ejpmr, 2016,3(9), 37-43.

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF SOME TRACE ELEMENTS (COPPER, SELENIUM, IRON, AND LEAD) IN PATIENTS WITH ACTIVE TUBERCULOSIS ATTENDING CENTRAL HOSPITAL BENIN CITY, EDO STATE.

***Festus, O.O., Omon, E., Dada, F.L. and Iweka, F.K.**

Nigeria.

Corresponding Author: Festus O. O.

Nigeria

Article Received on 22/06/2016 **Article Revised on 13/07/2016** **Article Accepted on 04/08/2016**

ABSTRACT

Pulmonary Tuberculosis (TB) is caused by the bacteria ***Mycobacterium tuberculosis*** – aerobic, non-motile bacillus. This disease is known as an ancient disease and still is one of the most serious health problems in the world. The biological role of trace elements, especially Copper, Selenium and Iron in different pathologic conditions has been extensively investigated in many diseases. The aim of this study was to evaluate serum concentrations of some trace elements Iron (Fe), Copper (Cu), Selenium (Se) and Lead (Pb) in patients with active tuberculosis (TB). A total of one hundred (100) samples were used in this study, comprising of sixty (60) patients with active tuberculosis (subjects) attending Central Hospital, Benin City and forty (40) apparently healthy individuals as control. The study population was made up of 26 (43.3%) males and 34 (56.7%) females, while the control population was 18 (45.0%) males and 22 (55.0%) females respectively. Serum levels of copper, selenium, iron and Lead were determined using Atomic Absorption Spectrophotometer. The results obtained showed that the concentrations of Iron and Selenium were significantly lower ($P<0.05$) while that of Copper and Lead were significantly higher ($P<0.05$) in the serum of patients with tuberculosis compared with the control. There was no significant difference ($P>0.05$) in serum concentrations of Iron, Copper and Selenium in TB patients in relation to sex. Furthermore, there was no significant difference ($p>0.05$) in the concentration of Copper and Lead in the different age groups. There was also no significant difference ($p>0.05$) in Iron and Selenium in ages 36-50years and 51-65 years, but there was significant increase ($p<0.05$) in Iron and Selenium in age 20-35years in both males and females. There was no difference in the mean Copper in ages 20-35years and 36-50years in both males and females but there was a decrease in copper level in age 51-65years in both males and females respectively. Finally, there was a decrease in selenium with increase in age in both males and females. There was also an increase in Lead with increase in age in both males and females respectively. Conclusively, the results indicated that patients with tuberculosis have altered profile of trace elements in their sera. This warrants the need for further investigations so that strategies for trace elements supplementation can be planned in addition to their potential as diagnostic parameters in monitoring responses to anti-TB chemotherapy.

KEYWORDS: *Mycobacterium tuberculosis* – aerobic, non-motile bacillus.

INTRODUCTION

Tuberculosis (TB) is a contagious disease caused by the organism *Mycobacterium tuberculosis* – aerobic, non-motile bacillus (Sharma, 2003). Tuberculosis spreads through air, when infectious people cough, sneeze, talk or spit; they propel TB germs known as bacilli into the air. Inhalation of very small numbers of these bacilli will lead to *M. tuberculosis* infection (Deveci, 2003). The tuberculosis bacteria can attack any part of the body, most commonly the lungs (known as pulmonary tuberculosis). Extra-pulmonary TB occurs when tuberculosis develops outside of the lungs, although extra-pulmonary TB may coexist with pulmonary TB, as well (Van-Lettow, 2004). Pulmonary TB is characterized by prolonged cough, hemoptysis, chest pain and dyspnea. Systemic manifestations of the disease include fever, malaise, chills, fatigue, anorexia, weight loss, weakness and night sweats (WHO, 2005).

