

Original Article

Assessment of Vitamin D 25(OH)D Status in Patients with Tuberculous Pleural Effusion in a Tertiary Care Hospital: A Cross-Sectional Study

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Abstract: Tuberculous pleural effusion (TPE) remains a significant health concern globally, particularly in countries like Bangladesh where the burden of tuberculosis is substantial. It has posed a great challenge to us in its management due to increasing drug resistance and the long duration of treatment. Vitamin D has a recognized role in immune modulation and body defense against infection. This study aimed to investigate the distribution of Vitamin D deficiency, insufficiency and sufficiency among tuberculous pleural effusion patients and its association with different socio-demographic as well as clinical characteristics. This cross-sectional study was conducted in the Department of Respiratory Medicine of Bangabandhu Sheikh Mujib Medical University (BSMMU), Bangladesh. A total of 40 participants diagnosed with tuberculous pleural effusion (TPE) were included in this study. TPE was confirmed by pleural biopsy. Comparison of categorical and continuous variables were done using the Chi square (χ^2) tests and one way analysis of variance (ANOVA) respectively. P-values of less than 0.05 were considered statistically significant. Informed written consent was obtained from the participants before starting the study, approval was taken from the institutional review board (IRB), BSMMU (BSMMU/2022/5159). The mean age of the 40 participants were 35.28 ± 14.01 years and the male-female ratio was about 2:1 (67.5% vs 32.5%). Fever, cough, anorexia, and weight loss were prevalent presenting symptoms (100%). In this study, the mean value of serum vitamin D was higher in males than females (16.9 ± 4.7 vs 15.6 ± 4.0 ng/ml). In this study, about 75% of participants had vitamin D deficiency and 15% participants had insufficient vitamin D. In the bi-variate analysis, age was found to be significantly associated with vitamin D status (p -value < 0.05) and participants from 31 to 40 years were more vulnerable to vitamin D deficiency. In this study, we concluded that serum vitamin D deficiency was higher in TPE participants. Therefore, adequate management of vitamin D and proper supplementation can enhance the immune system and reduce the vulnerability of tuberculosis.

Keywords: Vitamin D, Tuberculous Pleural Effusion, Serum Profile, Pleural Fluid, Bangladesh

