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RESEARCH PAPER

EVALUATION OF SOME TRACE ELEMENTS (ZINC, CHROMIUM, CADMIUM AND MANGANESE) IN PATIENTS WITH ACTIVE TUBERCULOSIS ATTENDING CENTRAL HOSPITAL BENIN CITY, EDO STATE

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ABSTRACT

Throughout the world, tuberculosis (TB) infection is on the increase and it has remained one of the most important causes of death among adults in developing countries. This study evaluated the serum concentrations of some trace elements -*Zinc (Zn), Manganese (Mn), Chromium (Cr) and Cadmium (Cd),* in 100 blood samples; comprising sixty (60) active TB patients (*test subjects*) and forty (40) apparently healthy individuals (*control*). Serum concentrations of the elements were determined with an Atomic Absorption Spectrophotometer, and the results showed significantly ($P<0.05$) lower concentrations of Zn, Mn and Cr, but significantly ($P<0.05$) higher levels of Cd in patients with TB than those of the control. In relation to sex, there was no significant difference ($P>0.05$) in Zn and Mn concentrations in TB patients, but a significant difference ($P<0.05$) was observed for Cr and Cd in the male and female subjects studied. On age, there was no significant difference ($p<0.05$) in the concentrations of Mn and Cd across the age groups studied, but an age dependent decrease in Cr and increase in Cd, was observed in both gender. Our results suggest that Zn, Mn and Cr concentrations are reduced in TB patients, while serum Cd level is increased.

Key words: Infectious disease, Serum concentration, Tuberculosis, Trace elements

INTRODUCTION

Tuberculosis (TB) is a widespread infectious disease caused by various strains of mycobacteria usually *Mycobacterium tuberculosis*. It is an airborne disease that typically attacks the lungs, but can also affect other parts of the body (Garner *et al.*, 2007). Most of the infections are asymptomatic (latent tuberculosis) and one-tenth of latent TB infections, eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected (WHO, 2005). The classic symptoms of active TB infection include chronic cough with tinged sputum, fever, night sweats, and weight loss. Infection of other organs causes a wide range of symptoms (Khan *et al.*, 2008).

A trace element is a dietary mineral needed in small quantities for the proper growth, development and proper functioning of the organism (Mills, 2005). The second most abundant trace element is Zinc and it acts in the body as a cofactor for 100 diverse zinc-dependant enzymes like DNA polymerase, alkaline phosphatase, carboxypeptidase etc. These enzymes mainly regulate normal growth, immune system, cell growth, collagen synthesis, wound healing, bone metabolism, reproduction, taste, smell and vision. Diet rich in zinc includes: red meat, vegetables, fruits, fish and sea food (Lawn *et al.*, 2005).

Manganese is another naturally occurring mineral in humans; though in very small amounts. It is an actual component of manganese super oxide dismutase enzyme and a powerful antioxidant that seeks out and neutralizes free radicals in the human body, thereby preventing many of the potential dangers they cause (Arnetz and Nicolich, 1999). It is also considered as an important metal for human health, being absolutely necessary for development, metabolism, and the antioxidant system (Arnetz and Nicolich, 1999).

Chromium is a mineral that humans require in trace amounts, although its mechanisms of action in the body and the amounts needed for optimal health are not well defined (Lettow, 2004). It is found primarily in two forms: 1) trivalent (chromium 3^+), which is biologically active and found in food, and 2) hexavalent (chromium 6^+), a toxic form that results from industrial pollution. Chromium is known to enhance the action of insulin, a hormone critical to the metabolism and storage of carbohydrate, fat, and protein in the body (Sharma, 2003). Chromium facilitates insulin at intracellular sites including the ribosomes, and it is known to stimulate some enzymes in vitro. Chromium is important for the structure and metabolism of nucleic acids (Arnetz and Nicolich, 1999).

