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Research Article

Evaluation of copper, zinc and ceruloplasmin concentrations in patients with brucellosis and their comparison with the control group in eastern Guilan province during 2022-2023

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ABSTRACT

Brucellosis is one of the most important and zoonosis common diseases between humans and animals. The metabolism of trace elements such as zinc, copper, and ceruloplasmin can influence the immune system response and activate the host's immunochemical mechanisms against this organism. Accordingly, this study aimed to evaluate copper, zinc, and ceruloplasmin concentrations in patients with brucellosis . This descriptive, cross-sectional study was conducted on 32 patients with brucellosis who had referred to medical centers in eastern Guilan province and 32 people from the control group. Copper levels in the serum of patients and controls were measured according to the biochemical protocol kit with code REF 10-564, zinc levels according to the biochemical protocol kit with code REF 10-517, and ceruloplasmin levels according to the biochemical protocol kit with code MAK 177. In this study, the average copper levels in test and control group were 98 ± 4.14 g/dl and 76 ± 5.12 g/dl respectively, the average zinc levels in test and control group were 44 ± 3.28 g/dl and 72 ± 3.15 g/dl respectively, the average ceruloplasmin levels in test and control group were 40 ± 8.10 g/dl and 31 ± 11.1 g/dl, and the copper to zinc ratio in the patients with brucellosis was 2.22 ± 0.5 and 1.05 ± 8 in the control group. Therefore, it was determined that there was a significant difference between the average levels of copper, zinc, ceruloplasmin, and the ratio of copper to zinc in people with brucellosis and the control group.

1. Introduction

Brucellosis is a zoonotic disease caused by bacteria of the genus *Brucella*. It is a significant public health threat, particularly among vulnerable populations in rural areas, with significant economic impacts (Dadar et al., 2021). Brucellosis is one of the most common zoonotic diseases, affecting 500,000 cases annually . The disease was previously known by names such as Malta fever, Mediterranean fever, Gibraltar fever, Cyprus fever, and unstable fever (Al Jindan et al.2021). The disease is transmitted to humans during milking time through direct contact with infected animals, as well as through consumption of raw milk (Lakew et al., 2019). Brucellosis has been reported worldwide, but has been effectively eliminated in many developed countries. However, it is still considered a significant disease in developing countries, and clinical cases of the disease are reported (Zhao et al., 2022). In Iran, as in other developing countries, brucellosis is an endemic disease that causes great economic damage to

