Original Research Article

Does ascorbic acid supplementation improve treatment outcomes in adults with pulmonary tuberculosis?

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Tuberculosis (TB) endemicity is rife in over one-third of the world's population. Globally, over 13% of new cases die yearly, making TB one of the deadliest infectious diseases. Nutritional supplementation, principally micronutrients may play significant role in reducing TB burden. The study assessed the effect of ascorbic acid supplementation on treatment outcomes in adults with pulmonary TB. Participants consisted of out-patients attending the Dr. Lawrence Henshaw Memorial Hospital, Calabar, Nigeria. Of 182 patients assessed for eligibility, 81 met inclusion criteria. Intervention group received anti-TB drugs plus oral supplement of ascorbic acid (50mg/day) for 60 days. Control group received only anti-TB drugs. Anthropometric, clinical, bacteriological and haematological parameters were assessed at baseline and two months post-treatment. Patients in the intervention group (14.6%) had positive acid fast bacilli at endline against 25% in the control group (RR: 059; CI 95% 0.23 to 1.46). Similarly, intervention group had significantly lower proportion of patients (7.3%) with serum ascorbate levels below lower ranges (< 0.4 mg/dl), compared to control group (25%);(p=0.023). Baseline figures; body weight (p = 0.04); total body fat (p = 0.028); Karnofsky index score (p = 0.04); and haematological parameters assessed increased in favor of intervention group. Observations suggest positive effects of supplementation of ascorbic acid on TB treatment outcomes irrespective of TB-HIV co-infection status.

Key words: Tuberculosis, ascorbic acid supplementation, tuberculosis treatment, Nigeria.

INTRODUCTION

Prevention and treatment of tuberculosis (TB) in settings where it is endemic is pivotal to achieving the sustainable development goal (SDG) three. TB endemicity is rife in over one-third of the world's population particularly in low-and middle-income countries with about 10 million new cases reported globally and consequential deaths of up to 1.3 million yearly (WHO, 2018). This colossal health burden is often further complicated by the huge cultural and socioeconomic dimensions of TB, making the requirement for urgent and extraordinary actions inevitable (Gholipour, 2013; WHO, 2013). Thus, the need for evidence-based technology and methods to control TB thus, presents important public health and development quest. It has been suggested that nutritional supplementation, principally micronutrients may play significant role in this quest (Bacelo et al., 2015; Zhuang et al., 2015).

Another perspective to this highly threatening TB burden is that, increasingly, tuberculosis has developed resistance

to current antibiotics that once dominated it, with the end result being antibiotic-resistant tuberculosis which is difficult to control. It is termed multi-drug resistant tuberculosis (MDR-TB). Among thoseactually infected, there is around a third of whom who do not manifest symptoms, but are actually carriers. And this multi-drug resistant tuberculosis bug is progressively more concerning (Vilchèze et al., 2013; Fassa, 2014). Fassa (2014) reported that an estimated 650,000 people have MDR-TB and even worse version ofextensive drug resistant tuberculosis (XDR-TB), which has infected close to 10% of the MDR-TB population.

The response to infection and the disease outcome depends on the balance between microorganism proliferation and host resistance. Nutrition is acknowledged as an important factor that can modify resistance and tip the balance towards morbidity and mortality (Scrimshaw and Sangiovanni, 1997; WHO, 2013). Serious complications and high deaths caused by infections such as TB particularly in low-/middle-income countries have often been attributed to malnutrition (WHO, 2013;Etim et al., 2017). This synergistic relationship between malnutrition and infection has long been established (WHO, 2013). Infectious illnesses such as TB are accompanied by a complex variety of nutritional and metabolic responses within the body. These have been recognized to be mediated through changes in dietary intake, absorption, excretion, requirement, etc. (Scrimshaw and Sangiovanni, 1997; Van Lettow et al., 2004; Bacelo et al., 2015). Nutritional supplementation particularly micronutrients such as vitamin C (ascorbic acid) have been muted as important in the pathogenesis, prevention, treatment, management and control of diseases (Cunningham et al., 1994; Jacob, 1999; Ketiku et al., 1999; Gupta et al., 2009; Zhuang et al., 2015).