1. INTRODUCTION

Tuberculosis (TB) is an ancient preventable, treatable disease and the major leading cause of mortality worldwide from a single infectious agent *Mycobacterium tuberculosis* (MTB). According to World Health Organization (WHO) reports 2020, Globally an estimated 10.0 million (range, 8.9-11.0) people are affected with TB. There were 1.2 million (range, 1.1-1.3 million) TB deaths among HIV-negative people, an additional 208000 deaths (range, 177000-242000) among HIV-positive. Though the mortality and morbidity have come down with the advent of DOTS, further research is needed to bring down the disease severity in future era. According to sustainable development (SDG) goals, the WHO adopted a strategy to end the TB epidemic by 2030. The Discovery of *Mycobacterium Tuberculosis* dates back to 150 million years ago [1]. Ancient Greeks described TB as "Phtisis", the works of Hippocrates document Phtisis to be a fatal disease in young adults and his work does elaborate classical signs and symptoms along with characteristic lung lesions [1]. It was in 1882 that Robert Koch isolated *Mycobacterium Tuberculosis* bacillus and started a new renaissance in the field of TB. In the pre antibiotic era TB was initially treated with the advent of sanatoriums where people were isolated. With the days many novel approaches like artificial pneumothorax, thoracoplasty, plombage and lung resection was used [2]. Vitamin D, a fat-soluble vitamin was one of the adjuncts in the TB treatment in pre antibiotic era. Administration of Cod liver oil which contained Vitamin D was associated with the increased cure rates [3]. About 80% of cases of tuberculosis are pulmonary and extrapulmonary tuberculosis (EPT) accounts for about 20-25% of all TB cases. Among EPT cases, pleural effusion is one of the most common sites of involvement and virtually every site of the body can be affected [4]. TPE is contributed to 30% to 80% of all pleural effusions [5]. Tuberculous pleural effusion (TPE) is a form of extrapulmonary tuberculosis that is frequently encountered everywhere as a cause of the exudative pleural effusion. Specifying the risk factors for TB is urgent to target the preventive programs. Various factors were documented including human immunodeficiency virus (HIV) infection, female sex, younger age associated with diabetes mellitus, smoking, alcohol use, under-nutrition [6], and vitamin D deficiency (VDD) [7]. Vitamin D is a fat-soluble vitamin that has a vital role in cell growth, immunity, and calcium homeostasis by intestinal absorption of calcium and phosphate. Vitamin D is produced in human skin from 7-dehydrocholesterol due to exposure to ultraviolet B rays (UVB: 280–315 nm range) from sunlight. The subcutaneous production by UVB exposure is the major source of vitamin D and dietary sources include dairy products or fish liver oil [8]. Vitamin D from skin and diet is metabolized in the liver to 25-hydroxyvitamin D (25(OH)D), which is used to assume the patient's vitamin D status. This 25(OH)D is transformed to its active form, 1,25-dihydroxy vitamin D {1,25(OH)₂D} in the kidney by the enzyme 25-hydroxyvitamin D-1 α hydroxylase [9]. Vitamin D deficiency is associated with the risk of tuberculosis (TB) infection [10]. Vitamin D deficient individuals have a greater susceptibility to developing TB [11] and worse disease progression if infected with TB [12]. The likely mechanism through which vitamin D may prevent or limit infection by *Mycobacterium Tuberculosis* is through the binding of the bioactive form of vitamin D (1,25-dihydroxycholecalciferol) to the vitamin D receptor (VDR), a polymorphic nuclear receptor that regulates the expression of genes important for immune function and involved in cytokine production [13] which have a protective effect against TB infection. The VDR is present in immune cells [14] and bronchial and pulmonary epithelial cells [15], and is up-regulated following the ligation of specific toll-like receptors (TLRs) during an antimicrobial response. The antimicrobial activity of Toll-like receptors (TLRs) depends on the presence of vitamin D [16]. Therefore, VDD and any structural or functional defect in its receptor can lead to an impairment of host immunity against MTB [17]. Through this mechanism, calcitriol induces several endogenous antimicrobial peptides, specifically cathelicidin LL-37 and β defensin, and suppresses matrix metalloproteinase enzymes that degrade the pulmonary extracellular matrix [18]. and accelerates Tuberculosis intracellular death [19]. Studies have shown that there is a relationship between the deficiency of vitamin D and extrapulmonary tuberculosis [20]. It is

estimated that 1 billion people worldwide are vitamin D deficient (i.e., circulating 25(OH)D concentrations <20 ng/mL) and 50% population have insufficient vitamin D status (i.e., 25(OH)D <30ng/dl). Aging decreases the ability of the skin to synthesize vitamin D and increased skin pigmentation reduces the efficacy of UV-B to stimulate the synthesis of vitamin D. The prevalence of vitamin D deficiency is highest in the elderly (61%), the obese (35% higher than in nonobese), nursing home residents (50–60% of nursing home and hospitalized patients), and those with higher melanin in their skin (40%). Vitamin D status was defined as follows: deficiency 25(OH)D is 0 to <20 ng/ml, insufficiency 20 to <30 ng/ml, sufficiency 30-100 ng/ml [21]. Thus, this study has been conducted to see the assessment of vitamin D 25(OH)D status in patient with tuberculous pleural effusion in a tertiary care hospital in Bangladesh.

2. MATERIALS & METHOD

A Cross-sectional study done at Department of Respiratory Medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka. Study population includes Patients diagnosed with tuberculous pleural effusion. Non-probable consecutive sample collection has been done. The serum concentration of 25(OH)D was measured by chemiluminescence microparticle immunoassay (LIAISON Analyzer). Vitamin D deficiency has been defined as 25(OH)D <20ng/ml. The sample size was calculated by using the following formula [22]:

$$N = \frac{\left\{u\sqrt{[\pi(1-\pi)]} + v\sqrt{[\pi_0(1-\pi_0)]}\right\}^2}{(\pi - \pi_0)^2}$$

Where,

N = estimated sample size

u = 0.52

v = 1.96

π = Population proportion (subjects with TB) with vitamin D deficiency = 32% or 0.32 [23]

