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EVALUATION OF SERUM CONCENTRATIONS OF Cu/Zn, Cu /Se, AND Cu/Fe RATIO DURING THERAPEUTIC RESPONSE IN TUBERCULOSIS PATIENTS ATTENDING SELECTED HOSPITALS IN UYO, NIGERIA

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ABSTRACT
Nutritional status is one of the most important determinants of immune response to infection. The aim of this study was to assess the levels of the concentration of the selected trace elements during and at the end of TB treatment. The serum concentration of some essential trace elements was assessed during therapy among patients attending Tuberculosis (TB) treatment centers in selected hospitals in Uyo, Akwa Ibom State, Nigeria. Written informed consent was obtained from the patients. Sputum samples were collected into wide mouth containers. This was repeated at two-month intervals for the period of six months for TB positive cases. Five millilitres of venous blood were collected into plain blood container and centrifuged at 3,000 rpm for 5 minutes at room temperature to separate sera. These were stored in the refrigerator at -20^{0}C until they were ready for analysis. Serum concentration of copper, zinc, iron, selenium and chromium were assessed by flame atomic absorption spectrometry method. A total of 155 persons participated in the study, 83 (53.5%) were females while 72 (46.5%) were males. Majority of the participants were in age group 31-50 years. The mean serum concentration of trace elements measured at different stages of TB treatment was statistically significant ($p<0.05$). The mean serum concentration of zinc, iron and selenium was significantly increased at 4th and 6th month of treatment compared to concentration at diagnosis and 2nd month of treatment. However, the mean serum concentration of copper and chromium significantly decreased at 6th month of treatment compared to concentration at initial diagnosis. The serum Cu/Zn ratio, Cu/Fe ratio and the Cu/Se ratio significantly increased at initial diagnosis, and significantly lower at 6th month of treatment ($p<0.001$). Assessment of the serum Zn, Fe, Cu, Se and Cr, and of the cu/Zn, Cu /Se and Cu /Fe ratio could serve as an indicator of the level of nutritional status and oxidative stress in tuberculosis patients. Findings from this study suggest a strong association of Zn, Fe, Cu, Se and Cr with TB before, during and after treatment. Therefore, measurements of trace elements can be used as tool to assess treatment indices during anti-tuberculosis therapy.
KEYWORDS: Tuberculosis, Trace elements, Serum Concentration, therapy, Therapeutic response, Uyo

INTRODUCTION
Tuberculosis (TB), an infectious disease that mostly affects the lungs but can also affect other parts of the body, is caused by the bacterium *Mycobacterium tuberculosis* (Garner *et al*., 2007). Common symptoms of active TB are cough with sputum and blood, chest pains, weakness, weight loss, fever and night sweats. In global estimation, 10.0 million people fell ill with TB in 2019 (WHO, 2020). Tuberculosis remains the top infectious killer in the world claiming close to 4,000 lives a day (WHO, 2020).
Tuberculosis mostly affects adults in their most productive years (WHO, 2021). However, all age groups are at risk (WHO, 2020). People with undernutrition are three times more at risk. Globally in 2019, there were 2.2 million new TB cases that were attributable to undernutrition (WHO, 2021). Alcohol use disorder increases the risk of developing TB disease by a factor of 3.50 (Lönnroth *et al*., 2008) as well as alcohol consumption accompanied with tobacco smoking is associated with increased risk of TB infection by a factor of 1.51 (Soh *et al*., 2017).

Nutritional status is one of the most important determinants of immune response to resist TB and undernutrition has been reported to be a risk factor for the progression of TB to active TB disease. Tuberculosis may cause malnutrition through increased metabolic demands and decreased nutrient intake (Sinclair *et al*., 2011). Food and nutritional care are essential for a successful TB prevention and health promotion.
Trace elements are elements that occur in nature or in perturbed environment in small amount, and that when present in sufficient bioavailable concentration are toxic to living organism (Wada, 2004). The immunological effects of these elements include immunomodulation, autoimmunity and allergy (Cannas *et al*., 2020). These elements can act as immunosuppressants or as immune adjuvants. Their effects depend on the dose. For example, low zinc levels and high levels of copper, manganese and iron participate in the activation of inflammatory responses and responses to oxidative stress induced by the reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Mezzaroba *et al*., 2019). Interactions between different oligoelements may play an important role in metabolic disease onset. For example, copper deficiency anemia can develop in people who consume high doses of zinc over a long period of time (Turnlund, 2006). Deficiencies in various essential trace elements have been associated with decreased immunity against tuberculosis; moreover, trace elements are believed to have an impact on clinical outcome and are thus related to disease control (Sinclair *et al*., 2011).