Vitamin C is well known for its anti-oxidant actions. As an immunological maintenance measure, vitamin C can enhance the function of the immune system in different ways such as T-lymphocyte proliferation and thus strengthens the cell mediated immunity (Niki, 1991; Higdon, 2006; Fassa, 2014). Chakraborty et al., (2014) reported that both lower dietary intake and lower blood concentration of vitamin C are associated with the higher incidence of tuberculosis and that in patients with active cavitary tuberculosis, the anti-oxidant vitamin C level gets substantially decreased with an increase in lipid peroxides.

Supplementation of vitamin C was found to improve components of the human immune system such as antimicrobial and natural killer cell activities, lymphocyte proliferation, chemotaxis, and delayed type hypersensitivity (Frei et al., 1989; Bacelo et al., 2015; Zhuang et al., 2015). Vitamin C contributes to maintaining the redox integrity of the cells and thereby protecting them against reactive oxygen species generated during the respiratory burst and inflammatory response (Wintergerst et al., 2006; Gholipour, 2013).

Vitamin C has also been observed to accelerate recovery from tuberculosis by healing decay cavity and turns sputum acid fast bacillus (AFB) negative (Fassa, 2014). Experimental research supports are there which show that an adequate dietary supplementation of vitamin C along with standard anti-tubercular antibiotic regimen contributes protection against tuberculosis, lowers the incidence, and accelerate healing of tuberculosis (Wintergerst et al., 2006; Vilcheze et al., 2013; Chakraborty et al., 2014; Lodha et al., 2014).

Since most of the reliable data on the role of micronutrient in immunity to tuberculosis has been generated on experimental animal models, the relevance for human of the conclusion drawn from such experiments must be interpreted cautiously. It is known that deficiency of Vitamin C can cause profound impairment of immunity and precisely the cell type that are critical to combat tuberculosis (Scrimshaw and Sangiovanni, 1997; Wintergerst et al., 2006). It is therefore not unreasonable to propose that supplementation of an important micronutrient such as Vitamin C may be an important determinant of tuberculosis treatment outcome. The paucity of evidence of the role of Vitamin C and its relationship to TB pathogenesis and treatment in this environment adds significantly to these narratives. This study therefore, aimed at assessing the effects of the Vitamin C supplementation on tuberculosis patients in the Cross River State, Nigeria.

MATERIALS AND METHODS

Ethics statement and study population

Ethical clearance for the conduct of this study was received from the Research Ethics Committee, Centre for Clinical Governance, Research and Training, Ministry of Health, Cross River State, Nigeria. The participants only agreed to be part of the study after they read and fully understood the objectives of the study as contained in the study information sheet and confidentiality and anonymity assured. Each participant provided written informed consent before the implementation of the intervention study. The participants consisted of out-patients attending the Dr. Lawrence Henshaw Memorial Hospital, Calabar, Nigeria; a health facility essentially devoted to the treatment of infectious diseases. It is located in Calabar, the capital city of Cross River state. The state lies within the tropical rain forest belt of Nigeria and between latitude 4°28′ and 6° 55′ north of the equator and longitude 7° 50′ and 9° 28′ east of the Greenwich meridian.

Study design

This study was designed as a prospective parallel randomized controlled trial. The sample size was calculated using the difference in mean formula for two independent groups approach as reported in Ejemot-Nwadiaro (2009);

$n = 2 \sigma^2 / (\mu_1 - \mu_0)^2 \; X \; \int(\alpha, \beta)$

n =the number required for each group

σ = the standard deviation(0.317 µmol/L – Karyadi et al., 2002)

μ_0 =the mean response for one treatment group

μ_1 = mean response for the other treatment group

$\mu_1 - \mu_0 = 0.31$ µmol/L

$f(\alpha,\beta)$ = (is the numeric value obtained from the table (related to the level of significance ($\alpha = 0.05$) and the power ($\beta = 0.01$).

Applying the formula, we arrived at having 22 patients for each arm of the study for which we used the estimated mean response in the control group and standard deviation as reported from previous similar study (Karyadi et al., 2002); at 5% level of significance(at which we wish to minimize the probability of falsely rejecting the null hypothesis) and 90% power (we wish to minimize the probability of not detecting a significant difference when there really is one). To account for 30% attrition rate and 30% of failure to meet the inclusion criteria, the desired minimum sample size was ≥72 patients with (36 per study group).