π_0 = Population proportion under the null hypothesis= 15% or 0.15

Taking alpha (significance level) at 0.05

So,

$$\begin{aligned} N &= \frac{\left\{u\sqrt{[\pi(1-\pi)]} + v\sqrt{[\pi_0(1-\pi_0)]}\right\}^2}{(\pi - \pi_0)^2} \\ &= \frac{\left\{0.52\sqrt{[0.32(1-0.32)]} + 1.96\sqrt{[0.15(1-0.15)]}\right\}^2}{(0.32-0.15)^2} \\ &= \frac{(0.2425+0.6998)^2}{0.0289} \\ &= \frac{(0.9423)^2}{0.0289} \end{aligned}$$

$$=30.72 \approx 31$$

According to the above formula, the samples size is 31. However, the final sample size was considered as 40. A forty patients fulfilling criteria. Data was collected in a structured data collection sheet. The patient was explained properly about the purpose, procedure, potential physical and psychosocial risks and right to refuse to participate. They were enrolled upon their voluntary agreement. Those who fulfilled the inclusion and exclusion criteria were taken as study populations for this study. Then after obtaining informed written consent, a thorough history and physical examination were done. Pleural fluid aspiration and pleural biopsy were done at sitting forward, leaning on a pillow over the table, with their arms folded in front of them. Skin, intercostal muscle and parietal pleural were infiltrated with 10 - 20ml (up to 3 mg/kg) of 2% lidocaine. A small (5mm) skin incision followed by intercostal muscle was dissected with blunt forceps. Abram's biopsy needle was inserted into pleural space then fluid was withdrawn by attaching a syringe to the needle and opening the port. The biopsy was taken by attaching a syringe to the needle. The biopsy port was opened and angled downwards, then pull the biopsy port firmly against the parietal pleura on the rib beneath the entry point. Close the biopsy port, pulling a parietal pleura sample into the needle. The biopsy needle was removed; opened the biopsy port and the biopsy sample was collected. This procedure was repeated 4-6 times in position 4-8 o'clock. The sample was sent in saline for analysis for tuberculosis and in formalin for histopathological examination. A dressing was applied to the biopsy site and if needed stitch was given. Pleural fluid was sent for the cytological and biochemical study which includes total protein, glucose, LDH, and ADA. With all aseptic precautions, 5 mL of venous blood was drawn from the antecubital vein of each participant in a disposable plastic syringe and delivered immediately into a clean tube, which was kept in a standing position till clot formation. The blood samples were centrifuged at 3000 rpm (about 2500 $\times$ g) for 10 min for isolation of serum. The serum samples were then stored at -20° C at the Department of Biochemistry, Bangabandhu Sheikh Mujib Medical University (BSMMU). Serum 25(OH)D was used to evaluate the vitamin D status among the study subjects. The laboratory analysis was carried out at the Department of Biochemistry, BSMMU. The serum concentration of 25(OH)D was measured by chemiluminescence microparticle immunoassay (LIAISON Analyzer). A structured questionnaire: a structured questionnaire containing items to elicit socio-demographic information and relevant information about physical illness. A checklist of investigation findings. The data was analyzed by using the SPSS Package (version 25).

3. RESULTS & DISCUSSION

Table 01: Frequency and percentage distribution of respondents' socio-demographic characteristics

Demographic characteristics	Frequency (N)	Percentage (%)
<i>Age group (in years)</i>		
<i>Mean ($\pm$ SD) = 35.28$\pm$ 14.01</i>		
18 – 30	18	45.0
31 – 40	8	20.0
41 – 50	8	20.0
51 – 60	3	7.5
61 and above	3	7.5
<i>Gender</i>		
Male	27	67.5
Female	13	32.5
<i>Marital Status</i>		
Married	29	72.5

Unmarried	11	27.5
<i>Educational level</i>		
Up to class 5	9	22.5
Class 6 to HSC	23	57.5
Graduate and higher	8	20.0
<i>Occupation</i>		
Housewife	10	25.0
Service holder	9	22.5
Day labor	9	22.5
Businessperson	6	15.0
Student/unemployed	6	15.0
<i>Ever smoking history</i>		
No	32	80.0
Yes	8	20.0

