RESEARCH ARTICLE

Elevated Serum Cathelicidin and Inverse Correlation with Vitamin D in Patients with Intestinal Tuberculosis

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Abstract

BACKGROUND: Intestinal tuberculosis (ITB) is a type of tuberculosis (TB) that affects the gastrointestinal tract. Cathelicidin, an antimicrobial peptide, contributes to defense against *Mycobacterium tuberculosis*, which causes TB. Its expression is tightly regulated by vitamin D through vitamin D receptor signaling and autophagy pathways. Despite the increasing evidence on TB, studies exploring the correlation between cathelicidin and vitamin D in the ITB remain poorly characterized and almost exclusively derived from pulmonary TB. Therefore, the present study was conducted to compare serum cathelicidin and vitamin D levels between subjects with ITB and without ITB, and to determine the correlation between these two biomarkers.

METHODS: This comparative cross-sectional study utilized stored serum samples obtained from participants enrolled in a previous ITB project. Twenty-two ITB and 22 non-ITB subjects aged >18 years who had undergone colonoscopy, histopathological examinations, and fulfilled predefined clinical and diagnostic criteria were included. Serum cathelicidin and vitamin D levels were measured using sandwich enzyme-linked immunosorbent assay (ELISA) and chemiluminescent microparticle immunoassay (CMIA), respectively.

RESULTS: Significantly higher levels of serum cathelicidin were identified in ITB subjects, with a median level of 3.67 (2.27–5.95) ng/mL, compared with non-ITB subjects with a median level of 2.04 (1.66–2.46) ng/mL (p<0.0001). Although the difference was not statistically significant (p=0.091), vitamin D level tended to be lower in subjects with ITB, with a median level of 11.05 (6.80–25.25) ng/mL, compared to non-ITB subjects with a median level of 17.95 (12.13–23.63) ng/mL. A significant moderate negative correlation was found between cathelicidin and vitamin D (r=-0.485, p=0.001).

CONCLUSION: The ITB is associated with elevated cathelicidin levels and a tendency toward lower vitamin D levels. The inverse correlation suggests a complex relationship between vitamin D status and cathelicidin expression, highlighting immunological mechanisms involved in ITB pathogenesis.

KEYWORDS: antimicrobial peptide, cathelicidin, innate immunity, intestinal tuberculosis, vitamin D

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Introduction

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* infection. In 2023, Indonesia was the second country with the highest TB incidence, accounting for 10% of the worldwide total.(1) In addition to affecting the respiratory system (pulmonary TB, PTB), TB can also affect other sites (extrapulmonary TB). The global prevalence of extrapulmonary TB is 16%,

while in Indonesia it ranges from 10–19%.(2) Among the extrapulmonary TB cases, 3–16% are intestinal tuberculosis (ITB).(3-5) Mortality rates for untreated ITB can exceed 40%, and even with treatment, there is a recurrence rate of 13–20% in cases.(6)

Moreover, management of ITB remains a diagnostic challenge due to its clinical symptoms mimicking other gastrointestinal disorders, such as colorectal cancer and Crohn's disease.(7,8) Misdiagnosis, missed diagnosis, and delayed diagnosis and treatments can significantly reduce quality of life, increase medical costs, and potentially worsen the disease and even be life-threatening.(9) In addition, the gold standard test for ITB is a culture of *M. tuberculosis* that utilizes intestinal mucosal tissue samples. However, because these bacteria are paucibacillary, it is difficult to detect *M. tuberculosis* using this method, which raises the possibility of obtaining false-negative results.(4) Therefore, making an accurate diagnosis at the earliest stage is crucial.

In the pathogenesis of TB, the initial barrier that counteracts *M. tuberculosis* is the innate immunity. This immunity is activated when pathogen-associated molecular patterns (PAMPs) on *M. tuberculosis* bind to pathogen recognition receptors (PRRs), specifically toll-like receptors (TLRs)-2 and TLR-4 on immune cells. The activation of TLR-2 and TLR-4 subsequently induces increases in pro-inflammatory cytokines, decreases in anti-inflammatory cytokines, and the secretion of antimicrobial peptides, such as cathelicidin.(10-12) Cathelicidin is an antimicrobial peptide with direct microbiocidal activity at micromolar concentrations. Cathelicidin exerts its antimicrobial effects primarily through two mechanisms, including the formation of transmembrane pores and the destruction of microbial membranes.(13) Based on these antimicrobial activities, cathelicidin has been recognized as a crucial effector molecule in host defense against *M. Tuberculosis*. *M. tuberculosis* infection induces cathelicidin expression, particularly in alveolar macrophages, suggesting its crucial role during early infection.(14) Consistently, higher cathelicidin levels have been observed in patients with PTB compared to those with healthy controls.(15) Although most studies have focused on PTB, similar innate immune mechanisms are presumed to operate in extrapulmonary sites, including the intestinal system.

