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(Mn) in Tuberculosis Patients

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Effect of Antituberculosis Medication on Serum Concentrations of Zinc (Zn), Copper (Cu), and Manganese (Mn) in Tuberculosis Patients

Godwin R.A. Okogun¹, Ebenezer O. Dic-Ijiewere^{2*}, Obadiah Okpokpo¹, Lucky E. Omolumen¹

¹Department of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Nigeria.

²Department of Chemical Pathology, Ambrose Alli University, Ekpoma, Nigeria.

*Correspondence: ebenexar@gmail.com, labheth@gmail.com

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Abstract

Tuberculosis (TB) is a well-known disease state caused by the bacteria *Mycobacterium tuberculosis*. The disease accounts for about 1.5 million deaths worldwide and is transmitted from person to person by the inhalation of respiratory droplets. The progression of tuberculosis from the latent phase to its active form has been reported to be associated with reduced immunity and occurs in about 10% of tuberculosis-infected population. Trace elements play a vital role in the maintenance of the immune system in humans, and therefore their concentration in the serum is important in the management of TB patients. The primary objective of this study was therefore to assess the serum concentrations of zinc (Zn), copper (Cu), and manganese (Mn) in tuberculosis patients and compare the results obtained against apparently healthy subjects as well as to compare the concentrations of trace element levels in TB patients already on antituberculosis drugs against those not yet exposed to antituberculosis drugs. A total of 62 TB patients were selected, and their blood samples were collected while controls were taken from 20 apparently healthy individuals. The results obtained showed that the serum concentration of the trace elements Zn and Cu was significantly lower and higher for Mn (1.58 ± 1.09 mg/l, 1.17 ± 0.16 mg/l and 0.78 ± 0.11 mg/l, respectively) in the control than in tuberculosis patients (2.59 ± 1.79 mg/l, 1.64 ± 0.47 mg/l, and 0.25 ± 0.35 mg/l). However, those on drugs had higher Zn and Cu levels than those not on drugs. It was therefore concluded and recommended that further studies should be carried out to ascertain the mechanism of action of antituberculosis drugs and their specific effects on serum trace elements of immunological significance, to improve the care for tuberculosis patients.

Keywords: Tuberculosis; Zinc; Copper; Manganese; Immunological.

1. INTRODUCTION

Tuberculosis (TB) is an infection caused by the *Mycobacterium tuberculosis*, which is typically spread from one person to another by the inhalation of respiratory droplets [1]. The World Health Organization (WHO) in 2013 estimated that nine million new cases of active tuberculosis worldwide exist resulting in 1.5 million deaths [2]. Nearly 10% of the latent infections get to active disease and if left untreated could result in the death of half of those infected [3]. When the tuberculosis infection is no longer contained by the immune system, active tuberculosis occurs. The chance of conversion from latent to active tuberculosis is around 5%–10% in an otherwise healthy population [1], although this can increase to around 50% in people with severe impairment of their immune system, similar to what occurs with the human immunodeficiency virus (HIV) infection [4]. Tuberculosis affects the lungs more commonly (pulmonary tuberculosis), although it can also spread to affect the central nervous system, lymphatic system, circulatory system, genitourinary system, and bones and joints. Active pulmonary tuberculosis presents with symptoms such as cough, fever, night sweats, chest pain, weight loss, and sometimes coughing up blood [1].

Tuberculosis is spread through air, when individuals who are infected cough, sneeze, or spit of which infections in humans result in asymptomatic, latent infection, and about 1 in 10 latent infections eventually progress to active disease, which if left untreated, kills more than 50% of its victims [5].

Tuberculosis is treated with a combination of antibiotic drugs (antituberculous therapy) consisting of rifampicin, isoniazid, pyrazinamide, ethambutol, and streptomycin administered orally for 6 months to ensure efficacy [6]; noncompliance or partial compliance will result in death of about half of the population with active tuberculosis [7].

Globally, poor nutritional status is often observed in people with active tuberculosis than in people without tuberculosis or latent tuberculosis [8], and weight loss, including loss of lean body mass, is a well-recognized symptom of the disease. Cohort

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and cross-sectional studies have suggested that active tuberculosis is commonly associated with low serum levels of important micronutrients such as zinc [9] and vitamins A, C, D, and E [10].