SD, standard deviation

Table 02: Frequency and percentage distribution of respondent's comorbidities

Comorbidities	Frequency (N)	Percentage (%)
<i>Body Mass Index (BMI) (kg/m²)</i>		
Underweight	15	37.5
Normal	23	57.5
Overweight	2	5.0
<i>Hypertension</i>		
No	38	95.0
Yes	2	5.0
<i>Diabetes Mellitus</i>		
No	36	90.0
Yes	4	10.0

Table 03: Frequency and percentage distribution of clinical characteristics of the respondents

Clinical characteristics	Frequency(N)	Percentage(%)
<i>Fever</i>	40	100.0
<i>Mean duration (in days) (±SD)</i>	41.2 ± 19.8	
<i>Cough</i>	40	100.0
<i>Mean duration (in days) (±SD)</i>	40.9 ± 20.1	
<i>Shortness of breath</i>	16	40.0
<i>Mean duration (in days) (±SD)</i>	38.3 ± 21.5	
<i>Chest pain</i>	21	52.5
<i>Mean duration (in days) (±SD)</i>	40.2 ± 21.9	
<i>Weight loss</i>	40	100.0
<i>Mean duration (in days) (±SD)</i>	4.6 kg in 40 days	
<i>Anorexia</i>	40	100.0
<i>Mean duration (in days) (±SD)</i>	40.4 ± 19.8	
<i>Anemia</i>		
Absent	29	72.5

Present	11	27.5
<i>Cervical lymphadenopathy</i>		
Enlarged	2	5.0
Not enlarged	38	95.0
<i>Pleural effusion</i>		
Left sided	21	52.5
Right sided	19	47.5

Table 04: Sex-specific mean distribution of the clinical variables of the respondents

Clinical variables (<i>mean</i>)	Male	Female	Reference*
<i>Pulse (per min)</i>	81.9	80.0	60 – 100
<i>Blood Pressure</i>			
Systolic	114.07	110.0	120
Diastolic	72.9	67.7	80
<i>Body temperature (F)</i>	99.2	99.0	98.6
<i>Respiratory rate (per min)</i>	18.4	18.9	12 – 16
<i>SpO₂ (per min)</i>	96.9	96.2	95 – 100%

* References obtained from American Board of Internal Medicine (ABIM) Laboratory Test Reference Ranges

Table 05: Sex-specific mean distribution of the laboratory variables of the respondents

Laboratory variables (<i>mean</i>)	Male	Female	Reference*
<i>Complete blood count</i>			
HB (grams/dL)	12.4	10.4	Male: 13.2 – 16.6 Female: 11.6 – 15
ESR (mm in 1 st hr.)	53.6	58.8	Male: <15 Female: <20
WBC (cells/m cL)	8.8×10 ³	9.99×10 ³	4.5 – 11×10 ³
Neutrophil	72.1	72.5	40 – 75%
Lymphocyte	19.4	19.7	20 – 50%
RBS (Random Blood Sugar) (mmol/L)	6.7	7.7	<7.8

* References obtained from American Board of Internal Medicine (ABIM) Laboratory Test Reference Ranges

Table 06: Sex-specific descriptive statistics (mean, SD) of the serum profile and pleural fluid profile of the respondents.

Serum Profile	Male (mean ±SD)	Female (mean ±SD)
Vitamin D (ng/dl)	16.9 ±4.7	15.6 ±4.0
Protein (gm/L)	74.4 ±29.1	73.1 ±27.6
LDH (U/L)	224.2 ±196.6	262 ±202.2
Creatinine (mg/dL)	0.80 ±0.31	0.74 ±0.28

ALT (Alanine Transaminase) (U/L)	35.9 ±16.4	26.9 ±12.1
Pleural fluid profile		
Lymphocyte	90.7 ±30.8	84.2 ±28.2
Neutrophil	9.3 ±3.8	15.9 ±5.5
Protein (gm/L)	57.3 ±21.2	58.3 ±21.9
Adenosine Deaminase (ADA)	54.5 ±20.5	47.8 ±18.7
LDH (U/L)	449.0 ±190.1	463.5 ±206.4
Glucose (mmol/L)	4.9 ±1.1	4.0 ±0.9

