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Original Article **Open Access**

Evaluation of serum concentration of essential trace elements during therapy among tuberculosis patients in Uyo, Nigeria

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Abstract:

Background: Nutritional status is one of the most important determinants of immune response to infection. The objective of this study was to assess the serum concentrations of selected trace elements in selected patients on anti-tuberculosis (TB) therapy in Uyo, Akwa Ibom State, Nigeria

Methodology: This was a prospective observational study of selected TB patients attending the TB treatment centers of selected hospitals in Uyo, Akwa Ibom State, Nigeria, for assessment of the serum concentrations of some essential trace elements during anti-TB therapy. First, participants with suspected pulmonary TB were consecutively selected and sputum samples were collected from each of them into wide mouth containers for GeneXpert TB analysis. Then, 5 millilitres of venous blood were collected from participants who tested positive for *Mycobacterium tuberculosis* (MTB) on GeneXpert test into plain specimen containers at the time of diagnosis, and at the 2^{nd}, 4^{th} and 6^{th} month of anti-TB therapy. Blood samples were also collected from randomly selected apparently healthy individuals as controls. The samples were centrifuged at 3,000 rpm for 5 minutes, and serum concentrations of copper (Cu), zinc (Zn), iron (Fe), selenium (Se) and chromium (Cr) were measured using flame atomic absorption spectrometry.

Results: A total of 155 participants with suspected TB were selected for the study, 83 (53.5%) were females while 72 (46.5%) were males. Majority of the participants were in age group 31-50 years. Thirteen (8.4%) participants were positive for MTB on GeneXpert analysis and placed on standard anti-TB therapy, while 1 participant defaulted. The mean serum concentrations of all the trace elements measured for the 12 positive participants at the different stages of anti-TB therapy was statistically significant ($p<0.05$). The mean serum concentrations of Zn, Fe and Se were significantly increased at the 4^{th} and 6^{th} month of therapy compared to the concentration at diagnosis and at 2^{nd} month of treatment. However, the mean serum concentrations of Cu and Cr significantly decreased at the 6^{th} month of treatment compared to their concentrations at initial diagnosis.

Conclusion: Assessment of the serum concentrations of Zn, Fe, Cu, Se and Cr could serve as indicator of nutritional status and oxidative stress, as well as serve as treatment indices to assess patients on anti-TB therapy.

Keywords: tuberculosis; trace elements; serum concentration; therapeutic response; Uyo; Nigeria

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Évaluation de la concentration sérique des oligo-éléments essentiels au cours du traitement chez les patients tuberculeux à Uyo, Nigeria

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Résumé:

Contexte: L'état nutritionnel est l'un des déterminants les plus importants de la réponse immunitaire à l'infection. L'objectif de cette étude était d'évaluer les concentrations sériques d'oligo-éléments sélectionnés chez des patients sélectionnés sous traitement antituberculeux (TB) à Uyo, dans l'État d'Akwa Ibom, au Nigeria.
Méthodologie: Il s'agissait d'une étude observationnelle prospective de patients tuberculeux sélectionnés fréquentant les centres de traitement de la tuberculose d'hôpitaux sélectionnés à Uyo, dans l'État d'Akwa Ibom, au Nigeria, pour l'évaluation des concentrations sériques de certains oligo-éléments essentiels pendant le traitement antituberculeux. Tout d'abord, les participants suspects de tuberculose pulmonaire ont été sélectionnés consécutivement et des échantillons d'expectorations ont été prélevés sur chacun d'eux dans des récipients à large ouverture pour l'analyse GeneXpert TB. Ensuite, 5 millilitres de sang veineux ont été prélevés sur des participants testés positifs pour *Mycobacterium tuberculosis* (MTB) sur le test GeneXpert dans des récipients d'échantillons simples au moment du diagnostic et aux 2e, 4e et 6e mois de traitement antituberculeux. Des échantillons de sang ont également été prélevés sur des individus apparemment en bonne santé choisis au hasard comme témoins. Les échantillons ont été centrifugés à 3 000 tr/min pendant 5 minutes et les concentrations sériques de cuivre (Cu), de zinc (Zn), de fer (Fe), de sélénium (Se) et de chrome (Cr) ont été mesurées par spectrométrie d'absorption atomique à flamme.
Résultats: Un total de 155 participants suspects de tuberculose ont été sélectionnés pour l'étude, 83 (53,5%) étaient des femmes et 72 (46,5%) étaient des hommes. La majorité des participants appartenaient à la tranche d'âge des 31 à 50 ans. Treize (8,4%) participants étaient positifs pour MTB lors de l'analyse GeneXpert et placés sous traitement antituberculeux standard, tandis qu'un participant a abandonné. Les concentrations sériques moyennes de tous les oligo-éléments mesurées pour les 12 participants positifs aux différentes étapes de la thérapie antituberculeuse étaient statistiquement significatives ($p<0,05$). Les concentrations sériques moyennes de Zn, Fe et Se ont été significativement augmentées au 4ème et 6ème mois de traitement par rapport à la concentration au moment du diagnostic et au 2ème mois de traitement. Cependant, les concentrations sériques moyennes de Cu et Cr ont significativement diminué au 6ème mois de traitement par rapport à leurs concentrations lors du diagnostic initial.
Conclusion: L'évaluation des concentrations sériques de Zn, Fe, Cu, Se et Cr pourrait servir d'indicateur de l'état nutritionnel et du stress oxydatif, ainsi que servir d'indices de traitement pour évaluer les patients sous traitement antituberculeux.

Mots clés: tuberculose; oligo-éléments; concentration sérique; réponse thérapeutique; Uyo; Nigeria

Introduction:

Tuberculosis (TB) is a chronic granulomatous infectious disease that mostly affects the lungs but can affect other parts of the body, caused by members of the *Mycobacterium tuberculosis* complex (1). It remains the top infectious killer in the world claiming close to 4,000 lives a day (2). Common symptoms of active TB are cough with sputum and blood, chest pains, weakness, weight loss, fever and night sweats. 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 latent TB to active TB disease. Tuberculosis may cause malnutrition through increased metabolic demands and decreased nutrient intake (3). Food and nutri tional 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 (4). The essential ones are known to play a variety of important roles, including acting as structural components of vitamins (e. g. cobalt), co-factors in metalloenzymes, glutathione peroxidase (e. g. selenium), catalytic components of numerous enzymes (e. g. zinc and copper), and as structural components of some proteins that are significant in immune reactions (5).

Deficiencies in various essential trace elements have been associated with decreased immunity against TB, moreover, trace elements are believed to have an impact on clinical outcome and are thus related to disease control (3). The World Health Organization (WHO) guideline 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 (6). 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 (5). The objective of this study is 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 of persons with clinical suspicion of pulmonary tuberculosis (TB) attending the TB treatment centers of selected hospitals in Uyo, Akwa Ibom State, Nigeria from November 2021 to July 2022. The selected hospitals were randomly selected and included Saint Luke’s Hospital Anua, and University of Uyo Teaching Hospital.

Study population, sample size & participants selection:

A total of 155 with clinical suspicion of pulmonary TB were consecutively selected as participants for the study. The sample size of 155 was calculated based on TB prevalence of 10.3% from a previous study carried out in Enugu, Nigeria by Kenneth et al., (7).

Patients with clinical diagnosis of pulmonary TB and co-infected with HIV, and patients who were yet to commence TB treatment after diagnosis were included in the study, while patients with another diagnosed pulmonary morbidity, who tested negative for TB and persons below 18 years of age were excluded.

Of the 155 suspected TB persons, 13 were positive for TB by GeneXpert MTB, one of whom defaulted, leaving only 12 confirmed TB patients for the trace elements determination at diagnosis, 2^{nd}, 4^{th} and 6^{th} month of TB treatment. Ten randomly selected apparently healthy individuals aged 18 years and above, formed the control group.

