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ORIGINAL ARTICLE

Vitamin D deficiency and its associated factors in active TB patients at a tertiary hospital and three primary health care level facilities in Lusaka, Zambia: A cross sectional analytical study

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ABSTRACT

Background: Tuberculosis (TB) remains a leading infectious cause of morbidity and mortality in Zambia. Vitamin D deficiency has been associated with increased TB incidence and severity but data for the Zambian setting is lacking, inclusive of the general population. We sought to determine the prevalence of vitamin D deficiency and its associated factors in active TB patients in comparison to matched adults from the general population without TB, and to compare clinical and radiological severity of TB based on vitamin D status.

Methods: We enrolled 89 TB patients and 78 matched adults from the general population. Demographic and clinical data was collected. The key findings on physical examination were body mass index (BMI), mid upper arm circumference (MUAC), and signs of undernutrition. Clinical severity was assessed using the TB I Score and the Karnofsky Performance Index (KPI).

Chest x-ray and measurement of serum vitamin D levels were done. Radiological severity was assessed using the Timika chest x-ray score. Vitamin D was measured using the *Cobas® E411* analyser from Roche Diagnostics (Germany) with vitamin D deficiency defined as <30 ng/ml and chi square used to analyse vitamin D deficiency as a categorical variable.

Results: The median (IQR) age was 27.5 (24-38) years in the TB patients and 32 (25-37) years in the non-TB adults (p=0.30). Sixty-nine (77.53%) of the TB patients and 57 (73.08%) of the comparison group were male (p=0.51). The prevalence of vitamin D deficiency was 33.71 % in the TB patients and 15.38% in the comparison group (p <0.01). Associations with vitamin D deficiency were active TB (AOR =2.27; 95% CI = 1.04- 4.95; p=0.04) and undernutrition (AOR 14.5; 95% CI 1.65-126.97; p=0.02). Median (IQR) KPI was 70 (60-80) in the vitamin D deficient patients compared to 80 (70-90) in non-vitamin D deficient patients (p=0.01). Median (IQR) Timika chest x-ray score was 75 (45-115) in the vitamin D deficient TB patients compared to 42.5 (20-75) in the non- vitamin D deficient patients (p<0.01).

Keywords: Tuberculosis; Vitamin D deficiency; Undernutrition

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Conclusions*:* We found a significant difference in the prevalence of vitamin D deficiency in active TB patients compared with matched non-TB adults. Vitamin D deficiency was associated with active TB and undernutrition. TB patients with vitamin D deficiency had more clinical and radiological severe disease than those with normal vitamin D levels. These findings warrant further studies on the role of vitamin D supplementation in TB in Zambia.

INTRODUCTION

Tuberculosis (TB), an infection caused by the Mycobacterium Tuberculosis complex is an ancient disease that remains a public health concern[1]. According to the World Health Organization, 5.8 million people contracted TB in 2020 with over a million deaths worldwide[2]. Africa continues to be one of the most affected continents. Zambia is a high burden TB country with an estimated TB incidence rate of 319 per 100,000 in 2021[3].

Vitamin D is a fat soluble vitamin that is acquired from dietary sources and synthesised in the skin under the influence of ultraviolet light[4]. Though it has classically been associated with calcium metabolism, research has linked vitamin D with immunity against TB[5]. Vitamin D receptors have been identified on macrophages and lymphocytes, cells that are involved in the immune response against TB[6]. The mechanism for this effect is thought to be the up-regulation of the synthesis of anti-mycobacterial peptides such as cathelicidin and β-defensin[7]. Research has validated a positive correlation between vitamin D levels and cathelicidin levels[8].

Consequently, low vitamin D status has been associated with an increased risk of contracting TB[9]. A study in Pakistan showed that close contacts of TB patients with low vitamin D levels were five times more at risk of TB than those with normal levels[10]. Ethiopian studies showed a higher prevalence of vitamin D deficiency in TB patients compared to controls[11,12]. Research has revealed increased severity of TB symptoms in vitamin D deficient TB patients[13]. In contrast, studies from Gambia and Zimbabwe found associations between elevated vitamin D levels and TB[14,15].