Table 07: Frequency and percentage distribution of Vitamin D status of the respondents

Vitamin D status	Frequency (n)	Percentage (%)
Deficiency (< 20 ng/dl)	30	75.0
Insufficiency (20 – 30 ng/dl)	6	15.0
Sufficiency (> 30 ng/dl)	4	10.0

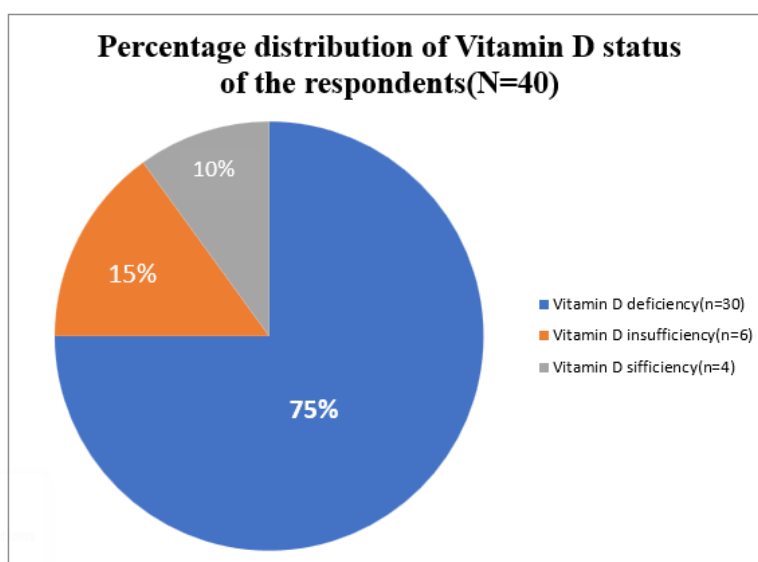


Figure 01: Vitamin D deficiency

Table 08: Age specific distribution of Vitamin D status in patients with tuberculous pleural effusion.

Age group (in years)	Vitamin D 25(OH)			P – value (χ^2)
	Deficiency	Insufficiency	Sufficiency	
18 – 30	83.3	11.1	5.6	0.026 ^s
31 – 40	100.0	0	0	
41 – 50	50.0	12.5	37.5	
51 – 60	66.7	33.3	0	
61 and above	33.3	66.7	0	

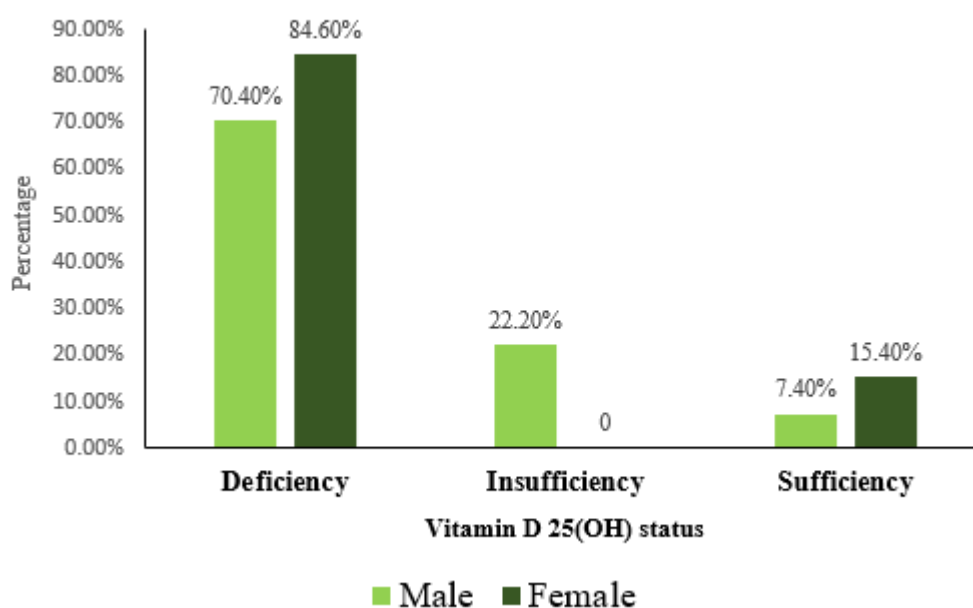


Figure 02: Sex-specific vitamin – D status of the participants

Table 09: Occupation and smoking habit specific vitamin D status of the participants.