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the livestock industry (Esmaeili et al., 2014).Since this condition is very destructive, timely diagnosis and appropriate and rapid treatment will save the patient from further disability and disability (Corbel et al., 2008). Brucellosis is also associated with numerous complications such as gastrointestinal complications, hepatobiliary system disorders, neurological complications, cardiovascular, respiratory, genitourinary, hematological, skin, eye, and bone and joint complications (Salata et al., 2012). The global prevalence of human brucellosis is unknown due to the inaccuracy of diagnostic methods and the inadequacy of reporting and monitoring systems in many countries. Approximately half a million cases of human brucellosis are reported to the World Health Organization worldwide annually, most of which are specific to third world countries (Dames et al., 2005). Copper (Cu) and zinc (Zn) are two of the most important nutrients for the human body. They affect many processes in the human body because they act as a structural ion, catalyst and regulator of enzymatic reactions. They participate in antioxidant processes, the immune system, aging processes and have anti-inflammatory effects (Costa et al., 2023). Both are important for the structure and function of many enzymes, including dehydrogenases, aldolase peptidases, phosphatases, and dismutases such as Cu-Zn SOD (superoxide dismutase) (Zastrow et al., 2005). Their content in the body depends, among other things, on lifestyle, age or environmental influences (Guo et al., 2011). Changes in the levels of copper and zinc in the body are implicated in various disease states. Due to their wide range of functions in the human body, the role of copper and zinc in the carcinogenesis process is also of interest. Zinc is considered the most important micronutrient in the human body. It is essential as a structural or functional element for at least 3000 proteins (Maret et al.2013). It is involved in DNA synthesis, RNA transcription, and cell division of the immune response (Bonaventura et al.2015). The amount of zinc in the body is estimated to be about 2-4 g (Franz et al., 2013), of which only 0.1% is present in plasma (plasma concentration 13.8-22.9 μmol/L) (Bonaventura et al., 2015), while the remainder is intracellular. It is estimated that about 10% of the human proteome contains zinc. Its highest levels are found in the retina and choroid, liver, bones, and skin (Karcioglu et al., 1982). In plasma, zinc is bound and transported by albumin and transferrin. The distribution, uptake and regulation of zinc activity are tightly controlled by metal transporters of the Zirt-/Irt-like (ZIP) family or by Zn transporter (ZnT) family and intracellular binding proteins - metallotinins (Kimura et al., 2016). In studies conducted in different places, it has been reported that trace elements such as copper and zinc affect the functioning of the immune system and activate the host's immunochemical mechanisms against this organism. It has been observed that serum levels of copper and zinc also change in patients with brucellosis. Given that brucellosis is an endemic and common disease and a health problem in Iran, a study was designed to compare serum copper and zinc levels in patients with brucellosis and healthy individuals. Serum copper levels have also been shown to be altered in patients with brucellosis. The immune response to Brucella is mediated by T lymphocytes, and changes in the Th1/Th2 ratio indicate susceptibility or resistance to Brucella infection. Cytokines secreted by Th1 confer resistance to Brucella, while activation of Th2 predisposes the individual to brucellosis. Several studies have shown that T cell proliferation and production of Th1 cytokines are reduced in brucellosis (Nakayama et al., 2002).A suitable sign and a good marker for distinguishing infectious from non-infectious diseases is the measurement of changes in serum levels of trace elements in patients. The problem is that these parameters are not always specific for the disease, and in any case, other serum parameters should also be used for accurate diagnosis of the disease. The body's immune system requires copper for activation and maturation. Copper reduces interferon alpha and affects the function of the immune system (Afzali et al., 2004) .In patients with sepsis, we see increased expression of cytokines, which seems to be mainly due to reduced transferrin saturation and increased bacteriostatic levels of elements such as copper. Zinc plays an important role in the immune system, and the important effects of zinc on lymphocytes include participation in activities such as cell division, antibody synthesis, activation of T cells and cellular immunity. Zinc deficiency causes damage to the immune system and thymus atrophy and allergies, which impairs the function of the immune system and increases

fungal, viral and bacterial infections. Zinc deficiency also causes an imbalance between Th1 and Th2 and reduces the killing effect of killer T cells (Bricker et al., 2000). One of the characteristics of Brucella is the presence of a zinc (Zn) uptake system that reduces intracellular growth, thus suggesting that the system could be a factor in the virulence of the microbe (Suzuki et al., 1996).Therefore, considering the above-mentioned issues, this study aimed to determine the serum concentration levels of zinc, copper, and ceruloplasmin, investigate the role of these elements in the recovery process of brucellosis, and determine the relationship between serum levels of zinc and copper with brucellosis and compare it with healthy individuals. Ceruloplasmin is a multifunctional copper-containing ferroxidase enzyme in serum. Ceruloplasmin is the main copper transporter in blood, transporting more than 95% of the copper in plasma. Increased ceruloplasmin levels have been observed in patients with celiac disease and Crohn's disease. Increased serum ceruloplasmin has also been associated with cancer, pregnancy, rheumatoid arthritis, schizophrenia, and Alzheimer's disease, but low levels have been associated with Wilson's and Menkes' diseases. Therefore, measuring ceruloplasmin activity in biological samples can help in better understanding various metabolic and pathological complications (Mobain et al., 2008). Serum agglutination testing is used to screen for brucellosis, and real-time PCR is used to detect Brucella DNA in serum samples (Hassan et al., 2021).Risk factors for acute brucellosis in patients on the day of admission at selected hospitals of Abbottabad, Pakistan. Front Public Health. (2021).

2. Materials and Methods

This study was conducted during 2022 and 2023 in individuals referring to medical health care centers, in eastern Gilan province, including Langrud, Rudsar, and Amlash counties. After obtaining a permit, 140 samples were collected from individuals with clinical findings and epidemiological evidence suspected of brucellosis. To confirm the disease in these individuals, Wright, Coombs Wright, 2ME, and Rose Bengal laboratory tests were performed, as well as CBC, ESR, and CRP tests, which ultimately resulted in 32 samples being positive for brucellosis. Sampling was conducted in such a way that 10 ml of fasting blood was collected from 140 individuals referring to laboratories for the diagnosis of brucellosis. Also, in this study, 32 blood samples from individuals whose laboratory results were negative for routine serological tests and clinical findings of brucellosis were examined as controls. The collected blood samples (in both groups) were centrifuged at 3500 rpm for 5 minutes at room temperature and the serum samples prepared in this way were stored at -80°C for subsequent experiments.