Meanwhile Zambia continues to grapple with the onslaught of TB. An estimated 15,000 Zambians succumbed to the disease in 2021. This is compounded by the rise in the amount of drug resistant TB[16]. Despite the link between TB and vitamin D, and Zambia being heavily burdened with the disease, vitamin D levels in Zambian TB patients and the general population remain unknown[17]. This study therefore aimed to determine the prevalence of vitamin D deficiency and its associated factors in active TB patients and matched adults without TB from the general population, and compare the clinical and radiological severity TB in patients, according to vitamin D status.

MATERIALS AND METHODS

Study Area

The study took place in Lusaka, Zambia between May 2020 and December 2020, at University Teaching Hospitals -Adult Hospital (UTH) and three first level hospitals levels (Chawama, Chipata and Kanyama) in Lusaka, Zambia. Recruitment of participants was initially scheduled to be held at the UTH only, but the coming of COVID-19 saw a drastic drop in TB patients presenting to the facility, so the three first level hospitals were added because of their relatively high TB numbers and their being in Lusaka. This enabled the sample size to be met and the study to be adequately powered.

UTH is the highest referral centre in Zambia with TB treatment at the facility being dispensed by the chest clinic. It attends to up to 900 patients yearly. Kanyama first level hospital is a 139-bed capacity facility with a catchment population of 96,465 people and a TB clinic with an average patient of 30 patients per week. Chawama first level hospital is a 74-bed capacity facility with a catchment population of 130,000 people and a TB corner dispensing treatment to around 300 patients weekly. Chipata first level hospital is a 71-bed capacity centre with a chest clinic that attends to up to 200 TB patients weekly.

Study Population

TB patients aged 18 and above were conveniently sampled from the chest clinics and medical wards at the recruitment centres. The participants in the comparison group were matched with the TB patients for age group, gender, and HIV status. They were recruited among blood donors at the UTH blood bank, outpatient clinics among those attending routine medicals for employment and

education purposes, and the Adult Centre of Infectious Diseases among stable HIV patients doing routine drug refills.

Case Definitions

An active TB patient was defined as one whose diagnosis had been confirmed by either sputum acid fast bacilli staining, Gene X-pert® or TB culture and had been on treatment for less than seven days. Patients that had been on Rifampicin based treatment for more than one week, were excluded from the study. The rationale behind this is the potential lowering effect of Rifampicin on serum vitamin D levels. Serum vitamin D has a half-life of two weeks, therefore excluding patients that had taken Rifampicin for one week or more guarded against its confounding effect. A non-TB adult was defined as a person above the age of 18 with no complaints at the time of recruitment and negative for the symptoms for TB and no radiological or microbiological evidence of TB.

Participants with a history of TB, vitamin D supplementation within three months, household contacts of TB patients (defined per national TB guidelines as a person that had shared the same enclosed living space with the index case 3 months before the diagnosis of TB), as well as those not consenting to an HIV test were excluded.

Collection of Data

Data was collected and entered onto a data entry tool. Demographics included the participant's age, gender, residential area, and employment status. The participants estimated the duration of their daily sunlight exposure. A history of alcohol and tobacco use was elicited. Karnofsky Performance Index (KPI) was graded by an experienced clinician to assess functional status. KPI is an assessment tool for functional impairment that has been used in Tuberculosis[18]. The parameters that are assessed include the ability to carry out normal work, ability for self-care and the need for hospitalisation. The scale ranges from 0-100 with a lesser score implying a greater degree of functional impairment.

All the study participants had their BMI and MUAC taken. Participants were assessed for undernutrition with markers being, wasting of temporal muscles, thin hair, scaling of the skin, angular stomatitis, and oedema[19].

Participants had their signs and symptoms aggregated into the TB I Bandim Score as another marker of disease severity[20]. This is a scoring system based on the number of symptoms in a TB patient. More symptoms denote a higher score and vice versa.

Investigations included serum albumin and a chest radiograph. Interpretation of the chest x-ray was carried out by a radiologist. The Timika Chest X-ray score was used to grade the radiological extent of disease[21]. This is a scoring system for x-rays that is based on the proportion of the lung fields that are involved in the disease process. A greater score indicates more lung involvement and contrarywise.

