Journal of Advances in Medicine and Medical Research

29(4): 1-13, 2019; Article no.JAMMR.48238
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Zinc Supplementation as Adjunctive Therapy in Adults with Tuberculosis in Calabar, Nigeria: A Randomized Controlled Trial

Regina I. Ejemot-Nwadiaro[1*], Edisua H. Itam[2] and Emmanuel N. Ezedinachi[3]

[1]Department of Public Health, Faculty of Allied Medical Sciences, University of Calabar, Nigeria.
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Nigeria.
[3]Calabar Institute of Tropical Diseases Research and Prevention, University of Calabar Teaching Hospital, Calabar, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors RIE, EHI and ENE made significant input to the design of this study. Author RIE performed the statistical analysis, wrote the protocol and the first draft of the manuscript. All authors managed the analyses and contributed to the interpretation of results of the study. Author RIE managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2019/v29i430079
Editor(s):
(1) Dr. Fatma Mohammad Nasr, Theodor Bilharz Research Institute, Warraq El-hadar, Imbaba, Giza, Egypt.
Reviewers:
(1) Ian James Martins, Edith Cowan University, Australia.
(2) Prof. Nitesh mohan, Bareilly International University, India.
Complete Peer review History: http://www.sdiarticle3.com/review-history/48238

Original Research Article

Received 10 January 2019
Accepted 25 March 2019
Published 01 April 2019

ABSTRACT

Introduction: Tuberculosis (TB) still remains a leading killer from a single infectious agent worldwide, especially in Asia and Africa. Achieving the Sustainable Development Goal (SDG) 3 will be contingent on addressing ways of reducing the impact of TB to the health, socio-economic and health system of populations most at risk. Micronutrients supplementation is increasingly being recognized as having great potentials to that effect.
Objective: This study thus assessed the potential benefits or otherwise of zinc supplementation on tuberculosis treatment outcomes in Calabar, Nigeria.
Methods: Eligible patients (81) out of the 182 assessed were randomized to receive anti-TB drug regimen plus oral administration of individual zinc, 25 mg daily for 60 days (intervention group),

**Corresponding author: E-mail: reginaejemot@yahoo.com, reginaejemot@gmail.com;*

while the control group received anti-tuberculosis drug regimen only for 60 days. Both qualitative and quantitative data were collected. Clinical examination, Karnofsky performance scale index, direct sputum examination, anthropometric measurements and blood collection for haematological and zinc assessment were carried out before and 2 months after anti-TB treatment began. Difference in treatment effects between and within groups for continuous variables was tested using *t*-test. Mantel-Haenszel summary estimate of the relative risk (RR) was used to test the clinical effectiveness of the intervention. The Pearson test was applied to determine the correlation between variables.

Main Results: The mean serum zinc levels at 2 months of TB treatment were significantly higher in the intervention group (14.4 ± 0.37µmol/L) in comparison with the control (12.9 ± 0.37 µmol/L); (p = 0.004). A significant difference (p = 0.010) in the serum concentrations of zinc was observed between the two groups when adjustments were made for TB-HIV co-infection. Risk reduction of about 41% for acid fast bacilli (AFB) positivity (RR: 0.59; 95% CI 0.23 to 1.46) was observed after 2 months of anti-TB treatment in favour of the intervention group. Similarly, intervention group had significantly (p = 0.005) lower proportion of patients with serum zinc levels < 10.7 µmol/L (intervention: 5; Control 10) and (p = 0.030) BMI < 18.5 kg /m^2 below the lower ranges ((intervention: 9; Control 16). There was a significant improvement in the haematological parameters as evidenced by significant higher proportion of patients in the intervention group than the control group with values above the lower ranges for these parameters with risk reductions in favour of the intervention group for lower ranges as 34%, 12%, 73% and 58% respectively for haemoglobin, albumin, serum total protein and globulins.

Conclusion: Irrespective of HIV status in individuals with tuberculosis, zinc micronutrient supplementation significantly increases clinical outcomes, haematological parameters, improves nutritional status as proxied by anthropometric indices and leads to faster sputum smear conversion. The study adds to the growing body of evidence in support of the beneficial role of zinc in TB control.

Keywords: Tuberculosis; micronutrients; zinc supplement; anthropometric indices; Nigeria.

1. INTRODUCTION

Tuberculosis (TB) is a contagious bacterial disease caused by *Mycobacterium tuberculosis*, which most commonly affects the lungs. It is the leading killer from a single infectious agent worldwide, especially in Asia and Africa. The WHO [1] reported that TB remains a major public health problem in the world. An estimated 10 million people were infected with TB in 2017, including 1.1 million people who have co-morbidity with human immunodeficiency virus (HIV). Each year, about 1.3 million people die of TB. More than 95% of the cases and deaths are in developing countries [2,3]. It has been suggested that achieving the Sustainable Development Goal (SDG) 3 will be contingent on addressing ways of reducing the impact of TB on the health, socio-economic and health system of populations most at risk.

Tuberculosis has long been associated with malnutrition [4,5]. Malnutrition and tuberculosis are both problems of very high gravity in most of the low-/middle-income countries [1,6]. Malnutrition may predispose people to the development of clinical disease and tuberculosis can contribute to malnutrition [7,8,9]. Research evidence shows that deficiencies of some micronutrients notably vitamin A, C, D, E; zinc, iron and selenium can cause significant impairment of immunity especially cellular immunity [6,7]. This becomes all the more complicit since TB is recognized to involve predominantly cellular immunity; particularly cell types such as cytokines – interferons, interleukins-1 (IL-1), tumor necrosis factor (TNF) – that are critical to fighting infections [8,10].