Iron deficiency anemia (IDA) affects 1–53% of tuberculosis (TB) patients, depending on the demographic and pertinent contributing factors (Hella *et al*., 2018, Kerkhoff *et al*., 2016, Isanaka *et al*., 2011). Low transferrin and hemoglobin, in addition to ferritin and hepcidin, and raised ferritin were associated with an increased incidence and susceptibility to tuberculosis in HIV patients. (McDermid *et al*., 2013).
Mycobacterium needs copper to survive, and it is also utilized by the bacteria to fight off infections (Edem *et al*., 2015). It is an important component of the immune system that protects against reactive oxygen species that are produced when a person contracts tuberculosis (Bozan *et al*., 2017).

Many facets of cellular metabolism involve zinc (Classen *et al.*, 2011). Cellular immune system uses zinc to destroy bacteria, including Escherichia coli and Tubercle bacilli (Botella *et al*., 2011, Stensland *et al.*, 2015). Zinc's antioxidant-like qualities allow it to hold onto biological macromolecules and stabilize them (Hennig *et al*., 1999). DNA regulates the creation of proteins in biological systems. Bacterial DNA molecules, namely those of *Mycobacterium tuberculosis*, include zinc-binding regions that prevent the DNA from synthesizing the necessary proteins for correct operation. Bacteria die as a result of this. Zinc helps the host cell become immune, which in turn kills the Escherichia coli and tuberculosis bacteria (Hennig *et al.,* 1999).

In almost all tissues and cell types, including those involved in innate and adaptive immunological responses, selenium, when it is integrated into selenoproteins, is involved in controlling oxidative stress, redox, and other critical cellular processes (Gromer *et al.,* 2005). Selenium has the ability to modulate tuberculosis due to its anti-inflammatory and antioxidant qualities as well as its critical function in immunological responses. Notably, translocation of the nuclear factor of T cells, oxidative burst, and calcium mobilization can all enhance the cellular immune response when selenium levels are appropriate (Huang *et al*., 2012). While selenium deficiency has been linked to decreased T cell proliferation and decreased NK cell activity—both of which are essential for antibacterial immunity—selenium supplementation may promote the growth of natural killer (NK) cells by up-regulating interleukin-2 (Baum *et al.*, 2000).

Additionally, research on animals has demonstrated that supplementing with selenium may decrease (Wang *et al*., 2013) or enhance (Xue *et al*., 2010) Tregs, which aid in the escape of Mycobacterium tuberculosis from the host immune system (Larson *et al.,* 2013). Chromium exists in divalent [Cr(II)], trivalent [Cr(III)], and hexavalent [Cr(VI)] oxidation states, with Cr(VI) and Cr(III) being the most stable forms, among which Cr(III) and Cr(VI) are insoluble and soluble forms, respectively. The total body content of chromium is relatively low and is about 0.006 g in an average healthy human adult (Krejpcio, 2001).

Chromium is an important trace element for overweight people as it is one of the key minerals in controlling blood sugar and lipid levels. Chromium [Cr(III)] increases the efficacy of insulin and stimulating glucose uptake from the muscles and other tissues being the main ingredient of glucose tolerance factor (GFT). In case of low serum levels of chromium, the circulating level of (GFT) is also less, and, consequently, insulin is less effective in reducing blood sugar. As a result, high blood sugar stimulates further release of ineffective insulin.

The WHO guidelines on nutritional care and support for TB patient states that their nutritional status should be assessed at diagnosis and throughout treatment, and they should receive appropriate counseling based on their nutritional status (WHO, 2013). Various direct and indirect assays such as measurement of enzymatic activity or nutrient metabolites are used to assess the quantity of nutrients in specimens such as blood, tissue, and urine (Burtis *et al*., 2012). The objective of this study was therefore to determine the serum concentration of selected trace elements in newly diagnosed TB cases attending some selected hospitals in Uyo, Akwa Ibom State, Nigeria.