Eligibility criteria for inclusion in the study were; (a) newly confirmed case of pulmonary tuberculosis (PTB). This was ascertained by acidfast bacilli (AFB) sputum and clinical examination conducted by a microbiologist and clinician respectively; (b) ≥18 years; (c) signed written informed consent; and (d) indication of intention to stay in the study area for the duration of active 2 months follow-up period. The following constituted the exclusion criteria; (i) individuals who had previously been on anti-TB treatment for more than four weeks in the past five years (ii) use of any drug that could suppress immunity (iii) pregnant women who would have received micronutrient supplements as part of routine prenatal services (iv) intake of supplements containing micronutrients of interest during the previous month (v) subjectis in prison or in police custody and (vi) subject is mentally incapable of understanding the information about the study.

One hundred and eighty-two patients were assessed for eligibility employing already specified inclusion criteria.The participants were recruited within the periods of July 2007 to January 2008. Only 81 patients met the criteria. Randomization was performed by an independent Statistician at the individual level using block randomization to generate allocation sequence. Allocation concealment was achieved through the use of serially numbered opaque bottles. Details of the study profile are indicated in Figure 1.

Description of intervention

Eligible patients were randomized to receive either micronutrient in addition to the anti-TB drugs (intervention group) or anti-TB drugs only (control group), prescribed by the clinician in line with World Health Organization (WHO) dosage recommendation. The type and dosage were recorded in the individual patient's case record form (CRF). The intervention group received anti-TB drug regimen plus oral administration of   individual supplement of vitamin C, 50mg (as ascorbic acid) daily for 60 days. The control group received only anti-tuberculosis drug regimen. Supplements were stored in dark bottles and each patient instructed to store in cool dark place. We followed the WHO recommended TB treatment strategy - direct observed treatment short-course (DOT). Each eligible patient was asked to take the micronutrient supplements as prescribed daily from the start of anti-TB treatment till two months post-treatment. Patient's compliance was assessed by comparing the number of remaining supplements with the number recorded in the logbook as they come to collect anti-TB drugs.

Data collection

We used diverse data collection tools to obtain both qualitative and quantitative data. Outcome measurements were taken at enrolment and eight weeks after start of the intervention. Clinical examination conducted included history of exposure to TB (contact investigation), measurement of body temperature and assessment of the presence of a bacilli Calmette-Guérin (BCG) scar.The Karnofsky performance scale index that allows for patients to be classified as to their functional impairment was also assessed. It compares effectiveness of different therapies and 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) (Schag et al., 1984).

About 5ml fasting whole blood was withdrawn by venipuncture into vaccutainers. They were protected from light and stored at –20°C, centrifuged and subsequently processed for analysis and estimation of ascorbic acid, heamglobin(Cyanmethaemoglobin technique), serum total protein(Biuret method of Doumas et al., 1981) and albumin (Bromocresol Green method) as described in Chawla (1999). Globulin was estimated by subtracting the albumin level from the total protein.HIV status screening was determined by Immunochromatographic method. The test involves the qualitative detection of antibodies to HIV-1 and -2 (Arai et al., 1999).

Serum ascorbic acid was estimated adapting the methods of Krishn and Ranjhan(1980). This method essentially depends on the reduction of 2:6-dichlorophenol indophenol by ascorbic acid at pH of about 4.0. The absorbance of the residual unreduced pink dye is measured at 520nm. Appropriate kits designed for estimation of these parameters were used.