TB infection begins when the mycobacteria reach the pulmonary alveoli, where they invade and replicate within endosomes of alveolar macrophages. Macrophages identify the bacterium as "foreign" and attempt to eliminate it by phagocytosis. During this process, the entire bacterium is enveloped by the macrophage and stored temporarily in a membrane-bound vesicle called a phagosome. The phagosome then combines with a lysosome to create a phagolysosome. In the phagolysosome, the cell attempts to use reactive oxygen species and acid to kill the bacterium. However,

M. tuberculosis has a thick, waxy mycolic acid capsule that protects it from these toxic substances. *M. tuberculosis* actually reproduces inside the macrophage and will eventually kill the immune cell (Gurumurthy, 2004).

Trace element is a dietary mineral that is needed in very minute quantities for the proper growth, development, and physiology of the organism (Mills, 2005). Selenium, a mineral found in the soil plays a key role in metabolism. Selenium salts are toxic in large amounts, but trace amounts are necessary for cellular function in many organisms (Hawkes *et al.*, 2003). The essential trace element selenium has an important function in maintaining the immune processes and thus may have a critical role in clearance of mycobacteria (Richie *et al.*, 2012).

Iron is an element essential to life. Iron is an essential component in the transfer of oxygen in the body (Jurado, 1997). The human body needs iron to make the oxygen-carrying proteins haemoglobin and myoglobin. Iron is a component of enzymes critical for the functioning of immune cells and is involved in the regulation of cytokine production and action (Mills, 2005). Copper is essential to all living organisms as a trace dietary mineral because it is a key constituent of the respiratory enzyme complex cytochrome C oxidase (Citci *et al.*, 2003). Copper is involved in the process of erythropoiesis, erythrocyte function and regulate erythrocyte survival (Rejali *et al.*, 2007). Copper is critical for energy production in the cells. It is also involved in nerve conduction, connective tissue, the cardiovascular system and the immune system (O'Dell, 2013).

Amongst known toxic heavy metals, "Lead" in any form seems to be a ubiquitous environmental poison to any form of life. Lead, having no beneficial role to the human body enters the body through multiple routes and gets distributed and stored in almost every organ resulting in the defective functions of the organ (Schwartz and Levin, 2001).

MATERIALS AND METHODS

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 subject who 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 in this study, comprising of sixty (60) patients with active tuberculosis (subjects) attending Central Hospital Benin City and forty (40) apparently healthy individuals (control). Five (5.0) mls of blood sample was collected from fasting subjects via venipuncture into a plain container without any additive to determine the serum trace metals (Fe, Cu, Se and Pb). It was allowed to stand for 1 hour to clot. It was then centrifuged at 3000g for 10 min and the serum was isolated and stored at 4^0c until required for analysis.

INCLUSION 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 TB.

EXCLUSION CRITERIA

All subjects with previous anti-TB treatment, pregnant and lactating women, subjects using imunosuppressive drugs and other diseases were excluded from this study.

SAMPLE ANALYSIS

Iron, Copper, Selenium and Lead concentrations in serum were estimated by Atomic Absorption Spectrophotometer. The 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. A portion 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 ± S.D (standard deviation). 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 demographic and clinical data of the Tuberculosis subjects and control. The mean age of both the subjects and control were 31.72 ± 8.72 and 31.02 ± 6.54 years respectively. Of the Sixty (60) subjects that

participated in the study, 26 (43.3%) were males while 34 (56.7%) were females. Similarly, of the forty (40) persons that made up the control group, 18 (45.0%) were males while 22 (55.0%) were females. All subjects who participated in this study were sputum positive for acid-fast bacilli by microscopy (100%). On the clinical signs, 98% of the subjects had cough, 75% had fever, 70% had experienced weight loss and 66% had night sweats respectively.

Table 2 shows the mean value of Iron, Copper, Selenium and Lead in the TB subjects and control. The results obtained shows that there was a significant decrease ($p<0.05$) in Iron (86.56±10.68) and Selenium (12.22±4.18) in the TB subjects compared with the control (122.19±20.36 and 28.85±4.81), while there was a significant increase ($p<0.05$) in copper (135.64±11.23) and Lead (0.57±0.95) of subjects compared with control (113.48±7.71 and 0.29±0.15).