Measurement of Vitamin D

Serum was extracted from 4 mls of collected blood and stored at -80˚C. Measurement of 25(OH) D was done using the *Cobas® e411* analyser from Roche Diagnostics (Germany) using the *Elecsys Vitamin D total II* assay which uses the mechanism of employing a vitamin D binding protein to capture 25-hydroxyvitamin D. It measures 25 (OH) D levels in the ranges of 3-100 ng/ml. Vitamin D deficiency was defined as a serum level of 25 hydroxyvitamin D of <30ng/ml.

Statistics

Statistical analysis for the study was carried out using the Stata® version 14 from the Stata Cooperation (USA). The outcome variable was the presence or absence of vitamin D deficiency. Independent variables showing disease severity were KPI, TB I Bandim Score and the Timika Chest X-ray score. Vitamin D deficiency was analysed as a categorical variable using chi square. KPI, TB I Bandim Score and Timika Chest X-ray score were analysed as continuous variables. The Shapiro -Wilk test was used to assess them for distribution pattern. The TB I Score was normally distributed while the KPI and Timika chest x-ray score had skewed distributions. We assessed the TB I Score using the t-test while the Mann Whitney test was used for the KPI and Timika Chest X-ray score.

The other independent variables were classified into categorical and continuous variables. The categorical variables included gender, HIV status, TB status, area of residence, season of recruitment, alcohol and tobacco use, and presence or absence of undernutrition. These were

expressed as proportions and analysed using the chi square or fisher's exact tests for those values that had a value of less than five in the two-by-two tables.

The main continuous variables were age, hours of sunlight exposure, BMI, MUAC, serum albumin and the creatinine clearance. The Shapiro-Wilk test was used to assess all the continuous variables for normality of distribution. The BMI, MUAC, serum albumin and creatinine clearance were normally distributed while the age and hours of sunlight exposure had skewed distributions. The normally distributed variables were analysed using the t-test while the skewed variables were analysed with the Mann Whitney test. Factors associated with vitamin D deficiency were assessed using a multivariate logistic regression model.The study was powered at 80%. For all the analysed variables, a p value of less than 0.05 indicated statistical significance.

Ethical Considerations

This study was approved by University of Zambia Biomedical and Research Ethics committee under approval number 398-2019, and the National Health Research Authority of Zambia. Written, informed consent was obtained from all the participants.

RESULTS

Study Process

A total of 180 participants were enrolled, among which 89 (49.44 %) were TB patients and 91 (50.56%) were adults without TB from the general population. All the enrolled TB patients and 78 (85.71%) of the comparison group were included in the analysis. We included 62 (69.70 %) of the 89 TB patients in the radiological analysis as 27 patients were missing chest x-ray images due to malfunction of chest x-ray machines at some of the study centres. Six (6.70%) of the comparison group were excluded based on clinical grounds while 7 (7.69%) denied consent after initially agreeing to participate (figure 1).
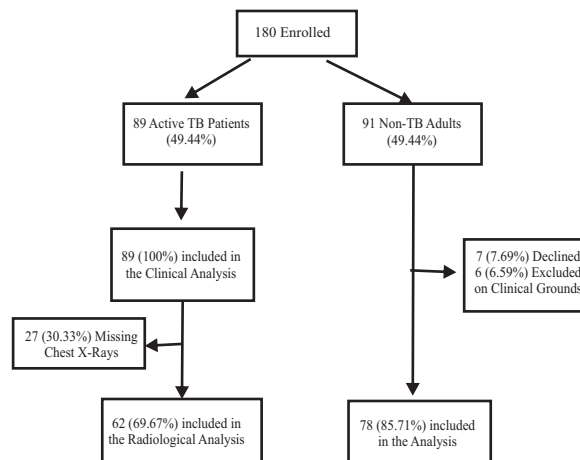

Figure 1. Participant Enrolment

Baseline Characteristics of Study Participants

Sixty-nine (77.53%) of the TB patients and 57 (73.08%) of the comparison group were male (p=0.51). The median (IQR) age for the TB patients was 27.5 (24-38) years and 32 (25-37) years in the comparison group (p=0.30). The number of employed participants was 68 (76.4%) in the TB group and 67 (85.90%) in the comparison group respectively (p<0.01). Thirty-one (34.83%) of the TB patients and 28 (35.90%) of the non-TB adults were HIV positive (p=0.89). The median (IQR) duration of sunlight exposure was 1.5(1-4) hours in the TB patients and 2 hours (1-4) in the comparison group (p=0.38).