Sputum examination was conducted using three specimen of early morning sputum taken from each patient. It was examined by direct microscopy to assess sputum conversion from positive to negative adopting the Ziehl-Nelson staining method for the number of AFB visible in oil immersion. Sputum smears grading was based on the following; 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(McMurray, 1996).
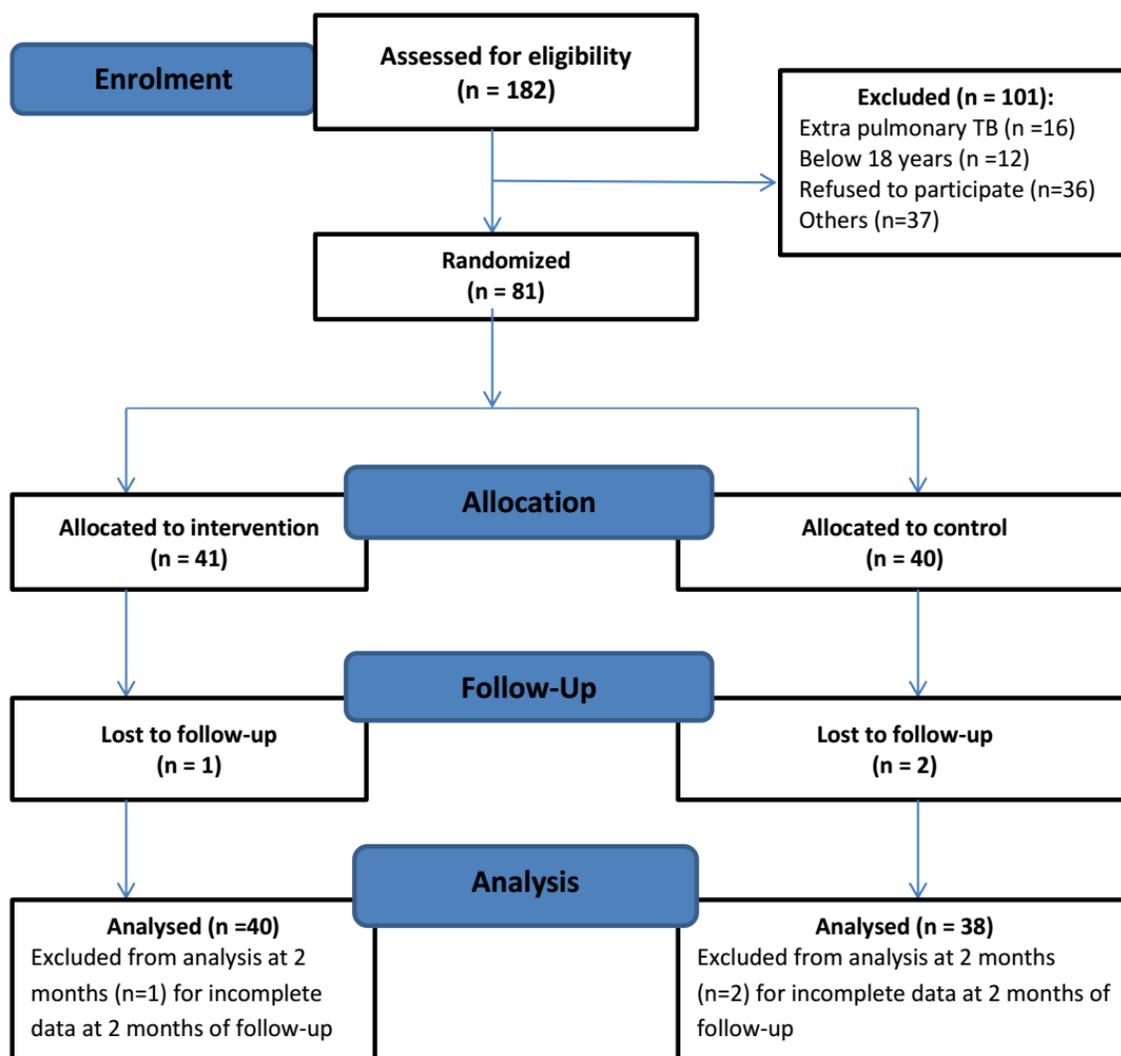
Figure 1: Study flow diagram

Anthropometric measurements assessed were age as at the last birthday; height was measured in centimeters and weight in kilogram. Body mass index (BMI) was determined from the values of height and weight for each subject. 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. The skinfold thicknesses were measured using ADIOPOMETER™ Skinfold Calipers of subcutaneous fat skinfold at the bicep, tricep, subscapular, and suprailliac sites. The proportion of total body fat (TBF) and fat-free-mass or lean body mass (LBM) were extrapolated from the skinfold measurements and calculated using Durnin and Womersley (1974) equation. Mid-upper arm circumference (MUAC) was measured with a plastic inelastic measurement tape. The measurement was recorded in centimeters (cm). All measurement followed the international standards for anthropometric assessment as published by International Society for Advancement of Kinanthropometry (2001).

