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Comparative Analysis of Serum Vitamin D Levels in Newly Diagnosed Tuberculosis Patients versus Healthy Individuals

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ABSTRACT

Vitamin D deficiency has been implicated in the susceptibility to tuberculosis due to its crucial role in immune regulation and host defense mechanisms. **Objectives:** To compare serum Vitamin D levels between newly diagnosed TB patients and healthy individuals and assess their association with the nutritional-inflammatory profile. **Methods:** This comparative cross-sectional study was conducted over 1 year from Dec 2021 to Dec 2022. A total of 224 participants, comprising 112 newly diagnosed tuberculosis patients as cases and 112 healthy individuals as controls, were recruited. Cases included patients aged over 18 years with confirmed tuberculosis diagnosis GeneXpert MTB/RIF assay. Controls were individuals without tuberculosis symptoms and with serum vitamin D levels available. **Results:** Vitamin D levels were found to be lesser in TB patients with median levels of 14.35 ng/mL (interquartile range (IQR): 8.65–25.48) versus 19.08 ng/mL (IQR: 13.92–26.17; p=0.029) in normal people. A higher proportion of TB patients exhibited severe deficiency (<10 ng/mL) at 35.7% compared to 13.4% in controls (p=0.002). Similarly, deficiency (10–20 ng/mL) was more prevalent among tuberculosis patients (42.9%) than controls (26.8%). Vitamin D levels in tuberculosis patients had a positive correlation with BMI and albumin levels. **Conclusions:** It was concluded that tuberculosis patients exhibited poorer nutritional status, with lower BMI, albumin, hemoglobin, and Vitamin D levels compared to healthy controls with a significantly higher proportion of tuberculosis patients having severe Vitamin D deficiency.

INTRODUCTION

Vitamin D deficiency is a public health concern, with around one billion people estimated to have insufficient levels [1]. The prevalence varies geographically due to differences in sun exposure, dietary intake, cultural practices, and skin pigmentation. In many regions, particularly in South Asia, vitamin D deficiency is endemic [2]. In Europe, nearly 40% of the population is deficient, with even higher rates in elderly populations [3]. This widespread deficiency underscores the need for increased awareness and intervention. Tuberculosis (TB) remains one of the leading infectious diseases globally, caused by Mycobacterium tuberculosis. Recent evidence highlights its potential role in combating infections, including tuberculosis (TB), due to its ability to enhance macrophage function and upregulate the production of antimicrobial peptides like "cathelicidin and defensins" [4, 5]. The interaction between vitamin D and TB is well documented, with vitamin D deficiency identified as a risk factor for TB susceptibility. Studies suggest that individuals with low vitamin D levels have impaired macrophage activation, resulting in suboptimal bacterial clearance [6, 7]. A meta-analysis revealed that vitamin D deficiency is significantly more prevalent among TB patients than in healthy individuals [8]. Furthermore, supplementation with vitamin D in TB patients has been

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explored as an adjunct therapy, showing promise in enhancing treatment outcomes [9]. In Pakistan, TB remains a major public health challenge, with the country ranking fifth among high-burden TB countries [10]. Simultaneously, vitamin D deficiency is alarmingly prevalent, affecting approximately 7090% of the population, including all age groups [11, 12]. A 2019 study reported that over 80% of TB patients had vitamin D deficiency compared to 45% in healthy controls, highlighting the correlation between low vitamin D levels and increased TB risk [13]. Factors contributing to this deficiency include limited sun exposure due to cultural practices, inadequate dietary intake, and high rates of malnutrition [14]. Studies indicate that the majority of TB patients in Pakistan have insufficient vitamin D levels, potentially impairing immune response and bacterial clearance. Variations in Mycobacterium tuberculosis strain types, including the Beijing and East African-Indian lineages, further impact disease severity and treatment outcomes. While standard treatment regimens achieve high success rates in drug-sensitive TB, the growing threat of Multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) necessitates prolonged, toxic therapies [13]. While global research has established a link between vitamin D deficiency and TB susceptibility, regional variations in prevalence and severity necessitate localized investigations. Pakistan's dual burden of TB and vitamin D deficiency offers a unique opportunity to explore this relationship further and help in the development of preventive and therapeutic strategies.