2.1. Wright Test

In this method, 11 sterile tubes were selected and numbered from one to eleven. Then 0.9 ml of physiological serum was added to the first tube and 0.5 ml of saline to the other tubes. Then 0.1 ml of serum was added to the first tube and the contents of the tube were mixed well and 0.5 ml of the contents of the first tube was added to the second tube and so on until the tenth tube in sequence and 0.5 ml was discarded from the tenth tube. Then 0.5 ml of BhuA antigen (product of Institute Pasteur - Iran) was added to all tubes and in this way the final titer was obtained from 1.20 to 1.10240. Finally, all tubes were incubated at 37 for 24 hours and the results were read. Thus, the last tube in which agglutination (at least 50%) was observed was reported as the final titer.

2.2. Coombs Wright

After performing the Wright test, the Wright tubes were centrifuged at 2000 rpm for 15 minutes, and the precipitate was separated and washed three times with sterile physiological serum. After the last wash, the supernatant was discarded and a drop of Coombs serum (Anti-human Globulin) was added to the precipitate of each tube. After placing the tubes at 37 degrees Celsius for 0.5 to 1 hour, they were centrifuged for 15 minutes at 2000 rpm. The result was reported as in the Wright test by gently tapping and examining the last tube in which agglutination was observed.

2.3. 2ME Test

This method is used to investigate the chronic or chronically active stage of brucellosis. The test was performed in the same manner as the Wright tube method, but 2ME buffer was used instead of physiological serum and 2ME antigen was used instead of Wright specific antigen. The final titer was determined as in the above methods.

2.4. Screening test

The test method was rapid, like Wright's, and the antigen was mixed with the patient's serum and agglutination was observed after a maximum of 4 minutes.

2.5. CRP

It was performed according to the colorimetric method. In addition, CBC and ESR tests were also performed on the patients' blood samples.

2.6. Measurement of copper levels in serum of patients and controls

The copper level in the serum of patients and controls was measured according to the biochemistry protocol kit with code REF 10-564. In this way, 100 microliters of the sample (serum without hemolysis) were mixed with 1000 microliters of reagent and too 100 microliters of calibrator were mixed with 1000 microliters of reagent, and these two mixtures were incubated at 37°C for 5 minutes. Then, the optical absorption of the sample (A sample) and calibrator (A calibrator) was measured at a wavelength of 578-600 nm against the blank (1000 microliters of reagent), and the copper level was calculated using the following formula:

$$Copper(\mu g/dl) = \frac{A\ sample}{A\ calibrator} * Calibrators\ Value$$

Normal copper levels in men are 70 to 145 µg/dl and in women are 80 to 155 µg/dl.

2.7. Measurement of zinc levels in serum of patients and controls

Serum zinc levels were measured in the two aforementioned groups according to the biochemistry protocol with the code REF 10-517. First, serum was centrifuged for a maximum of two hours after sampling and stored in special Trace Element-Free blood collection tubes that have a blue cap and contain EDTA as an anticoagulant. Then, 50 microliters of the sample were mixed with 1000 microliters of R1 reagent. Also, 50 microliters of calibrator were mixed with 1000 microliters of R1 reagent and then the two were incubated at 37°C for 5 minutes and then the optical absorption of the sample (A sample) and calibrator (A calibrator) was measured at a wavelength of 546 to 578 nm against the blank (1000 microliters of R1 reagent) and the zinc level was calculated using the following formula:

$$Zinc(\mu g/dl) = \frac{A\ sample}{A\ calibrator} * Cal.\ Conc$$

Normal zinc levels are 73 to 127 µg/dl in men, 70 to 114 µg/dl in women, 64 to 110 µg/dl in children, and 50 to 100 µg/dl in newborns.