Data analyses

Reporting of results for this study followed consolidated standards of reporting trials (CONSORT). Statistical analyses were performed using EPI-Info statistical software version 3.3.2. Data management, cleaning, and descriptive analysis were done using the same statistical software. No imputation for missing data was done. 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 summarize skewed data. T-test was employed for comparing difference in treatment effects between and within groups for continuous data. Chi-square and Fisher's exact tests were

Table 1. Baseline characteristics of study participants

Parameters	Intervention group (n= 41)	Control group (n=40)	Test Statistic	P-value
Sex				
Male	23	22	χ^2= 0.0099	0.9208
Female	18	18		
Presence of BCG Scar (n)				
Male	11 (26.8)	09 (22.5)		
Female	08 (19.5)	09 (22.5)	OR: 1.10	0.492
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)		
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
Serum albumin < 3.2 g/dl (n)	10 (24.4)	07 (17.5)	RR: 1.39	0.432
Height (cm) mean ± SD	165.0 ± 7.8	161.9 ± 9.1		
range	148.0 – 177.0	138.0 – 180.0	1.671^{ɫ}	0.099
BMI < 18.5 kg /m^2 (n)	14 (34.2)	13 (32.5)	RR: 1.05	0.304
Sputum smear grade (n)				
+ 1	09 (22.0)	08 (20.0)		0.431
+ 2	15 (36.6)	13 (32.5)	r = 0.10^	0.524
+ 3	10 (24.4)	10 (25.0)		0.839
Not seen	07 (17.1)	09 (22.5)		0.633
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)		

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; ɫ = t-independent test; r = coefficient of regression

used to compare categorical data sets. Mantel-Haenszel summary estimate of the relative risk (RR) was used to test the clinical effectiveness of the intervention. In determining the correlation between variables Pearson's test was used. Further analyses adjusted for HIV co-infection for major variables in focus. Data were presented with 95% confidence interval and statistical significance based on *P*-value <0.05.

RESULTS

Description of study population

Of the 81 eligible subjects, 41 were randomized to the intervention group, while 40 randomized to the control group. The final analysis had a total of 78 patients with one excluded from analysis at two months of follow-up for incomplete data from the intervention group and two from the control group respectively. This gives the attrition rate at 3.7% (Figure 1).

The baseline characteristics of the study participants show that the intervention and the control groups were comparable at the start of the study as indicated in Table 1.

About 33.3% of the participants were malnourished at baseline using the Body Mass Index (BMI) widely acknowledged as the best simple and quantitative anthropometric indicator of body composition and thus nutrition state in adults. However, no sub-group differences were noted in both groups (p = 0.304).

Baseline versus endline status of key parameters in study participants

Compared with the control, the intervention group had higher mean serum ascorbate of 0.61 mg/dl as against 0.57 mg/dl at 2 months after start of anti-TB treatment (Table 2). This difference was however not statistically significant (p = 0.33). The intervention group had an increase in mean serum ascorbate of 0.07 mg/dl from mean of 0.54 mg/dl at baseline to 0.61 mg/dl 2 months post-treatment as against 0.05 mg/dl mean value for the control group, from 0.52 mg/dl to 0.57 mg/dl.

The intervention group had a mean weight gain of 1.4 kg from mean weight of 55.8 kg at baseline to 57.2 kg two 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 =

Table 2. Comparison of key parameters in patients with pulmonary tuberculosis at baseline and two months of anti-tuberculosis treatment

Characteristics	Intervention group	Control group	Test statistics	p-value
Serum Ascorbate (mg/dl)				
0 month	0.54 ± 0.03	0.52 ± 0.03	0.5223	0.600
2 months	0.61 ± 0.03	0.57 ± 0.03	0.9911	0.330
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
Karnofsky 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
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
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

‡ 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

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 two 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 micronutrient supplementation group that had varying categories of positive sputum smear for AFB reducedfrom 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).

Comparison of haematological parameters at baseline and end of intervention

There was slight improvement in the mean haemoglobin concentration from 10.9 g/dl at baseline to 11.3 g/dl for the intervention group whereas converse was the case for the control group as there was 0.1 g/dl drop from the mean baseline value of 11.0 g/dl (Table 3). This difference was not statistically significant (p = 0.12).

Similarly, serum albumin concentration appreciated from a mean value of 3.6 g/dl at baseline to 3.8 g/dl within two months of anti-TB treatment in the intervention group while it slightly depreciated in the control group by a difference of 0.10 g/dl (p = 0.91). There was marginal increase of 0.10 g/dl respectively in serum total protein concentration from baseline to two months post-TB treatment for both groups. However, the intervention group had higher mean values of 7.5 g/dl and 7.6 g/dl at baseline and 2 months post-TB treatment as against 6.9 g/dl and 7.0 g/dl respectively for the control group. These differences were found to be statistically significant (p = 0.007).