Micronutrient environments are key contributors to immune function and cytokine kinetics. Thus, such environments have been increasingly suggested to play an essential role in the individual response to infectious diseases [11]. This study was therefore aimed at determining the extent of correlation between the infection of TB with serum concentrations of zinc (Zn), copper (Cu), and manganese (Mn) as well as comparing and contrasting between those patients already exposed to therapy for at least 3 months against patients not yet exposed to antituberculosis therapy.

2. METHOD(S)

2.1. Study Area

This study was conducted among patients visiting a Central Hospital in Benin City, Edo State. Benin City is the capital of Edo State in southern Nigeria. It is a city approximately 25 miles north of the Benin River. It is the center of Nigeria's rubber industry, but processing palm nuts for oil is also an important traditional industry. Benin City is situated at 6.34° North latitude, 5.63° East longitude, and at an 80 m elevation above sea level [12].

2.2. Population of the Study

The study was conducted on 62 persons having tuberculosis in Benin City and was matched against 20 apparently healthy individuals (controls). Among these patients, 34 were males of which 22 were under multidrug-resistant (MDR) TB therapy for at least 3 months while 12 were new cases to whom drugs were not yet administered. Among the TB patients, 28 were females of which 12 were already under MDR-TB therapy for at least 3 months, and the remaining 16 were new cases, yet to be exposed to antituberculosis therapy.

2.3. Biological Sample

Blood was obtained from each patient and sediment to obtain serum to evaluate the serum levels of trace elements (zinc, copper, and manganese) in patients with tuberculosis (both those on drugs and those whom yet to be exposed to drugs) and apparently healthy individuals as control subjects. Only those tuberculosis patients who had been on drugs for at least 3 months were selected for this study.

2.4. Treatment Administered in the Hospital

TB patients on drugs were administered anti-Tb multidrug regimen consisting of rifampicin, isoniazid, pyrazinamide, ethambutol, and streptomycin.

2.5. Sample Analysis

The trace element (zinc, copper, and manganese) concentrations assessment in the sample was performed using an air/acetylene flame atomic absorption spectrometer Pg instrument AA500F (AAS).

The sample digest is first aspirated into the flame whose high temperature converts the analyte ions into atoms in vapor state. Absorption occurs when a ground state atom absorbs energy in the form of light at a specific wavelength and is elevated to an excited state. The relationship between the amount of light absorbed and the concentration of the analyte present is a known standard and can be used to determine the unknown concentration by measuring the amount of light absorbed.

2.6. Procedure for Sample Analysis

Moreover, 1 ml of blood sample was pipetted into a conical flask, and 5 ml of the nitric perchloric acid was then added, mixed, and left to stand overnight. A small glass funnel was inserted to act as a reflux condenser and heated for 1 h at 150°C, and the temperature of the system was gradually increased to 235°C. When a dense-white fume emerged, the heating was intensified until a colorless solution was obtained. Furthermore, 100 ml of the mixture was poured into a volumetric flask and rinsed five times with water each time added to the mixture being washed to make up the volume in the flask. A blank was prepared using the same procedure without any sample. The sample filtrates and the blank were analyzed for trace elements using AAS.

2.7. Ethical Consideration

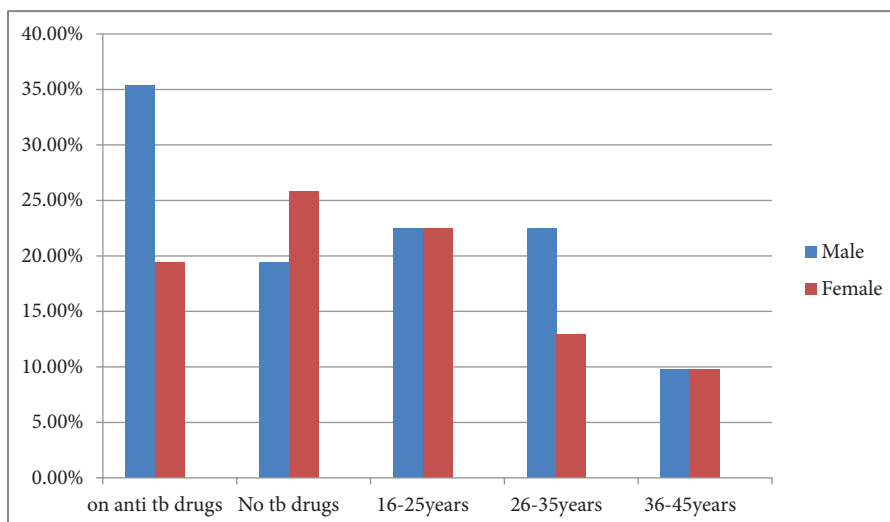
Ethical approval for this study was obtained from the Ambrose Alli University Ethical and Research Committee before the research was conducted, and informed consent was obtained from each patient before the sample was obtained.

