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The Relationship of Vitamin D and IL-17 Levels with Disease Severity in Pediatric Tuberculosis Patients

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Abstract: Tuberculosis (TB) remains a significant global health burden, particularly in children, where immune responses play a crucial role in disease progression. The objective of this research was to analyze the relationship of vitamin D and IL-17 levels with the severity of pediatric TB. This observational analytic study was conducted at Dr. Saiful Anwar Hospital, Malang, involving 33 pediatric patients diagnosed with TB, divided into mild (n = 8), moderate (n = 18) and severe (n = 7) groups. Severity was determined using the score developed in this study. Vitamin D levels were measured using the Cobas c501 analyzer, and IL-17 levels were measured using the Human IL-17 ELISA Kit. While Vitamin D level was significantly lower in microbiologically confirmed patients (p = 0.038), it was not significantly different among severity groups (p = 0.799). Meanwhile, IL-17 levels were significantly higher in patients with more severe respiratory distress (p = 0.01), although the difference between severity groups was not statistically significant (p = 0.966). Furthermore, there was no correlation between vitamin D levels and IL-17 levels (r = 0.178, p = 0.322). These findings indicate the need for further research to explore vitamin D supplementation as an adjunctive therapy and to evaluate IL-17 as a biomarker for disease activity.

Keywords: Pediatric TB; Vitamin D Deficiency; Interleukin-17; TB Severity; Biomarkers; Immune Modulation

1. Introduction

Tuberculosis (TB) affected 10.8 million people in 2023, including 12% who were children and young adolescents. Between 2015 and 2023, the incidence rate was reduced by only 8.3%, leaving a substantial gap from the End TB Strategy goal of a 50% reduction by 2025 [1]. However, 17.5% of HIV-negative and HIV-positive patients who died from TB were children aged <15 years. Case notifications were significantly lower in children aged 5–14 years and 0–4 years (48% and 61%, respectively) compared to that in adults (71%) [2]. This suggests that more children with TB were diagnosed late and come to health facilities in a serious condition. Indeed, children are more likely to have extrapulmonary and disseminated TB [3]. Furthermore, clinicians are often unaware that a seemingly non-severe patient may have the potential to progress to a serious condition, especially in young children [4].

After primary infection, *M. tuberculosis* can spread via the bloodstream before the immune system mounts a full response to kill the bacteria. This allows early dissemination and persistence of bacteria into the lungs and other organs. Depending on the balanced interaction between host immunity and pathogen virulence, these new focuses may progress to active disease. In children under 2 years of age, the primary disease frequently leads to

severe disease without obvious prior symptoms, generally occuring within 12 months after contact with source case. Primary infection in children over 10 years of age usually develop to adult type TB [4].

The spectrum of the disease is varied from asymptomatic to severe pulmonary lesion. Non-severe TB in children encompasses intrathoracic lymph node TB without significant airway obstruction, pulmonary TB confined to one lobe without cavitation or a miliary pattern, or uncomplicated pleural effusion without pneumothorax or empyema. It is defined by a negative bacterial confirmation and mild symptoms that do not require hospitalization [5]. In contrast, extensive diseases, such as complicated pleural effusion, cavitary lung disease, and bilateral lung involvement on chest X-ray, indicate severe TB [6, 7]. Miliary TB and central nervous system TB are the two most serious conditions of extrapulmonary TB in children [8]. High bacterial burden or severe systemic symptoms requiring hospitalization are also considered indicators of severe TB [5].

The role of T lymphocyte orchestration is pivotal in the pathogenesis of TB. Following phagocytosis of mycobacterium by macrophage, Th1 T cells play important role by activating alveolar macrophage. Granuloma is formed when bacteria could not entirely be eliminated. While Th1 cytokines have direct effect on activating macrophage within granulomas, IL-17 is involved in the formation and stability of granulomas by increasing chemokine production, which helps recruit inflammatory cells migrating to the Mtb-infected sites. However, overactivation can lead to excessive IL-17 production and heightened inflammation, which may disrupt granuloma integrity and result in tissue damage [9–11].