Table 3 shows the Mean±S.D of Iron, Copper, Selenium and Lead in male and female subjects studied. The results obtained shows that there was no significant difference ($p>0.05$) in Iron, Copper and Selenium in both males and females in the subjects studied. Though, the mean value of Iron and Copper was slightly higher in females than in males, the differences is not statistically significant ($p>0.05$). The mean value of Iron in females and males were 89.04±8.40ug/dl and 84.27±12.32ug/dl, while that of Copper were 135.17±7.95ug/dl and 134.76±7.80ug/dl respectively. Selenium was higher in males (14.63±4.28ug/dl) than in females (9.61±1.98ug/dl), but not statistically significant ($p>0.05$). On the other hand, there was a significant difference ($p<0.05$) between the mean Lead of males and females. The values were higher in females (0.32±0.17ug/dl) than in males (0.25±0.13ug/dl).

Table 4 shows the Mean distribution of Iron, Copper, Lead and Selenium in subjects studied in the different age groups. The result shows that there was no significant difference ($p>0.05$) in Copper and Lead in the different age groups. There was also no significant difference ($p>0.05$) in Iron and Selenium in ages 36-50 years and 51-65 years, but there was significant increase ($p<0.05$) in Iron and Selenium in age 20-35years in both males and females. There was a decrease in Iron in the different age groups in both males and females respectively. There was no difference in the mean Copper in age's 20-35years and 36-50years in both males and females but there was a decrease in copper level in age 51-65years in both males (127.72 ± 5.55ug/dl) and females (125.67 ± 4.53ug/dl). Furthermore, there was a decrease in Selenium with increase in age in both males and females. There was also an increase in Lead with increase in age in both males and females respectively.

APPENDIX

TABLE 1: DEMOGRAPHIC AND CLINICAL DATA OF SUBJECTS AND CONTROL

PARAMETERS	SUBJECTS (N=60)	CONTROL (N=40)
Age (Mean ± S.D in years)	31.72 ± 8.72	31.02 ± 6.54
Sex		
Male (%)	26 (43.3)	18 (45.0%)
Female (%)	34 (56.7)	22 (55.0%)
Clinical Signs		
Smear positive (%)	100.0	-
Cough (%)	98.0	-
Fever (%)	75.0	-
Weight loss (%)	70.0	-
Night sweats (%)	66.0	-

Key: S.D – Standard deviation; % - Percentage

TABLE 2: MEAN VALUE OF IRON, COPPER, SELENIUM AND LEAD IN SUBJECTS STUDIED AND CONTROL

Parameters	Subjects(n=60) Mean ± S.D	Control(n=40) Mean ± S.D	F Value	P Value
Iron (ug/dl)	86.56 ± 10.68	122.19 ± 20.36	0.176	0.001*
Copper (ug/dl)	135.64 ± 11.23	113.48 ± 7.71	0.186	0.001*
Selenium (ug/dl)	12.22 ± 4.18	28.85 ± 4.81	0.215	0.002*
Lead (ug/dl)	0.57 ± 0.25	0.29 ± 0.15	0.134	0.001*

*The mean difference is significant at p-value<0.05. Values are in Mean ± Standard Deviation (S.D)

TABLE 3: MEAN DISTRIBUTION OF IRON, COPPER, SELENIUM AND LEAD IN MALE AND FEMALE SUBJECTS STUDIED

Parameters	Male Mean ± S.D (n=26)	Female Mean ± S.D (n=34)	F Value	P Value
Iron (ug/dl)	84.27 ± 12.32	89.04 ± 8.40	0.823	0.344
Copper (ug/dl)	134.76 ± 7.80	135.17 ± 7.95	0.995	0.543
Selenium (ug/dl)	14.63 ± 4.28	9.61 ± 1.98	0.820	0.345
Lead (ug/dl)	0.25 ± 0.13	0.32 ± 0.17	0.203	0.004*

*The mean difference is significant at p-value<0.05. Values are in Mean ± Standard Deviation (S.D)