2.8. Statistical Analysis

Data obtained were analyzed using the SPSS version 20 statistical software package. Results generated were expressed as mean \pm SD, and a *p*-value of <0.05 was considered significant. The significant difference among the groups was assessed by repeated measures of analysis of variance (ANOVA).

3. RESULTS

Figure 1: Histogram showing demographics of the Tb patients in the study by percentage.



From the demographic characteristics of the population, a total of 62 tuberculosis patients and 20 apparently healthy individuals (control) were used. From Fig. 1, 35.4% of the Tb patients on anti-Tb drugs are males, whereas 19.4% are females. Moreover, 19.4% of those not exposed to drugs are males, whereas 25.2% are females. With respect to age range, 22.5% of the entire population are males between the ages of 16 and 25, which is the same for females (22.5%). In addition, 22.5% are males between the ages of 26 and 35, whereas 12.5% are females between these ages. The other 19.6% are males and females (9.8% each) between the ages of 36 and 45.

From Table 1, the Zn level of control (1.58 ± 1.09 mg/l) when compared with that of Tb patients (2.59 ± 1.79 mg/l) was significantly lower (p -value = 0.021). The Cu level of control (1.17 ± 0.16 mg/l) was significantly lower (p -value = 0.003) than Tb subjects (1.64 ± 0.47 mg/l). The Mn level of Tb subjects (0.25 ± 0.35 mg/l) was significantly lower (p -value = 0.033) than that of the control.

From Table 2, the mean Zn level of female Tb patients (2.94 ± 1.49 mg/l) was significantly higher than the control (1.58 ± 1.09 mg/l) (p -value = 0.021), but it was not statistically significant in comparison with male Tb patients (2.30 ± 1.97 mg/l)

Table 1: Mean \pm standard deviation of Zn, Cu, and Mn of tuberculosis (Tb) patients in comparison with healthy control subjects.

Parameter	Control (n = 20)	Tb-infected patients (n = 62)	p-value	Significance
Zn (mg/l)	1.58 ± 1.09	2.59 ± 1.79	0.021*	Significant
Cu (mg/l)	1.17 ± 0.16	1.64 ± 0.47	0.003*	Significant
Mn (mg/l)	0.78 ± 0.11	0.25 ± 0.35	0.033*	Significant

Key: Tb = tuberculosis; Zn = zinc; Cu = copper; Mn = manganese

Table 2: Mean \pm standard deviation of Zn, Cu, and Mn levels of male Tb subjects compared with female Tb subjects against the controls using ANOVA with necessary post hoc comparison

Parameters	Control (n = 20)	Male subjects infected with Tb (n = 34)	Female subjects infected with Tb (n = 28)	p-value	Significance
Zn (mg/l)	1.58 ± 1.09^a	2.30 ± 1.97^{ab}	2.94 ± 1.49^b	0.021*	Significant
Cu (mg/l)	1.17 ± 0.16^a	1.46 ± 0.47^b	1.85 ± 0.38^c	0.002*	Significant
Mn (mg/l)	0.78 ± 0.11^a	0.30 ± 0.36^{ab}	0.19 ± 0.34^{ab}	0.041*	Significant

Note: Values with different superscripts are statistically significant at $p < 0.05$.

($p > 0.05$). The mean Zn levels of male Tb patients were not statistically significant in comparison with female Tb patients and control. The mean Cu levels of male Tb patients (1.46 ± 0.47 mg/l) were significantly higher than the control and significantly lower than females (1.85 ± 0.38 mg/l) (p -value = 0.002). The mean Mn level of Tb males (0.30 ± 0.36 mg/l) was significantly lower than the control (0.78 ± 0.11 mg/l) ($p = 0.01$). Although it was higher than the Mn levels of the females (0.19 ± 0.34 mg/l), it was not statistically significant ($p > 0.05$).

