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Essential Trace Element Levels in Patients with Cutaneous Anthrax

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ABSTRACT

The causative microorganism in anthrax is *Bacillus anthracis* and this disease is more common in some regions of Türkiye. Changes in trace elements other than iron in anthrax infection have not been studied. In this study, iron, copper, lead, zinc, manganese, magnesium, cadmium and cobalt levels were investigated in cases with cutaneous anthrax.

Fifteen patients with cutaneous anthrax and 15 healthy individuals wereincluded in the study. The groups were similar to each other in terms of age and gender. Anthrax was diagnosed according to contact status with animals, symptoms, examination, and microbiological results. We performed our study with Atomic Absorption Spectrophotometer (UNICAM-929 spectrophotometer).

Serum iron, lead, and cadmium levels were significantly higher in the patients than in the control subjects ($p<0.05$). Serum magnesium, manganese, zinc, copper, and cobalt levels were significantly lower in the patients than in the control subjects ($p<0.05$). The copper/zinc molar ratio was not significantly increased in the patients with cutaneous anthrax than in the control subjects.

It has been reported that iron, cadmium, and lead levels are low and copper level is high in infectious diseases. But we determined the opposite situation in the patients with cutaneous anthrax. As a result, it can be said that the detection of high lead and cadmium levels in the cell in anthrax disease suppresses the immune system. Also, zinc can be used as a marker for this disease.

Keywords: Anthrax, *Bacillus anthracis*, cutaneous anthrax, serum, trace element

Introduction

Although anthrax cases are rare, they are still common in places where animal husbandry is common, such as Türkiye and Georgia. The causative microorganism of this disease is *Bacillus anthracis (B. anthracis)* and it is more common in some regions of Türkiye. *B. anthracis* is a non-motile, Gram-positive bacillus and it forms spores in aerobic environments (1, 2). Anthrax may be transmitted through the wool, skin, meat, and other materials of infected animals. Cutaneous anthrax is the type seen in over 95% and the disease is transmitted from animals with anthrax. The first skin lesion usually occurs on an exposed part of the body. After a while, the lesion takes on a bullous appearance. Hemorrhagic eschar occurs. The lesion turns into an ulcerated appearance. A vesicular ring may occur. Such images were also observed in Bacillus species other than *B.anthracis* (3, 4).

Two exotoxins are effective in the pathogenesis of anthrax; edema toxin (ET) and lethal toxin (LT). There is necrosis with edema, fibrin extravasation, and haemorrhage in the lesion area. The blood vessels are dilated more than normal. Occasionally, a fibrin thrombus may form. Sometimes, cellulitis may occur with an abscess.

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Gram-positive staining of *B.anthracis* can be seen in the microscopic sample taken from the exudate. Culture can be used to confirm the diagnosis. Fatal septicemia may occur if diagnosis and treatment are delayed. Penicillin can be used in the treatment, but it is possible to encounter strains that produce β-lactamase. This possibility should be known in such a case and appropriate antibiotic therapy should be selected. Ciprofloxacin and amoxicillin can also be used in the treatment (1).

Trace elements divide into "essential" (necessary to maintain life: including zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), magnesium (Mg), and cobalt (Co)) and "toxic" (have skewed distribution and toxic in excess: including cadmium (Cd), and lead (Pb)) categories (5). Trace elements are necessary for the immune system's adequate and effective development and functioning. Therefore, they are effective in protecting the organism against infections (6). Some essential trace elements are very important for many proteins and are located as co-factors in enzymatic reactions (7). These elements are necessary for immune system defence functions. Non-essential trace elements can be found more intensely in infected organs, and changes in blood levels may indicate related organ lesions (8). In addition to this, it has been reported that the levels of trace elements are influenced by infectious diseases (9). However, changes in trace elements except for Fe in anthrax infection have not been studied. In this study, the changes in the levels of some trace elements were investigated in patients with acute cutaneous anthrax.

Materials and Methods

The study protocol and patients: Fifteen patients diagnosed with cutaneous anthrax based on clinical and laboratory findings were included in this study. Fifteen healthy age- and sex-matched persons were included as a healthy control group in the study.