Occupation	Vitamin D 25(OH) (%)			P – value (χ²)
	Deficiency	Insufficiency	Sufficiency	
Housewife	90.0	0	10.0	0.583
Service holder	66.7	22.2	11.1	
Day labor	55.6	33.3	11.1	
Businessperson	83.3	0	16.7	
Student /unemployed	83.3	16.7	0	
Smoking habit				
No	78.1	12.5	9.4	0.626
Yes	62.5	25.0	12.5	

P – values obtained from chi-square estimation

Table 10: Comorbidity and clinical feature-specific vitamin D status of the participants.

Comorbidities	Vitamin D 25(OH)			P – value (χ^2)
	Deficiency	Insufficiency	Sufficiency	
<i>Body Mass Index (BMI)</i> (kg/m ²)				
Underweight	66.7	20.0	13.3	0.852
Normal	78.3	13.0	8.7	
Overweight	100.0	0	0	
<i>Hypertension</i>				
No	76.3	13.2	10.5	0.349

Yes	50.0	50.0	0	
<i>Diabetes Mellitus</i>				
No	77.8	13.9	8.3	0.435
Yes	50.0	25.0	25.0	
<i>Anemia</i>				
Absent	72.4	17.2	10.3	0.795
Present	81.8	9.1	9.1	
<i>Cervical Lymphadenopathy</i>				
Enlarged	100.0	0	0	0.704
Not enlarged	73.7	15.8	10.5	

Table 11: Bivariate association of serum and pleural fluid profile with vitamin D status of the participants.

Serum and Pleural fluid profile	Vitamin D 25(OH) Mean (± SD)			P – value (ANOVA)
	Deficiency	Insufficiency	Sufficiency	
<i>Serum Profile</i>				
LDH (U/L)	242.6±56.5	217.7±42.4	220.0±59.3	0.496
Creatinine (mg/dL)	0.78 ±0.18	0.86±0.15	0.66±0.05	0.231
Protein (gm/L)	74.5±7.4	74.3±6.1	69.2±6.3	0.386
ALT (Alanine Transaminase) (U/L)	33.6±16.2	30.5±11.3	33.0±10.1	0.905
<i>Pleural fluid profile</i>				
Protein (gm/L)	58.1±19.6	60.7±11.5	49±4.2	0.574
Adenosine Deaminase (ADA)	51.4±16.6	61.9±25.3	45.5±20.4	0.334
LDH (U/L)	465.5±302.4	353.5±141.5	516.1±260.8	0.611
Glucose (mmol/L)	4.4±2.6	5.4±0.6	4.8±2.3	0.652

DISCUSSION

An early assessment of Vitamin D status and early Vitamin D supplementation have the potentiality to reduce both mortality and morbidity in Bangladesh. However, 40 patients with tuberculous pleural effusion from one of the tertiary hospitals in Bangladesh, Bangabandhu Sheikh Mujib Medical University (BSMMU), were included in this study. In terms of descriptive findings from the demographic and clinical profile of the patients with tuberculous pleural effusion, this study reveals that the mean age of the 40 respondents was 35.28 $\pm$ 14.01 years and the male-female ratio of this study was about 2:1. More than half of the patients (57.5%) completed education of class 6 to HSC level and 25% of the patients were housewives. 20% of the participants have smoking habit. All the patients had been suffering from fever, cough, anorexia and weight at the time of survey and their mean duration in days were 41.2 $\pm$ 19.8 days, 40.9 $\pm$ 20.1 days, 40.4 $\pm$ 19.8 days and 4.6 kg in 40 days respectively. Nearly half of the respondents had chest pain for 40.2 $\pm$ 21.9 days on average. Only 5% of the patients had hypertension and 10% had diabetes mellitus. Though 37.5% of the respondents were underweight, 5% of the respondents had overweight. For both sexes, the mean respiratory rates were higher, compared to the reference range (around 18.4 and 18.9 breath per min). But the pulse rate and SpO₂ were normal for both sexes. Additionally, the mean values of serum protein, s. creatinine, ALT were in normal range for both sexes