Because of diverse metabolic characteristics and functions, micronutrients have presently been accepted as essential for optimum human health. Micronutrients deficiency is considered to be the most frequent cause of secondary immunodeficiency and infection related to morbidity including tuberculosis [3]. Malnutrition has a negative impact on the immune system [11,12]. Micronutrients like Zinc play an essential role in immune function [4,7]. Zinc deficiency affects the host defences in a variety of ways. It results in decreased phagocytosis and leads to a reduced number of circulating T-cells and reduced tuberculin reactivity, at least in animals. *In vitro* cellular killing by macrophages was found

to be reduced during zinc deficiency and rapidly restored after zinc supplementation [6].

Study conducted with patients being treated for tuberculosis, which followed dietary prescription exclusively or received supplemental food donation, showed early BMI improvement [3,7]. Additional parameters with evidence of improvement with this intervention were: total caloric intake, serum protein, sanitary requirements for food preparation and clinical response. This intervention also determined earlier improvement on acid-fast bacilli detection on sputum smears and reduced bronchial secretion [5].

Several studies have reported that zinc status influences the incidence and severity of infections. Bhutta et al. [13] found a 41% and 25% risk reduction in childhood pneumonia and diarrhea respectively in a pooled analysis of randomized-controlled trials done in the developing countries involving supplementation with zinc. Similarly, Bhandari et al. [14] in their study in a slum in New Delhi, India reported that zinc supplementation substantially reduced the incidence of pneumonia in children who had received vitamin A by about 26% (Odds ratio 0.74, 95% confidence interval 0.56 to 0.99). There is evidence that malarial morbidity is reduced by zinc supplementation [12,15].

Gupta et al. [6] studies on patients with TB had shown significantly lower plasma zinc level than those without TB, irrespective of their nutritional status. These findings are comparable with those of previous studies conducted in Ethiopia, Italy, and Indonesia [2]. There was a significant rise in zinc level at the end of six- months of anti-tuberculosis therapy (ATT). Thus, it may be suggested that plasma zinc status is likely a marker for monitoring the severity of disease and response to therapy. Zinc deficiency in tuberculosis is likely due to redistribution of zinc from plasma to other tissues or reduction of the hepatic production of the zinc carrier protein a2-macroglobulin and to a rise in the production of metallothionein, a protein that transports zinc to the liver [6].

Reduction in plasma zinc concentration was shown in tuberculosis patients after two months of treatment. This phenomenon may be because during the intensive phase of ATT, the anti-TB drugs were used to kill the population of replicating bacilli and zinc may play an important role in the macrophage contribution to host defenses at the site of infection. The other possible mechanisms could be the effect of anti-TB drugs on zinc absorption [6,16]. Zinc supplementation of patients with pulmonary tuberculosis and bacterial pneumonia was shown to increase immune function. In a study, it was found that PPD indurations were larger in children receiving zinc and zinc increases the PPD induration size in children irrespective of nutritional status [6].

This study thus aimed to describe the convalescence of patients with tuberculosis who received Zinc supplementation in comparison to those that did not during the course of tuberculosis treatment in Calabar, Cross River State, Nigeria and to evaluate the effects of Zinc supplementation in enhancing convalesce from tuberculosis.

2. METHODOLOGY

2.1 Study Setting

The study was undertaken at the Dr Lawrence Henshaw Memorial Hospital (DLHM) formerly Infectious Disease Hospital (IDH), located in Calabar South Local Government Area (LGA) one of the 18 LGAs in Cross River State, Nigeria. Most of the state lies within the tropical rain forest belt of Nigeria. It lies between latitude 4^0 28´ and 6^0 55´ north of the equator and longitude 7^0 50´ and 9^0 28´ east of the Greenwich meridian. The health facility is primarily delineated to the treatment and control of infectious diseases.

2.2 Population/Study Design

This study was a randomized-controlled supplementation trial. Patients attending the health facility with clinically diagnosed cases of pulmonary tuberculosis (PTB) were the targeted population. Patients that were eligible to participate in the study were those fulfilling the inclusion criteria.

2.2.1 Inclusion criteria

- Newly confirmed case of PTB. This was ascertained by acid-fast bacilli (AFB) sputum and clinical examination conducted by a microbiologist and clinician respectively.
- Patients aged 18 years and above
- Subject who grants informed consent to participate

- Intention to stay in the study area for the duration of active follow-up (2 months from the start of treatment).

2.2.2 Exclusion criteria

- Individuals who had previously been on anti-TB treatment for more than four weeks in the past five years.
- Pregnant women who would have received iron and folate supplements as part of routine prenatal services.
- Use of any drug that could suppress immunity.
- Intake of supplements containing micronutrients of interest during the previous month.
- Subject is mentally incapable of understanding the information about the study.
- Subject is in prison or in police custody.

2.3 Sample Size

Sample size was calculated using the formula for two independent groups (Difference in means) [17]. Employing this formula (for continuous data) requires the specification of the estimated mean response in the control group, (0.31µmol/L) and standard deviation (0.317) from Karyadi et al. [18]; using the level of significance (5%) at 90% power (1 – probability of a type II error). Therefore 22 patients for each arm were included in the study. However to account for 30% attrition rate and 30% of failure to meet the inclusion criteria, we had 36 patients in each group. Thus a minimum of ≥72 patients were included.

An independent statistician employed block randomization to generate allocation sequence for both the intervention group and the control group. Allocation concealment was achieved through the use of serially numbered dark bottles. One group received anti-TB drug regimen plus oral administration of individual treatment drugs of 25mg (as zinc gluconate) daily for 60 days while the other group received anti-tuberculosis drug regimen only. Each eligible patient was asked to take the micronutrient supplement as prescribed daily from the start of anti-TB treatment till 2 months post-treatment. Patient's compliance was assessed by comparing the number of remaining supplements with the number recorded in the logbook as they came to collect anti-TB drugs. This is in line with directly observed treatment short-course (DOT) strategy recommended by World Health Organization [19]. Field assistants (nurses) assisted in this monitoring and evaluation of the treatment.