The diagnosis of anthrax: The microbiological diagnosis was based on conventional methods such as large non-haemolytic greyish-white colonies on sheep blood agar, encapsulated Gram-positive bacilli, aerobic or facultatively anaerobic, nonmotile, spore-forming and bamboo shoots shaped chains under the microscope, and sensitivity of penicillin.

Blood samples: Blood samples were collected in tubes and samples were centrifugated at 5000 rpm for 10 minutes serum samples were placed at -80°C until the study and used for the analysis of serum trace element levels.

Measurements of serum trace elements: Determination of the trace elements and heavy metals (Fe, Zn, Cu, Mn, Mg, Cd, Co, and Pb) levels were performed by Atomic Absorption Spectrophotometer (AAS) (UNICAM-929 spectrophotometer, Unicam Ltd, York Street, Cambridge, UK). The AAS method is used to detect the concentrations of metal elements in a liquid solution. This method is extremely suitable for the determination of concentrations of trace elements. In this method, the relevant substance must first be ionized. For this, the flame is used in the relevant system. Spectral transitions, either absorption or emission, are produced. Spectral lines are separated for use in the analysis process. A decrease or increase in the amount of radiation is detected. The data is recorded by the system (10,11)

Ethics approval: The ethics committee's decision was taken from the Faculty of Medicine, Non-Pharmaceutical Clinical Research Ethics Committee of our University with the decision dated 11.09.2013 and numbered 10.

The principles of the Declaration of Helsinki were complied with at every stage of this study.

Statistical Analysis: The values are expressed as a minimum, maximum, mean ± SD (standard deviation), and the difference between mean values for the cutaneous anthrax patients and control groups, separately. For the comparison of groups was performed by using Student's *t*-test. Correlation analyses were performed by using Pearson's correlation test. A *p*-value ≤ 0.05 was accepted as significant. Data were analyzed by using the Minitab® for Windows computing program (version 16).

Results

Data regarding the age and gender of the patient and healthy control groups are given in Table 1. There were no significant differences between the groups with respect to age and sex ($p>0.05$).

Serum Fe, Pb, and Cd levels were significantly higher in patients than in the control subjects ($p<0.05$). Serum Mg, Mn, Zn, Cu, and Co levels were significantly lower in patients than in the control subjects ($p<0.05$) (Table 2). The Cu/Zn molar ratio, which is a well-known serum marker of infectious and inflammatory conditions, was not significantly increased in patients than in the control subjects.

Table 1. Demographic Parameters of the Cases and Control Groups

	Cutaneous Anthrax Patients Mean values ± St.D. N=15	Control Groups Mean values ± St.D. N=15	P value
Ages	36.17 ± 17.28	35.65 ± 17.60	0.910
Sex			
Male	7	7	0.999
Female	8	8	

N: Number of volunteers, **St. D.:** Standard Deviation

Table 2. Serum Trace Element Levels In Patients With Cutaneous Anthrax and Controls

Parameters	Study groups	N	Ranges	Mean ± St.D.	The difference between mean values	P value
Fe (µg/dL)	Case	15	1.031-1.781	1.345 ± 0.256	+0.824	<0.05
	Control	15	0.363-0.611	0.521 ± 0.069		
Zn (µg/dL)	Case	15	2.977-3.995	3.202 ± 0.255	-4.772	<0.05
	Control	15	6.228-10.021	7.974 ± 1.111		
Cu (µg/dL)	Case	15	3.772-4.342	4.027 ± 0.138	-5.019	<0.05
	Control	15	7.443-11.651	9.046 ± 1.231		
Mg (µg/dL)	Case	15	13.033-15.994	14.592 ± 0.964	-22.185	<0.05
	Control	15	34.055-39.554	36.777 ± 1.392		
Mn (µg/dL)	Case	15	0.278-0.308	0.298 ± 0.008	-0.451	<0.05
	Control	15	0.612-0.923	0.749 ± 0.107		
Co (µg/dL)	Case	15	0.650-0.940	0.777 ± 0.080	-0.061	<0.05
	Control	15	0.750-0.930	0.838 ± 0.061		
Cd (µg/dL)	Case	15	0.060-0.090	0.078 ± 0.010	+0.071	<0.05
	Control	15	0.004-0.009	0.007 ± 0.002		
Pb (µg/dL)	Case	15	2.845-3.003	2.932 ± 0.045	+1.990	<0.05
	Control	15	0.812-1.065	0.942 ± 0.077		
Cu/Zn (µg/dL)	Case	15	1.013-1.412	1.265 ± 0.104	0.110	>0.05
	Control	15	0.828-1.482	1.155 ± 0.224		