Cadmium is a trace element that is not believed to play any role in higher biologic systems or in human nutrition (Schwartz and Levin, 2001). It occurs naturally with zinc and is a by-product in the smelting of zinc and some lead ores. Cadmium is toxic in moderate doses and is a potent antagonist of several essential minerals including calcium, iron, copper and zinc (Lettow, 2004). Cadmium also causes destruction of the immune system, thus, predisposes the consumer to infectious diseases like tuberculosis (Khan *et al.*, 2008).

The biological role of trace elements, especially Zinc (Zn), Manganese (Mn) and Chromium (Cr) in different pathologic conditions has been extensively investigated in many diseases. The aim of this study therefore, is to evaluate serum concentrations of some trace elements (Zn, Mn, Cr and Cd) in patients with tuberculosis (TB).

MATERIALS AND METHODS

Study Setting: This study was carried out in Central Hospital, Benin City, Edo State, Nigeria. Edo State is an inland state in South-South Nigeria. It is bounded in the north and east by Kogi State, in the south by Delta State and in the west by Ondo State. English is the official language of the state. The major tribal languages spoken in the state are Igarra, Edo, Esan and Okpamheri. As at 2014, the population of Edo State was estimated to be 5 million, while Benin City, the capital of Edo State, has a population of 1,147,188 (NPC, 2014).

Ethical approval and informed consent: Ethical approval for the collection of sample was obtained from the Ministry of Health, Edo State. Informed consent was also obtained from each of the subjects that participated in the study, before the collection of blood sample.

Sample size and sample collection: This study was carried out in Central Hospital, Benin City, Edo State. A total of one hundred (100) samples were used for this study. It comprised samples from sixty (60) active TB patients (subjects) attending Central Hospital Benin City and forty (40) apparently healthy individuals (control). From fasting subjects, 5mls of blood sample was collected via venipuncture into a plain container without any additive to determine the serum trace metals (Zn, Cr, Mn and Cd). It was allowed to stand for 1 hour to clot and then centrifuged at 3000g for 10 min. The serum was thereafter, isolated and stored at 4^0c until required for analysis.

Inclusion criteria and Exclusion criteria: Selection of these subjects was based on the following criteria: age 20–65 years, sputum specimens positive for acid-fast bacilli by microscopy and clinical and radiographic abnormalities consistent with pulmonary tuberculosis. All subjects with previous anti-TB treatment, pregnant and lactating women, subjects using imuno-suppressive drugs and other diseases were excluded from this study.

Sample Analysis: Zinc, Chromium, Manganese, and Cadmium concentrations in serum were estimated by Atomic Absorption Spectrophotometer using the Beck 20 (AAS) machine. Working standard solution were prepared by diluting the stock standard with deionized water and the required PPM used for the standardization of the corresponding trace metals. 2mls of the thawed samples was taken after ensuring thorough mixing, and added to a clean 10ml centrifuge tube and diluted to 10ml with 0.1M hydrochloric acid. The diluted serum sample was then centrifuged (3000rev/min) to remove cellular debris and aspirated directly into the flame for analysis and data recording. All analysis were carried out in the Clinical Chemistry Laboratory at University College Hospital, Ibadan, Oyo State, Nigeria.

Principle of the test: Serum trace metals were determined with flame Atomic Absorption Spectrophotometer (AAS) using direct method as described by Kaneko (1991). The atoms of the elements, when aspirated into the AAS, vapourized and absorb light of the same wavelenght as that emitted by the metal when in the excited state i.e. in the vapourized ground state (unexcited) atom of a trace metal in the excited state. The amount of light absorbed is proportional to the trace metal in the solution.

Statistical analysis: The results were presented using tables. Data was presented as mean ± standard deviation (S.D). Comparison was made between test subjects and control groups using one-way analysis of variance (ANOVA) and the student's t-test. Significant difference was accepted at $p<0.05$.

RESULTS

Table 1 shows the mean value of Zinc, Manganese, Chromium and Cadmium in the subjects and control. The results obtained shows that the mean zinc of subject and control were 75.11±10.68ug/dl and 88.34±10.21ug/dl, while Manganese was 3.61.±0.45ug/dl and 9.62±1.60ug/dl respectively. Similarly, the mean chromium of the subjects and control were 0.96±0.33ug/dl and 1.14±0.19ug/dl, while cadmium was 0.21± 0.21ug/dl and 0.15±0.31ug/dl respectively. There was a significant decrease ($p<0.05$) in Zinc, Chromium and Manganese in the subjects compared with the control, while there was a significant increase ($p<0.05$) in Cadmium concentration of subjects compared with control.