TABLE 4: MEAN DISTRIBUTION OF IRON, COPPER, LEAD AND SELENIUM 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
Iron (ug/dl)				
20-35 years	85.36 ± 11.78	79.25 ± 7.45	0.412	0.004*
36-50 years	84.64 ± 11.52	88.34 ± 8.21	0.995	0.441
51-65 years	83.48 ± 6.49	85.65 ± 6.88	1.663	0.301
Copper (ug/dl)				
20-35 years	132.92 ± 8.38	131.23 ± 9.45	0.885	0.671
36-50 years	132.94 ± 7.53	132.45 ± 8.32	0.772	0.364
51-65 years	127.72 ± 5.55	125.67 ± 4.53	1.067	0.365
Selenium (ug/dl)				
20-35 years	15.33 ± 3.67	8.51 ± 1.98	0.305	0.004*
36-50 years	12.68 ± 1.51	9.61 ± 2.00	1.075	0.537
51-65 years	10.44 ± 2.45	6.67 ± 1.22	0.559	0.223
Lead (ug/dl)				
20-35 years	0.28 ± 0.16	0.25 ± 0.12	0.785	0.571
36-50 years	0.29 ± 0.14	0.31 ± 0.15	0.672	0.264
51-65 years	0.31 ± 0.21	0.33 ± 0.24	1.167	0.465

*The mean difference is significant at p-value<0.05. Values are in Mean ± Standard Deviation (S.D).

DISCUSSION

The high serum copper concentrations observed in the TB patients in this study were also in line with previous reports (Bogden *et al.*, 1977; Ahmad *et al.*, 1985; Ciftci *et al.*, 2003; Koyanagi *et al.*, 2004). The elevated serum levels of copper may reflect a nonspecific increase in serum concentration of copper-binding protein, ceruloplasmin. Plasma concentrations of ceruloplasmin and copper increases as an acute-phase response in a variety of infections and inflammatory conditions (Bogden *et al.*, 2000). High serum levels of copper were also reported in TB/HIV co-infected patients suggesting its possible role as a useful marker of HIV activity and progression to AIDS (Moreno *et al.*, 1998; Bogden *et al.*, 2000).

Elevated copper was reported in patients with different pathological conditions such as intestinal amebiasis and giardiasis (Karakas *et al.*, 2001), lymphoma and leukemias (Rosas *et al.*, 1995), gastric cancer (Hua-Dong *et al.*, 1999), and breast cancer (Kuo *et al.*, 2002) and considered to have diagnostic and prognostic values. In the present study too, copper was higher in serum of TB patients compared to that of healthy subjects.

The low serum Iron concentration observed among TB subjects is in line with that reported by Juarado (1997), who reported a low serum Iron concentration among TB patients in Northern Nigeria. Lawn *et al.*, (2000) also reported similar result in Ghana. Similar result were observed in a study carried out in the Eastern part of Nigeria where Iron level was lower in TB patients as compared with healthy controls (Harries *et al.*, 2004). In Uganda, mean haemoglobin concentrations were lower among HIV-infected adults with TB than in HIV-infected adults without TB (Taylor and Smith, 1998). Low plasma transferrin concentrations have been described among HIV-infected adults with TB (Niyongabo *et al.*, 1999).

There are two explanations for the association of low iron status with infection. One is that anaemia results from chronic inflammation. In line with this, Monteiro *et al.*, (2000) suggested the implication of acute-phase response (APR) on the etiology of anaemia in TB patients by demonstrating higher serum ferritin levels among APR-positive subjects compared to those which are negative for acute-phase response.

This might indicate that the hypoferraemia in TB patients with or without HIV co-infection may be induced by the shift of iron from a transferrin-bound available state to a ferritin-incorporated storage state. The condition may have evolved as a cytokine-mediated defense against microbial pathogens, effectively withholding iron from microbes, which incidentally also deprives erythroid precursors of their iron supply (Jurado, 1997). The other, which is more speculative, is that iron deficiency would increase susceptibility to an infection such as TB. In this context, it may be argued that cell-mediated immunity is compromised in iron deficiency (Dallman, 1987) before anaemia becomes apparent. It is worth mentioning that subjects with TB infection manifest iron deficiency, which may be ascribed to intestinal malabsorption (Castaldo *et al.*, 1996).