Vitamin D is synthesized in the skin from 7-dehydrocholesterol under ultraviolet B light or obtained from foods like fatty fish and mushrooms. The main circulatory form, 25-hydroxyvitamin D3 [$25(OH)D_3$], is considered the primary indicator of vitamin D status, with level below 20 nmol/L classified as deficient [12]. Vitamin D has been known to have protective role in TB as modulator of the immune response. The active form enhances macrophage function by promoting the production of antimicrobial peptides such as cathelicidin [13, 14]. Furthermore, vitamin D regulates inflammatory responses, reducing the risk of excessive tissue damage [12]. Vitamin D deficiency commonly occurs in children with TB and has been associated with increased susceptibility to infection and more severe disease outcomes [15]. Conversely, other findings indicate that vitamin D level are not associated with the risk of TB infection and disease severity [16, 17]. However, the interaction between vitamin D status, IL-17 levels, and TB severity remains poorly understood in pediatric population.

Given the complex interplay between cytokines such as IL-17, vitamin D status, and the immune response to TB, understanding their roles in pediatric TB is essential. This study aims to elucidate the relationship between vitamin D, IL-17 levels, and the severity of TB in children, providing insight into potential biomarkers of disease severity and therapeutic strategies to improve outcomes in this vulnerable population.

2. Materials and Methods

This was a cross-sectional study conducted at Dr. Saiful Anwar Hospital in Malang using consecutive sampling. In this study, consecutive sampling was used to recruit eligible pediatric TB patients over a one-year period. Consecutive sampling was chosen due to the limited number of patients diagnosed with TB in the study setting, ensuring that all eligible patients who presented during the period were included.

This study included children below 18 years of age who were diagnosed with TB based on the WHO diagnostic approach for pediatric TB. This age limit is in accordance with the WHO definition of children, which includes individuals under 18 years old. Moreover, the clinical spectrum and immune responses in those aged 15–18 can still differ significantly from adults. The diagnosis was established using combination of criteria, including documented close contact with an active TB case; clinical signs of TB, such as persistent non-resolving cough, prolonged fever with or without night sweats, poor appetite or anorexia, weight loss or failure to thrive, and unusual fatigue or decreased activity as well as clinical symptoms specific to the affected site of extrapulmonary TB (EPTB); chest X-ray findings suggestive of TB; a positive tuberculin skin test (TST); and microbial confirmation with a positive molecular test (GeneXpert MTB/RIF, Cepheid, Sunnyvale, CA, USA) for *Mycobacterium tuberculosis* [5]. The inclusion criteria required a constellation of these diagnostic features to confirm TB. Patients with multi-drug resistant TB (MDR-TB) or TB-HIV co-infection were excluded from the study. Patients with known primary immunodeficiency disorders, autoimmune disease, or conditions receiving immunosuppressive therapy were also excluded from the study, as these factors could independently alter IL-17 responses.

TB severity was determined by clinical, radiological, and laboratory findings at the time of patient's initial

presentation and diagnosis of TB. Severe TB was defined as having extensive disease manifestations, such as complicated pleural effusion, cavitary lung disease, or bilateral lung involvement on chest X-ray. Miliary TB and central nervous involvement were classified as severe TB. Furthermore, high bacterial burden or severe systemic symptoms necessitating hospitalization were considered severe TB [5–8]. To stratify cases into mild, moderate, and severe categories, we develop a severity scoring system specifically for this study. Five parameters including chest X-ray findings, nutritional status, extrapulmonary manifestation, the presence of respiratory distress, and microbiological test results were considered to assess the severity of the disease. The maximum achievable score was 12 and the classification was determined according to the data distribution in this study. Scores of 1–3 indicated mild severity, 4–6 indicated moderate severity, and scores greater or equal to seven were categorized as severe (**Table 1**).

Table 1. TB Severity Assessment Used in The Study.

Clinical criteria: Respiratory distress	
No respiratory distress	0
Mild respiratory distress	1
Severe respiratory distress a	2
Chest X-ray findings	
No lesion	0
Mild lesions	1
Moderate lasions	2
Severe lesions	3
Nutritional status	
Well nourished	1
Moderate malnutrition	2
Severe malnutrition	3
Extrapulmonary manifestations	
No or mild extrapulmonary lesions	1
Moderate extrapulmonary lesions	2
Severe extrapulmonary lesions	3
Microbiology test results	
Negative	0
Positive	1
Total score	12

Blood samples were collected from the subjects at the time of diagnosis. The samples were stored at −20 oC in the central laboratory of Dr. Saiful Anwar Hospital. Serum vitamin D levels were analyzed using the Cobas c501 analyzer (Roche Diagnostics GmbH, Mannheim, Germany), while serum IL-17 levels were assessed using Human IL-17 ELISA Kit (Catalog No. EO142Hu, Bioassay Technology Laboratory [BT Lab], Shanghai, China). Data was analyzed using SPSS.26. Categorical variables were analyzed using chi-square or Fisher's exact test when appropriate. Continues variables were tested for normality (Shapiro-Wilk test) and analyzed using ANOVA for normally distributed data or Kruskal-Wallis test for non-normally distributed data. Correlations were assessed using Pearson's or Spearman's test as appropriate. A p-value < 0.05 was considered statistically significant.