2.4 Methods of Data Collection

Both qualitative and quantitative data were collected. Clinical examination, direct sputum examination, anthropometric measurement and blood collection was carried out before and 2 months after anti-TB treatment began.

2.5 Qualitative Data

A clinician with either basic medical training or specialist training as a pulmonologist attending to the patients at the study site carried out a detailed clinical examination on patients including history of exposure to TB (contact investigation) and assessment of the presence of a bacilli Calmette-Guérin (BCG) scar. A well-designed semi-structured questionnaire was employed to collect part of the qualitative data. It consisted of Section A which elicited information on socio-demographic factors that impact on TB treatment and control and Section B dwelt on obtaining diet history which got information on general food patterns and dietary habits over a long period of time. The Karnofsky Performance Scale Index was also used. It allows for patients to be classified as to their functional impairment. This can be used to compare effectiveness of different therapies and to assess the prognosis in individual patients. The lower the Karnofsky score, the worse the survival for most serious illnesses. The score ranges from 0 (dead) to 100 (normal) [20,21] and recorded in the case record form (CRF).

2.6 Quantitative Data

2.6.1 Sputum examination

Three specimen of early morning sputum was taken from each patient weekly and examined by direct microscopy (by a microbiologist; using the Ziehl-Nelson staining method) to assess sputum conversion from positive to negative. Sputum smears grading was based on the number of acid fast bacilli (AFB) visible in oil immersion by microscopy. Grade +1, ≥ 3 AFB found in 15 minutes; grade +2, 1 – 20 AFB in 10 fields; grade +3, 20 – 60 AFB in 10 fields; grade +4, 60 – 120 AFB in 10 fields; and grade +5, >120 AFB in 10 fields [22].

2.6.2 Anthropometric measurements

The parameters assessed were; age (age at last birthday in years), height (cm), weight (kg) and body composition as judged by the principal soft tissues; subcutaneous fat skinfold at the bicep, tricep, subscapular and suprailliac sites. The skinfold thicknesses were measured using ADIOPOMETER™ Skinfold Calipers. All measurement followed the international standards for anthropometric assessment as published by International Society for Advancement of Kinanthropometry [23]. The proportion of total body fat (TBF) and fat-free-mass or lean body mass (LBM) were extrapolated from the skinfold measurement and calculated using Durnin and Womersley (1974) equation. Body mass index (BMI) widely used as the best simple and quantitative anthropometric indicator of body composition and thus nutrition state was used. The standard reference for people within the normal range is 18.5 – 25 $Kg/m^{2.}$ Any value below 18.5 Kg/m^2 would be regarded as underweight while values above 25 Kg/m^2 would be regarded as varying degrees of overweight and obesity. Mid-upper arm circumference (MUAC) was measured with a plastic inelastic measurement tape. The measurement was recorded in centimetres (cm).

2.7 Blood Collection and Analyses

About 5ml fasting whole blood was withdrawn by venipuncture into vacutainers and centrifuged at 3,000 rpm for 10 minutes. It was stored at -20ºC and subsequently processed and prepared for analysis and estimation of zinc, measurement of blood haemoglobin, serum albumin and total protein, using appropriate methods (Enzyme-linked immunosorbent assay (ELISA), Kits designed for estimation of these parameters and Atomic Absorption Spectrophotometric technique for assay of the trace element) [24]. All chemicals used were of analytical grade.

2.8 Statistical Analysis and Reporting

Data were manually checked and cleaned on hard copy (register, case record form (CRF), Log books, etc) for entry completeness and to ensure that information collected made sense in terms of scientific correctness. Data entry and analysis were done using EPI-Info. Result was expressed as mean values ± standard deviation (SD) or mean values ± standard error of mean (SEM) for unskewed data set, while median/mode and inter quartile range (IQR) were used to present skewed data set. Cross tabulations were done for categorical data and comparisons made using chi-square and Fisher's exact tests. Independent *t*-test was used to compare normally distributed variables between the groups. Difference in treatment effects within groups for continuous variables was tested using paired *t*-test. Mantel-Haenszel summary estimate of the relative risk (RR) was used to test the clinical effectiveness of the intervention. The Pearson test was applied to determine the correlation between variables. Statistical significance was based on *P*-value < 0.05. Data were presented with 95% confidence interval. Analysis was based on intention-to-treat (ITT). Reporting was in line with consolidated standards of reporting trials (CONSORT).

2.9 Ethical Considerations

Research Ethics Committee, Centre for Clinical Governance, Research and Training, Cross River State Ministry of Health granted ethical approval for conduct of this study. Informed written consent for participation in the study was sought from eligible patients after their full understanding of the information sheet detailing the nature, objective, methods, anticipated benefits and potential hazards of the intervention. Anonymity of participants and confidentiality of data were ensured in accordance with the principles laid down by the World Health Assembly, Helsinki Declaration of 1975 on ethics on human experimentation. Good clinical practice (GCP) and standard operating procedures (SOP) were employed.

3. RESULTS

3.1 Study Profile

Of the 182 patients with clinically diagnosed cases of pulmonary tuberculosis (TB) attending Dr Lawrence Henshaw Memorial Hospital, Calabar during the study period assessed for eligibility, only 81 (44.5%) met the inclusion criteria and were enrolled in the study (Fig. 1).

Forty-one (50.6%) patients of the 81 that were enrolled in the study were randomly assigned to the Zinc supplementation group and they received the allocated intervention (micronutrient supplement: zinc plus conventional anti-TB drugs), and 40 (49.4%) were assigned to control

group; (they received only conventional anti-TB drugs). The participants in the two groups were basically similar in the key parameters assessed at baseline as shown in Table 1.