2.8. Measurement of ceruloplasmin levels in serum of patients and controls

The measurement of ceruloplasmin in the serum of patients and controls was performed according to the biochemical protocol kit code MAK177 Product of Aldrich Company, USA
The vial was centrifuged shortly before opening and the ceruloplasmin activity buffer was placed in a water bath for 30 minutes at 25°C before use. The kit was kept in a refrigerator and away from light. A standard curve was prepared by non-enzymatic oxidation using a chemical oxidant. 10 µL of the 100 mM oxidizer in the kit was mixed well with 180 µL of the buffer and 10 µL of the stabilizer to prepare a 5 mM standard solution. Blank values and 2, 4, 6, 8, and 10 µL of 15 mM oxidizer were added to 96-well wells to prepare blank values (0) and standards of 10, 20, 30, 40, and 50 nmol in each well. Then, kit buffer was added to each well to make a volume of 100 µL. One hundred microliters µl of saturated ammonium sulfate solution was added to 100 µL of serum and mixed gently, then placed on dry ice for 5 min, then centrifuged at 10,000 rpm at room temperature for 5 min to precipitate proteins. Then 160 µL of the oily supernatant was pipetted and 160 µL of water

was added to dissolve the precipitate. Alternatively, the serum sample was dialyzed with 1000 times its volume for 1 hour to remove chlorine. Five to 20 µL of the chlorine-free serum samples were poured into 96-well wells and the sample was brought to a final volume of 100 µl with the kit buffer.

One hundred microliters µl of the reaction mixture was added to each of the sample and standard wells and then mixed thoroughly using a horizontal mixer or pipette. The plate reader was set at 25°C. The samples were read in kinetic mode at A560 for 15 min.

2.9. Calculations

1. Chemically oxidized standards were used for 15 minutes to draw a standard curve at a wavelength of 560A, and then the slope of the curve was calculated. (Ss).

Note: A new standard curve must be prepared for each experiment. For accurate results, absorbance values of 560A less than 1 were used to calculate the slope of the standard curve.

Number of moles of substrate oxidized / absorbance value = 560A (Ss) Slope of the curve

2. The linear range of the kinetic curve was determined for each sample. The slope of the curve for each sample in the linear range was calculated as follows:

$$S_k = {}^{(\Delta A560)}/_{min} = \frac{[(A560)_2 - (A560)_1]}{T2 - T1}$$

So in this equation the absorption number is at the end of the linear range and the absorption value is at the beginning of the linear range.

T1 = Time step at the beginning of the linear range

T2 = Time step at the end of the linear range

The ceruloplasmin activity of a sample was determined with the following equation:

$$C_A = \frac{S_k}{V} * \frac{S_s}{b}$$

SK=Slope of the kinetic curve of the two linear parts of the sample /min( )

Ss=Slope of the standard curve /nmole

V=Volume of sample added to the well (Ml)

b = sample dilution factor for samples precipitated with ammonium sulfate (not required for dialyzed samples).

One unit of ceruloplasmin is the amount of enzyme that can oxidize 1 micromole of substrate in one minute at 25°C. Ceruloplasmin activity is expressed in mu/mL or U/L.

All data collected from patients and controls were analyzed using SPSS 17.0 statistical software. One-way ANOVA was used to compare data between patients with brucellosis and controls, with P values <0.05 considered as positive results.

3. Results

In this study, which was conducted during 2022-2023, 140 cases of suspected brucellosis and 32 people as controls were examined. Among the 140 cases of suspected brucellosis, 32 were positive in terms of blood tests and serology, and all of the above cases were consistent with the clinical and epidemiological findings of brucellosis.

3.1. Distribution of patients with Malta fever in eastern Guilan province by age groups

These people were divided into several age groups. In the age group of 6-14 years, 4 positive cases were diagnosed, all of whom were males. In the age group of 15-23 years, 5 cases of brucellosis were diagnosed, 3 of whom were males and 2 of whom were females. In the age group of 24-32 years, 12 of the samples were positive, 9 of whom were males and 3 of whom were females. In the age group of 33-41 years, there were 7 cases of brucellosis, of which 5 were male and 2 were female, and in the age group of 42-50 years, 4 cases of brucellosis were observed (Table 1).

3.2. Distribution of control samples in eastern Guilan province by age groups and gender

In this study, 32 samples were also examined as control cases (negative brucellosis), 75% of whom were men and 25% were women, and their frequency varied according to age groups (Table 2.).

