracture, and cause of fracture. The height and weight of the participants were measured while they wore light clothing, and the body mass index was calculated. Nutritional intake was determined by 3 day food records. Fasting blood samples were collected and were drawn into acid wash trace mineral free tubes. The blood samples were centrifuged at 3000 μg for 10 minutes to get serum, and the serum zinc concentration was measured by using the atomic absorption spectrophotometer (CAT-2000, Chem. Thech Co., USA) and alkaline phosphatase by Pars Azemon kit (kit no. 86001-1) and spectrophotometer (Metrolab 1600 DR, Argentina). Patients were visited monthly, the supplement intake and the treatment process were controlled for 60 day. Dietary intake, anthropometric data, blood samples, and radiography of bone were again obtained in the end of the study. Callus formation was defined as a radiological finding of fracture healing in this study.

Statistical Package for Social Sciences version 11.5 was used for all statistical procedures. Anthropometric variables, dietary intakes, and serum zinc and alkaline phosphatase concentration are shown as mean ± SEs. Pearson correlation coefficients were used to evaluate the associations. The significance of the difference between values was estimated by student t-test. *P* values of <0.05 was considered statistically significant.

Results. From 60 patients, 39 people (65%) were male and 21 people were female (35%). Mean age of participant was 30.64 years old (20-50 years). The baseline characteristics of the patients are shown in **Table 1**. Fifty-eight percent of patients had light physical activity and only 4% of patients had severe physical activity. The anatomical distribution of fractures was: tibia (56.8%), femur (24%), radius (12.2%), and homarus (6.8%). There was no significant difference in gender, age, anthropometric variables, history of fracture, energy, and zinc intake, and anatomical distribution of fractures between case and controls, and the change of mean anthropometric variables and nutrient intake from diet during the trial also was not significant (**Table 1**).

The mean of serum zinc concentration (70.6±33 μg/dl) in patients with bone fractures was significantly lower than normal range (75-125 μg/dl), although serum alkaline phosphatase activity (104±20 IU) was in normal range (80-306 ul/l). As shown in **Figures 1 & 2**, the administration of zinc sulfate for 60 days caused a significant elevation of serum zinc (133±60 μg/dl versus 77±21 μg/dl) and alkaline phosphatase activity (137.58±24.94 ul/l versus 103±19.9 ul/l), however, changes in the control group were not significant. Assessment of bone radiographs by expert in radiology showed significant change in callus formation if the group will compare to the control group after 60 days (**Figure 3**), and fracture healing was faster in the supplement group than control group. The mean of serum zinc and alkaline phosphatase activity in patients that callus was seen in their radiographs was higher than patients without callus, however, this difference was not significant (**Figure 4**).

Table 1 - Physical and dietary intake characteristics of subjects.

Variables	Supplement Group (n=30)	Control group (n=30)
Weight (kilogram)		
Baseline		
Final	65.65±3.11	72.51±2.35
	66.97±4.02	72.67±2.27
Body mass index (kg/m²)		
Baseline		
Final	24.07±1.28	24.96±0.79
	24.27±1.32	25.09±0.78
Energy intake (kilocalorie/day)		
Baseline	2400.15±150.97	2414.56±133.73
Final	2401.0 ±151.50	2403.66±135.86
Zinc intake (milligram/day)		
Baseline	4.60±0.32	4.93±0.36
Final	5.83±1.08	5.02±0.36

Each value are expressed as mean ± SEM.

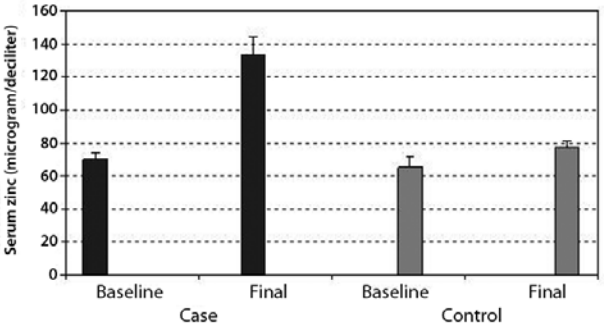

Figure 1 - Effect of zinc sulfate on serum zinc concentration in humans with bone fracture after 60 days. Each value is expressed as mean±SEM.