Figure 1
TRIAL PROFILE

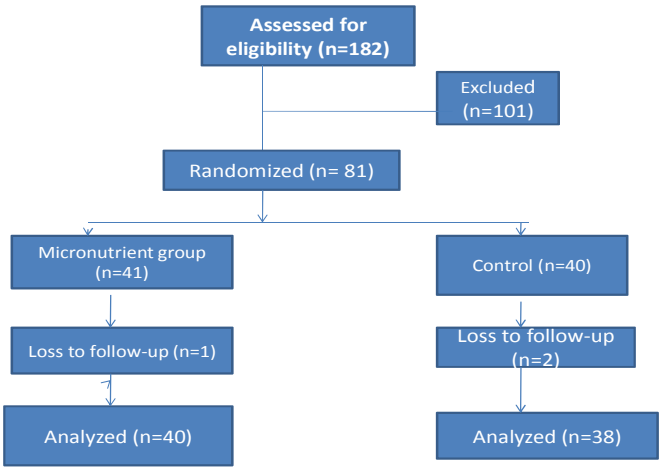

Table 1. Baseline parameters of study participants

Parameters	Intervention group (n= 41)	Control group (n=40)	Test Statistic	P-value
Presence of BCG Scar (n)				
Male	11 (26.8)	09 (22.5)	OR: 1.10	0.492
Female	08 (19.5)	09 (22.5)		
Sputum smear grade (n)				
+ 1	09 (22.0)	08 (20.0)		0.431
+ 2	15 (36.6)	13 (32.5)		0.524
+ 3	10 (24.4)	10 (25.0)	r = 0.10^	0.839
Not seen	07 (17.1)	09 (22.5)		0.633
Body weight (kg) Mean ± SEM	55.8 ± 1.96	52.3 ± 1.78	1.31ǂ	0.190
Height (cm) mean ± SD	165.0 ± 7.8	161.9 ± 9.1		
range	148.0 – 177.0	138.0 – 180.0	1.671ǂ	0.099
BMI < 18.5 kg /m^2 (n)	14 (34.2)	13 (32.5)	RR: 1.05	0.304
HIV Positive (n)				
Male	02 (04.9)	07(17.5)	OR:0.508	0.430
Female	06 (14.6)	06 (15.0)		
Karnofsky score (<50%)				
Male	03 (07.3)	03 (07.5)	RR: 0.49*	0.142
Female	02 (04.9)	07 (17.5)		
Serum albumin < 3.2 g/dl (n)	10 (24.4)	07 (17.5)	RR: 1.39	0.432
Serum total protein < 6.0 g/dl (n)	06 (14.6)	11(27.5)	RR: 0.53	0.081
Globulin < 2.3 g/dl (n)	05 (12.2)	08 (20.0)	RR: 0.61	0.101
Haemoglobin (g/dl)				
Male (<13) (n)	15 (36.6)	17 (42.5)	RR: 0.86*	0.963
Female (<12) (n)	14 (34.2)	16 (40.0)		

Figures in parenthesis represent percentages.
BMI: Body Mass Index; HIV: Human immuno-deficiency virus
IQR: Inter quartile range; OR: Odds ratio; RR: Relative risk
** = Pooled figure for male and females; ǂ = t-independent test; r = coefficient of regression*

3.1.1 Comparisons of baseline and endline parameters

The mean serum zinc levels at 2 months of TB treatment were significantly higher in the intervention group in comparison with the control (p = 0.004). However there was a marginal increase in both groups from the baseline values following TB chemotherapy. The mean values of anthropometric indices are presented in Table 2. It shows almost a consistent increase in the parameters studied from their baseline values in favour of the intervention group. There was a marginal increase of 0.4 kg/m2 in mean value of body mass index (BMI) for the intervention group; whereas the reverse was the case for the

Table 2. Comparison of baseline and endline parameters of patients two months after start of TB treatment

Parameters/Time of assessment	Intervention group	Control group	Test Statistic	p-value
Serum Zinc (µmol/L)				
0 month	13.8 ± 0.48‡	12.6 ± 0.43	1.8688*	0.060
2 months	14.4 ± 0.37	12.9 ± 0.37	2.9489*	0.004
Karnosfsky score#				
0 month	59.0 ± 2.21	55.5 ± 2.10	1.16	0.25
2 months	65.0 ± 1.94	59.0 ± 2.27	2.03	0.04
Sputum positive (n)				
0 month	34 (82.9)	31 (77.5)	0.38§	0.55
2 months	05 (12.5)	08 (21.1)	1.03	0.14
Body Weight (kg)				
0 month	55.8 ± 1.96‡	52.3 ± 1.78	1.31*	0.19
2 months	57.2 ± 1.85	52.1 ± 1.78	2.01*	0.04
MUAC (cm)				
0 month	24.3 ± 0.72	23.7 ± 0.54	0.70	0.487
2 months	24.9 ± 0.69	23.2 ± 0.60	1.86	0.066
Total body fat^ (kg)				
0 month	13.0 ± 1.13‡	10.7 ± 0.78	1.64*	0.105
2 months	13.5 ± 1.03	10.6 ± 0.78	2.23*	0.028
Lean body mass^ (kg)				
0 month	42.8 ± 1.20‡	41.6 ± 1.29	0.70	0.485
2 months	43.7 ± 1.21	59.0 ± 1.27	1.28	0.203
BMI (kg/m^2)				
0 month	20.5 ± 0.72	19.8 ± 0.53	0.75*	0.455
2 months	20.9 ± 0.65	19.7 ± 0.55	1.45*	0.150
Haemoglobin (g/dl)				
0 month	10.9 ± 0.21	11.0 ± 0.02	0.0455	0.96
2 months	11.3 ± 0.16	10.9 ± 0.15	1.5616	0.12
Serum Albumin (g/dl)				
0 month	03.6 ± 0.10	03.7 ± 0.09	0.1413	0.89
2 months	03.8 ± 0.08	03.6 ± 0.08	0.3781	0.91
Serum Total Protein (g/dl)				
0 month	07.5 ± 0.13	06.9 ± 0.16	2.7594	0.007
2 months	07.6 ± 0.15	07.0 ± 0.16	2.7703	0.007
Globulin (g/dl)				
0 month	03.8 ± 0.12	03.2 ± 0.17	2.8452	0.006
2 months	03.9 ± 0.13	03.3 ± 0.17	2.6776	0.009
Albumin / Globulin Ratio				
0 Month	01.0 ± 0.05	01.3 ± 0.12	2.6354	0.010
2 Months	0.90 ± 0.05	01.2 ± 0.10	2.1160	0.038