Table 2 shows the distribution of Zinc, Manganese, Chromium and Cadmium in male and female subjects studied. The mean value of Zinc in males and females were 72.81±12.32ug/dl and 77.59±8.40ug/dl, while that of Manganese were 3.51±0.51ug/dl and 3.71±0.35ug/dl respectively. The results obtained showed that there was no significant difference ($p>0.05$) in Zinc and Manganese in both male and female subjects studied even though the mean concentration of Zinc and Manganese was slightly higher in females than in males. On the other hand, chromium in females (1.01±0.33) is significantly higher (p0.05) than in males (0.81±0.33) while cadmium in males (0.24±0.19) was significantly higher ($p<0.05$) than in females (0.18±0.12).

Table 3 shows the distribution of Zinc, Manganese, Chromium and Cadmium in subjects studied in the different age groups. The result showed that there was no significant difference ($p<0.05$) in Manganese and Cadmium in the different age groups. There was also no significant difference ($p<0.05$) in Zinc and Chromium in ages 36-50 years and 51-65 years, but there was significant increase ($p>0.05$) in Zinc and Chromium in age 20-35years in both males and females. There was a decrease in Zinc with increase in age in the different age groups in both males and females respectively. There was also a slight decrease in the mean Manganese and Chromium with increase in age in both male and female subjects respectively, but the values were higher in females than in males. Similarly, Cadmium increased with increase in age and it was higher in males than in females.

Table 1: Mean value of zinc, manganese, chromium and cadmium in subjects studied and control

Parameters	Subjects (n=60) Mean ± S.D	Control (n=40) Mean ± S.D	F Value	P Value
Zinc (ug/dl)	75.11±10.68	88.34 ± 10.21	0.246	0.002*
Manganese (ug/dl)	3.61±0.45	9.62 ± 1.60	0.186	0.001*
Chromium (ug/dl)	0.96 ± 0.33	1.14 ± 0.19	0.215	0.004*
Cadmium (ug/dl)	0.21 ± 0.12	0.15 ± 0.13	0.134	0.001*

*The mean difference is significant at p-value<0.05. Values are inMean ± Standard Deviation

Table 2: Distribution of zinc, manganese, chromium and cadmium in male and female subjects studied

Parameters	Male Mean ± S.D (n=26)	Female Mean ± S.D (n=34)	F Value	P Value
Zinc (ug/dl)	72.81 ± 12.32	77.59 ± 8.40	0.823	0.344
Manganese (ug/dl)	3.51 ± 0.51	3.71 ± 0.35	1.182	0.621
Chromium (ug/dl)	0.81 ± 0.33	1.01 ± 0.33	0.317	0.004*
Cadmium (ug/dl)	0.24 ± 0.19	0.18 ± 0.12	0.203	0.002*

*The mean difference is significant at p-value<0.05. Values are as Mean±S.D

Table 3: Distribution of zinc, manganese, chromium and cadmium in subjects studied in the different age groups