N: Number of volunteers, **St. D.:** Standard Deviation

A positive correlation was observed between Fe and Cu ($r=0.496$, $p<0.05$); Mn and Pb ($r=0.546$, $p<0.05$); Pb and Cd ($r=0.509$, $p<0.05$) levels in patients. A positive correlation was also observed between Cu and Cd ($r=0.469$, $p<0.05$); Cu and Co ($r=0.534$, $p<0.05$); Co and Cd ($r=0.465$, $p<0.05$) levels in the control subjects. There was no correlation between the other serum trace element levels in the two groups ($p>0.05$) (Tables 3 and 4).

Discussion

According to the way the spores enter the organism, anthrax appears in three clinical forms: skin, inhalation, and gastrointestinal. Skin anthrax constitutes 95% of anthrax that is seen in humans and the disease is transmitted by the spore form of the microorganism with minor traumas such as cuts or scratches (12). Initially, a red macule occurs at the inoculation site and then a papule is formed. A few days later, the lesion turns into a vesicle and turns into a blue-black colour with the clouding of the fluid in the lesion. Cutaneous anthrax disease is endemic in our country and it is frequently observed in Africa, Asia, and Eastern Europe. However, it has been reported that its frequency has gradually decreased in our country in recent years (4, 12, 13).

LT and OT are two important factors in the formation of the disease pattern. Protective

Table 3. Correlation Coefficients of The Anthrax Group and Comparison Results

	Fe	Zn	Cu	Mg	Mn	Co	Cd	Pb
Fe (µg/dL)	1							
Zn (µg/dL)	-0.443	1						
Cu (µg/dL)	0.496*	-0.134	1					
Mg (µg/dL)	0.304	-0.052	-0.147	1				
Mn (µg/dL)	0.274	-0.368	0.371	0.011	1			
Co (µg/dL)	-0.074	-0.119	-0.125	-0.082	0.019	1		
Cd (µg/dL)	-0.347	0.034	-0.210	0.067	-0.263	0.179	1	
Pb (µg/dL)	0.067	-0.187	0.203	0.264	0.546*	0.405	0.509*	1

*p<0.05

Table 4. Correlation Coefficients of The Control Group and Comparison Results

	Fe	Zn	Cu	Mg	Mn	Co	Cd	Pb
Fe (µg/dL)	1							
Zn (µg/dL)	0.047	1						
Cu (µg/dL)	0.267	0.089	1					
Mg (µg/dL)	0.039	0.008	0.174	1				
Mn (µg/dL)	0.265	-0.014	0.005	0.291	1			
Co (µg/dL)	0.259	0.116	0.534*	0.204	-0.057	1		
Cd (µg/dL)	-0.077	0.272	-0.469*	-0.167	-0.077	-0.465*	1	
Pb (µg/dL)	0.088	0.078	0.049	0.160	0.041	0.005	-0.168	1