‡ Mean ± SEM; #Range: 0 (dead) to 100 (normal); §: Chi-square test
** Independent t-test; ^ extrapolated; MUAC = Mid-upper arm circumference*
Statistically significant based on P-value < 0.05

control group as the mean body mass index value dropped by 0.10kg/m2 from its baseline figures. These differences were however not statistically significant (p = 0.150). There was a borderline statistical significance (p = 0.06) for the mean value of mid-upper arm circumference between the two groups at two months of anti-TB treatment, with a mean difference of 1.7 cm.

There were significant differences from baseline values within the same group (paired t-test; p< 0.05) for variables assessed. Two months after commencement of TB treatment, the intervention group had improved clinical outcomes. The intervention group similarly had a mean weight gain of 1.4 kg from mean weight of 55.8 kg at baseline to 57.2 kg 2 months post-treatment as against 0.2 kg weight loss for the control group, from 52.3 kg to 52.1 kg (Table 2). This difference was found to be statistically significant (T-test = 2.01; p = 0.04). However, there was no statistically significant difference in mean weight at baseline for both groups (T-test = 1.31; p = 0.19). Significant statistical difference (T-test = 2.03; p = 0.04) was observed for karnofsky performance score at 2 months of anti-TB treatment reflecting a mean difference of 6.0, whereas no such difference was observed at baseline (p > 0.05).

The number of patients in the intervention group that had varying categories of positive sputum smear for AFB reduced from 34 at baseline to 5 two months post-intervention, as against 31 to 8 for the control group (Table 2), indicating a percentage difference of 11%. This difference was however not statistically significant (Chi-square = 1.03; p = 0.14).

3.1.2 Correlations among variables

The relationships between some selected variables are shown in Table 3. Low serum zinc (< 10.7 µmol/L) was independently associated with low BMI. This association was statistically significant (p < 0.0001). The association tended to be stronger between zinc and BMI (r^2 = 0.23), reflecting that 23% of the variance in serum zinc concentrations can be explained by the BMI. Similarly, lower serum zinc level was independently associated with lower LBM, total body fat and MUAC. Inverse relationships between zinc levels and sputum smear for AFB was observed (p < 0.0211).

Table 3. Relationship between variables in patients with pulmonary tuberculosis using multiple regression or Pearson correlation

Parameter	Constant	Co-efficient (r)	Co-efficient (r^2^)	*P*-value
Zinc vs BMI	11.26	0.42	0.18	0.0001
Zinc vs AFB	13.62	-0.14	-0.02	0.0211
Zinc vs LBM	9.642	0.30	0.09	0.0077
Zinc vs TBF	11.51	0.42	0.18	0.0001
Zinc vs MUAC	6.288	0.52	0.27	0.000001
Zinc vs HIV	14.26	-0.43	-0.19	0.0005

HIV: Human immuno deficiency virus; BMI: Body mass index
AFB: Acid fast bacilli (sputum smear for AFB); TBF: Total body fat (fat mass)
LBM: Lean body mass; MUAC: Mid-upper arm circumference

Table 4. Proportion of patients in the groups with sputum smears not converting to negative at two months of anti-tuberculosis treatment

Parameter	Intervention group (n = 41)	Control group (n = 40)	Relative Risk (RR)	95% CI
Sputum smear status (Unadjusted for HIV positive)	06 (14.6)	10 (25.0)	0.59	0.23 to 1.46
Sputum Smear Status (Adjusted for HIV positive)	04 (12.5)	06 (23.1)	0.54	0.17 to 1.72

Analysis by intention-to-treat (ITT)
Figures in parenthesis represent percentages.
CI: Confidence interval

3.1.3 Intervention effect on AFB sputum conversion

The intervention appeared to reduce the time of AFB sputum smear conversion from positive to negative as the proportion of patients in the intervention group (85%) was higher than the control (75%). With a relative risk (RR) of patients sputum smear test for acid fast bacilli (AFB) converting to negative after 2 months of anti-tuberculosis treatment for the intervention group was 0.59 of the control group with 95% confidence interval (CI) which ranges between 0.23 to 1.46 (Table 4), representing a 41% risk reduction for AFB positivity. Thus this shows that there is sufficient evidence against the null hypothesis, indicating that the difference observed may not have been due to chance.

Similar risk reduction was also observed when adjustments were made for HIV positivity (RR: 0.54; 95% CI 0.17 to 1.72). This thus represents a 46% risk reduction for sputum smear positive at 2 months of anti-TB treatment. This therefore strengthens the position that the observed difference is not due to chance and that micronutrient supplementation has effect on sputum smear conversion irrespective of HIV status.

The intervention group exhibited greater increase in weight than the control group (Table 2). This difference was found to be statistically significant ($p = 0.04$). Also the intervention group had significant lower proportion of patients with serum zinc levels and BMI below the lower ranges (Table 5). Equally BMI at 2 months TB treatment was higher than the control though this difference was not statistically significant ($p = 0.150$), there was increase from the baseline mean value whereas the control group had a lower value from the baseline. Since nearly all the anthropometric parameters had significant difference thus the null hypothesis is rejected, signifying that these differences are not due to chance. In addition the findings that lower concentrations of serum zinc was significantly independently associated with lower values for Lean body mass, fat mass and, MUAC (Table 3), lend further credence to the possibility that this could not have occurred by chance.