Parameters	Male 20-35 years (n=14) 36-50 years (n=8) 51-65 years (n=4) Mean ± S.D	Female 20-35 years (n=22) 36-50 years (n=10) 51-65 years (n=2) Mean ± S.D	F Value	P Value
Zinc (ug/dl)				
20-35 years	75.05 ± 11.10	69.25 ± 6.45	0.312	0.003*
36-50 years	72.08 ± 11.57	68.34 ± 8.21	0.895	0.331
51-65 years	70.49 ± 6.56	66.65 ± 6.88	1.603	0.521
Manganese (ug/dl)				
20-35 years	3.69 ± 0.48	3.23 ± 0.45	0.775	0.571
36-50 years	3.58 ± 0.48	3.01 ± 0.32	0.627	0.364
51-65 years	2.56 ± 0.50	2.67 ± 0.53	1.110	0.765
Chromium (ug/dl)				
20-35 years	1.11 ± 0.20	0.91 ± 0.48	0.405	0.003*
36-50 years	0.88 ± 0.33	0.81 ± 0.20	1.205	0.737
51-65 years	0.84 ± 0.44	0.78 ± 0.22	0.694	0.383
Cadmium (ug/dl)				
20-35 years	0.24 ± 0.13	0.18 ± 0.12	0.585	0.271
36-50 years	0.28 ± 0.14	0.26 ± 0.15	0.872	0.441
51-65 years	0.30 ± 0.15	0.28 ± 0.24	1.067	0.650

*The mean difference is significant at p-value<0.05. Values are as Mean ± Standard Deviation

DISCUSSION

The low zinc concentrations in serum samples of TB patients (75.11±10.68ug/dl) as compared to apparently healthy controls (88.34 ± 10.21ug/dl) was in agreement with the reports from India (Ahmad *et al.*, 1985), Indonesia (Karyadi *et al.*, 2000), Africa (Ciftci *et al.*, 2003) and Northern Nigeria (Koyanagi *et al.*, 2004). The decline was probably due to redistribution of zinc from serum to other tissues (Cousins and Leinart, 1988) or the reduction of zinc-carrier proteins and/or a rise in the production of metallothionein -a protein that transports zinc to the liver (Gabay and Kushner, 1999). It is worth noting that the low level of zinc in patients with TB may be indicating strong association of hypozincaemia with impaired immune response and degree of malnutrition as reported previously from TB patients (Graham *et al.*, 1991).

Zinc deficiency was shown to decrease the production of tumour necrosis factor –α (TNF-α) and gamma interferon (IFN-γ) from peripheral mononuclear cells (Beck *et al.*, 1997). It was also suggested that administration of anti-TNF-α to patients may lead to reactivation of TB (Mayordomo *et al.*, 2002), and a mutation in the gene coding for the IFN-γ receptor resulted in an increased susceptibility to mycobacterial infections (Newport *et al.*, 1996). It therefore seems possible that zinc deficiency is implicated in the activation of TB. It is interesting to note that supplementation of zinc (Pant *et al.*, 1987) or zinc and vitamin-A to adult TB patients can result in faster clearance of acid fast bacilli from sputum and faster resolution of chest-X-ray pathology (Karyadi *et al.*, 2002).

The results of this study showed that there was a significant decrease in the serum levels of manganese in TB patients as compared with the control. This result is in agreement with other studies carried out in India (Ahmad *et al.*, 1985), Indonesia (Karyadi *et al.*, 2000), Africa (Ciftci*et al.*, 2003) and Northern Nigeria (Koyanagi *et al.*, 2004) respectively. Manganese activates enzymes associated with fatty acid metabolism and protein synthesis, and is involved in neurological function. Manganese (Mn) forms part of the antioxidant superoxide dismutase (SOD), which helps prevent free radical damage. Free radical generation and oxidative stress are features of TB patients (Newport *et al.*, 1996) therefore high levels of Mn in subjects with TB is needed for the formation and proper functioning of SOD to neutralize the free radical being generated. Therefore, the structure and function of the mitochondria are particularly affected by manganese status (Wilson *et al.*, 1997).

Interestingly, manganese deficiency does not affect the functions of most of these enzymes, presumably because magnesium may substitute for manganese in most instances. In animals, manganese is required for normal bone growth,

lipid metabolism, reproduction, and CNS regulation. Manganese has an important role in the normal function of the brain, primarily through its effect on biogenic amine metabolism. This effect may be responsible for the relationship between brain concentrations of manganese and catecholamines. In some cases, calcium and iron are believed to interfere with the appropriate use of manganese in the human body. Eye problems, sweating, fast heartbeats, weakness, and severe cramps may be some of the deficiency symptoms. Severe deficiency may cause infertility in women, pancreatic damage, heart ailments and osteoporosis and increase susceptibility to infectious disease (Soeters, 2005).