Ethical approval for this study was obtained from the Ethics Committee of Dr. Saiful Anwar Hospital, with approval number 400/179/K.3/102.7/2022.

3. Results

3.1. Baseline Characteristics

A total of 33 pediatric patients diagnosed with TB were included, with a median age of 14 years. More than half were aged between 14–18 years. The most common symptoms were fever, weight loss, and cough. Respiratory distress occured in 13 cases, of which three were classified as severe (**Table 2**).

Twenty-seven (81.8%) patients had moderate to severe malnutrition. Chest X-ray revealed moderate and severe lesions in 20 (60.6%) subjects, while microbiology tests were positive in 13 (39.4%) subjects. Two patients were presented with meningitis TB, and one of them passed away after two months of hospitalization. One patient required surgical intervention for peritonitis TB. One patient had TB coxitis resulting in loss of ambulation, while another developed TB spondylitis with large paravertebral abscess and paraplegia.

One patient with severe malnutrition, extensive bilateral lung consolidation, and bilateral pleural effusion eventually passed away despite admission to the PICU and support with mechanical ventilation.

Table 2. Demographic and Clinical Characteristics of Subjects.

Characteritics	**n = 33**	**Characteristics**	**n = 33**
Age, years, median (IQR)	14.0 (10.99)	Nutritional status, n (%)	
Age group, n (%)		Normal	6 (18.2)
0–<2 years	2 (6.1)	Moderate malnutrition	13 (39.4)
2–<5 years	7 (21.2)	Severe malnutriton	14 (42.4)
5–<10 years	3 (9.1)	Chest X-ray lesion, n (%)	
10–<14 years	4 (12.1)	No lesion	6 (18.2)
14–18 years	17 (51.5)	Mild	7 (21.2)
Male sex, n (%)	12 (36.4)	Moderate	11 (33.3)
Contact, n (%)	12 (36.4)	Severe	9 (27.3)
Positive tuberculin skin test, n (%)	21 (63.6)	Extra pulmonary lesion, n (%)	
Symptoms, n (%)		No lesion	21 (63.6)
Cough	26 (78.8)	Mild	1 (3.0)
Fever	28 (84.8)	Moderate	6 (18.2)
Weight loss	28 (84.8)	Severe	5 (15.2)
Lymphnode enlargement	18 (54.5)	Positive molecular test, n (%)	13 (39.4)
Dyspnea	13 (39.4)	Disease severity, n (%)	
Respiratory distress, n (%)		Mild	8 (24.2)
No distress	20 (60.6)	Moderate	18 (54.5)
Mild	10 (30.3)	Severe	7 (21.2)
Severe	3 (9.1)	Vit D level, ng/dL, md (IQR)	9.2 (13.75)
		IL-17 level, ng/dL, md (IQR)	94.3 (292.22)

3.2. Comparative Analysis of Disease Severity Groups

Of the 33 subjects, 8 were categorized as mild TB, while 18 and 7 were categorized as moderate and severe TB, respectively (**Table 3**). The median age of the severe TB group tended to be younger compared to mild and moderate TB groups. However, there was no clear statistically significant trend of decreasing age with increasing disease severity in this study population ($r = -0.188$, $p = 0.294$).

Respiratory distress was observed in 13 cases, of which severe distress occurred in one case of the moderate and two cases of severe TB groups. No significant association was found between TB disease severity and respiratory distress ($p = 0.072$). However, significant linear trend ($p = 0.012$) suggests a progressive increase in respiratory distress with higher TB severity category. Spearman correlation revealed a moderate positive relationship ($r = 0.433$, $p = 0.012$)

Although the overall association between TB severity and nutritional status was not statistically significant ($p = 0.165$), there was a significant linear trend ($p = 0.013$) indicating that severe TB is more likely associated with severe malnutrition. This finding aligns with moderate correlation ($r = 0.439$, $p = 0.011$) observed in the data.