Table 5. Proportion of pulmonary tuberculosis patients with low concentrations/ranges of parameters at baseline and two months of anti-tuberculosis treatment

Parameter/Time of Assessment	Intervention group (n=41)	Control group (n=40)	Relative Risk (95% CI)	P-value
Serum Zinc < 10.7 µmol/L				
0 month	10(24.4)	12(30.0)	0.81	0.120
2 months	05(12.2)	10(25.0)	0.49	0.005
BMI < 18.5 kg/m^2				
0 month	14(43.2)	13(32.5)	1.05	0.304
2 months	09(22.0)	16(40.0)	0.55	0.030
Serum Albumin <3.2g/dl				
0 month	10(24.4)	07(17.5)	1.39	0.432
2 months	09(22.0)	10(25.0)	0.88	0.512
Serum Total Protein <6.0g/dl				
0 month	06(14.6)	11(27.5)	0.53	0.081
2 months	05(12.2)	10(25.0)	0.49	0.002
Globulin <2.3g/dl				
0 month	05(12.2)	08(20.0)	0.61	0.101
2 months	03(07.3)	07(17.5)	0.42	0.041
Heamoglobin g/dl				
0 month				
Male (<13)	15(36.6)	17(42.5)	0.86*	0.963
Female (<12)	14(34.2)	16(40.0)		
2 months				
Male (<13)	08(19.5)	13(32.5)	0.66*	0.035
Female (<12)	09(22.0)	12(30.0)		

Analysis is by intention-to-treat (ITT)
Figures in parenthesis represent percentages
** Pooled relative risk*

There was a significant improvement in the haematological parameters – haemoglobin (Hb), protein, albumin and globulins two months after start of anti-tuberculosis treatment (Table 2; Table 5). This is evidenced by significant higher proportion of patients in the intervention group than the control group with values above the lower ranges for these parameters with risk reductions in favour of the intervention group for lower ranges as 34%, 12%, 73% and 58% respectively for haemoglobin, albumin, serum total protein and globulins.

3.1.4 Adjustments for HIV sera status

After adjustments for HIV sero status, it was observed that those in the intervention group still had significantly gained weight (p = 0.02) and had better prognosis predictor as proxied by higher mean Karnofsky performance score (Table 6) though this was of borderline statistical significance (p = 0.07). Similarly, the intervention group had higher levels of parameters assessed at 2 months of anti-tuberculosis treatment (Tables 6). These differences were mostly

Table 6. Parameters adjusted for HIV positive at baseline and two months of anti-TB treatment

Parameters/time of assessment	Intervention group*	Control group*	*T*-Test	p-value
Serum Zinc (µmol/L)				
0 month	14.5 ± 0.45	13.3 ± 0.48	1.8906	0.064
2 months	14.9 ± 0.38	13.4 ± 0.42	2.6524	0.010
Body weight (kg)				
0 month	57.5 ± 2.14	51.8 ± 2.21	1.84	0.07
2 months	59.5 ± 1.95	52.0 ± 2.26	2.50	0.02
Karnofsky score# (%)				
0 month	62.1 ± 2.12	58.9 ± 2.46	0.9880	0.33
2 months	68.8 ± 0.17	63.5 ± 2.48	1.8260	0.07
Haemoglobin (g/dl)				
0 month	11.3 ± 0.17*	11.5 ± 0.14*	0.5400	0.59
2 months	11.6 ± 0.14	11.3 ± 0.15	1.0933	0.28
Serum Albumin (g/dl)				
0 month	03.7 ± 0.11	03.7 ± 0.11	0.4767	0.640
2 months	03.7 ± 0.08	03.7 ± 0.11	0.5918	0.560
Serum Total Protein (g/dl)				
0 month	07.5 ± 0.14	06.9 ± 0.21	2.3790	0.021
2 months	07.6 ± 0.16	07.1 ± 0.20	2.1686	0.007
Globulin (g/dl)				
0 month	03.7 ± 0.12	03.2 ± 0.22	2.0906	0.041
2 months	03.9 ± 0.14	03.4 ± 0.20	1.9951	0.051
Albumin / Globulin Ratio				
0 Month	01.0 ± 0.06	01.4 ± 0.15	2.0438	0.046
2 Months	01.0 ± 0.06	01.2 ± 0.12	1.4324	0.158
BMI (kg / m^2)				
0 month	21.0 ± 0.81*	19.8 ± 0.69*	1.0811	0.284
2 months	21.5 ± 0.73	19.8 ± 0.71	1.6951	0.100
MUAC (cm)				
0 month	25.2 ± 0.79	23.6 ± 0.72	1.4360	0.156
2 months	25.8 ± 0.74	23.3 ± 0.80	2.2455	0.028
Total body fat β (kg)				
0 month	13.5 ± 1.28	10.7 ± 1.09	1.6405	0.105
2 months	14.0 ± 1.19	10.8 ± 1.07	2.0098	0.042
Lean body massβ (kg)				
0 month	44.1 ± 1.25	41.2 ± 1.44	1.5094	0.137
2 months	45.4 ± 1.21	41.3 ± 1.48	2.1923	0.033

** Mean ± Standard error of mean (SEM)*
 # Range: 0 (dead) to 100 (normal)
Statistically significant based on a two-tailed P-value < 0.05
β extrapolated

statistically significant ($p < 0.05$). Furthermore, though the multivariate analysis (Table 3) among tuberculosis patients showed that HIV positivity was a strong predictor of and independently associated with lower serum zinc concentrations; zinc versus HIV ($r = -0.44$), result still showed that irrespective of HIV sero-positive that zinc supplementation improved haematological parameters (Table 5), anthropometric indices (Table 2), and increased serum zinc concentrations ($p= 0.010$) (Table 6) in favour of the intervention group.