Ethical consideration:

Ethical approval was obtained from the Akwa-Ibom State Ethical Review Committee of the Ministry of Health. Written informed consent was obtained from patients, stating that they understood and agreed to participate in the research.

Collection of blood sample:

Five millilitres of venous blood were collected from the 12 selected participants by venipuncture into plain blood container at the time of diagnosis, and at follow up in the 2^{nd}, 4^{th} and 6^{th} month (completion) of TB 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. Approximately, 5 mls of blood were also collected from each of the 10 control participants once.

Measurement of serum concentration of trace elements:

Serum concentrations of copper, zinc, iron, selenium and chromium were measured using Varian AA240 Atomic Absorption Spectrophotometer at the Michael Okpara University of Agriculture Research Institute laboratory. Reference ranges of each of the selected trace elements are copper 1.6-2.4 μmol/L, zinc 0.66-1.10 μmol/L, iron 10.09-16.82 μmol/L, selenium 110-165 μg/L, and chromium 0.05 to 0.16 μg/L

Statistical analysis of data:

The data were analysed using IBM SPSS Version 20.0. Continuous variable was expressed as mean and standard deviation (mean ± SD). The mean difference between more than 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-value less than 0.05 was considered statistically significant difference/association between group(s).

Results:

The demographic distribution of the study participants is shown in Table 1. Of the total 155 participants included in the study, 83 (53.5%) were females, while 72 (46.5%) were males. Forty-one (20%) were 20 years or below, 54 (34.8%) were aged 31-30 years, 42 (27.1%) were 31-40 years, 19 (12.3%) were 41-50 years, 5 (3.2%) were 51-60 years and 4 (2.6%) were 60 years or above. The mean age of the participants is 25.83±17.21 years.

Table 1: Demographic distribution of the study participants

Characteristics	Frequency	Percentage
Gender		
Female	83	53.5
Male	72	46.5
Age group (years)		
≤ 20	31	20.0
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**
Mean age (years)	25.83±17.21	

The mean serum concentrations of trace elements for the 12 confirmed pulmonary TB participants at the various stages of TB treatment compared with the controls (n=10) are shown in Table 2. The difference in the mean serum concentrations of all the trace elements between the various stages of treatment was statistically significant ($p<0.05$) by ANOVA test.

However, the mean serum concentration of copper at the time of initial diagnosis (Cu0, 1.03±0.27) and at the 2^{nd} month of treatment (Cu1, 0.99±0.25) was not significantly different, but was significantly higher than the mean serum concentration at the 4^{th} (Cu2, 0.25±0.10) and 6^{th} month (Cu3, 0.33±0.14), and in the control subjects (0.63±0.13). From the ANOVA post-hoc analysis, the mean serum concentrations of copper at the 4^{th} and 6^{th} month were significantly lower than the mean serum concentration at initial diagnosis and in the controls ($p<0.001$).

Similarly for zinc, the mean serum concentration at diagnosis (Zn0, 0.59±0.26) and at the 2^{nd} month (Zn1, 0.53±0.19) was not significantly different from each other and from the controls (0.72±0.11), but was significantly lower than the mean serum concentrations at the 4th (Zn2, 1.28±0.27) and 6th month (Zn3, 1.29±0.19) of the treatment ($p<0.001$). For iron, the mean serum concentration at diagnosis (Fe0, 0.37±0.29) and at the 2nd month (Fe1, 0.34±0.1) was not significantly different, but was significantly lower than the controls (0.94±0.18), and at the 4th (Fe2, 0.87±0.33) and 6th month (Fe3, 0.94± 0.18) of the treatment regimen ($p<0.001$).