Tuberculosis remains a major infectious disease burden in developing countries, and emerging evidence suggests that vitamin D deficiency may contribute to increased susceptibility and poor disease outcomes due to its role in immune regulation. However, in Pakistan, there is limited comparative data evaluating serum vitamin D levels between newly diagnosed TB patients and healthy individuals, particularly in relation to nutritional-inflammatory status. Existing studies show inconsistent findings regarding the strength of this association and its clinical relevance in local populations. This study aimed to perform a comparative analysis of serum vitamin D levels between newly diagnosed TB patients and healthy individuals and assess their association with the nutritional profile.

METHODS

This comparative cross-sectional study was conducted over 1 year from Dec 2021 to Dec 2022 at Liaquat University Hospital, Hyderabad. A total of 224 participants, comprising 112 newly diagnosed TB patients as cases and 112 healthy individuals as controls, were recruited. The sample was calculated via an open epi sample size calculator by taking the percent of Vitamin D deficiency in exposed (TB patients) with the outcome as 68.96% percent of unexposed (healthy controls) with the outcome as 51.72% with 80% power of study and 90% CI [15]. The study was approved by the ERC of Liaquat University of Medical and Health Sciences, Jamshoro vide letter No. LUMHS/REC/-164. Cases included patients aged over 18 years with confirmed TB diagnosis through AcidFast Bacilli (AFB) smear microscopy or GeneXpert MTB/RIF assay. Controls were individuals without TB symptoms and with serum vitamin D levels available. Participants with chronic liver disease, chronic kidney disease, HIV-positive status, or ongoing vitamin D supplementation were excluded from the study. Pregnant individuals were also excluded to eliminate potential confounding factors related to pregnancy-induced changes in vitamin D metabolism. Vitamin D status was classified based on the clinical practice guidelines of the Endocrine Society Task Force [16]. Informed written consent was taken from every participant (cases and controls) before enrollment in the study. The study outcomes were assessed in terms of measuring the serum Vitamin D levels and their correlation with nutritional (BMI, Vitamin B12, Serum Ferritin, Hemoglobin, and Albumin) and inflammatory markers (ESR, Total Leucocyte Count, and Neutrophil-to-Lymphocyte Ratio) in TB patients and healthy controls. Data analysis was performed using SPSS version 21.0. Descriptive statistics were used to summarize demographic and clinical characteristics. An Independent t-test was applied to compare continuous variables between cases and controls, while the chi-square test assessed the relationship of the level of Vitamin D in cases and controls. Spearman's correlation was used to measure the association between Vitamin D levels and nutritional-inflammatory parameters. A p-value<0.05 was considered statistically significant.

RESULTS

The mean age was similar between TB patients (50 ± 16.46 years) and controls (49 ± 11.38 years) (p=0.43). A greater ratio of males was observed among TB patients (71.43%) compared to controls (55.36%). TB patients had a significantly lower mean BMI (21.34 ± 3.16 kg/m^2) compared to controls (26.53 ± 2.14 kg/m^2) (p=0.04). Mean albumin levels were lower in TB patients (3.03 ± 1.32 mg/dL) than in controls (4.3 ± 0.53 mg/dL) (p=0.013). Likewise, hemoglobin levels were lower in cases (10.52 ± 2.19 g/dL) than in controls (13.32 ± 1.56 g/dL) (p = 0.045). Differences in mean calcium, total WBC count, and platelet count were not significant (Table 1).