4. DISCUSSION

The connection between tuberculosis and malnutrition has long been acknowledged. However, understanding the effects of micronutrient supplementation in reducing the burden of this relationship remains a quest. This study thus assessed the effects of zinc supplementation in TB treatment outcomes. On completion of the 2 months intensive treatment period, the Karnofsky performance score a measure of functional impairment [20,21] was higher in the intervention group than the control ($p = 0.04$) an indication of better prognosis. This may in part be as a result of improved nutritional status and haematological parameters in the micronutrient supplementation group such as albumin. Albumin is responsible for the transport of important blood constituents such as drugs and hormones [3]. Thus hypoalbuminemia may have implications for anti-TB chemotherapy by its reduction in bactericidal activities of TB drugs. This may in part explain the higher proportion of patients in the control group with AFB sputum smear positive at 2 months of anti-TB treatment. The better prognosis observed in the intervention group than in the control may have also been as a result of reduction in bacteriological load occasioned by the intake of zinc supplements.

The findings of this study is in consonance with Karyadi et al. [18] in their supplementation trial in persons with tuberculosis in Indonesia that found supplementation with zinc and vitamin A improved the effects of tuberculosis medication after 2 months of anti-tuberculosis treatment and resulted in earlier sputum conversion. Ejemot-Nwadiaro et al. [7] also reported earlier AFB sputum conversion with vitamin A supplementation. Similarly, Cuevas and Koyangi [15] cited zinc supplementation as adjuncts to TB treatment studies done in India and Ethiopia that equally reported earlier smear sputum conversion.

There was a significant difference in body weight at 2 months of TB treatment ($p = 0.04$). Similar differences were also observed for other anthropometric indices. The implication of this is that individuals with low weight and low anthropometric indices not only present with features of malnutrition, but also have increased risk of disease and death [6,8,25]. The results of this study that show 33% of the patients below the lower range of BMI (18.5 kg/m^2) tends to be in agreement with reports of Van Lettow et al. [8] and WHO [1] that wasting is common in TB patients. Conversely, Lodha et al. [7] reported that micronutrient supplementation did not modify the weight gain or clearance of lesions on Chest X-ray in children with intrathoracic tuberculosis but that it may have improved height gain. This difference could be due to the different targeted populations in both studies. Whereas this study had adults with TB, theirs was among children.

Serum zinc concentrations were significantly higher in the intervention group than the control ($p = 0.004$), a difference that may have contributed to the 40% risk reduction for sputum smear conversion. The observation of inverse relationship between serum zinc and sputum smear for AFB ($p = 0.021$) strengthens this position. This may not be unrelated to zinc's role in the maintenance of the integrity of biological membranes, resulting in the protection against oxidative damage, structural strains and alterations in specific receptor sites and transport systems [3,10,15,26]. This may also be due to the possible role of zinc supplementation in restoring rapidly in vitro cellular killing of macrophages usually reduced in zinc deficiency [2,5,16]. This observed benefit of zinc in TB treatment is consistent with the results of Karyadi et al. [18] that a combination of zinc and vitamin A supplementation improved the effects of TB medication after 2 months of anti-TB treatment and results in earlier sputum conversion.

The findings of this study show that irrespective of the TB-HIV co-morbidity, that the intervention group had higher serum zinc concentration ($p= 0.010$) and improvements in clinical outcomes, haematological parameters, and nutritional status as proxied by anthropometric indices. This seems to be in consonance with the study of Van Lettow et al. [8] that Zinc supplementation produced higher CD4+ lymphocyte cell counts and reduced the incidence of bacterial infection. These thus suggest better treatment outcomes effects favouring the intervention.

A significant difference in the serum concentrations of zinc was also observed between the two groups when adjustments were made for TB-HIV co-infection. This suggested that irrespective of HIV status in individuals with tuberculosis, zinc micronutrient supplementation significantly increases clinical outcomes, haematological parameters, improves nutritional status as proxied by anthropometric indices and may lead to faster sputum smear conversion.

5. CONCLUSION

The results of this study points to the benefits of zinc supplementation in improving the treatment outcomes in patients with pulmonary TB. However, the interpretation of the findings of this study would be strengthened by evidence of the effects of this intervention in children with TB and supporting data on other immunological markers such as cytokines (interferons, interleukins-1 (IL-1), tumor necrosis factor (TNF), etc), acute phase protein (C-reactive protein, haptoglobin, etc.) critical to fighting infections; TB drug susceptibility and direct dietary evaluations. This study therefore adds to the growing body of evidence that zinc supplementation as adjunct to TB chemotherapy potentiates the effects of anti-TB drugs in this environment.