From Table 3, the mean Zn level of female Tb subjects on drugs (3.05 ± 0.88 mg/l) was significantly higher than the control (1.17 ± 0.16 mg/l) ($p = 0.010$), but when compared with female Tb subjects not on drugs (2.85 ± 1.85 mg/l), it was not statistically significant ($p = 0.70$). The mean Zn level of female Tb subjects not on drugs (2.85 ± 1.85 mg/l) was significantly higher than the control ($p = 0.010$). The mean Cu level of female tuberculosis subjects on drugs (1.97 ± 0.22 mg/l) was significantly higher than the control (1.17 ± 0.16 mg/l) ($p = 0.001$). Female tuberculosis subjects not on drugs also had significantly higher Cu levels (1.76 ± 0.45 mg/l) in comparison with control ($p = 0.001$). There was no significant difference between female Tb subjects on drugs and those not on drugs ($p = 0.080$), although those on drugs had higher Cu levels than those not exposed to drugs. In addition, there was no significant difference between the mean Mn levels of female Tb subjects on drugs (0.092 ± 0.14 mg/l) when compared with those not yet on drugs (0.26 ± 0.23 mg/l) ($p = 0.100$).

From Table 4, the Zn level of the female tuberculosis subjects and male tuberculosis subjects on drugs (3.05 ± 0.88 mg/l and 2.67 ± 2.34 mg/l) was higher than female and male tuberculosis subjects not on drugs (2.85 ± 1.85 mg/l and 1.63 ± 0.69 mg/l, respectively), although it was not statistically significant ($p > 0.05$). The mean Cu level of female subjects on drugs (1.97 ± 0.22 mg/l) was not statistically significant in comparison with female subjects not on anti-Tb drugs (1.76 ± 0.45 mg/l) ($p = 0.210$), but it was significantly higher than Cu levels of male subjects not on drugs (1.54 ± 0.49 mg/l) ($p = 0.010$) and male subjects not on drugs (1.31 ± 0.41 mg/l) ($p = 0.001$). The mean Cu level of male subjects on drugs (1.54 ± 0.49 mg/l) although higher than that of male subjects not on drugs (1.31 ± 0.41 mg/l), it was not statistically significant ($p = 0.140$). The mean Mn level of female subjects on drugs (0.92 ± 0.14 mg/l) was higher than female subjects not on anti-Tb drugs (0.6 ± 0.23 mg/l), but it was not statistically significant. The mean Mn level of male subjects on drugs (0.36 ± 0.21 mg/l) was higher than that of male subjects not on drugs (0.20 ± 0.12 mg/l), but it was not statistically significant.

From Table 5, the mean serum Zn level of male Tb subjects on drugs (2.67 ± 2.34 mg/l) was significantly higher than the control ($p = 0.040$), it was also higher than that of those not on drugs (1.63 ± 0.69 mg/l), but the difference was

Table 3: Comparison between mean \pm standard deviation in Zn, Cu, and Mn of female Tb subjects on drugs and female Tb subjects not on drugs

Parameter	Control (n = 20)	Female tuberculosis subjects on drugs (n = 12)	Female tuberculosis subjects not on drugs (n = 16)	p-value	Significance
Zn (mg/l)	1.58 ± 1.09^a	3.05 ± 0.88^b	2.85 ± 1.85^b	0.010*	Significant
Cu (mg/l)	1.17 ± 0.16^a	1.97 ± 0.22^b	1.76 ± 0.45^b	0.001*	Significant
Mn (mg/l)	0.78 ± 0.11^a	0.092 ± 0.14^a	0.26 ± 0.23^a	0.100*	Not Significant

Table 4: Mean \pm Standard deviation of Zn, Cu, and Mn levels of male and female tuberculosis subjects on anti-Tb drugs and male/female subjects not on anti-Tb drugs.