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Table 1: Baseline Characteristics of Tb Patients (n=112) and Healthy Controls (n=112)

Variables	TB Patients (n=112)	Controls (n=112)	p-Value
Male	80 (71.43%)	62 (55.36%)	0.120
Female	32 (28.57%)	50 (44.64%)	
Mean BMI (Kg/m^2)	21.34 ± 3.16	26.53 ± 2.14	0.040*
Mean Albumin (mg/dL)	3.03 ± 1.32	4.3 ± 0.53	0.013*
Mean Calcium (mg/dL)	8.15 ± 1.27	10.21 ± 0.13	0.860
Mean ESR (mm/h)	67 ± 12.76	14.53 ± 6.31	0.003*
Mean Hemoglobin (g/dL)	10.52 ± 2.19	13.32 ± 1.56	0.045*
Mean Total WBC Count (10^3/L)	13642 ± 3281	6324 ± 1138	0.080
Mean Platelets (100,000/dL)	3.23 ± 1.51	2.21 ± 0.62	0.130

*Statistically significant

Vitamin D levels were lesser in TB patients in comparison to healthy controls, with median levels of 14.35 ng/mL (IQR: 8.65–25.48) versus 19.08 ng/mL (IQR: 13.92–26.17; p=0.029). A higher proportion of TB patients exhibited severe deficiency (<10 ng/mL) at 35.7% compared to 13.4% in controls (p=0.002). Similarly, deficiency (10–20 ng/mL) was more common among TB patients (42.9%) than controls (26.8%). Opposite to that, Vitamin D sufficiency (>30 ng/mL) was observed in only 8% of TB patients compared to 24.1% of controls (Table 2).

Table 2: Comparison of Vitamin D Status Between Tb Patients (n=112) and Healthy Controls (n=112)

Variables	TB Patients (n=112)	Controls (n=112)	p-Value
Median (IQR) Serum Vitamin D3 Levels (ng/mL)	14.35 (8.65-25.48)	19.08 (13.92-26.17)	0.029*
Severe Deficiency (<10 ng/mL)	40 (35.7%)	15 (13.4%)	0.002*
Deficiency (10-20 ng/mL)	48 (42.9%)	30 (26.8%)	
Insufficiency (21-30 ng/mL)	15 (13.4%)	40 (35.7%)	
Sufficiency (>30 ng/mL)	9 (8.0%)	27 (24.1%)	

*Statistically significant

A significant positive correlation was observed between Vitamin D and BMI (r=+0.40, p=0.032) and albumin (r=+0.55, p=0.018), suggesting that better nutritional status may be associated with higher Vitamin D levels. While hemoglobin (r=+0.48, p=0.076) and Vitamin B12 (r=+0.15, p=0.162) showed positive correlations, they were not statistically significant. Conversely, ESR (r=-0.65, p=0.091), serum ferritin (r=-0.08, p=0.247), and NLR (r=-0.09, p=0.225) exhibited negative correlations with Vitamin D, though these associations lacked statistical significance (Table 3). While correlation of Vitamin D3 with Nutritional-Inflammatory Profile in Healthy Controls was not found to be statistically significant.

Table 3: Correlation Between Vitamin D3 and Nutritional-Inflammatory Profile in Tuberculosis Patients and Healthy Controls

PARAMETER	TB PATIENTS		CONTROLS	
	Correlation Coefficient (r)	p-Value	Correlation Coefficient (r)	p-Value
BMI	+0.40	0.032*	+0.22	0.148
Vitamin B12	+0.15	0.162	+0.09	0.312
Serum Ferritin	-0.08	0.247	-0.12	0.284
Albumin	+0.55	0.018*	+0.38	0.079
Hemoglobin	+0.48	0.076	+0.29	0.092
ESR	-0.65	0.091	-0.15	0.056
Total Leucocyte Count	+0.12	0.198	+0.07	0.367
Neutrophil-to-Lymphocyte Ratio (NLR)	-0.09	0.225	-0.05	0.419