While half (66.6%) of moderate TB subjects had moderate-severe lesions in their CXR, most of severe TB group (71.4%) had severe lesions. There was a statistically significant association between TB severity and CXR lesions severity ($p = 0.022$). Moderate correlation (Spearman's $r = 0.519$, $p = 0.002$) aligning clear linear trend (linear $p = 0.005$) indicates that severe TB is predominantly associated with severe lesions.

Both extrapulmonary lesions and microbiology test results showed no significant association with TB severity. Extrapulmonary lesions demonstrated no linear trend with TB severity (linear $p = 0.085$).

Table 3. Analysis of Comparison Between Severity Category.

Parameter	Mild TB (n = 8)	Moderate TB (n = 18)	Severe TB (n = 7)	p
Age, years, median (IQR)	14.56 (11.45)	14.10 (11.49)	12.95 (10.33)	0.533 *
Age group				0.688
0–<2 years	1 (12.5)	1 (5.6)	0	
2–<5 years	1 (12.5)	4 (22.2)	2 (28.6)	
5–<10 years	0	2 (11.1)	1 (14.3)	
10–<14 years	1 (12.5)	1 (5.6)	2 (28.6)	
14–18 years	5 (62.5)	10 (55.6)	7 (28.6)	
Respiratory distress				0.121
No distress	7 (87.5)	11 (61.1)	2 (28.6)	
Mild	1 (12.5)	6 (33.3)	3 (42.9)	
Severe	0	1 (5.6)	2 (28.6)	
Nutritional status				0.072
Normal	3 (37.5)	3 (16.7)	0	
Moderate malnutrition	3 (37.5)	9 (50.0)	1 (14.3)	
Severe malnutrition	2 (25.0)	6 (33.3)	6 (85.7)	
Chest X-ray				0.022
No lesion	2 (25.0)	4 (22.2)	0	
Mild	4 (50.0)	2 (11.1)	1 (14.3)	
Moderate	2 (25.0)	8 (44.4)	1 (14.3)	
Severe	0	4 (22.2)	5 (71.4)	
Extra pulmonary lesion				0.654
No lesion	7 (87.5)	10 (55.6)	4 (57.1)	
Mild	0	1 (5.6)	0	
Moderate	1 (12.5)	3 (16.7)	2 (28.6)	
Severe	0	4 (22.2)	1 (14.3)	
Positive tuberculine skin test	5 (62.5)	14 (77.8)	2 (28.6)	0.071
Positive microbiology results	1 (12.5)	7 (38.9)	5 (71.4)	0.066
Vitamin D level, median (IQR)	12.4 (13.33)	9.0 (11.0)	1.5 (18.5)	0.799 *
IL-17 level, median (IQR)	74.36 (500.58)	108.61 (290.57)	84.51 (255.71)	0.966 *

Data presented in n (%); * Kruskal-Wallis test.

3.2.1. The Association between Vitamin D Level with TB Severity

While median values of vitamin D levels across TB severity groups showed a decreasing trend from mild to severe TB, statistical analysis did not indicate a significant difference ($p = 0.799$) or correlation ($p = 0.520$) among the groups, suggesting random fluctuation rather than meaningful pattern (**Figure 1A**). This may partly be caused by large variability within each group. Further analysis revealed that vitamin D levels were not significantly associated with respiratory distress ($p = 0.272$), nutritional status ($p = 0.841$), chest x-ray findings ($p = 0.738$), or the presence of extrapulmonary lesions ($p = 0.155$). However, vitamin D level were significantly lower in microbiologically positive patients ($p = 0.038$) (**Figure 2A**). Spearman's correlation analysis revealed moderate correlation ($r = -0.367$, $p = 0.036$), indicating that lower vitamin D levels may be associated with microbiological confirmation of TB. However, given the small sample size, further studies with larger cohorts are necessary to validate these findings.

3.2.2. The Association between IL-17 Level with TB Severity

IL-17 levels did not show a statistically significant difference ($p = 0.966$) or correlation ($p = 0.935$) among TB severity groups (**Figure 1B**). The median IL-17 level was highest in the moderate TB group, while mild TB group exhibit the widest variation suggesting that IL-17 levels may not correlate directly with TB severity in this cohort.

IL-17 levels were not associate significantly with nutritional status ($p = 0.094$), chest x-ray findings ($p = 0.204$), or presence of extrapulmonary lesions ($p = 0.505$). While IL-17 levels seemed to be lower in microbiologically positive cases, it was not statistically significant ($p = 0.161$) (**Figure 2B**). However, IL-17 levels were significantly higher in patients with more severe respiratory distress ($p = 0.01$) (**Figure 3**). Furthermore, Spearman's correlation revealed significant correlation ($r = 0.538$, $p = 0.001$). This suggests that IL-17 levels may contribute to worsening respiratory distress in pediatric TB patients.