Furthermore, this study shows that the mean chromium concentration of TB patients (0.96±0.33ug/dl) is lower than the mean concentration of chromium in healthy control (1.14±0.19ug/dl). This report is in agreement with other study (Graham *et al.*, 1991; Gabay and Kushner, 1999; Koyanagi *et al.*, 2004). The main physiological role of chromium is as a cofactor for insulin. In its organic form, chromium potentiates the action of endogenous and exogenous insulin, presumably by augmenting its adherence to cell membranes. The organic form is in the dinicotinic acid-glutathione complex or glucose tolerance factor (GTF). Chromium is the metal portion of GTF; with insulin, GTF affects the metabolism of glucose, cholesterol, and triglycerides. Therefore, chromium is important for glucose tolerance, glycogen synthesis, amino acid transport, and protein synthesis. Chromium is also involved in the activation of several enzymes.

Chromium is also required for the biosynthesis of some vitamins and is also an essential micronutrient for both *Mycobacterium tuberculosis* and its host. A recent study reported a novel anti-tuberculosis treatment that employed metal-based drugs by utilizing coordination complexes of chromium and other metals with the pyrophosphate ligand. This study reported notable selectivity and marked potency against *M. tuberculosis*, which implicates these metals as important for controlling tuberculosis infection (Gabay and Kushner, 1999). However, our knowledge of the role of chromium in tuberculosis is incomplete.

Blood Cadmium (Cd) reflects both recent and cumulative exposures; therefore, older people tend to have higher blood Cd level (Soeters, 2005). This is in agreement with the findings of this study where Cadmium (Cd) concentration was not only significantly higher in the subjects (0.21±0.21ug/dl) compared with the control (0.15±0.31ug/dl), but the increase in the mean concentration of Cd also increased with age. As TB risk increases with age, these changes in trace elements exposure may further increase TB risk. This is supported by the present study where Cadmium was increased; Zinc and Manganese were reduced in subjects with TB. The involvement of high Cd in the pathogenesis of TB could be linked with fact that Cd affects the immune system thus, raised levels of Cd in our subjects with TB is thus attributed to the progression and development of TB.

Chronic cadmium exposure also can cause pulmonary emphysema and bone diseases (osteomalacia and osteoporosis). Cadmium toxicity can also result in Diarrhoea, stomach pains and severe vomiting, Bone fracture, Reproductive failure and possibly even infertility, Damage to the central nervous system, Damage to the immune system, Psychological disorders and Possibly DNA damage or cancer development as well as increase susceptibility to Tuberculosis infection as seen in this study due to damage of the immune system (Paton *et al.*, 2004).

CONCLUSION

The results of this study showed that the serum concentration of Zinc, Manganese and Chromium was significantly lower in the subjects compared to the control, while cadmium was higher in the subjects compared to the control. The decrease in zinc, manganese and chromium in serum samples from patients with tuberculosis was probably induced by inflammatory processes contained in these patients. Furthermore, the low concentrations of zinc, chromium and manganese observed in this study could result from preceding deficiencies that enhanced susceptibility to infection.

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REFERENCES

Ahmad, P., Garg, R. and Salahuddin, A. (1985): Serum and copper in tuberculosis.*Indian J Pediatr* 22(10): 786–788.

Arnetz, B.B. and Nicolich, M.J. (1999): Modelling of environmental Manganese contributors to manganese blood in humans. *Arch. Occup. Environ. Health* 55(62):397-402.

Beck, K.W., Schramel, P., Hedl, A., Jaeger, H. and Kaboth, W. (1997): Serum trace element levels in HIV-infected subjects. *Biol Trace Elem Res* 25 (15):89–96.

Ciftci, T.U., Yis, O., Guney, Y., Bilgihan, A. and Ogretensoy, M. (2003): Changes in serum selenium, copper, zinc levels and Cu/Zn ratio in patients with pulmonary tuberculosis during therapy. *Biol Trace Elem Res* 95 (40): 65–71.