Among the TB patients, 51(57.30%) admitted to using alcohol while this number was 33 (42.31%) in the non-TB adults (p =0.06). Regarding tobacco use, 42 (47.19%) of the TB patients and 5 (6.41%) from the non -TB group admitted to using tobacco respectively (p<0.01). The mean (±SD) BMI in the TB patients was 18.22 (± 2.55) $kg/m^2$and 23.10 (± 3.74) kg/m^2in the comparison group (p<0.01). The mean (±SD) MUAC was 23.46 (± 3.67) cm in the TB patients and 27.73 (± 3.25) cm in the comparison group (p<0.01).TB patients had a mean (±SD) serum albumin of 32.22 (± 5.57) g/l in comparison to the adults from the general population that had a mean serum albumin of 40.35 (± 4.18) g/l (p<0.01) (Table 1).

Table 1. Baseline characteristics of study participants

	TB Patients		**Non-TB adults**		
	n (%)	Mean (±SD)	n (%)	Mean (±SD)	p-value
	89 (100)	Median [IQR]	78 (100)	Median [IQR]	
Gender					
Male	69 (77.53)		57 (73.08)		0.51
Female	20 (22.47)		21 (26.92)		
Age (years)		27.5[24- 38]		32 [25-37]	0.30
HIV Positive					
No	58 (65.17)		50 (64.10)		0.89
Yes	31 (34.83)		28 (35.90)		
Area of Residence					
UnplannedHigh Density	70 (78.65)		26 (33.33)		<0.01
Non-Unplanned High Density	19 (21.35)		52 (66.67)		
Median (IQR) Sun Exposure (hrs.)		1.5 [1-4]		2 [1-4]	0.38
Nature of Occupation					
Indoor	25 (28.09)		49 (62.82)		<0.01
Outdoor	43 (43.31)		18 (23.08)		
Unemployed	21 (23.60)		11 (14.10)		
Alcohol					
No	38 (42.70)		45 (57.69)		0.06
Yes	51 (57.30)		33 (42.31)		
Smoking					
No	47 (52.81)		73 (93.59)		<0.01
Yes	42 (47.19)		5 (6.41)		
Nutritional Status					
Mean (±SD) BMI (kg/m^2)		18.22 (±2.55)		23.1 (±3.74)	<0.01
Mean (±SD) MUAC (cm)		23.46 (±3.67)		27.73(±3.25)	<0.01
Undernutrition*					
No	82 (92.13)		78 (100)		0.02
Yes	7 (7.87)		0 (0.00)		

*Defined by the presence of temporal muscle wasting, thin hair, scaling skin andoedema

Prevalence of Vitamin D Deficiency in TB Patients and Matched non-TB Adults from the general population.

When analysed for the prevalence of vitamin D deficiency, 30 (33.71 %) of the TB patients and 12 (15.38%) of the comparison group were vitamin D deficient respectively (p<0.01) (Figure 2).
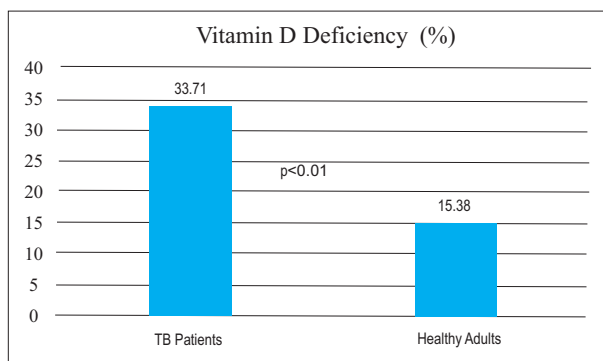

Figure 2. Prevalence of vitamin D deficiency in TB patients and healthy adults from the general population.

Comparison of TB Patients based on vitamin D status.

