# Assessment of Serum Levels of Copper, Magnesium, and Zinc in Patients Infected with *Trichinella Britovi*

## *Trichinella britovi* ile Enfekte Hastalarda Serum Bakır, Magnezyum ve Çinko Seviyelerinin Değerlendirilmesi

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**ABSTRACT Objective:** *Trichinella britovi* is found in many species of carnivores and omnivores. T.britovi occurs in Eurasia and there are also studies on trichinellosis in Turkey showing the outbreak of trichinellosis, which occurred in Izmir, between January and March 2004. It was aimed to investigate the changes of level of the essential elements of copper, magnesium and zinc status in cases of trichinellosis. **Material and Methods:** This research has focused mainly on the relationship of copper, magnesium and zinc contents in human serum with Trichinella infection. Subjects were selected from anti-Trichinella IgG ELISA positive patients (48 patients, 24 controls). The parasite species was diagnosed from the deltoid muscle biopsy specimen using PCR and it was confirmed as T.britovi. The copper, magnesium and zinc concentrations of serum samples were determined using Zeeman atomic absorption spectrometry. **Results:** There was significantly higher serum magnesium concentration in both female and male patients than in controls. However, there was no significant difference in female and male patients' serum copper and zinc concentrations as compared with the controls. **Conclusion:** The increased magnesium levels clearly suggest that pathophysiology of trichinellosis may effect on element status and accordingly in human health.

**Key Words:** Trichinella; copper; magnesium; zinc

**ÖZET Amaç:** *Trichinella britovi* karnivor ve omnivor canlıların bir çok türünde bulunur. T.britovi, özellikle avrasya bölgesinde görülür, bununla birlikte Türkiye'de trichinellosis çalışmaları İzmir de Ocak-Mart 2004 yılları arasında T.britovi vakalarının görülmesinden sonra artmıştır. Bu çalışmada amaç temel olarak trichinellosisli hastalarda meydana gelen eser element seviyelerindeki değişiklikler bakır, magnezyum ve çinko elementleri üzerinden değerlendirmektir. **Gereç ve Yöntemler:** Çalışmaya alınanlar anti-Trichinella IgG ELISA pozitif hastalardan seçilmiştir (48 hasta, 24 kontrol). Parazit türü deltoid kas biyopsi numunelerinin örneklerinin PCR ile analizi sonucu T.britovi olarak belirlenmiştir. Serum örneklerindeki bakır, magnezyum ve çinko seviyeleri Zeeman atomik absorbsiyon spektrofotometresi kullanılarak tayin edilmiştir. **Bulgular:** Serum magnezyum konsantrasyonları erkek ve kadın hastalarda kontrol grubuna göre istatistiksel olarak anlamlı yüksek bulunmuştur ($p<0.05$). Bununla birlikte, hasta kadın ve erkeklerin serum bakır ve çinko konsantrasyonları kontrol grubu ile karşılaştırıldığında istatistiksel olarak anlamlı bir fark gözlenmemiştir ($p>0.05$). **Sonuç:** Trichinellosisli hastalarda yükselmiş magnezyum seviyesi bu hastalığın patofizyolojisinin element seviyesi ve dolayısıyla insan sağlığı üzerine etkili olabildiğini göstermektedir.

**Anahtar Kelimeler:** *Trichinella britovi*; bakır; magnezyum; çinko

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Trace elements have been extensively studied in recent years to assess whether they have any modifying effects in the aetiology of diseases.[1,2] Although it has been previously reported that some other diseases change the concentration of trace elements in humans,[3-6] there are no

reports on the effects of trichinellosis on the serum levels of any elements.

*Trichinella britovi* is a parasitic nematode. The life cycle of the parasite begins when the infectious capsules are eaten with the flesh of any meat-eating animal. The capsules are digested and the liberated larvae invade the small intestine. The female hatches larvae are carried by the blood and lymph to the muscles.[7,8]

Trichinellosis is probably best known as a parasitic infection that humans contract from eating raw or undercooked meat. Trichinellosis is caused by the colonisation of *Trichinella* larvae in muscles where they grow in encapsulated. Severe symptoms are likely if the levels exceed 100 larvae per gram of muscle.[9] Many cases are never diagnosed, but, nausea, dysentery, colic can be observed. In addition, migrating larvae causes pain as they invade muscle tissue, there may also be oedema, delirium, cardiac and pulmonary difficulty, pneumonia, nervous disorders, deafness and delayed or lost reflexes due to invasion.[10]