as per reference values but the mean value of serum LDH (262 ± 202.2 U/L) was higher among females. The pleural fluid profile shows that the mean values of lymphocyte (90.7% and 84.2%) was reported to be higher than neutrophil (9.3% and 15.6%) for both sexes. In this study, while investigating the vitamin D status of the patients with tuberculous pleural effusion, it was found that 75% patients had vitamin D deficiency (< 20 ng/dl), 15% patients had insufficient vitamin D, whereas only 10% patient had sufficient vitamin D level. The mean value of serum vitamin D was 16.9 ± 4.7 and 15.6 ± 4.0 ng/dl for male and female respectively. As for the prime findings, the mean vitamin D value (16.25 ± 4.1) was found to be lower among the patients with tubercular pleural effusion, than that of healthy population (20.25 ± 13.1) of study [24]. Supporting this finding, a recent meta-analysis reported that vitamin D deficiency was highly found among the patients who were suffering from tuberculous pleural effusion [25]. This is concordant with our results. The prevalence of vitamin D deficiency among TB patients reported in our study is comparable to that reported in Tunisia (80%) [26], Pakistan (78%) [27], South Africa (63%) [28]. On the contrary, lower prevalence of vitamin D deficiency was reported in other studies of Bangladesh which was conducted on apparently healthy population such as in study of [29] found the vitamin D deficiency among 50% high income urban women, and 38% low-income rural women. Additionally, similar studies found vitamin D deficiency around 6% [30], 18.6% [31], and 54.2% [32]. This further supports finding of our study. Vitamin D deficiency among the patients with tuberculous pleural effusion is more common in regions where tuberculosis is endemic, such as South Asia, and African regions [33]. Additionally, our study found that mean difference between the value of vitamin D among the patients with tubercular pleural effusion, and healthy population was significant. Supporting this finding, a study reported that Vitamin D and TB remain strongly linked and low vitamin D levels were associated with a five-fold increased risk for progression to tuberculous pleural fluid [34]. The reason behind the vitamin D deficiency related to tuberculosis is because vitamin D plays a role in the immune system's response to infection. Vitamin D can activate the expression of antimicrobial peptides, such as cathelicidin that can kill intracellular *Mycobacterium tuberculosis*, the bacteria that causes tuberculosis [35]. Vitamin D can also modulate the inflammatory response and prevent tissue damage caused by excessive inflammation [36]. However, people from Asian region have low levels of vitamin D due to lack of sun exposure, skin pigmentation, dietary factors or genetic variations. But the comparison of vitamin D deficiency among healthy and TB patients yielded in different studies indicate that vitamin D deficiency can increase the likelihood of tuberculosis within the healthy population along with the confounding factors such as - dark skin color, homebound and sedentariness, insufficient sunlight exposure, atmospheric pollution, clothing style, obesity, use of sunscreen and no supplementation [37]. Therefore, vitamin D deficiency is a potential risk factor for tuberculosis and vitamin D supplementation may be a beneficial adjunct therapy for tuberculosis patients. In terms of demographic characteristics, this study found that the 31 to 40 years patients with tuberculous pleural effusion suffered from more vitamin D deficiency (100%), followed by 18-30 years old (83.3%), than other age groups and the male patients are more vulnerable to vitamin-D insufficiency. Supporting these findings, studies have found that tuberculous pleural effusion typically affects young adults, with a median age of 30 to 40 years [38]. However, in countries with low incidence of tuberculosis, such as the United States and Europe, tuberculous pleural effusion may occur more frequently in older adults, especially immigrants [39]. On the other hand, tuberculous pleural effusion is more common in males than females, with a male to female ratio of approximately 3:2 – which is very close to the findings of this study. This may be due to biological, social, or environmental factors that influence the exposure and susceptibility to tuberculosis infection [40]. More specially, Vitamin D is mainly synthesized in the skin by exposure to sunlight. Young adults and males may have less sun exposure than older adults and females due to their lifestyle, occupation or clothing preferences. For example, young adults may spend more time indoors studying or working, while males may wear more covering clothes than females in some cultures [41]. Overweight and the people with anemia also found to be vitamin D deficient in this