Like Iron, a significantly low level of serum selenium was observed in Tuberculosis patients as compared with healthy control. Selenium is known to be an essential component of antioxidative selenoenzymes such as glutathione peroxidase which are known to protect host cells from oxidative damage in inflammatory conditions (McKenzie *et al.*, 1998). Although the mechanisms involved have yet to be elucidated, the important role of selenium in normal immune responses is well established (McKenzie *et al.*, 1998). Selenium also has immunopotentiating effects as its deficiency appears to result in immunosuppression, whereas supplementation with low doses of selenium appears to result in augmentation and/or restoration of immunologic functions (Roy *et al.*, 1994).

An inverse correlation between this micronutrient and TB/HIV co-infection has been reported (Dworkin, 1994) substantiating our present finding of its significantly low level in TB patients. Increased susceptibility to TB was seen in HIV-infected individuals with low plasma concentrations of selenium (Shor-Posner *et al.*, 2002). The trace element appears to be important in reducing the virulence of chronic infections and slowing the progression of TB-related disease (Rayman, 2000). It is worth noting that, deficiencies in antioxidants during TB infection were shown to induce chronic oxidative stress (Palme *et al.*, 2002), which has been linked to apoptosis of T-lymphocytes during TB disease (Dobmeyer *et al.*, 1997) and increased rates of HIV replication by activating the nuclear factor-α B (NF-α B) gene (Schreck *et al.*, 1991).

Lead (Pb) is a non-essential toxic metal that have affinity for free sulphuhydryl active site of enzymes and proteins. Reports have shown that Selenium interacts with Pb in vivo. These interactions are part of natural metal detoxification process, which result in the metabolic inactivation of Selenium (Se). However, at sufficiently high exposure levels, Pb may overtime produce a state akin to Se deficiency thereby aborting the protecting effects of Se. This study showed a significant increase ($p<0.05$) in the serum levels of Lead in tuberculosis patients as compared with the controls. This toxic trace metal has been reported to be involved in the generation of reactive oxygen species resulting in lipid peroxidation, DNA damage and altered gene expression (Whanger, 2004). The significant increase in the levels of Lead in tuberculosis patients observed in this study could have contributed to the pathogenesis of tuberculosis.

The high level of Lead observed in this study would be a risk factor rather than protective agent for tuberculosis patients. Other researchers have indeed found increased levels of Lead in tuberculosis patients, implying the increase in serum level could have resulted from sequestration to the site of increased cellular activity. It has been suggested that Lead compete for the binding sites in the cell, change its enzymatic activity and exert direct or indirect action on the immune system, thereby, accelerating the pathogenesis of tuberculosis and other related infection (Rejali *et al.*, 2007).

CONCLUSION

In conclusion, this study shows that the serum concentrations of trace elements in patients with tuberculosis were altered. It is interesting to note that an association between acute-phase response to infection and alteration in dynamics of many trace elements, particularly iron, selenium and copper, has been recognized for many decades. The low concentrations of iron and selenium observed in this study could result from preceding deficiencies that enhanced susceptibility to infection, and/or from their high demands in overt tuberculosis. The fall in serum Iron and Selenium, and rise in serum Copper and Lead of tuberculosis patients, is brought about by changes in the concentration of specific tissue proteins such as C-reactive protein (CRP) that are controlled by cytokines.

REFERENCES

1. Ahmad, P., Garg, R. and Salahuddin, A. (1985): Serum and copper in tuberculosis. *Indian J Pediatr* 22(10): 786–788.
2. Bogden, J., Lintz, D., Joselow, M., Charles, J. and Salaki, J. (1977): Effect of pulmonary tuberculosis on blood concentrations of copper and zinc. *Am J Clin Pathol* 67(34): 251–256.
3. Bogden, J.D., Kemp, F.W., Han, S., Li, W., Bruening, K. and Denny, T. (2000): Status of selected nutrients and progression of human immunodeficiency virus type 1 infection. *Am J Clin Nutr* 72(35): 809–815.
4. Castaldo, A., Tarallo, L., Palomba, E., Albano, F., Russo, S. and Zuin, G. (1996): Iron deficiency and intestinal malabsorption in HIV disease. *J Pediatr Gastroenterol Nutr* 12(22): 359–363.
5. 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.