Serum globulin concentrations in both groups appreciated by 0.1 g/dl respectively at 2 months of anti-TB treatment, though the intervention group had higher mean serum concentration both times of assessment and these differences were found to be significant (Table 3). In contrast, the control group had higher albumin-globulin ratio at baseline and two months of TB treatment than the micronutrient group. These differences were found to be significant (p < 0.05).

The intervention group had significant lower proportion of patients (7.3%) with serum ascorbate levels below the lower ranges (< 0.4 mg/dl), indicating deficiencies, than the

Table 3. Hematological parameters in patients with pulmonary tuberculosis at baseline and two months of anti-tuberculosis treatment

Parameter / time of assessment	Intervention group	Control group	Test statistics	P-value
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
Serum Ascorbate< 0.4 mg/dl				
0 month	08 (19.5)	08 (20.0)	0.98#	0.804
2 months	03 (07.3)	10 (25.0)	0.29#	0.023

All test statistics are independent T-test except for Serum Ascorbate
#Relative Risk (RR)
*Figures: Mean ± Standard Error Mean (SEM)
Statistically significant based on a one-tailed P-value < 0.05

Table 4. Correlation between variables in patients with pulmonary tuberculosis

Parameter	Constant	Co-efficient(r)	Co-efficient(r^2)	P-value
Ascorbatevs HIV	00.62	-0.20	-0.04	0.0700
Ascorbatevs BMI	17.30	0.22	0.05	0.0390
Ascorbatevs AFB	00.60	0.00	0.00	0.7000
Albumin vs AFB	03.70	-0.22	-0.05	0.214
Karnofskyvs AFB	62.34	-0.17	-0.03	0.337
Ascorbatevs LBM	0.386	0.17	0.03	0.1053
Ascorbatevs TBF	0.408	0.43	0.19	0.000053
Ascorbatevs MUAC	0.081	0.42	0.18	0.00009
Hbvs AFB	11.21	-0.17	0.03	0.182
Albumin vs AFB	03.70	-0.22	-0.05	0.214
Protein vs AFB	07.26	-0.20	-0.04	0.041
Globulins vs AFB	03.56	-0.36	-0.13	0.005

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

control group (25%). This difference was statistically significant (p = 0.023).

Association between variables

The relationships between some selected variables shown in Table 4 indicate that low serum ascorbate (< 0.4 mg/dl) was independently associated with low BMI. This association was statistically significant (p = 0.03). The association between ascorbate and BMI (r^2 = 0.05) reflected that only 5% of the variance in serum ascorbate concentration can be explained by the BMI. Similarly, lower serum ascorbate levels were independently associated with lower LBM, total body fat, and MUAC. All were statistically significant except for the association between serum ascorbate level and lean body mass (p = 0.1053).

Table 5. Endline acid fast bacilli sputum smearstatus of participants

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

No independent association was observed between serum ascorbate and AFB (r = 0.000). Inverse relationships were also observed for the haematological parameters and AFB except, for albumin and haemoglobin. These associations were found to be statistically significant (p < 0.05). Thirteen percent variance (strongest) in globulin tended to explain the variance in AFB (Table 4).

Effects of intervention

The results indicate that the relative risk (RR) of patients' sputum smear test for acid fast bacilli (AFB) converting to negative after two months of anti-TB 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 5), signifying a 41% risk reduction for AFB positivity. The intervention appeared to reduce the time of AFB sputum smear conversion from positive to negative as 14.6% of patients in the intervention group had positive AFB at two months of treatment as against 25% in the control group. When adjustments were made for HIV sera positive, similar risk reduction was also observed (RR: 0.54; 95% CI 0.17 to 1.72). This thus represents a 46% risk reduction for sputum smear positive at two months of anti-TB treatment.