The mean serum concentration of selenium at the initial diagnosis (Se0, 20.42± 6.61) and at the 2nd month (Se1, 20.17± 6.58) was not significantly different, but significantly lower than the controls (38.60± 6.43), and at 4th (Se2, 33.42±7.81) and 6th month (Se3, 34.17±10.36) of the treatment regimen ($p<0.001$). The mean serum concen trations of chromium were high in the control subjects (5.60±5.03) but not significantly different from the mean serum concentrations of the TB patients at initial diagnosis (Cr0, 8.0±5.69) and at 2nd (Cr1, 3.75±2.6) and 4th month (Cr2, 3.92±3.12), however, it was significantly higher than at the 6th month (Cr3, 2.17±1.53) of the treatment regimen ($p=0.02$).

Discussion:

Micronutrients play a crucial role in the pathophysiology of tuberculosis (8). Its 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 (9). 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 (10).

In this study, the TB prevalence was 8.4%, which is lower compared to the rate reported in Uyo by Ibokette et al., (11) with TB prevalence of 12.4%. It is also lower than 13.7% reported by Itah and Udofia (12) in another study in Uyo on epidemiology and endemicity of pulmonary TB in South-Eastern Nigeria. A study by Ulasi et al., (13) in Enugu, Nigeria reported prevalence of 6.8%, which is lower than the rate in our current study. In Lagos a higher prevalence of 23.4% was reported by Adejumo et al., (14).

Trace elements levels have been reported to be associated with diseases (15). The present study shows that mean serum concentration of Zn at diagnosis (Zn0) was significantly low, but with progression of therapy at the 4th (Zn2) and 6th month (Zn3), there was significant increase in Zn concentration compared to control group. The same findings were reported in a study by Pourfallah et al., (16) in Iran and Festus et al., (17) in Edo, Nigeria. Decreased level of Zn could be due to pre-existing malnutrition and increased usage of Zn by the tuberculosis bacteria itself. Thus, these parameters directly reflect the pathophysiological state of the disease process. Zinc insufficiencies among MDR-TB patients have a negative impact on the immune system. In effect, zinc deficiency is responsible for an alteration in the macrophage function and reduction in production of tumor necrosis factor (TNF-ɑ) and interferon-γ (INF-γ). These low zinc concentrations could also be responsible for decrease in proliferation and differentiation of T and B lymphocytes (18), which are at the forefront of immune system defenses and protection against active TB (9). In addition, it was reported that zinc and vitamin A supplementation in adult patients with active TB could allow elimination of *Mycobacterium,* thus leading to quick cure of these patients (19).

This study also shows that mean serum iron concentration in patients with TB was significantly lower at initial diagnosis (Fe0) and at second month of therapy (Fe1) compared to control, but serum Fe increased significantly in the 4th and 6th month of therapy. These findings are consistent with the work of Pourfallah et al., in Iran (16). Lower serum concentration was also reported among TB patients by Lawn et al., (20) in Northern Nigeria (20). Iron is a micronutrient that is important for both the host and MTB metabolism. Pathogenic mycobacteria compete with the host for iron, either by directly depleting intracellular iron from the host cytoplasm or by synthesizing siderophores and micromolecules, including transferrin, ferritin or lactoferrin, which have high affinity to capture extracellular ferric ion (21). Anaemia, as a result of inflammation caused by MTB infection, is predominantly caused by an iron delivery problem, where erythrocyte iron is poorly used and dietary iron is enriched in intestinal enterocytes (22).

Table 2: Comparison of the mean serum concentrations of different trace elements at various stages of TB treatment for the twelve participants with confirmed pulmonary TB and the controls