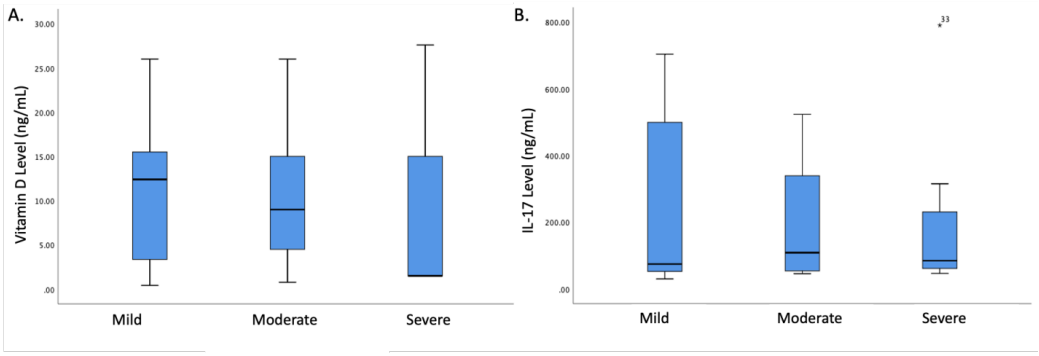

Figure 1. Vitamin D (**A**) and IL-17 level (**B**) between, mild, moderate and severe TB groups. There was no significant difference of vitamin D or IL-17 level between groups (p = 0.779 and 0.966, respectively).

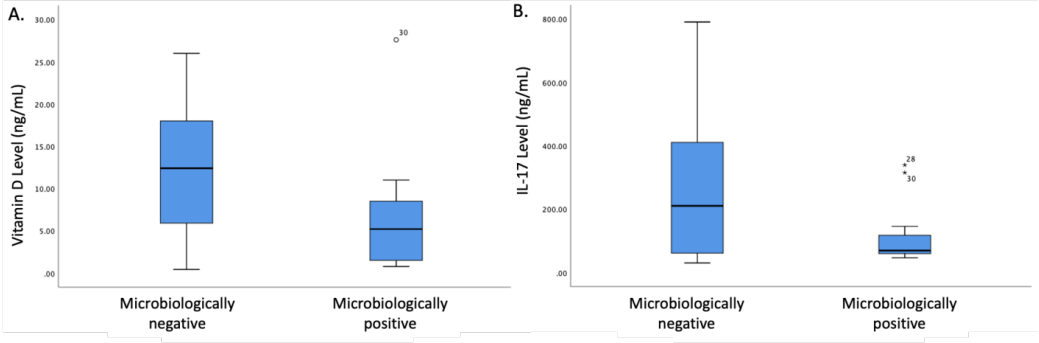

Figure 2. Vitamin D level (**A**) and IL-17 level (**B**) between subjects with negative and positive rapid molecular test results. Significant difference in vitamin D level was observed between two groups (p = 0.038), whereas no significant difference was found in IL-17 level (p = 0.161).

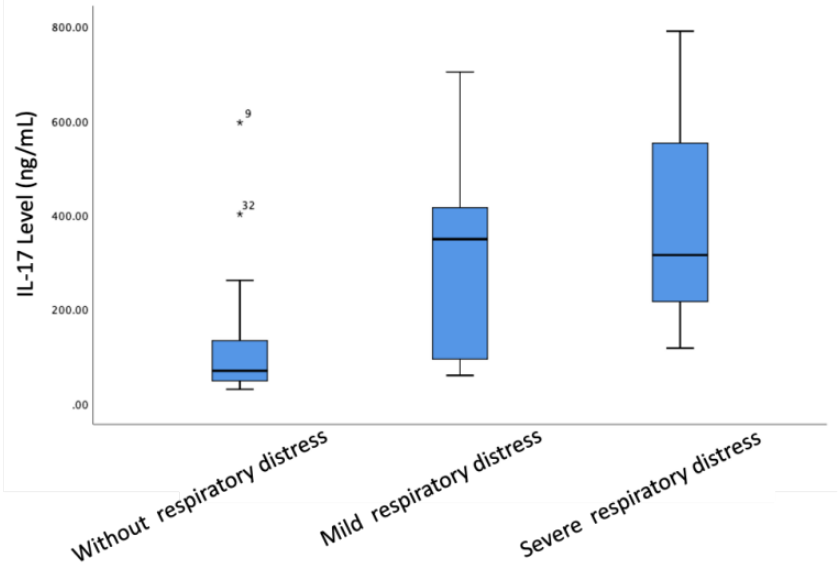

Figure 3. IL-17 level between subjects without respiratory distress, with mild, and severe respiratory distress. Significant difference in IL-17 level was observed between groups (p = 0.01).