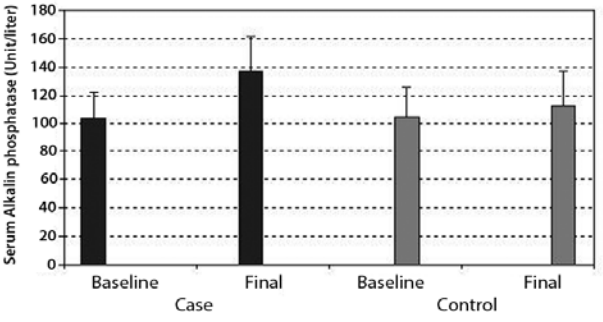

Figure 2 - Effect of zinc sulfate on serum alkaline phosphatase activity in humans with bone fracture after 60 days. Each value is expressed as mean±SEM.

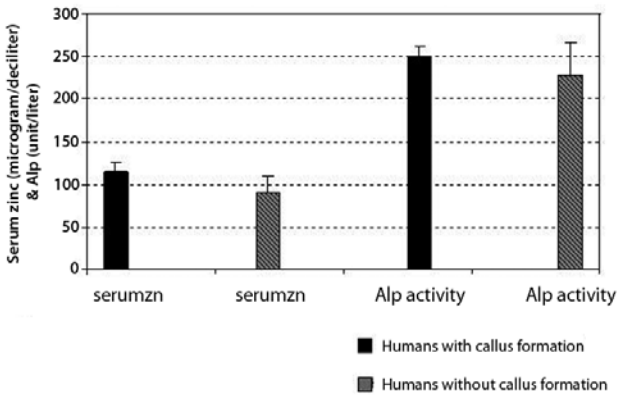

Figure 3 - Alteration in serum zinc and alkaline phosphatase (Alp) activity during fracture healing. Each value is expressed as mean±SEM.

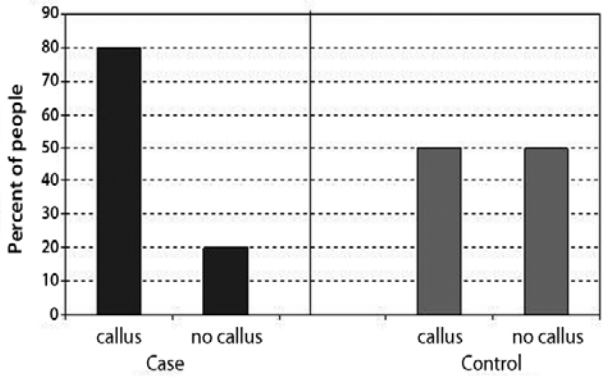

Figure 4 - Effect of zinc sulfate on callus formation in humans with bone fracture after 60 days. Each value is expressed as mean±SEM.

Discussion. The effects of zinc in bone health and fracture healing in humans are not well clarified, and most of the studies are limited to animal study. The present study demonstrated that serum zinc concentration was significantly lower in patients with bone fracture than normal range, which is similar to the results from the second National Health and Nutrition Interview Survey in 1976-1980,[14] and epidemiologic survey conducted in Rome.[15] Low serum zinc concentrations may be a result of low dietary intake of zinc (**Table 1**), or inflammation, or hospitalization.[16] Besides, several groups of investigators reported a higher urinary zinc excretion[16-17] in these groups of patient. Zinc supplementation increased serum levels of zinc and alkaline phosphatase activity, that are similar to the result of Igarashi et al[18] in rats, Yan et al[19] in rats, and Clark et al[20] in humans, and Dimai et al[21] in mice. It is supposed that an increase in serum zinc and alkaline phosphatase activity my be stimulated by fracture healing, and can be defined as biochemical marker of fracture healing, as clinical studies have shown that the amount of skeletal alkaline phosphatase activity in serum can provide a useful index of the rate of bone formation.[22]

Our results indicate that supplementation with zinc for 60 days had a stimulatory effect on callus formation under condition of fracture healing in humans. Zinc is an essential trace metal, has been demonstrated to stimulate protein synthesis due to activation of amino acyl-tRNA synthetase in osteoblastic cells and bone tissues.[11] The oral administration of zinc caused a significant increase in bone components, calcium

content, alkaline phosphatase activity, and protein content of rats with fracture healing.[19] It has been suggested that zinc supplementation may be a useful tool as a stimulatory factor for fracture healing.[18] Wang et al[23] reported that zinc promotes osteoblast formation and reduces secretion of cytokines, which may inhibit osteoclast formation and activation. Bone mineral density is appreciably increased by the administration of zinc in fracture healing in rats.[19] The above mentioned mechanisms are supposed that in the supplement group of our study the stimulatory effect of zinc on callus formation was in bone. A good pharmacological tool for the therapy of bone fracture is not found, and zinc compound may have a role as a pharmacological tool in the healing of bone fracture in humans.

Further studies are needed to evaluate the role of zinc and its mechanism on fracture healing in human which control our study limitation. In our study, gradation of callus formation did not determined and its relation with serum zinc level was not considered.

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