CONSENT AND ETHICAL APPROVAL

Research Ethics Committee, Centre for Clinical Governance, Research and Training, Cross River State Ministry of Health granted ethical approval for conduct of this study. Informed written consent for participation in the study was sought from eligible patients after their full understanding of the information sheet detailing the nature, objective, methods, anticipated benefits and potential hazards of the intervention. Anonymity of participants and confidentiality of data were ensured in accordance with the principles laid down by the World Health Assembly, Helsinki Declaration of 1975 on ethics on human experimentation. Good clinical practice (GCP) and standard operating procedures (SOP) were employed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization (WHO) (2018). Global Tuberculosis Report 2018. Geneva: World Health Organization; 2018 Licence: CCBY-NC-SA 3.0 IGO
2. Choi R, Tae Kim H, Lim Y, Min-JiKim, O JungK won, Jeon K, Park HY, Jeong B, Koh WJ, Lee SY. Serum concentrations of trace elements in patients with tuberculosis and its association with treatment outcome. Nutrients. 2015;7:5969-5981.
3. Zhuang LS, Ling LK, Xu BS, Yuan ZZ. Adjuvant efficacy of nutrition support during pulmonary tuberculosis treating course: Systematic review and meta-analysis. Chinese Medical Journal. 2015; 128(23):3219-3230
4. Lodha R, Mukherjee A, Singh V, Singh S, Friis H, Faurholt-Jepsen D, Bhatnagar S, Saini S, Kabra SK, Grewal HM. Effect of micronutrient supplementation on treatment outcomes in children with intrathoracic tuberculosis: A randomized controlled trial. The American Journal of Clinical Nutrition. 2014;100(5):1287-1297.
5. Bacelo AC, Ramalho A, Brasil PE, Cople-Rodrigues CD, Georg I, Paiva E, Argolo SV. Nutritional supplementation is a necessary complement to dietary counseling among tuberculosis and tuberculosis-HIV patients. Plos One. 2015;1:10(8).
6. Gupta KB, Gupta R, Atreja A, Verma M, Vishvkarma S. Tuberculosis and nutrition. Lung India. 2009;26:9-16.
7. Ejemot-Nwadiaro RI, Itam EH, Ezedinachi EN. Effects of vitamin A supplementation on tuberculosis treatment in Calabar, Nigeria: A randomized controlled trial. World Journal of Pharmacy and Pharmaceutical Sciences. 2017;6(2):243–258.
8. Van Lettow M, Harries AD, Kumwenda JJ, Zijlstra EE, Clark TD, Taha TE, Semba RD. Micronutrient malnutrition and wasting in adults with pulmonary tuberculosis with and without HIV-co-infection in Malawi. BioMed Central (BMC) Infectious Disease. 2004;4:61-69.
9. Akhtar S, White F, Hasan R, Rozi S, Younus M, Ahmed F, Husain S, Khan BS. Hyperendemic pulmonary TB in Peri-urban areas of Karachi, Pakistan. Public Health. 2007;7:70-80.
10. Solomon NW. Mild human zinc deficiency produces an imbalance between cell-

mediated and humoral immunity. Nutrition Review. 1998;56:27-28.
11. Ketiku A, Ejemot RI, Fasanmade A. The relationship between anti-oxidants and blood lipids in diabetes mellitus. West African Journal of Biomedical Research. 1999;2:177-122.
12. Wintergerst ES, Maggini S, Hornig DH. Immune enhancing role of vitamin C and zinc and effects on clinical conditions. Annals of Nutrition and Metabolism. 2006;50:85-94.
13. Bhutta ZA, Black RE, Brown KH, Nhdari IL. Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: Pooled analysis of randomized controlled trials. Journal of Pediatrics. 1999;135:689-697.
14. Bhandari N, Bahl R, Taneja S, Strand T, Molbak K, Ulvik RJ. Effect of routine zinc supplementation on pneumonia in children aged 6 months to 3 years: Randomized controlled trial in an urban slum. British Medical Journal. 2002;324(7350):1359-1364
15. Cuevas LE, Koyanagi A. The effect of Zinc on childhood infection: Review. Annals of Tropical Paediatrics. 2005;25(3):149-160.
16. Shankar A, Prasad A. Zinc and immune function: The biological basis of altered resistance infection. Am J. of Clin Nutri. 1998;68:447S-463S
17. Ejemot-Nwadiaro RI. A guide to Biostatistics and Health Research Methods. DataPro Publishers, Calabar; 2009. ISBN 978-051-089-3
18. Karyadi E, West CE, Schultink W, Nelwan RH, Gross R, Amin Z. A double-blind placebo- controlled study of vitamin A and Zinc supplementation in persons with tuberculosis in Indonesia: Effects on clinical response and nutritional status. American Journal of Clinical Nutrition. 2002;75(4):720-727.
19. World Health Organisation/ United Nations on Acquired Immuned Deficiency Syndrome (WHO/UNAIDS) (2001) WHO recommended strategies for the prevention and control of communicable disease. WHO/CDS/CPE/SMT/2001.13:107-10. Geneva: World Health Organisation.
20. Schag CC, Heinrich RL, Ganz P. Karnofsky performance status revisited: Reliability, validity, and guidelines. Journal of Clinical Oncology. 1984;2:187-193.
21. Crooks V, Waller S, Block BD, Wallis BC. The use of the Karnofsky Performance Scale in determining outcomes and risk in geriatric outpatients. Journal of Gerontology. 1991;46, M139-M144.
22. McMurrary DN. Mycobacteria and Nocardia. In S. Baron (Ed). Baron's Medical Microbiology (4th ed). Texas: University of Texas Medical Branch. 1996;910-950.
23. International Society for the Advancement of kinanthropometry. International standards for anthropometric assessment; London; 2001.
24. Chawla R. Practical clinical biochemistry: Methods and interpretations (2nded.). Bangalore: JAYPEE Brothers Medical Publishers Ltd; 1999.
25. Ramachandran G, Santha T, Garg R, Baskaran D, Iliayas SA, Venkatesan P, Fathima R, Narayanan PR. Vitamin A levels in Sputum-positive pulmonary tuberculosis patients in comparisons with household contacts and healthy 'normals'. International Journal of Tubercle Lung Diseases. 2004;8(9):1130-1133.
26. Kassu A, Yabutani T, Mahmud ZH, Mohammad A, Nguyen N, Huing BT, Hailemariam G, Diro E, Ayele B, Wondmikun Y, Motonaka J, Ota F. Alterations in serum levels of trace elements in tuberculosis and HIV infections. European Journal of Clinical Nutrition. 2005;60:580-586.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/48238