3.3. Distribution of Brucellosis Cases in Eastern Guilan Province by Transmission route

In this study, the variable of disease transmission routes was also examined, which included fresh milk with a frequency of 38.9%, contact with animals with a frequency of 88.46%, consumption of fresh cheese with a frequency of 12.28%, and 62.15% of cases where the disease transmission route was unknown (Table 3.).

3.4. Distribution of the frequency of brucellosis cases in eastern Guilan province according to the level of education

Another variable in this study was the relationship between the frequency of cases of Malta fever and the level of education. In this study, the frequency of illiterate, primary school, secondary school, diploma and higher education individuals was 31.25%, 50%, 37.9% and 37.9%, respectively (Table 4.).

3.5. Distribution of the frequency of cases of brucellosis in eastern Guilan province by place of residence

Another issue examined in this study was the relationship between the frequency of Malta fever and the place of residence of the infected individuals, with 25% of the infected individuals being urban and 75% being rural (Table 5).

3.6. Mean levels of copper, zinc, and copper to zinc ratio in serum

As mentioned, the main aim of this study was to evaluate the levels of copper and zinc and their ratio in the serum of patients with Malta fever, as well as to evaluate the level of ceruloplasmin and compare their levels with the control subjects under study. In this study, the level of copper in the serum of 32 people with brucellosis was measured according to the biochemical protocol kit with the code REF 10-564, and the results showed that the mean levels of copper in patients with malta fever (32 people) and the control group (32 people) were 14.98 and 12.76 micrograms per deciliter, respectively. In this study, the ratio of copper to zinc in individuals with brucellosis and the control group was also evaluated, so that this ratio was evaluated as 2.22 to 0.5 in the affected individuals and 1.05 to 0.8 in the control group (Table 6). As shown in the above results and according to the ANOVA statistical method with a 95% confidence level and $P < 0.001$, it was determined that there was a significant difference between the mean values of copper and zinc between the affected individuals and the control group, and it was also determined that there was a significant difference between the ratios of copper and zinc between the two groups ($P < 0.001$) (Table 6).

Also, in this study, the mean levels of zinc in the serum of patients with malta fever and the control group were 44.3 ± 28.44 and 72.33 ± 15 micrograms per deciliter, respectively (Table 6).

Table 1. Distribution of patients with Malta fever in eastern Guilan province by age groups and gender during 2022-2023

Age Groups (Years)	Gender				Total	
	Male		Female			
6-14	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
	4	12.5	0	0	4	12.5
15-23	3	9.37	2	6.25	5	15.62
24-32	9	28.13	3	9.37	12	37.50
33-41	5	15.62	2	6.26	7	21.88
42-50	3	9.38	1	3.12	4	12.50
Total	24	75	8	25	32	100

Table 2. Frequency distribution of control samples in eastern Guilan province by age groups and gender during 2022-2023

Age Groups (Years)	**Gender**				**Total**	
	Male		**Female**			
6-14	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
	4	12.5	0	0	4	12.5
15-23	3	9.37	2	6.25	5	15.62
24-32	9	28.13	3	9.37	12	37.50
33-41	5	15.62	2	6.26	7	21.88
42-50	3	9.38	1	3.12	4	12.50
Total	24	75	8	25	32	100

Table 3. Distribution of Brucellosis Cases in Eastern Guilan Province by Transmission route 2022-2023

Transmission route	Frequency	
	Number	Percentage
Fresh milk	3	9.38
Contact with animals	15	46.88
Fresh cheese	9	28.12
Uncertain	5	62. 15
Total	32	100

Table 4. Distribution of the frequency of brucellosis cases in eastern Guilan province according to the level of education during the years 2022-2023

Literacy Level	Frequency	
	Number	Percentage
Illiterate	10	31.25
Primary	16	50
Secondary	3	9.37
Diploma and above	3	9.37
Total	32	100

Table 5. Distribution of the frequency of cases of brucellosis in eastern Guilan province by place of residence during the years 2022-2023

Residence	Frequency	
	Number	Percentage
Urban	8	25
Rural	24	75
Total	32	100

3.7. Paraclinical parameters of patients with brucellosis

Normal ESR limit: Between 0 and 16 mm in the first hour. According to Table 8, it was shown that the median obtained in the leukocyte count of patients with malta fever was 11700 and in the control group was 9100, so that a significant statistical difference was found between the two groups studied (P < 0.03). Also, unlike the control group, in all cases, the CRP test of the patients was positive and the mean erythrocyte sedimentation rate (ESR) in the patient group was 11.48. This value was shown to be within the normal range for the control group (Table 4-8).