Copper is needed by a variety of key systems in the body. It is required for numerous enzymes necessary for reproduction, immunity and growth. In addition, copper is necessary for proper metabolism of iron, maintenance of connective tissue, pigmentation of skin and hair, maturation of hoof tissue, and many other functions.[11] Magnesium is needed for more than 300 biochemical reactions in the body. It helps to maintain normal muscle and nerve function, bone strength and to keep heart rhythm steady. It is also involved in energy metabolism and protein synthesis.[1] Zinc is an essential element found in almost every cell. It stimulates the activity of several enzymes, which promote biochemical reactions in the body. Zinc supports a healthy immune system, is needed for wound healing, helps to maintain sense of taste and smell, and is needed for DNA synthesis. Zinc also supports normal growth and development during pregnancy, childhood, and adolescence.[12]

There are limited number of studies available on element level changes relating to the parasite infections. This will be the first study evaluating the levels of elements' changes during the *T.britovi* infection.[13-15] The aim of this study was to determine a possible relationship between trichinellosis and copper, magnesium and zinc level changes, i.e., whether trichinellosis might have a modifying effect on these trace elements level. For this we investigated serum concentrations of copper, magnesium and zinc levels in 48 patients with trichinellosis and compared the results with those of 24 healthy controls.

## MATERIALS AND METHODS

### PATIENTS

It was assayed copper, magnesium and zinc levels of 72 subjects in human serum aged between 3 and 62 years (39 males and 33 females). Patients were included the history of raw pork eaten subjects (via eating raw meatball, a special Turkish food which was normally made of raw beef, but the patients were had these raw meatballs without knowing that they have made of pork) and with the symptoms of trichinellosis during the outbreak of trichinellosis in Izmir between January and March 2004. Diagnosis of patients were confirmed with myalgia, weakness, malaise, arthralgia, jaw pain, fever, periorbital and/or facial oedema, oedema at the trunk and limb, abdominal pain, nausea and vomiting and diarrhoea complaints as indicated previous study.[16]

Serum copper, magnesium and zinc levels were studied in patients with *T. britovi* between the twentieth-thirtieth days after they had eaten raw meatballs deceptively made of pork.

These patients had tested positive for serologic and laboratory results for trichinellosis. All patients were positive for anti-*Trichinella* IgG ELISA (Ridascreen-Biopharm). The parasite species detection were performed from the deltoid muscle biopsy specimen PCR by Dr. Edoardo Pozio (International *Trichinella* reference center, Rome, Italy) and confirmed as *T.britovi.*

Serum samples for control group were obtained from healthy people who had come to the different departments of Ataturk Training Hospital for regular check-up and students or employees of the hospital. All subjects fasted after midnight be-

fore blood collection in the next morning. Forty-eight patients and 24 controls were examined in this study. The mean age of the patient group was 25 men (aged 27±13 years) and 23 women (aged 33±14 years). The mean age of the control group was also 14 men (aged 31±10 years) and 10 women (aged 29±16 years).

### ASSAY

All venous blood samples were taken between 8 am and 9 am after 8 h of fasting and collected in polystyrene tubes. The tubes were centrifuged at 400 x g for 15 min. Sera were then removed and stored at -20ºC until analysis.

The copper, magnesium and zinc concentrations of serum samples were determined using Zeeman atomic absorption spectrometry (Hitachi Z-8000 mode). Standard solutions were freshly made from standard stock solutions containing 1 g Cu/L, 1 g Mg/L and 1 g Zn/L. Serum samples were prepared by dilution with deionised distilled water (serum in a dilution of 1:5). We matched the viscosity of the standard solutions to viscosity of diluted serum by adding an appropriate amount of glycerol. The total levels of copper, magnesium and zinc in the samples were determined by the regression analysis of the sample absorption data on the standard curve.

### STATISTICAL ANALYSIS

Statistical analysis was performed with SPSS software package (Version 10.0 for Windows). Data were expressed as mean ± standard deviation (SD). For comparison of 2 groups of continuous variables, independent samples t-test was used. A probability value of $p < 0.05$ indicated a statistically significant difference.

## RESULTS

Magnesium levels were comparable in both male and female patients and controls. Magnesium was above the normal range in female patients (5,8±1,2) and male patients (6,1±1,4) compared to female controls (4,4±0,9) and male controls (4,5±0,6), respectively ($p < 0.05$). Copper, magnesium and zinc scores are given in Table 1.