DISCUSSION

Our study on the supplementation of ascorbic acid in the treatment of adult patients with pulmonary tuberculosis (TB) indicates that, there appears to be improvement in the clinical outcomes assessed. The intervention group had greater proportion (85%) of patients that had sputum smear acid fast bacilli (AFB) conversion to negative after two months of treatment against 75% in the control group (Relative Risk: 0.59; 95% CI 0.23 to 1.46). When adjustments were made for TB-HIV co-infections, the relative risk reduction was slightly higher (46%) (RR 0.54; 95% CI 0.17 to 1.72). These observations may in part be related to the role of vitamin C in maintaining the redox integrity of the cells and thereby protecting them against reactive oxygen species generated during the respiratory burst and inflammatory response (Wintergerst et al., 2006; Gholipour, 2013; Vilcheze et al., 2013). The improved

Karnofsky score in favor of the intervention group (p = 0.04) lend further support to possible positive intervention effects. Since Karnosfsky performance scale index is used to classify patients as to their functional impairment, compare effectiveness of different therapies and to assess the prognosis in individual patients (Schag et al., 1984).

Similarly, there were statistical significant appreciations above baseline figures for body weight (p = 0.04), total body fat (p = 0.028) and the haematological parameters assessed except for serum albumin (p = 0.07), suggesting probable intervention contributions to risk reduction. These are in consonance with reports that, supplementation of vitamin C was found to reduce wasting often associated with TB (Van Lettow et al., 2004; Lodha et al., 2014; WHO, 2018) and improve components of the human immune system such as antimicrobial and natural killer cell activities, lymphocyte proliferation, chemotaxis and delayed type hypersensitivity (Frei et al., 1989; Fassa, 2014; Bacelo et al., 2015; Zhuang et al., 2015). Fassa (2014), in support of the aforementioned further observed that vitamin C caused iron to react with other molecules, creating a reactive oxygen type that was too much for the MDR-TB and XDR-TB strains that usually withstand all antibiotics.

Though there was a statistically significant lower proportion of patients in the intervention group (p = 0.023) with lower range of serum ascorbate (< 0.4 mg/dl) considered deficient, mean serum vitamin C level was observed to be similar and not statistically significant for both the intervention and control groups at baseline (p = 0.600) and two months after start of treatment (p = 0.330).This observation is further supported by no variance between ascorbate and sputum smear for AFB (p = 0.700). This may not be unrelated to the observation that vitamin C may act as pro-oxidant *in vitro* in the presence of metals (Niki, 1991; Podmore et al., 1998); and as antioxidant Frei et al., 1989; Ketiku et al., 1999; Reddy et al., 2004). Thus, the plausible explanation is that there was probable balance in vitamin C role in this respect.

The role of vitamin C in haematopoiesis particularly as co-factor in reactions requiring reduced metals such as Fe^{2+}(Podmore et al., 1998; Higdon, 2006; Gupta et al., 2009); may have also impacted the failure to observe clinically important differences within groups (p>0.05) and between groups (p > 0.05). This may be through a probable

increase in demand of vitamin C for this role. Irrespective of this, there was increase in most of the haematological parameters assessed for the intervention group than the control.

Furthermore, the failure to observe clinically important difference may have also been due to unreported lifestyle risk factors for low serum concentrations of vitamin C such as smoking and alcoholism. Smokers are known to require higher amounts of vitamin C up to 35mg/day above non-smokers, since smokers have been recognized to be under increased oxidative stress from toxins in cigarette smoke (Jacob, 1999; Higdon, 2006), exacerbating the already high free radical activity observed in TB (Reddy et al., 2004; Chakraborty et al., 2014; Zhuang et al., 2015). This thus, tilts the pro-oxidant-antioxidant balance in favour of the former. This is thought to increases the requirement for ascorbic acid since it is recognized as the body's broad-spectrum free radical scavenger Scrimshaw and Sangiovanni, 1997; Ketiku et al., 1999; Higdon, 2006; Wintergerst et al., 2006).

The results of this study suggest that vitamin C supplementation along with anti-TB therapy exhibited mycobactericidal activities that positively influenced TB treatment outcomes. However, the paradoxical observations of non-clinically differences in serum ascorbate level between the intervention and control groups probable occasioned from vitamin C inherent competing function requires further study.

Conclusion

The observations from this study show that the proportion of patients with sputum smear conversion to negative for acid fast bacilli was higher in the intervention group than in the control by about 11% with a risk reduction of about 40% for AFB sputum positivity irrespective of whether the patient has TB-HIV co-infection or not. This is thus suggestive of positive clinical effects of supplementation of vitamin C on tuberculosis treatment outcomes in this environment. However, scaled community-based studies in both children and adults supported by dietary consumption studies may provide better evidence of this intervention.

Acknowledgments

Conflict of interest

We declare that we have no conflict of interest regarding this study.

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