MATERIALS AND METHOD

Study setting and Design:

This study was a prospective observational study involving persons who attended tuberculosis treatment centers from November 2021 to July 2022 for suspected cases of TB in selected tuberculosis treatment hospitals in Uyo, Akwa Ibom State, Nigeria. The selected hospitals included Saint Luke's Hospital, Anua and University of Uyo teaching hospital. Selection was done randomly.

Study population, Sample size and Participants selection:

During the study period, 155 participants who attended the selected TB treatment centers in Uyo took part in this study. The sample size was calculated based on prevalence of 10.3% from the study carried out in Enugu, Nigeria by Kennethe *et al*., 2021. Participants Inclusion Criteria included; Patients who have been diagnosed of pulmonary tuberculosis, patients who were co-infected with HIV and patients who did not commence TB treatment after diagnosis, while patients with another diagnosed pulmonary morbidity, who tested negative for TB infection and persons aged below 18 years were excluded from participating in the study. Participants were choosen out of convenience with simple random selection process. Out of 155 persons who registered for TB test, 13 persons were positive for TB by GeneXpert. Diagnosis of TB by GeneXpert was done in the hospitals selected for this study. 1 patient defaulted from the study, while a total of 12 patients were tested for some selected trace elements at diagnosis, at 2nd month, 4th month and 6th month of TB treatment. 10 participants formed the control group. The participants were randomly selected from apparently healthy individuals of age 18 and above.

Ethical Consideration

Ethical approval was obtained from Akwa-Ibom State Ethical Review Committee of the Ministry of Health, HREC No. AKHREC/10/2/22/052.

Written informed consent was obtained from participants. The consent letter highlighted the research topic, purpose of the research, brief description of the research procedures, possible risk and benefits to the participants. Participants were made to understand that their identity will be concealed in the course of the research, and that the data obtained from the research will be opened to their doctors and also will be published. There was no conflict of interest regarding the publication of this article. Participants who consented to take part in the research were made to sign the consent form, indicating that they understood and agreed to participate in the research.

Collection of blood sample

Five millilitres of venous blood were collected from 12 consented patients by venipuncture into plain blood container at the time of diagnosis, and 5 mls of blood samples were also collected during follow-up at the second, fourth and sixth month (completion) of treatment regimen. The blood samples were centrifuged at 3,000 rpm for 5 minutes at room temperature to separate the sera. Serum samples were stored at -20^{0}C until ready for trace element analysis. Five millilitres venous blood were collected from 10 participants of the control group into plain blood containers for the analysis of trace elements. The evaluation of the serum concentration of the trace elements was assessed once at any point in time.

Measurement of serum concentration of Trace Elements

Serum concentrations of copper, zinc, iron, selenium and chromium were measured using Varian AA240 Atomic Absorption Spectrophotometer in Micheal Okpara University of Agriculture Research Institute laboratory. Table 1 shows the reference ranges of each of the selected trace elements.

Table 1: Reference Ranges of the Selected Trace Elements

Trace Element	Reference Range
Copper	1.6 to 2.4 µmol/l
Zinc	0.66 to 1.10 µmol/l
Iron	10.09 to 16.82 µmol/l
Selenium	110 to 165 µg/l
Chromium	0.05 to 0.16 µg/l

Statistical Analysis of Data

The data obtained from this study were analysed using IBM SPSS Version 20. The output of the continuous variable was expressed in mean and standard deviation (Mean±SD). The mean difference between two groups was analysed by One-way ANOVA. The categorical variables results were expressed in frequency and percentages. Comparison of proportion of distribution was analysed by chi-square. P-values less than 0.05 was considered a statistically significant difference/association between group(s).

RESULTS

The demographic distribution of the study participants is shown in Table 2. Out of 155 participants included in the study, 83 (53.5%) were females while 72 (46.5%) were males. Forty-one (20.0%) were 20 years or below, while fifty-four (34.8%) participants were aged 31-30 years, forty-two (27.1%) participants were 31-40 years, nineteen (12.3%) participants were 41-50 years, five (3.2%) participants were 51-60 and 4 (2.6%) participants were 60 years or above. The mean age (±SD) was 25.83±17.21.