In terms of gender, 24 (80%) of the deficient patients and 45 (76.27%) in the non- deficient patients were male (p=0.69). The median (IQR) age in the patients with vitamin D deficiency was 32 (28-32) years, compared to 29 (25-37) years in the patients with normal vitamin D levels (p=0.58). Ten (33.33 %) of the deficient patients were in-patients while 3 (5.08%) of non-deficient patients were admitted respectively (p<0.01).

Twelve (40 %) of the patients with suboptimal vitamin D levels had HIV in comparison to 19 (32.20%) from the patients with normal vitamin D levels (p=0.47). The median (IQR) symptom duration in the deficient TB patients was 3 (2-12) weeks, while this number was 4 (2-9) weeks in the non-deficient patients (p=0.14).With regards to nutritional status, 19 (63.33%) of the vitamin D deficient patients had a BMI less than 18.5 kg/m^2 in comparison to 28 (47.46%) in the non-deficient group (p=0.16). A MUAC of less than 22 cm was present in 12 (40%) of vitamin D deficient patients and 14 (23.73%) of non-vitamin D deficient TB patients (p=0.11). Six (20%) of the vitamin D deficient patients and 1 (1.69%) of the non-deficient patients were found with signs of undernutrition (p <0.01).

Regarding disease severity, the mean (±SD) TB I score in the vitamin D deficient group was 5.98 (±2.17) and 6.36 ((±2.19) in the non-deficient group (p=0.43). The median (IQR) Karnofsky Performance Index was 70 (60-80) in the deficient patients and 80 (70-90) in the non-deficient patients respectively (p < 0.01). The median (IQR) Timika chest x-ray score was 75 (45-115) in the deficient patients and 42.5 (20-75) in the non-deficient patients respectively (p=0.01) (Table 2).

Table 2. Analysis of TB Patients based on Vitamin D Status **.**

	Vitamin D Deficient TB Pts (<30 ng/ml)		Non-Vitamin D Deficient TB Pts (30 ng/ml)		
	n (%) 30 (100)	Mean(±SD), Median [IQR]	n (%) 59 (100)	Mean(±SD), Median [IQR]	p-value
Demographics					
Gender					
Male	24 (80.00)		45(76.27)		0.69
Female	6 (20.00)		14 (23.23)		
Age		32 [28-38]		29 [25-37]	0.58
Patient Setting					
Outpatient	20(66.67)		56 (94.92)		<0.01
In-Patient	10 (33.33)		3 (5.08)		
HIV Status					
Negative	18 (60.00)		40 (67.80)		0.47
Positive	12 (40.00)		19 (32.20)		
Median (IQR) Symptom Duration (weeks)		3 [2-12]		4 [3-9]	0.14
Physical findings					
BMI (kg/m$^{2)}$)					
18.5	11 (36.67)		31(52.54)		0.156
<18.5	19 (63.33)		28 (47.46)		
MUAC (cm)					
22	18 (60.00)		45(76.27)		0.11
<22	12 (40.00)		14(23.73)		
Overt Malnutrition					
No	24 (80.00)		58 (98.31)		<0.01
Yes	6 (20.00)		1(1.69)		
Clinical Severity					
Mean (±SD) TB I Score		5.98 (± 2.17)		6.36 (± 2.19)	0.43
Median (IQR) KPI		70 [60-80]		80 [70-90]	0.01

Factors Associated with Vitamin D Deficiency

In the unadjusted model for all predictor variables, the factors associated with vitamin D deficiency were active TB (COR= 2.80; 95% CI=1.31-5.96; p <0.01), a BMI less than 18.5 kg/m^2 (COR=2.50; 95% CI = 1.21-5.20 p=0.01) and undernutrition (COR=20.66; 95% CI = 2.4-177.2 p<0.01). After controlling for other variables, the associated factors with vitamin D deficiency were active TB (AOR=2.27; 95% CI = 1.04-4.95; p=0.04) and undernutrition (AOR=14.5; 95% CI = 1.65-126.97; p=0.02) (Table 3).

Table 3. Associated Factors with Vitamin D Deficiency.