Overall, the analysis showed a very weak and statistically non-significant correlation between vitamin D level and IL-17 levels (Spearman's $r = 0.178$, $p = 0.322$).

4. Discussion

4.1. Disease Severity

Tuberculosis remains a significant health issue in children, with its manifestations ranging from asymptomatic latent TB infection to severe disseminated form of the disease. The severity of pediatric TB is influenced by several factors, including age, immune status, and access to timely diagnosis and treatment. Younger children are particularly vulnerable to severe TB due to their underdeveloped immune systems, with higher risk of disseminated diseases such as meningitis TB or miliary TB [4, 18]. To our knowledge, this preliminary study is the first to investigate the association between vitamin D levels, IL-17 concentration, and the severity of TB disease in pediatric patients.

Assessing TB severity in children is critical for guiding management and predicting outcomes. Current classification systems primarily rely on clinical, radiological, and microbiological findings [19, 20]. In this study, a novel severity scoring system was developed to classify and assess TB severity in pediatric patients. This system incorporates five key components: respiratory distress, nutritional status, chest X-ray findings, presence and severity of extra-pulmonary lesions, and microbiological test results. By integrating these parameters, the scoring system enables comprehensive evaluation of disease severity across study subjects, allowing for a more nuanced understanding of clinical spectrum of pediatric TB. Moreover, this classification approach has the potential for broader application in routine clinical settings, offering a standardized tool for assessing TB severity in pediatric patients and guiding appropriate management strategies.

The findings showed that respiratory distress and severe malnutrition were more prevalent in severe TB cases, with significant linear trends indicating their association with higher TB severity. Studies found that malnutrition increases the risk of unfavorable TB clinical outcomes, including treatment failure and death [21–24]. Chest X-ray findings also correlated strongly with disease severity, reinforcing the importance of radiological assessment in TB management [7, 20]. Conversely, extra-pulmonary lesions and microbiological results were not significantly associated with severity, suggesting that while these findings are clinically important, they may not always reflect the overall disease burden.

4.2. The Relationship of Vitamin D with Disease Severity

Vitamin D is critical regulator of immune responses, with well-documented roles in antimicrobial defense and modulation of inflammation. In TB, vitamin D has been shown to enhance macrophage activation and promote the production of antimicrobial peptides such as cathelicidin [25]. Vitamin D also downregulates pro-inflammatory cytokines in pulmonary TB [12]. Several studies have linked low vitamin D levels with increased susceptibility to TB, but its relationship with disease severity remains less clear [26–28]. A study in subjects aged $\geq$15 years demonstrated an association between vitamin D level and TB score, which classifies severity of TB based on clinical conditions [29].

In this study, vitamin D level demonstrated a decreasing trend with increasing TB severity, with median levels being lowest in the severe TB group. However, this trend did not reach statistical significance, likely due to the small sample size and high variability in the severe group. The skewed distribution in this group, with median nearly overlapping with lower range, indicates that significant proportion of severe TB group has low vitamin D levels, while a few outliers exhibit markedly high level. These findings imply that the relationship between vitamin D level and severity may not be linear and could be influenced by confounding factors. This indicates that other factors may influence the severity of TB disease, such as bacterial virulence and individual differences in immune response [23, 24, 30, 31]. Further analysis with larger sample size is required to clarify these observations and to identify potential modifiers of vitamin D levels in severe pediatric TB.

Interestingly, lower vitamin D levels were more common in subjects with positive microbiological results, suggesting a potential link between vitamin D insufficiency and microbial activity of *M. tuberculosis* in pediatric TB. A study showed vitamin D level decreased gradually with an increasing number of AFB per high power field at microscopy level [32]. This implies that vitamin D plays a more important role in infection control, as evidenced by its

association with microbiological outcomes, rather than in determining the clinical severity of the disease. However, this finding highlights the possible influence of vitamin D on preventing TB disease progression, warranting further investigation into its role and critical implication in managing pediatric TB [16, 33–35].