**TABLE 1:** Copper, magnesium and zinc levels of patients infected with T. britovi and control group.

| | Patients | | | |
|---|---|---|---|---|
| | Age | Copper (µg/L) | Magnesium (mg/L) | Zinc (µg/L) |
| Female (23) | 33 ± 14 | 150 ± 21 | 5,8 ± 1,2 | 91 ± 15 |
| Male (25) | 27 ± 13 | 155 ± 32 | 6,1 ± 1,4 | 111 ± 25 |
| | Controls | | | |
| Female (10) | 29 ± 16 | 139 ± 37 | 4,4 ± 0,9 | 98 ± 16 |
| Male (14) | 31 ± 10 | 128 ± 33 | 4,5 ± 0,6 | 101 ± 26 |
| p value | | p> 0.05 | p< 0.05 | p> 0.05 |

There was no significant difference in serum copper and zinc concentrations between patients and control groups both in females and males ($p > 0.05$).

## DISCUSSION

Human growth and development is strictly dependent on copper, magnesium and zinc. The nervous, reproductive and immune systems are particularly influenced by copper, magnesium and zinc deficiency, as well as by increased levels of copper, magnesium and zinc.[1,2,11] In the present study, serum levels of copper, magnesium and zinc were estimated in 48 patients with trichinellosis and 24 age- and sex-matched controls.

*T. britovi* occurs in Eurasia. There are also studies on trichinellosis in Turkey such as showing the outbreak of trichinellosis, which occurred in Izmir, between January and March 2004.[16] It is common in sylvatic carnivores, and occasionally infects domestic animals. Trichinellosis can occur at 2 stages of infection: in the muscle and in the intestines. Trichinellosis is caused by the colonisation of *Trichinella* larvae in muscles where they grow in encapsulated. Severe symptoms are likely when the number of parasites increases. Capsules may be difficult to see with the naked eye; however, active muscles such as the tongue, diaphragm and muscles of the jaw usually contain the highest concentrations of infective larvae. Muscle tissue containing larval capsules was used for diagnosis.[9]

The most effective way to prevent trichinellosis is cooking meat thoroughly. Freezing could be effective if carried out properly. Several safe and effective prescription drugs are available to treat trichinellosis. Treatment should begin as soon as possible and the decision to treat is based on symptoms, exposure to raw or undercooked meat, and laboratory test results.[10]

Magnesium is an important electrolyte needed for proper muscle, nerve, and enzyme function. It also helps regulate energy production in cells and is needed to move other electrolytes (potassium and sodium) into and out of cells.[4] Copper and zinc are required for the normal functioning of humans. They are incorporated into a variety of organics, which perform specific metabolic functions. Because they are essential elements, daily dietary requirements have been recommended by a number of agencies.[5,17,18]

In compare to our results with the results from the other parasite infections show that elements status changes show difference from one parasite infection to other; such as; in patients with cystic echino-coccosis the mean serum levels of zinc and magnesium were lower and the mean serum level of copper was higher in the preoperative period when compared with the control. It has been found that as duration of symptoms increased, serum zinc and magnesium levels decreased but copper levels increased.[13]

However, the mean concentration of copper, magnesium, and zinc in blood has also showed no statistically difference in *Taenia saginata*-positive patients and in their controls.[14] In addition, investigation of zinc, iron, copper, cobalt, magnesium, and selenium levels in patients infected with intestinal parasites, *Giardia intestinalis* and *Enterobius vermicularis* have demonstrated that element status were either increasing or decreasing and also some of element status were correlated each others but some of them uncorrelated.[15]

The present study shows that both copper and zinc but not magnesium, have seems as biomarkers of disease progression. However, although the exact mechanism responsible for the alteration of magnesium levels is not clear, it is clear that there is an obvious increase in magnesium levels in patients compared to controls and a detailed study on a large sample size is therefore needed. As a result, in the present study, the rise of magnesium in the blood is probably due to insufficient uses of magnesium in body. Signs of excess magnesium can be similar to magnesium deficiency and include psychological changes, nausea, diarrhoea, appetite loss, muscle weakness, breathing difficulties, extremely low blood pressure, and irregular heartbeat. Inhibition of consumption of magnesium by *T. britovi* may also play a role in serum magnesium increase. These findings clearly suggest that trichinellosis may affect element metabolism.

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