Trace element	Time of measurement	Serum concentration (μmol/L) (Mean ± SD)	F value (ANOVA)	*p* value
Cu 0	At diagnosis	1.03±0.27[a]	39.52	<0.001
Cu 1	2 months	0.99±0.25[a]		
Cu 2	4 months	0.25±0.10[b]		
Cu 3	6 months	0.33±0.14[b]		
Cu control (n=10)		0.63±0.13[c]		
Zn 0	At diagnosis	0.59±0.26[a]	32.43	<0.001
Zn 1	2 months	0.53±0.19[a]		
Zn 2	4 months	1.28±0.27[b]		
Zn 3	6 months	1.29±0.19[b]		
Zn control (n=10)		0.72±0.11[a]		
Fe 0	At diagnosis	0.37±0.09[a]	25.29	<0.001
Fe 1	2 months	0.34±0.1[a]		
Fe 2	4 months	0.87±0.33[b]		
Fe 3	6 months	0.94±0.18[b]		
Fe control (n=10)		0.94±0.18[b]		
Se 0	At diagnosis	20.42±6.61[a]	11.27	<0.001
Se 1	2 months	20.17±6.58[a]		
Se 2	4 months	33.42±7.81[b]		
Se 3	6 months	34.17±10.36[b]		
Se control (n=10)		38.60±6.43[b]		
Cr 0	At diagnosis	8.0±5.69[a]	4.14	0.02
Cr 1	2 months	3.75±2.6[a,b]		
Cr 2	4 months	3.92±3.12[b]		
Cr 3	6 months	2.17±1.53[b]		
Cr control (n=10)		5.60±5.03[a,b]		

a, b, c values with same superscript are not significantly different at pairwise comparison; SD = Standard deviation ANOVA = Analysis of Variance

Our study also shows significant decrease in mean serum selenium concentration in patients with TB at diagnosis (Se0) compared to control participants. This decrease was found to cut across the second month (Se1) of therapy. However, there was significant increase in selenium concentration at the 4th (Se2) and 6th month (Se3) of therapy. This is because in 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. These findings are consistent with the report by Moraes et al., (23) in Brazil and Kassu et al., (24) in Ethiopia. Selenium deficiency induces a systematic redox imbalance and inflammation in the blood and causes pathological changes in the liver (25). Reduced selenium status in turn can pathologically increase inflammation cascade, thus contributing to disease manifestation, forming a vicious circle (26).

In this study, we found that mean serum concentration of chromium at TB diagnosis (Cr0) was high but not significantly different from the control. At month 2 (Cr1) and month 4 (Cr2) of TB therapy, there was also no significant difference in concentration of chromium. However, at the final stage (month 6, Cr3) of therapy, there was significant decrease in the chromium level compared to the level at diagnosis, but no significant difference compared to the control. No reported work on serum concentration of chromium in TB patients was found in the literature to support these findings. However, Festus et al., (17) reported a decrease in chromium concentration in TB patients (17). Nevertheless, a study by Akkas et al., (27) on serum trace elements and heavy metal levels in patients with sepsis reported an increase in the concentration of chromium in patients with sepsis.

Also reported in this study is the fact that the serum concentration of copper was significantly higher than that of the control group at diagnosis (Cu0) and at the second month of therapy (Cu1). At month 4 (Cu2) and month 6 (Cu3) of TB treatment, there was significant decrease in serum copper concentration compared to that at diagnosis and the control subjects. The findings on Cu level in this study can be compared with study done by Reza et al., (28), which reported a rise in serum Cu level in TB patients than in normal subjects. Copper is used as a bactericidal agent within macrophages, accumulating in phagolysosomes during infection (29), and both systemic and localized copper concentrations in mammals are increased during infection (30). However, Edem et al., (31) showed decreased serum copper level in PTB patients due to utilization by both macrophages and *Mycobacterium* (31). Another study reported that mycobacteria require copper for survival (32).

Conclusion:

The analysis of levels of the elements Fe, Cu, Zn, Se and Cr may help in the understanding the contributions 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. There is need for periodical nutrition supplementation during TB treatment.

Serum trace elements analysis should be included as one of the diagnostic procedures for TB since malnutrition and TB 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:

UAN was involved in conceptualization of the study, supervision, and writing of the manuscript draft; JNF was involved in methodology, investigation, and project administration; ASA was involved in formal analysis, reviewing and editing of the manuscript. All authors read and approved the manuscript submitted for publication.

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Conflict of interest:

No conflict of interest is declared.

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