Clinically, these results underscore the importance of assessing vitamin D levels in pediatric patients, particularly those with severe disease. Supplementation could be considered as an adjunctive therapy, although further research is needed to establish optimal dosing and timing [36, 37].

4.3. The Relationship of IL-17 with Disease Severity

IL-17, a pro-inflammatory cytokine produced by Th-17 cells, plays a complex role in TB pathogenesis. While IL-17 contributes to granuloma formation and containment of *M. tuberculosis*, excessive or dysregulated IL-17 responses can lead to tissue damage and exacerbate disease severity [38]. This study found no significant differences in IL-17 levels among TB severity groups, with the highest median levels observed in the moderate TB group. These findings align with previous studies showing inconsistent associations between IL-17 levels and TB severity [39, 40]. Furthermore, none of these patients in this study had known autoimmune or immunodeficiency conditions, minimizing potential confounding effects on IL-17 levels. Some research suggests that while IL-17 is essential for early immune responses, its role diminishes as the disease progresses, potentially explaining the lack of significant trends in the cohort [39]. However, while IL-17 contributes to the immune response against TB, its role is less pronounced in determining disease severity compared to the IFN–mediated activation of macrophage and T cell response [41, 42].

In this study, children with respiratory distress had notably higher IL-17 levels compared to those without respiratory distress. This indicates that IL-17 plays an important role in the inflammatory processes underlying respiratory distress in TB, potentially contributing to lung injury and immune dysregulation [43, 44]. Another study showed increased circulating IL-17 in adult patients with acute respiratory distress syndrome [45]. The pronounced elevation in IL-17 in the presence of respiratory distress underscores its potential as a biomarker for disease activity in TB patients. However, IL-17 levels showed lack of association with chest x-ray findings. This might reflect the complex, multifactorial nature of TB pathology, where local and systemic immune responses do not always align with radiological and clinical findings. The variability in IL-17 levels across these parameters warrants further investigation to determine whether localized IL-17 activity plays a distinct role in specific manifestations of TB.

We also found that the median of IL-17 levels was higher in those with negative microbiology results, although not statistically significant. This appears counterintuitive, as bacteriologically confirmed TB is often associated with heightened immune activation [43]. However, Feng *et al*. have reported lower IL-17 level in adults with positive microbiological results [46]. One possible explanation is that patients with microbiologically positive results represent cases where bacterial load has overwhelmed the immune system, leading to dysregulation or suppression of certain inflammatory pathways, including IL-17 production [47]. Alternatively, the difference may arise from intrinsic variations in host immune responses or the timing of sample collection during the disease course [39].

M. tuberculosis has mechanisms to evade the host immune response, including suppressing signaling pathways critical for IL-17 production, leading to reduced IL-17 levels despite active infection [38]. Vitamin D, a key modulator of immune responses in TB, regulates IL-17 production. Lower vitamin D levels in microbiologically positive patients may reflect pre-existing immune deficiencies or increased consumption due to the immune response to active infection. This deficiency can impair macrophage activation and bacterial control, exacerbating the active disease [48]. In microbiologically positive patients, the disease may be more advanced, contributing to reduced IL-17 and vitamin D levels due to chronic immune dysfunction [46, 49]. Conversely, microbiologically negative patients may have earlier-stage disease or a better-controlled immune response. Low levels of vitamin D and IL-17 levels in microbiologically positive patients could indicate a higher risk of progressive disease or complications, warranting more intensive clinical attention.

We found there was no correlation between vitamin D and IL-17 levels observed in this study, indicating that their interaction may be indirect or influenced by other immunological pathways. Vitamin D modulates the pro-inflammatory immune response, primarily by influencing macrophage activation and IFN- production, rather than directly affecting IL-17. While some studies indicate that vitamin D can inhibit IL-17 production, this effect is more pronounced in autoimmune diseases rather than in tuberculosis [50]. In TB, IL-17 levels are more di-

rectly influenced by *M. tuberculosis* infection and the host immune response, whereas vitamin D primarily enhances macrophage activation and bacterial elimination. Moreover, the heterogeneity in IL-17 responses suggests that additional factors, such as genetic variability, may modulate this relationship [9, 51, 52].