Table 2: Demographic Distribution of the Study Participants

Variable	Cate-gories	Freq-uency	Percen-tages	Mean± SD
Gender	Female	83	53.5	77.5±5.5
	Male	72	46.5	
	Total	155	100	
Age groups	≤20	31	20	25.83±17.21
	21-30	54	34.8	
	31-40	42	27.1	
	41-50	19	12.3	
	51-60	5	3.2	
	>60	4	2.6	
	Total	155	100	

The demography of control subjects in the study is indicated on Table 3. A total of 10 apparently healthy individuals were registered for control, 4 (40%) of the subjects were female, while 6 (60%) were male. 1 (10%) of the study population were 20 years or less (≤20), whereas 2 (20%) were aged 21-30, 2 (20%) were aged 31-40, 4 (40%) were aged 41-50, and 1 (10%) of the population were 50 years or more (≥50).

Table 3: Demographic Distribution of the control Participants

Variable	Cate-gories	Freque ncy	Percen-tages	Mean±SD
Gender	Female	4	40	5.0±1.0
	Male	6	60	
	Total	155	100	
Age groups	≤20	1	10	2.0±1.1
	21-30	2	20	
	31-40	4	40	
	41-50	2	20	
	>50	1	10	
	Total	10	100	

The mean comparison of all the trace elements at various stages of TB treatment are shown in Table 4.

Table 4: Mean Comparison of the Different Trace Elements at various stages of TB Treatment (n=12)

Element (µmol/l)	Mean±SD	F-value	p-value
Cu0	1.03±0.27^{a}	39.52	<0.001
Cu1	0.99±0.25^{a}		
Cu2	0.25±0.10^{b}		
Cu3	0.33±0.14^{b}		
Cu control	0.63±0.13^{c}		
Zn0	0.59±0.26^{a}	32.43	<0.001
Zn1	0.53±0.19^{a}		
Zn2	1.28±0.27^{b}		
Zn3	1.29±0.19^{b}		
Zn control	0.72±0.11^{a}		
Fe0	0.37±0.09^{a}	25.29	<0.001
Fe1	0.34±0.1^{a}		
Fe2	0.87±0.33^{b}		
Fe3	0.94±0.18^{b}		
Fe control	0.94±0.18^{b}		
Se0	20.42±6.61^{a}	11.27	<0.001
Se1	20.17±6.58^{a}		
Se2	33.42±7.81^{b}		
Se3	34.17±10.36^{b}		
Se control	38.60±6.43^{b}		
Cr0	8.0±5.69^{a}	4.14	0.02
Cr1	3.75±2.6^{a,b}		
Cr2	3.92±3.12^{b}		
Cr3	2.17±1.53^{b}		
Cr control	5.60±5.03^{a,b}		

a, b, c values with same superscript are not significantly different at pairwise comparison. Serum concentrations of the selected trace elements were analysed from twelve patients who consented for the study. The mean difference in the serum concentrations of all the trace elements between various stages of treatment was statistically significant ($p<0.05$). From the ANOVA post-hoc analysis, the serum concentrations of copper at month 4 and month 6 were significantly lower than the initial diagnosis and the control ($p<0.001$). The mean zinc in the initial diagnosis stage (Zn0) and 2nd month of treatment stage (Zn1) of the TB positive subjects was not significantly different from control, but significantly lower than 4th month of treatment stage (Zn2) and 6th month of treatment stage (Zn3). The mean iron in the initial diagnosis (Fe0) and 2nd month of treatment stage (Fe1) of the TB positive subjects was not significantly different but significantly lower than control, 4th month of treatment stage (Fe2) and 6th month of treatment stage (Fe3). The mean selenium in the initial diagnosis (Se0) and 2nd month of treatment stage (Se1) of the TB positive subjects was not significantly different but significantly lower than control, 2nd month of treatment stage (Se2) and 6th month of treatment stage (Se3). The level of chromium was higher in the control subject (5.60±5.03) but not significantly different from initial diagnosis (Cr0) and the other stages of the TB subject. The chromium level decreases with increased duration.

Table 5 shows the mean copper/zinc ratio, mean copper/selenium ratio, and the mean copper/iron ratio. The mean comparison shows that there was statistically significant difference ($p<0.05$) between TB positive subject and the control subject. The mean Cu/Zn ratio at diagnosis (2.15±1.15) was significantly higher than ratio at 4th month (0.2±0.08), 6th month (0.26±0.11), and control (0.89±0.22). The mean copper/selenium ratio (Cu/Se) of the 2th month treatment stage (Cu/Se2) and 6th month treatment stage (Cu/Se3) of the TB positive was significantly lower ($p<0.05$) than the initial stage (Cu/Se0) and stage 1 (Cu/Se1). The mean copper/iron ratio (Cu/Fe) of the initial diagnosis stage (Cu/Fe0) and 2nd month of treatment (Cu/Fe1) of the TB positive patients were significantly higher ($p<0.001$) than the control, 4th month treatment stage (Cu/Fe2) and 6th month treatment stage (Cu/Fe3).