*Statistically significant

DISCUSSION

The findings in the current study revealed a median serum Vitamin D level of 14.35 ng/mL among TB patients, significantly lower than the 19.08 ng/mL observed in controls (p=0.029). Severe Vitamin D deficiency was more prevalent in TB patients (35.7%) compared to controls (13.4%, p=0.002). Similarly, Vitamin D sufficiency was less common in TB patients (8%) than in controls (24.1%). A study by Balcells et al., showed that Vitamin D deficiency is consistently linked to active TB, likely owing to the immunomodulatory role in macrophage activation and granuloma formation [17]. TB patients had significantly lower BMI (21.34 ± 3.16kg/m^2) and serum albumin levels (3.03 ± 1.32mg/dL) compared to controls (26.53 ± 2.14kg/m^2 and 4.3 ± 0.53mg/dL, respectively, p=0.013). BMI and albumin were positively associated with Vitamin D levels in TB patients. This suggests that malnutrition exacerbates Vitamin D deficiency, which may worsen TB outcomes. Similar findings have been reported in India, where malnourished TB patients exhibited lower Vitamin D levels than their well-nourished counterparts, underscoring the need for nutritional interventions [18]. Malnutrition in TB patients likely arises from increased metabolic demands, poor appetite, and systemic inflammation. Low albumin levels, indicative of protein energy malnutrition, correlate with diminished Vitamin D binding protein, causing lower bioavailability of active vitamin D3 [19]. Elevated ESR levels in TB patients (67 ± 12.76mm/h) compared to controls (14.53 ± 6.31mm/h, p=0.003) indicate heightened systemic inflammation. However, no significant correlation was observed between Vitamin D levels and ESR (r=0.65, p=0.091). This contrasts with a study from China, where lower Vitamin D levels were linked to more severe inflammatory responses in TB patients [20]. The absence of such an association in our study could be due to variability in disease severity or differences in the inflammatory markers assessed. TB patients demonstrated lower hemoglobin levels (10.52 ± 2.19g/dL)

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compared to controls (13.32 ± 1.56g/dL, p=0.045), reflecting anemia of chronic disease. A positive relation between Hb levels and Vitamin D was observed (r=+0.48) but was non-significant (p=0.076). Anemia in TB is multifactorial, driven by chronic inflammation, nutritional deficiencies, and impaired erythropoiesis [21]. Studies from India have highlighted the synergistic impact of Vitamin D and iron deficiencies in exacerbating anemia among TB patients [22]. These findings reinforce the need to deal with Vitamin D deficiency and malnutrition in TB management. The WHO End TB Strategy emphasizes integrated nutritional support for TB patients, aligning with our observation that malnourished TB patients are prone to severe Vitamin D deficiency [23]. Vitamin D supplementation as adjunctive therapy has shown promise in enhancing sputum conversion rates and reducing treatment duration in randomized controlled trials conducted in India and Pakistan [24]. Variations in deficiency rates are influenced by dietary intake, sun exposure, genetic factors, and comorbidities. Comparative studies indicate that countries with higher latitudes experience more pronounced seasonal variations in Vitamin D3, potentially exacerbating TB risk during winter months [25].

This study was limited by its cross-sectional design, which prevents establishing a causal relationship between vitamin D deficiency and tuberculosis. Being a single-center study with a relatively small sample size may limit generalizability to the broader population. Additionally, potential confounding factors such as dietary intake, sunlight exposure, and socioeconomic status were not fully controlled. Future research should include large-scale multicenter longitudinal studies to establish causality and explore the effect of vitamin D supplementation on TB treatment outcomes. It is also recommended to integrate nutritional screening, including vitamin D assessment, into routine TB management protocols to improve patient outcomes.

CONCLUSIONS

Tuberculosis patients exhibited poorer nutritional status, with lower BMI, albumin, hemoglobin, and Vitamin D levels compared to healthy controls. A significantly higher proportion of TB patients had severe Vitamin D deficiency. Positive correlations were observed between Vitamin D levels and BMI as well as albumin, indicating a link between nutritional status and Vitamin D. However, inflammatory markers showed no significant association with Vitamin D levels. These findings suggest that nutritional deficiencies, particularly in Vitamin D, may be linked to TB, highlighting the importance of nutritional support in TB management.

Authors' Contribution

Conceptualization: MAR
Methodology: MAR, MN, IJ, MS
Formal analysis: MAM, MZ
Writing and Drafting: MN, IJ, MNA
Review and Editing: MN, IJ, MNA, MAR, MN, IJ, MS

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

The authors declare no conflict of interest.

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