Table 6. Mean levels of copper, zinc, and copper to zinc ratio in serum of patients with brucellosis in both patient and control groups

Trace Elements	Brucellosis (n=30)	Control Group (n=30)	P Value
Copper (mg/dl±SD)	98.4 ± 14	76.5 ± 12	< 0.001
Zinc (mg/dl±SD)	44.3 ± 28	72.3 ± 15	< 0.001
Copper to Zinc Ratio	2.22 ± 0.5	.05 ± 0.8	<0.001

Table 7. Mean ceruloplasmin levels (mg/dl) in serum of patients with malta fever in both patient and control groups

Brucellosis (n=32)	Control group (n=32)	P Value
40.08±10.12	31±1.11(۹۰-۱۴)	0.001<

As shown from the above results and according to the ANOVA statistical method with a 95% confidence level and P < 0.001, there was a significant difference between the mean ceruloplasmin levels between the affected individuals and the control group (P < 0.001).

Table 8. Study of paraclinical parameters of patients with brucellosis in eastern Guilan province during 2022-2023

Variable	Blood leukocyte count (median/mm3)	CRP	ESR
Brucellosis (n=32)	11700	+	48 ± 11
Control (n=32)	9100	-	22 <

4. Discussion

The highest prevalence of brucellosis has been reported in countries, such as West Asia, India, the Middle East,Southern Europe, and Latin America. The main cause of the disease is *B. melitensis*, which has been eradicated in countries, such as Australia, Canada, Israel, Japan, and New Zealand (Al Jindan et al., 2021). A number of countries have also attempted to eradicate MMR by implementing the following measures: establishing a robust system for continuous livestock monitoring and recording positive cases; close surveillance of slaughterhouses, markets, and herds for rapid and timely identification of positive cases; close monitoring of herd movements to prevent the spread of infection; ongoing and formal training of veterinarians, veterinary supervisors, veterinarians, and supervisors. Farmers should be notified in the event of animal destruction; and establishing laws to support eradication programs to prevent non-compliance with official measures (Zamri-Saad et al., 2016). According to the present study, out of 32 infected patients, 24 were male and 8 were female. The increased prevalence of the disease in men could have several reasons such as grazing and slaughtering of livestock by these people. About 75% of these people lived in rural areas and 25% in cities. The disease was more

common in the age group of 24 to 32 years. About 47% of the patients had a history of contact with animals and 38% of them had a history of consuming milk and fresh cheese, but 15% were infected for unknown reasons. Also, about 19% of the people had a secondary education level or higher and 81% of the people were illiterate or had primary education level, which indicates the effect of literacy on people's awareness of health tips and preventive measures. It was also shown in this study that the average serum concentrations of copper, zinc and ceruloplasmin and the copper to zinc ratio in people with brucellosis were statistically significantly different from the control group, such that the amounts of copper and ceruloplasmin in the patients increased and the amount of zinc decreased.