DISCUSSION

Micronutrients play a crucial role in the pathophysiology of tuberculosis (Lonnroth *et al*., 2010). It deficiency suppresses immune functions by affecting the innate T-cell-mediated immune response and adaptive antibody response, and this leads to dysregulation of the balanced host response and also increases the susceptibility to infections, with increased morbidity and mortality (Wintergest *et al*., 2007). Undernutrition has been reported to be a risk factor for the progression of latent TB infection (LTBI) to active TB disease (ATBD), and its presence at the initial diagnosis of active TB has been reported to be a predictor of increased risk of death and TB relapse (Hood, 2013).

The present study shows that concentration of Zn at diagnosis (Zn0) was significantly low (0.59±0.26) compared to the concentration of the Zn at the end of TB treatment (1.29±0.19). As therapy progressed at 4th month (Zn2) and 6th month (Zn3), there was significant increase in Zn concentration compared to control group. Low Zn level reported in TB patients is in consonace with reports from Pourfallah *et al.* (2011) in Iran, Ghulam *et al*. (2009) in India and Festus *et al*. (2016) in Edo, Nigeria, Edem *et al*. (2015) in Ibadan, Nigeria and Bozan *et al*., 2017 in Turkey. The low serum Zn in TB patients was perhaps due to redeployment of Zn from blood circulation to other tissues, reduction in hepatic production of a2-macroglobulin (a Zn carrier protein) or a increase in the manufacture of metallothionein, which is a protein that helps in transportation of Zn to the liver (Cifti *et al*., 2003, Kassu *et al.*, 2006, Koyanagi *et al*., 2004).

In this study, the serum iron concentration in patients with tuberculosis was significantly lower at initial diagnosis (Fe0) (0.37±0.09) and at second month of therapy (Fe1) compared to control. From Table 4, serum Fe was seen to increase significantly in 4th month (Fe2) and 6th month (Fe3). These findings are consistent with the work of Pourfallah *et al*. (2011) in Iran. Lower serum concentration was also reported from tuberculosis patients by Harris *et al*. (2004) in Eastern part of Nigeria, and by Lawn *et al*. (2000) in Northern

Nigeria. Pathogenic mycobacteria compete with the host for iron, either by directly depleting intracellular iron from the host cytoplasm or by synthesising siderophores and macromolecules, including transferrin, ferritin or lactoferrin, which have high affinity to capture extracellular ferric ion (Fang *et al*., 2015, Boelaert *et al.*, 2007

The result from Table 4 Shows significant reduction in selenium concentration in patients with tuberculosis at diagnosis stage (Se0) (20.42±6.61µg/l). This decrease was found to cut across the second month (Se1) of therapy stage. Rohini *et al*., 2013, and Van *et al.*, 2014 reported decreased selenium in pulmonary tuberculosis patients. However, there was significant increase in selenium concentration at 4th month (Se2) and 6th month (Se3) of treatment. This is because in case of inflammation, selenoenzymes are translocated as a result of increased vascular permeability, and selenium passes into the tissues. Therefore, serum selenium levels may not represent the actual level of selenium in the body (Forceville *et al.,* 2007). These findings are consistent with the report by Moraes *et al*. (2014) in Brazil, Kassu *et al*. (2006) in Ethiopia, who reported lowered serum selenium in tuberculosis patients as compared to controls.

In this study also, it was found that serum concentration of chromium at diagnosis (Cr0) (8.0±5.69) of tuberculosis was significantly higher than the concentration at the end of TB treatment (2.17±1.53). At month two (Cr1) and four (Cr2) of TB therapy, there was no significant difference in concentration of chromium. However, at the final stage (6th month (Cr3)) of treatment there was significant decrease in the chromium level compared to the level at diagnosis, but no significant difference compared to control. A work by Akkas *et al*. (2020) on serum trace element and heavy metal levels in patients with sepsis reported an increase in the concentration of chromium in patients with sepsis. The baseline value of copper at the initial diagnosis of active TB shows significant increase in the serum concentration (1.03±0.27) compared to serum copper at the end of TB treatment (0.33±0.14). The findings on Cu level in this study can be compared with study done by Ciftci *et al*. (2003), Reza *et al*. (2007). They found a rise in serum Cu level in tuberculosis than in normal subjects.