6. Dallman, P.R. (2007): Iron deficiency and the immune response in Tuberculosis patients.*Am J Clin Nutr* 23(46): 329–334.
7. Deveci, F. and Ilhan, L. (2003): Plasma malondialdehyde and serum trace element concentrations in patients with active pulmonary tuberculosis. *Biol. Trace. Elem. Res.* 45(95): 29-38.
8. Dobmeyer, T.S., Findhammer, S., Klein, S.A., Raffel, B. and Hoelzer, D. (1997): *Ex vivo* induction of apoptosis in lymphocytes is mediated by oxidative stress: role for lymphocyte loss in HIV infection. *Free Radic Biol Med* 22(10): 775–785.
9. Dworkin, B.M. (1994): Selenium deficiency in HIV infection and the acquired immunodeficiency syndrome (AIDS). *Chem Biol Inter* 9(1): 181–186.
10. Gurumurthy, P., Ramachandran, G., Hemanth, F.L. and Kumar, A.K. (2004):
11. Malabsorption of rifampin and isoniazid in HIV-infected patients with and without tuberculosis. *Clin Infect Dis.* 38(2): 280-283.
12. Harries, C.C., Whalen, T.E., Taha, N., Kumwenda, C., Kang, S.K. and Semba, S.D. (2004): Malnutrition and the severity of lung disease in adults with pulmonary tuberculosis in Nigeria. *Int. J. Tuberc. Lung. Dis.* 8(2): 211-217.
13. Hua-Dong, L.U., Zhi-Qiang, W., Yu-Rong, P., Tian-Shu, Z., Xi-Zhu, X. and Tian-Wang, K. (1999): Comparison of serum Zn, Cu and Se contents between healthy people and patients in high, middle and low incidence areas of gastric cancer of Fujian Province. *World J Gastroenterol* 5(6): 84–86.
14. Hawkes, J.G., Karyadi, E., Schultink, W.R., Nelwan, H.H., Gross, R., Amin, Z., Dolmans,
15. W.M., Meer, J.M. and Keim, N.L. (2003): Dietary selenium intake modulates thyroid hormone and energy metabolism in men. *J Nutr.* 105(133): 3443-3448.
16. Jurado, R.I. (1997): Iron, infection, and anaemia of inflammation. *Clin Infect Dis* 25 (12): 888–895.
17. Karakas, Z., Demirel, N., Tarakcioglu, M. and Mete, N. (2001): Serum zinc and copper levels in Southeastern Turkish children with giardiasis or amebiasis. *Biol Trace Elem Res* 84(15): 11–18.
18. Koyanagi, A., D. Kullo, L. Gresley, A. Shenkin and L.E. Cuevas, (2008): Relationships between serum concentrations of C-reactive protein and micronutrients in patients with tuberculosis. *Ann. Trop. Med. Parasitol.* 80(98): 391-400.
19. Kuo, H.W., Chen, S.F., Wu, C.C., Chen, D.R. and Lee, J.H. (2002): Serum and tissue trace elements in patients with breast cancer in Taiwan. *Biol Trace Elem Res* 8(9):1–12.
20. Lawn, S.D., Obeng, J., Acheampong, J.W. and Grifin, G.E. (2000): Resolution of the acute-phase response in West African patients receiving treatment for pulmonary tuberculosis. *Int J Tuberc Lung Dis* 4(8): 340–344.
21. Lettow, M., Harries, A.D., Kumwenda, J.J., Zijlstra, E.E., Clark, T.D. and Taha, T.E. (2004): Micronutrient malnutrition and wasting in adults with pulmonary tuberculosis with and without HIV co-infection in Malawi. *BMC Infect Dis* 4(8): 61–68.
22. McKenzie, R.C., Rafferty, T.S. and Beckett, G.J. (1998): Selenium: an essential element for immune function. *Immunol* 19(12): 342–345.
23. Mills, C.F. (2005): Biological roles of trace elements. In: Nutrition in health and disease. New York, Alan R. Liss. Pp. 179-188.