Cousins, R.J. and Leinart, A.S. (1988): Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. *FASEB J*; 2(18): 90-94.

Gabay, C. and Kushner, I. (1999): Acute-phase proteins and other systemicresponses to inflammation. *N Engl J Med*; 3(4): 448–454.

Garner, P., Smith, H., Munro, S. and Volmink, J. (2007): Promoting adherence to tuberculosis treatment. *Bull World Health Organ.* 85(5):404-406.

Graham, N.M., Sorensen, D., Odaka, N., Brookmeyer, R., Chan, D. andWillett, W.C. (1991). Relationship of serum copper and zinc levels to HIV-1 seropositivity and progression to AIDS. *J Acquir Immune Defic Syndr*; 4(5): 976–980.

Karyadi, E., Schultink, W., Nelwan, R.H., Wilson, B.O. and Perez, A.S. (2000): Poor micronutrient status of active pulmonary tuberculosis patients in Indonesia. *J Nutr.*130 (12):2953-2958.

Karyadi, E., Schultink, W.R., Nelwan, H.H., Gross, R., Amin, Z., Dolmans,W.M., Meer, J.M., Hawkes, J.G. and Keim, N.L. (2002): Dietary selenium intake modulates thyroid hormone and energy metabolism in men. *J Nutr.* 105(133):3443-8.

Khan, A., Sterling, T.R., Reves, R., Vernon, A. and Horsburgh, C.R. (2008): Lack of weight gain and relapse risk in a large tuberculosis treatment trial. *Am J RespirCrit Care Med.* 4(3):344-348.

Koyanagi, A., Kullo, D., Gresley, L., Shenkin, A. and Cuevas, L.E. (2004): Relationships between serum concentrations of C-reactive protein and micronutrients in patients with tuberculosis. *Ann. Trop. Med. Parasitol.* 80(98):391-400.

Mills, C.F. (2005): Biological roles of trace elements. In: Nutrition in health and disease.New York, Alan R. Liss. Pp. 179-188.

Newport, M.J., Huxley, C.M., Huston, S., Hawrylowicz, C.M., Oostra, B.A.and Williamson, R. (1996): A mutation in the interferongamma-receptor gene and susceptibility to mycobacterial infection. *N Engl J Med*; 33(15): 1941–1949.

Pant, K., Biswas, S., Chawla, R., Shah, A. and Singh, M. (1987): Zinc in active tuberculosis. *Indian J Chest Dis Allied Sci;* 29(5): 144–149.

Paton, N.I., Chua, Y.K., Earnest, A. and Chee, C.B. (2004): Randomized controlled trialof nutritional supplementation in patients with newly diagnosed tuberculosis and wasting. *Am J Clin Nutr*; 80(2):460-465.

Schwartz, J. and Levin, R. (2001): The risk of lead toxicity in homes with Cadmium paint hazard. Environ. Res. 15(54):1-7.

Sharma, S.K. and Mohan, A. (2003): Multidrug Resistant Tuberculosis: Current Trends. *J. Clin. Microbiol* 20(13):131-135.

Soeters, M., de Vries, A.M., Kimpen, J.L., Donald, .PR. and Schaaf, H.S. (2005): Clinical features and outcome in children admitted to a TB hospital in the Western Cape--the influence of HIV infection and drug resistance. *S Afr Med J.* 95(8):602-606.

Wilson, R.J., Llewelyn, M., Toossi, Z. and Duke, E.T. (1997): Influence of vitamin Ddeficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. *Lancet*; 355 (9204):618-621.

World Health Organization (2005): Global tuberculosis control: surveillance, planning, financing: *WHO report*. www.WHO/HTM/TB/379

AUTHORS' CONTRIBUTIONS

All authors involved in this study, actively took part in this study and presentation. Below are the specific contributions of the authors.
FESTUS, O.O.: Chief Investigator and Team Leader
DADA, F.L.: Literature search and write up
EKUN, V.: Laboratory analysis
IWEKA, F.L.: Statistical analysis and proof reading.
EIDANGBE, G.: Review of literature and proof reading