*p<0.05

antigen (PA) is common in both toxins and plays a role in the passage of other components into the cell. The lethal factor is the other component of LT is a zinc metalloprotease which causes selective protein kinase inactivation. The edema factor is the other component of ET that increases intracellular cAMP (cyclic adenosine monophosphate) levels too much, leading to impairment of intracellular water metabolism and the development of massive edema (4, 12). Microorganisms need metal ions to survive. The lack of metal ions in the human body and their unusable presence in the organism creates an obstacle to bacterial growth. This is supported by the fact that iron supplementation increases the frequency of recurrence in patients recovering from active tuberculosis and that iron-binding proteins in the blood inhibit the growth of bacteria (14). As mentioned above, trace elements are necessary for microorganisms as well as many acute phase proteins and immune system cells in the organism (8). If there is Zn deficiency, low CD4 + / CD8 + (cluster of differentiation 4/cluster of differentiation 8) ratio, T-cell dysfunction, delayed wound healing, defective phagocytosis, and abnormal cytokine release may occur (15, 16). The decrease in serum Zn level is one of the non-specific host defence mechanisms against bacterial infection. Fe is necessary for the growth and reproduction of most microorganisms and some negative effects related to the immune system occur in Fe deficiency. Another element that is important for the immune system and causes a decrease in Fe absorption in its deficiency is Cu. Cu intoxication is a primary defence strategy against microbial pathogens. The antimicrobial properties of copper have been known for thousands of years. Recent studies have found that Cu is activated by the innate immune system. In experimental studies conducted in various species, changes in serum Cu levels were observed (14, 15, 17, 18).

The increase in metal-binding protein synthesis and the exchange of trace elements between blood and tissue affect trace element levels (19). This situation generally results in a decrease in Fe and Zn levels in plasma, while an increase in Cu levels (9, 19, 20). However, there are different studies in the literature that do not comply with this rule (8, 21). Middleton et al. (18) monitored changes in serum trace element concentrations in cattle with experimentally induced *Staphylococcus aureus* mastitis. As a result of the study, they found that mean serum Cu, Zn, and Fe concentrations

decreased by 89%, 83%, and 81% at the 24th hour of infection. Erskine and Bartlett (22) reported that mean serum concentrations of Zn, Fe, and Cu decreased by 28%, 35%, and 52% in experimental *E.coli* mastitis in six Holstein cows. Ertan et al. (23) reported that giardiasis increased serological Cu levels but decreased Zn and Fe levels after an intestinal protozoan infection caused by *Giardia lamblia*. They also reported that malabsorption caused a decrease in Zn and Fe levels. Koçak et al. (24) found statistically low serum Zn concentrations in the COVID-19 patient group. In addition, the increase in the Cu/Zn ratio in the blood is known as a well-known indicator of active infection and can be detected before the infection develops (21, 25). In our study, serum Fe levels increased in cutaneous anthrax patients compared with the control group, while Zn and Cu levels decreased. Consistent with the literature, an increase in Cu/Zn ratio was found in cutaneous anthrax patients, but it was not statistically significant. Therefore, decreased serum Zn levels in anthrax patients may pose a serious risk. Low Zn levels may affect the etiopathogenesis of the disease.

Mg is a good modulator of the immune system and Mg levels increase in infected tissues. An increase in anti-inflammatory response and immune system cell proliferation in cases where serum Mg levels are decreased can be considered as proof of this (26). On the other hand, Mn is the cofactor of the metalloenzymes superoxide dismutase and pyruvate carboxylase enzymes. Thanks to these enzymes provide a protective effect on the organism against oxidants formed as a result of oxidative stress (27, 28). There are studies reporting that these two trace elements decrease in cases of infection (19, 21). While Ilback et al. (8) detected a decrease in serum Mg level in the sera of mice infected with *Coxsackievirus B*, they found an increase in Mn level on the third day of infection and a decrease in the following days compared to the control level. Taghipour et al. (29) in their systematic review of leishmaniasis and trace elements; reported changes in Mg, Zn, Cu, Fe, and selenium (Se) levels in visceral leishmaniasis, cutaneous leishmaniasis and canine leishmaniasis cases. In cutaneous leishmaniasis, zinc-based supplementation has demonstrated increased wound healing and reduced scar formation in humans and experimentally infected animals. Edvinsson et al. (19) monitored trace element changes in mice experimentally infected with *Chlamydophila pneumoniae*. They found an increase in serum Cu/Zn ratio and a decrease in Fe levels in response to infection. Besides, on the second day of infection; They found a decrease in serum levels of Mg, aluminium (Al), vanadium (V), Mn, Co, Cd, and mercury (Hg); an increase in arsenic (As) and Se levels. In our study, it was observed that Mg and Mn levels were decreased. In addition, it was determined that the trace element with the greatest decrease in serum level was Mg. In the study, the decreased Mn sera level compared to the control group gives an idea about the accumulation of Mn in this area as a result of the increase in oxidative stress in the lesion area. Low Mg may affect the etiopathogenesis of the disease.