Therefore, from this study, the mean copper concentration at the time of the initial diagnosis (Cu0, 1.03±0.27), and at 2nd month of treatment (Cu1, 0.99±0.25) was significantly higher than the mean copper concentration at 4th month (Cu2, 0.25±0.10), 6th month (Cu3, 0.33±0.14), and in the control subjects (0.63±0.13).

Copper is used as a bactericidal agent within macrophages, accumulating in phagolysosomes during infection (White *et al*., 2009, Achard *et al*., 2012, Wagner *et al*., 2005) and both systemic and localized copper concentrations in mammals are increased during infection Hyre *et al*., 2017, Wolschendorf *et al*., 2011). However, Edem *et al.* (2015) showed decreased serum copper level in pulmonary tuberculosis patients due to utilization by macrophages and mycobacterium. Another report showed that mycobacteria require copper for survival (Rowland and Niederweis *et al*., 2012).

The Cu/Zn ratio, Cu/Se ratio and Cu/Fe ratio was significantly higher at the level of TB diagnosis and at second month of therapy compared with that of control. At month 4 and 6 of therapy, there was significant in ratios of those elements.

High serum Cu/Zn ratio has been reported in patients with TB. These high values of the Cu/Zn ratio were due to the drop in the zinc concentrations; that means a high level of oxidative stress in these MDR-TB patients and negatively affect the immune system (Ananda, 2009). Determination of the ratio of copper/zinc is useful in diagnosing many diseases, controlling their transformation, and in reversing the nutritional status of zinc in the human body better than its content in the serum. Elevated Cu/Zn ratio was reported in patients with different pathological conditions in other studies (Lin, 2006).

Mohan *et al*., 2007 observed an initial significant increase in Cu/Zn ratio prior to treatment and significant decrease after anti-tuberculosis therapy thereby suggesting that Cu/Zn ratio can be used as an important laboratory marker for the diagnosis of tuberculosis. Previous work by Bahi *et al.* (2017) in Cote d᾽ Ivoire shows increase in Cu/Zn ratio before treatment, and reduction in Cu/Zn ratio during treatment. Also, Ciftci *et al.* (2003) found an initial increase in Cu/Zn ratio in patients prior to treatment and on follow up of same group after 2 months with treatment, there was a significant decrease in Cu/Zn ratio.

In the work on essential and toxic metals in serum of individuals with active pulmonary tuberculosis in an endemic region by Sepehri *et al*. (2017), Cu/Se ratio was reported to be significantly higher in TB patients compared with control group. Minon`czuk *et al*. (2021) also reported high concentration of Cu/Se ratios in patients with Acute Ischemic stroke (AIS). Cu/Fe ratio is reported to be involved in cellular respiration and electron transport. An elevated Cu/Fe ratio leads to increased free radical production, particularly lipid peroxidation that can lead to mitochondrial damage (David, 2010).

CONCLUSION

The analysis of levels of the elements Fe, Cu, Zn, Se, Cr and Cu/Zn, Cu/Fe, Cu/Se ratio may help in the understanding of the contribution of these elements to immune function during development of TB disease. The enhancement or reduction in the level of these elements may be linked with typical symptoms of TB (Pasha *et al*., 2010). Serum Cu/Zn, Cu/Fe, Cu/Se ratio is better indicator of oxidative stress and should be assessed regularly in tuberculosis cases and other related inflammatory diseases. There is need for periodical nutrition supplementation during TB treatment. Serum trace element analysis should be included as one of the diagnostic procedures for tuberculosis, since malnutrition and tuberculosis are both problems of considerable magnitude in most of the underdeveloped regions of the world. It is important to consider how these two problems tend to interact with each other. Awareness programs should be created in high-risk areas on need for good feeding habits and general nutrition.

Contribution of Authors:

Umoh, S. G. and Jimmy N. F.; Methodology, investigation, project administration, Umo, A. N.; Conceptualization, supervision, Agbulu, B. F., and Adie, S. A writing- original draft preparation, Formal analysis, manuscript writing -

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