Parameters	Female tuberculosis subjects on drugs (n = 12)	Female tuberculosis subjects not on drugs (n = 16)	Male tuberculosis subjects on drugs (n = 22)	Male tuberculosis subjects not on drugs (n = 12)	p-value	Significance
Zn (mg/l)	3.05 ± 0.88^a	2.85 ± 1.85^a	2.67 ± 2.34^a	1.63 ± 0.69^a	0.200*	Not Significant
Cu (mg/l)	1.97 ± 0.22^a	1.76 ± 0.22^a	1.54 ± 0.49^b	1.31 ± 0.41^b	0.001*	Significant
Mn (mg/l)	0.92 ± 0.14^a	0.26 ± 0.23^a	0.36 ± 0.21^a	$0.2. \pm 0.12^a$	0.210*	Not Significant

Table 5: Mean \pm standard deviation of Zn, Cu, and Mn levels of male tuberculosis patients on drugs and those not on drugs.

Parameters	Control	Male tuberculosis subjects on drugs (n = 22)	Male tuberculosis subjects not on drugs (n = 12)	p-value	Significance
Zn (mg/l)	1.58 ± 1.09^a	2.67 ± 2.34^b	1.63 ± 0.69^{ab}	0.040*	Significant
Cu (mg/l)	1.17 ± 0.16^a	1.54 ± 0.49^b	1.31 ± 0.41^{ab}	0.010*	Significant
Mn (mg/l)	0.78 ± 0.11^a	0.36 ± 0.21^b	0.20 ± 0.12^b	0.010*	Significant

not statistically significant ($p = 0.940$). The mean serum Cu level of male Tb subjects on drugs was significantly higher than the control ($p = 0.010$), but in comparison with male Tb subjects not on drugs, it was not statistically significant ($p = 0.300$). The mean Mn level of male tuberculosis patients on drugs (0.36 ± 0.21 mg/l) was significantly lower than that of the control (0.78 ± 0.11), and mean Mn level of those not on drugs (0.20 ± 0.12 mg/l) was significantly lower than that of the control.

4. DISCUSSION

From this study, although not sufficiently correlative, the levels of zinc appeared to be lower in tuberculosis patients not on drugs than those on drugs. These results are consistent with those of Edem *et al.* [13] who reported reduced zinc concentration during tuberculosis with an increase in these concentrations after 4 and 6 months of treatment, respectively. These authors indicated that the lower zinc concentration observed compared to normal values was due to a redistribution of zinc flowing in other tissues including the liver tissue; this could be explained due to a reduced liver's production of α_2 -macroglobulin (a protein carrier of zinc in the blood) for the benefit of high production of metallothionein, a protein carrying zinc to the liver [13]. Zinc insufficiencies among MDR-TB patients have a negative impact on the immune system. Although a recent systematic review reported that the plasma concentrations of zinc and selenium can be improved by supplementation during the early stages of tuberculosis treatment, a consistent benefit from supplementation on tuberculosis treatment outcome and/or nutritional recovery has not been demonstrated [14].

The copper levels were higher in tuberculosis patients than in the control subjects and were significantly higher in subjects who were already undergoing antituberculosis therapy. This agreed with previous studies by Kassu *et al.* [15], Cernat *et al.* [16], and Moraes *et al.* [17] in Ethiopia, Italy, and Canada, respectively. The negative correlation between copper and zinc concentrations observed in this study was comparable to the results presented in the previous literature and could be explained by the ability of zinc ions to block copper absorption, possibly by formation of intestinal metallothionein, which strongly binds copper [18].

The serum levels of the manganese among various groups showed higher levels in tuberculosis patients already on drugs than those yet to be exposed to drugs; although there was not much statistical significance, they appeared lower when compared with that of the controls.

5. CONCLUSION

This study has proven that there is not just a significant correlation between the infection of *M. tuberculosis* and serum concentrations of the trace elements, zinc (Zn), copper (Cu), and manganese (Mn) but that there also lies an important correlation between the drug combination choices for MDR-TB and the serum concentration of these elements, although this study did not include the specific drug combination and criteria of drugs that were being used for treatment.

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Author Contributions

This research work was conducted with the collaboration and contribution of all the authors. GRAO was responsible for the design and supervision; EOD was responsible for the development of the manuscript and statistical analysis; OO was responsible for sample collection, literature search, and preparation of the research report; and LEO was responsible for research logistics.

Conflict of Interest

None.

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