Apart from these elements, Co is included in the vitamin B12 structure, which is essential for the organism. The effects and optimum levels of Cd, which is among ultra-trace elements, are not known enough (28). Potential toxic trace elements such as Cd compete metabolically with essential trace elements such as Cu and Zn and can accumulate in the target organ (21). Changes in blood Pb levels indicate organ-specific lesions (30). Studies have found that serum Cd levels decrease in cases of organ damage due to infection (8,19,21). There are different studies that reported there is a decrease and increase in the Co level. Choi et al. (31) compared serum trace element concentrations of 141 adult patients with tuberculosis and 79 uninfected controls. In patients with tuberculosis; significantly higher serum Co and Cu concentrations compared with controls; Zn and Se concentrations were found to be significantly lower. Akkaş et al. (32) investigated serum Fe, Cu, Zn, Co, chromium (Cr), Se, V, nickel (Ni), Cd, and Al levels in patients with sepsis and found significantly higher levels of Cr, Fe, Ni, Cu, and Cd. Hu et al. (33) investigated serum concentrations of 15 elements (calcium, Mg, Fe, Zn, Cu, Co, Ni, Pb, Cd, Hg, As…etc.) in *H.pylori*-infected individuals at one centre. Serum Co was found at higher levels in people infected with *H.pylori* than in those not infected with *H.pylori*. However, they did not find a statistically significant difference in serum levels of other elements. Ilback et al. (8) reported that there was an increase in Pb level on the third day of infection and a decrease in the following days. In this study, it was determined that there were an increase in Cd and Pb levels and a decrease in Co levels compared to the control group. High Pb and Cd levels can create a toxic effect for anthrax and initiate the oxidative damage process.

There is no specific range in the data obtained by the AAS method, a clear range cannot be found in the amount of elements detected. Therefore, the obtained values may differ from the literature. However, the measurement values we obtained are within reasonable ranges. The aim of the study with this method is to determine whether there is a statistically significant difference between the healthy control and patient groups. The same results cannot be expected between the measurements in hospital auto analyzer devices and the measurements in AAS devices. Because the measuring ranges of the auto analyzers used in hospitals are defined for the devices, and the working methods of both devices are quite different from each other.

It has been determined that cutaneous anthrax causes some changes in the trace element balance due to the activation of the immune system and the use of these elements by the bacteria, as in other infections reported in the literature. Zn, Cu, Mg, Mn, and Co levels were found to be decreased, whereas Fe levels increased and Pb, and Cd which are toxic trace elements levels increased too. In addition, the increase in Cu/Zn ratio, as reported in acute infections was not found to be significant in this patient group. While it was expected to decrease in Fe, Cd, and Pb levels and an increase in Cu levels as reported in other studies, it was revealed that the opposite was observed in the serum of cutaneous anthrax patients.

As a result, it can be said that the detection of high Pb and Cd levels in the serum in anthrax disease suppresses the immune system. In addition, the presence of low Zn levels is an indication of a decrease in antioxidant agents in anthrax patients. Because Zn is a very powerful antioxidant substance. A decrease in this substance can initiate cellular damage. Also, Zn can be used as a marker for this disease. In conclusion, Zn, Cu, Fe, Pb, Cd, Mg, Mn, and Co levels may play an important role in the etiopathogenesis of anthrax disease. Therefore, we believe that this study will contribute to the literature. This study is interesting and original study. More studies should be done on this disease.

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