24. Monteiro, J.P., Cunha, D.F., Cunha, S.F., Santos, V.M., Silva-Vergara, M.L. and Correia, D. (2000): Iron status, malnutrition and acute phase response in HIV-positive patients. *Rev Soc Bras Med Trop* 33(9): 175–180.
25. Moreno, T., Artacho, R., Navarro, M., Perez, A. and Ruiz-Lopez, M.D. (1998): Serum copper concentration in HIV-infection patients and relationships with other biochemical indices. *Sci Total Environ* 21(7): 21–26.
26. Niyongabo, T., Henzel, D., Idi, M. and Njong, A.L. (1999): Tuberculosis, human immunodeficiency virus infection, and malnutrition in Burundi. *Nutri.* 15(4): 289-293.
27. O'Dell, B.L. (2013): Biochemical basis of the clinical effects of copper deficiency. In: Clinical, biochemical and nutritional aspects of trace elements. New York, Alan R. Liss, Publisher. Pp. 301-313.
28. Palme, I.B., Gudetta, B., Bruchfeld, J., Muhe, L. and Giesecke, J. (2002): Impact of human immunodeficiency virus 1 infection on clinical presentation, treatment outcome and survival in a cohort of Ethiopian children with tuberculosis. *Pediatr Infect Dis J.* 21(11): 1053-1061.
29. Rayman, M.P. (2000): The importance of selenium to human health. *Lancet* 35(6): 233–241.
30. Rejali, L., Jaafar, M.H. and Ismail, N.H. (2007): Deficiency of some trace metals in *M.tuberculosis. Environ Health Prev Med*. 12(3): 105–110.
31. Richie, A.L., Soeters, M., de Vries, A.M., Kimpen, J.L., Donald, .PR. and Schaaf, H.S. (2012): 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.
32. Rosas, R., Poo, J.L., Montemayor, A., Isoard, F., Majluf, A. and Labardini, J. (1995): Utility of the copper/zinc ratio in patients with lymphoma or acute or chronic leukemias. *Rev Invest Clin* 47(20): 447–452.
33. Roy, M., Kiremidjian-Schumacher, L. and Wishe, H.I. (1994): Supplementation with selenium and human immune cell functions. *Biol Trace Elem Res* 41(30): 103–114.
34. Schwartz, J. and Levin, R. (2001): The risk of lead toxicity in homes with lead paint hazard. Environ. Res. 15(54): 1-7.
35. Schreck, R., Rieber, P. and Baeuerie, P.A. (1991): Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B

transcription factor and HIV-1. *EMBO J.*, 10(22): 47–58.
36. Sharma, S.K. and Mohan, A. (2003): Multidrug Resistant Tuberculosis: Current Trends.*J. Clin. Microbiol* 20(13): 131-135.
37. Shor-Posner, G., Miguez, M., Pineda, L., Rodriguez, A., Ruiz, P. and Castillo, G. (2002):
38. Impact of selenium status on the pathogenesis of mycobacterial disease in HIV-1 infected drug users during the era of highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2(9): 169–173.
39. Taylor, B. and Smith, P.J. (1998): Does AIDS impair the absorption of antituberculosis agents? *Int J Tuberc Lung Dis.* 2(8): 670-675.
40. Van-Lettow, M., Kumwenda, J.J., Harries, A.D. and Kupter S.L. (2005): Malnutrition and the severity of lung disease in adults with pulmonary tuberculosis in Malawi. *Int J Tuberc Lung Dis.* 8(2):211-217.
41. Whanger, P.D. (2004): Lead and its relationship to *M. tuberculosis*. *B J Nutr*. 91(1): 11–28.
42. World Health Organization (2005): Global tuberculosis control: surveillance, planning, financing: *WHO report*. www.WHO/HTM/TB/379.