4.4. Implication and Study Limitations

Given the observed results, routine screening and supplementation for vitamin D deficiency in pediatric TB patients should be considered. Future studies should explore the impact of supplementation on clinical outcomes and disease progression. The variability in IL-17 levels highlights the need for personalized approaches to modulate immune responses in severe TB cases. Research into the therapeutic potential of targeting IL-17 pathway in TB is warranted. Further studies are needed to elucidate the mechanisms linking vitamin D and IL-17 with TB severity, particularly in pediatric populations. Interventions into other immunological markers and their interactions with vitamin D and IL-17 could provide deeper insights into TB pathogenesis.

This study has several limitations that should be acknowledged. First, the small sample size limits the generalizability of the findings. While trends were observed, the statistical power may have been insufficient to detect significant differences in some analyses. Future studies with larger, multicenter cohorts are necessary to confirm these findings, improve generalizability, and provide more robust analysis.

Second, the study utilized a cross-sectional design, which precludes the ability to draw causal inferences between vitamin D levels, IL-17 levels, and TB severity. Longitudinal studies tracking changes in these parameters over the course of treatment would provide better insights into their roles in disease progression and recovery.

Third, while the novel severity scoring system developed in this study provided comprehensive framework for assessing TB severity, it has not been externally validated. The scoring system should be tested in larger, independent cohorts to confirm its reliability and clinical utility.

Fourth, there was heterogeneity in the study population, with a wide range of ages, nutritional statuses, and comorbidities that could confound the relationships observed between vitamin D, IL-17, and TB severity. Stratified analyses or adjustments for these variables in future studies could help clarify their individual contributions.

Finally, the study did not assess other potential confounding factors that could influence vitamin D and IL-17 levels, such as genetic polymorphisms, co-infections, and variations in treatment regimens. Comprehensive evaluation of these factors in future research could provide a more nuanced understanding in their roles in pediatric TB.

Despite these limitations, the findings provide important insights into the potential roles of vitamin D and IL-17 in pediatric TB and highlight areas for further investigation. These data underscore the need for larger, more rigorous studies to validate these findings and explore their clinical implications.

5. Conclusions

This preliminary study provides novel insights into the relationship between vitamin D and IL-17 levels with disease severity in pediatric TB. While vitamin D levels were significantly lower in microbiologically confirmed cases, no significant association was found between vitamin D levels and TB severity. However, a decreasing trend in vitamin D levels with increasing severity suggests a potential role in TB pathogenesis that warrant further investigation.

IL-17 levels were significantly higher in children with respiratory distress, indicating its role in the inflammatory response contributing to disease severity. However, no significant difference in IL-17 levels was observed among severity groups. Interestingly, microbiologically negative patients exhibit higher IL-17 levels, suggesting complex immune interaction that requires further exploration.

The study highlights the potential for vitamin D screening and supplementation in pediatric TB patients as an adjunctive strategy to optimize clinical outcomes. Moreover, the findings suggest that IL-17 may serve as biomarker for disease activity, particularly in patients with respiratory distress. However, further research is needed to establish the precise role of these biomarkers in TB severity and their implications for treatment strategies.

Given the study's limitations, including small sample size and cross-sectional design, larger and longitudinal studies are necessary to confirm these findings and explore the dynamic interaction between vitamin D, IL-17, and TB severity. However, future research may consider focusing on a single immunological marker to minimize con-

founding and allow for more precise mechanistic understanding. Future research should also focus on validating the novel severity scoring system proposed in this study and investigating additional immunological markers that may contribute to TB pathogenesis in pediatric patients.

Recognizing these limitations is essential for transparency and scientific rigor. We believe that acknowledging potential confounding factors reflects the integrity of the study and provides valuable direction for future research.

Author Contributions

Conceptualization, E.O. and A.I.; methodology, E.O., and A.I.; software, M.A.; validation, E.O., and A.I.; formal analysis, E.O, M.A., A.I.; investigation, M.A.; resources, M.A. and A.I.; data curation, A.I.; writing—original draft preparation, M.A.; writing—review and editing, E.O. and A.I.; visualization, E.O.; supervision, A.I.; project administration, A.I.; funding acquisition, A.I. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and ethical approval for this study was obtained from the Ethics Committee of Dr. Saiful Anwar Hospital, with approval number 400/179/K.3/102.7/2022. Ethical clearance ensured that all procedures involving human participants adhered to the highest standards of research integrity and patient safety.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The datasets generated and/or analyzed during this study are not publicly available due to institutional regulations but may be obtained from the corresponding author upon reasonable request and with appropriate institutional approvals.

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Conflicts of Interest

The authors declare no conflict of interest. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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