According to a study conducted by Hassanzadeh and colleagues over a seven-year period on the epidemiology of brucellosis in Mobarakeh, Isfahan, from 2003 to 2010, it was shown that the median incidence of the disease in Mobarakeh during these years was 12 cases per 100,000 people, with the highest incidence in 2005. Of the 139 affected patients, 45 were female and 94 were male. About 50% lived in the countryside and the same number in the city. The disease was most common in the 11-20 age groups and was observed most frequently in spring and summer. About 70% of patients had a history of milk consumption and about 60% of patients had a history of contact with animals (55). The present study is consistent with the above study in terms of frequency by gender, and no correlation is seen in the other cases mentioned.In a study by Mobain et al. in 2006, regarding the epidemiological situation of 3880 patients with malaria in Kurdistan province, it was shown that there were about 2000 men and 1860 women with high titers. About 18% were urban and about 83% were rural. The highest incidence rate was in 2003 with 89 cases per 100,000 people and the lowest incidence rate was in 2000 with 17 cases per 100,000. In terms of occupation, the highest frequency was related to housewives with about 39% of the incidence. There was a relationship between age and gender in terms of malaria with the chi-square test ($p<0.001$). There was a significant relationship between gender and place of residence ($p<0.002$). In this study, men in villages and housewives in cities were considered as one of the most common groups involved. Qorveh and Bijar were identified as two cities at risk for malaria. (Mobain et al., 2010). A cross-sectional study by means of Esalatmanesh was conducted on 100 patients with brucellosis and the subjects were divided into two groups: case and control (50 subjects in each group). Serum copper and zinc levels were measured using an automatic absorption spectrophotometer. Of the total 100 patients, 31 patients (31%) were female and 69 (69%) were male. The mean age of patients in the case group was 32.74±13.7 years and in the control group was 33.44±13.6 years. The results showed that serum copper levels were significantly increased and zinc levels were significantly decreased in patients with brucellosis ($P<0.001$) (Esalatmanesh et al.2018). In a 2014 study by Aini and colleagues, serum copper and zinc levels were assessed before and after treatment in malaria patients and compared with healthy individuals in Yazd city. Of the 26 patients, 13 were female and 13 were male, with no significant differences in gender and age compared with the control group. The mean serum copper concentration in patients before and after treatment was 100.31 mg/dl and 92.81 mg/dl, respectively ($P=0.495$). The mean serum copper concentration in the control group was 97.96 mg/dl, indicating a slight increase in copper in patients compared with healthy individuals. The mean serum zinc concentration in patients was 93 mg/dl and in the control group was 96.38 mg/dl ($P=0.625$). The mean serum zinc concentration in patients after treatment was 90.27 mg/dl.In the aforementioned study, significant changes were observed in the serum concentrations of these two elements in brucellosis patients compared to the control group, but they were not statistically significant, and there was also no significant change in the concentrations of these elements at the end of treatment in the patients (Cesur et al., 2005). This study seems to be similar to the studies of Mobin et al. in Hamedan and Ghayur-Mobarhan in Mashhad, who reported that in patients with brucellosis, an increase in serum copper concentration and a decrease in serum zinc were observed. It is likely that the same geographical, racial, and dietary habits can explain this similarity (Kalkan et al., 2000).Similar results were also obtained in the studies of Cik et al. in Munich and Kalkan et al. in Turkey on infected

individuals. In these studies, it was shown that age in brucellosis patients increased and zinc levels decreased (Geyik et al.2008;, Sullivan et al., 1979).In an objective study, serum copper and zinc concentrations were compared before and after treatment based on gender, but no significant relationship was observed between gender and serum levels. Regarding the results, the increase in serum copper levels was related to inflammation, but it is not possible to comment on whether the change in these elements is a risk factor for infection or whether these changes occur under the influence of the disease itself. In this study, significant changes were also observed in serum copper and zinc concentrations before treatment in Brucella patients, but they were not statistically significantly different compared to the control group, and no statistically significant change was observed in the concentrations of these elements at the end of treatment compared to the beginning of treatment in patients (Cesur et al., 2005).Since there has been interest in investigating changes in serum micronutrients during the course of various diseases, many studies have been conducted to investigate the relationship between serum copper and zinc levels and various diseases, especially infectious diseases. This study appears to be the first to examine serum zinc and copper ratios in patients with brucellosis and in two different control groups. There are generally two types of abnormalities in micronutrient levels. The first abnormality is inadequate or unbalanced dietary intake of copper and zinc, and the second is the presence of a disease that can affect the patient's serum levels. Sullivan et al. reported that serum copper and zinc levels are significantly altered in many diseases, including infections, malignancies, diabetes, arthritis, ulcers, psychosis, cirrhosis, pancreatitis, hypertension, and chronic obstructive cardiovascular disease (Sfars et al.2009). Asfar and Mazzetti (2009) reported that aging may alter serum copper and zinc levels. Although statistically significant changes in serum copper and zinc levels are found across a wide range of diseases, these changes are not specific to any disease, including infectious diseases. An increase in the copper: zinc ratio above 0.2 is considered significant in patients with acute or sub-acute chronic disease compared with controls. This situation may indicate that a copper to zinc ratio greater than 0.2 may be helpful in both diagnosing the acute or sub-acute form and assessing the course of the disease, and that the serum copper to zinc ratio may also be useful in monitoring the therapeutic response of patients with brucellosis (acute or sub acute) (Sfars et al.2009;, Mezzetti et al.1998).

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