study. But there was no significant association found among the people with high BMI and anemia and vitamin D deficiency. One of the meta-analysis studies revealed that the prevalence of vitamin D deficiency was 35% higher in obese subjects compared to the eutrophic group (PR: 1.35; 95% CI: 1.21–1.50) and 24% higher than in the overweight group (PR: 1.24; 95% CI: 1.14–1.34) [42]. The study [43] argued that the factors for vitamin D deficiency among the obese tuberculosis (TB) patients can occur due to factors such as the sequestration of vitamin D within adipose tissue, reduced bioavailability in the bloodstream, immune system modulation disruption, chronic inflammation, and altered hormonal levels. On the other hand, anemia often results in reduced red blood cell mass, which can affect the distribution and availability of vitamin D in the body [44]. On the other hand, the reasons behind the vitamin D insufficiency among the TB patients with cardiovascular diseases are 1) hypertensive patients may avoid outdoor activities due to their condition or medication side effects and 2) Poor dietary intake of vitamin D-rich foods, such as fatty fish, egg yolks, cheese, or fortified cereals, since hypertensive patients may follow a low-salt or low-fat diet that limits their consumption of these foods [45]. In terms of serum profile investigation, the bivariate association of serum profile and pleural fluid profile with vitamin D status of the patients with tuberculous pleural effusion was not significant. The mean values of S. LDH was higher for the vitamin D deficient patients in this study. Similar findings have been observed in one of the studies of Taiwan [46], which showed that S.LDH value was 20% higher among the TB patients who had vitamin D deficiency. [47] argued that deficiency in vitamin D can hinder the body's ability to repair damaged tissues, leading to ongoing inflammation and consequently elevated S. LDH. Similarly, the mean values of Pleural ADA, P. protein, P. LDH were higher since they were from TB patients for both sexes in this study. But there was no statistical association found between the higher presence of P. ADA for the TB patients and their vitamin D status. Supporting this finding, the study [48] disclosed that there isn't a direct connection between vitamin D deficiency and P. ADA levels. But vitamin D deficiency can impact the immune response and TB infection, the elevation of P. ADA levels in TB patients is mainly a result of the immune activation and granuloma formation associated with the infection itself, rather than the deficiency of vitamin D [49].

4. CONCLUSIONS

In this study the mean age of the 40 participants were 35.28 ± 14.01 years and the male-female ratio was about 2:1 (67.5% vs 32.5%). Fever, cough, anorexia, and weight loss were prevalent presenting symptoms (100%). In this study, the mean value of serum vitamin D was higher in males than females (16.9 ± 4.7 vs 15.6 ± 4.0 ng/ml). In this study, about 75% of participants had vitamin D deficiency and 15% participants had insufficient vitamin D. In the bi-variate analysis, age was found to be significantly associated with vitamin D status (p -value < 0.05) and participants from 31 to 40 years were more vulnerable to vitamin D deficiency. we concluded that serum vitamin D deficiency was higher in TPE participants. Therefore, adequate management of vitamin D and proper supplementation can enhance the immune system and reduce the vulnerability of tuberculosis. serum vitamin D level was lower in patients with TPE and most of the patients were in deficiency level (75%) whereas vitamin D insufficiency was 15% and sufficiency was only 10%. A longitudinal approach would enable the tracking of vitamin D deficiency rate among the TB patients as well as to determine the optimal dose, duration, and timing of vitamin D supplementation for tuberculosis prevention and treatment. Collaborating with multiple medical centers or hospitals across different regions can increase the study's sample size and diversity. Adequate management of vitamin D deficiency can help support immune function and contribute to better outcomes in TB treatment. Additionally, for the Bangladeshi TB patients, to increase their vitamin D level, regular contact with outdoor sunshine for 10–15 min with exposed arm, feet, and face (at least 2 to 3 times per week) must be made.

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