Guinea Bissau (9%), but it is lower than reports from London by Ustianowski et al (40%) and Workineh et al in Ethiopia (86%). Possible explanations for this finding are discrepancies in the diets of the populations, variations in the climate they are exposed to, different vitamin D measurement methodologies and thresholds of vitamin D deficiency[24]. There are varying definitions of vitamin D deficiency. The threshold of 30 ng/ml was used in this study because the vast majority of experts agree that levels below this number are suboptimal[22]. Among the comparison group, 15.38% had vitamin D deficiency. This finding is at odds with findings by Jaimni et al in India (26%) and Taseem et al in Ethiopia (38%). Apart from the above reasons, an additional cause for this difference could be the inclusion of some contacts of the TB patients as a comparison group in their studies.

Variable	COR	p-value	AOR	p-value
TB Status				
Non-Active TB	1		1	
Active TB	2.80 (1.31-5.96)	<0.01	2.27 (1.04-4.95)	0.04
Nutritional Status				
BMI (kg/m^2)				
18.5	1		1	
<18.5	2.50 (1.20 -5.20)	0.01	1.34 (0.54- 3.29)	0.52
Undernutrition*				
No	1		1	
Yes	20.66 (2.4- 177.2)	<0.01	14.5 (1.65-126.97)	0.02

*Defined by the presence of temporal muscle wasting, thin hair, scaling skin and oedema[19]

DISCUSSION

Our findings reveal a higher prevalence of vitamin D deficiency in TB patients in comparison to matched adults without TB from the general population. This is in agreement with most literature[6]. This study augments the findings of Lungu et al in a Zambian case series where TB patients were found to be vitamin D deficient[23]. A prevalence of vitamin D deficiency of 33.71% in the TB patients from our study is higher than the findings of Nouri Vaskeh et al in Iran (13.3%) and Wejste et al in

The association between vitamin D deficiency and active TB in our study concurs with literature linking low vitamin D to impaired immunity against TB[25]. Vitamin D has been shown to aid anti-mycobacterial activity by inducing the production of antimicrobial peptides from the cathelicidin family and enhancing the capacity of macrophages to kill MTB[7]. The other associated factor with vitamin D deficiency was undernutrition. This is in agreement of the findings by Meker et al on the high prevalence of vitamin D deficiency in undernourished TB patients[26]. This could be explained by the fact that TB patients with malnutrition have both poor intake of food

and malabsorption of nutrients, among which is vitamin D[27]. A breakdown of the TB patients based on vitamin D status found a significant association between being admitted in hospital and being vitamin D deficient. Banda et al in Malawi had similar findings[28]. A plausible reason for this finding is that patients requiring admission are not exposed to as much sunlight as outpatients[29].

TB patients with vitamin D deficiency had a lower Karnofsky Performance index than the non-deficient TB patients and a lower (though not statistically significant) TB I Score. These findings add up to a higher level of clinical severity which agrees with findings by Farazi et al that showed a higher disease severity in TB patients with lower vitamin D levels[13]. We also noted that the vitamin D deficient TB patients had more radiological involvement compared to the non-vitamin D deficient patients. This could be as a result of the link between vitamin D deficiency with a more damaging inflammatory response and less repairing immunomodulatory response[6]. We did not find a significant difference in HIV status between the deficient and non-deficient TB patients which was contrary to findings in literature[30]. This may be because *Efavirenz* an antiretroviral which is has been implicated in low vitamin D levels has been phased out of the 1st line regimen in Zambia[31].

Study limitations

The cross-sectional design of our study means causation cannot be proved. We did not match the TB patients and the comparison group for social economic status which is a proxy for factors that could affect vitamin D levels such as access to nutrition. Another limitation is that some of our participants from the TB group were in-patients in contrast to the comparison group. Weighting to the sample size calculation was not added for the different recruitment centres so this may have skewed the results.

CONCLUSIONS

We found a significant difference in the prevalence of vitamin D deficiency in active TB patients compared with matched non-TB adults from the general population. Vitamin D deficiency was associated with active TB and undernutrition. Vitamin D deficiency in active TB was associated with more severe disease clinically and radiologically. The above findings warrant further studies on the role of vitamin D and TB possible vitamin D supplementation in TB in a high burden setting like Zambia.

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