Interestingly, the most effective inducer of cathelicidin is vitamin D. Vitamin D is a fat-soluble vitamin whose signaling and metabolism play crucial roles in bone and calcium homeostasis and is recognized as a pivotal immunomodulator in infectious diseases such as TB. Stimulation of dendritic cells with heat-killed *M. tuberculosis* strongly downmodulates cathelicidin expression, and vitamin D counteracts this by upmodulating cathelicidin expression.(16) Both the vitamin D receptor (VDR) and the vitamin D-1 hydroxylase gene are upregulated by the activation of TLRs, thereby increasing the conversion of inactive vitamin D to its active form. This process subsequently induces the expression of cathelicidin and killing of intracellular *M. Tuberculosis*.(17) This mechanism highlights how adequate vitamin D status can directly influence the host's innate immune capacity against *M. tuberculosis* through cathelicidin induction.

Despite the increasing evidence on TB, studies exploring the association between cathelicidin and vitamin D in the ITB remain poorly characterized. Existing studies on cathelicidin and vitamin D are almost exclusively derived from patients with PTB (15,18-20), whereas the immunological profile of ITB may differ due to the distinct mucosal immune environment of the gastrointestinal tract. A study has demonstrated elevated circulating cathelicidin levels in PTB and pleural TB, suggesting that increased serum cathelicidin may reflect systemic acute-phase immune responses rather than localized antimicrobial activity at the site of infection. In contrast, local cathelicidin expression in lymph node TB has been associated with higher vitamin D levels. These findings suggest that the relationship between cathelicidin and vitamin D may vary across different clinical forms of TB.(21) One study has identified a weak negative correlation between cathelicidin and vitamin D (18), while other studies have not found a correlation (15,19,20,22). In contrast, a linear correlation was observed between these two biomarkers in TB patients compared with healthy controls.(23) Considering Indonesia's high TB burden and the clinical relevance of the ITB, investigating the correlation between cathelicidin and vitamin D in the ITB is essential. Therefore, the present study was conducted to compare serum cathelicidin and vitamin D levels between subjects with ITB and without ITB, and to determine the correlation between these two biomarkers.

Methods

Study Design and Participants

This comparative cross-sectional study was performed at the Clinical Pathology Laboratory of Dr. Cipto Mangunkusumo Central General Hospital from June to November 2024. This study utilized samples obtained from participants enrolled in a previous ITB project, for which the methods,

details of informed consent, and ethical approval have been reported previously.(24) The current study explores the levels of serum cathelicidin and vitamin D, as well as their correlation in ITB. The original ITB project analyzed a total of 143 subjects, comprising 22 subjects diagnosed with ITB and 121 with non-ITB conditions (including hemorrhoids, non-specific ileocolitis, inflammatory bowel diseases, and malignancy). For the present study, a sample size calculation was performed using a two-sided test with 80% statistical power and a 5% significance level, resulting in a minimum required sample of 21 subjects in each group. To optimize analysis, this study included all available ITB subjects (n=22) and 22 non-ITB subjects, who were selected by simple random sampling from the original cohort.

The inclusion and exclusion criteria applied in the original ITB project were maintained. Inclusion criteria of subjects were: Adult patients aged >18 years; fulfilled the 3 of 4 major clinical criteria: 1) weight loss, 2) non-specific abdominal pain, 3) fever, 4) diarrhea or chronic constipation, and 1 of 3 additional histories: 1) PTB history, 2) active TB with ongoing anti-tuberculosis therapy (ATT) <3 months, 3) contact with a positive TB patient; performed colonoscopy and histopathology examinations; agreed and signed the informed consent form. All diagnosed subjects with ITB fulfilled the 2 of 4 criteria or positive stool TB polymerase chain reaction (PCR) only: 1) summary of colonoscopy result showed ITB suspect, 2) histopathological result showed granulomatous inflammation, 3) positive stool TB PCR, and 4) subjects showed a good response to ATT with clinical manifestation consistent with active TB. Meanwhile, the exclusion criteria were: Subjects who were on ongoing ATT for >3 months and post-treatment with ATT <6 months. Additional exclusion criteria specific to the present study included: 1) samples that were hemolyzed, icteric or lipemic, 2) individuals who received vitamin D supplementation, 3) those with chronic liver disease, and 4) those with chronic kidney disease. Subject characteristics, including age, sex, body mass index (BMI), and categorized BMI, were collected for analysis.

Collection of Study Specimens

Approximately 2 mL of serum samples were derived from the study population involved in the ITB project. These samples were stored at -80°C for a duration of 1–2 years. Measurements of cathelicidin and vitamin D levels were performed on these samples. Previous studies have shown that both cathelicidin and vitamin D in serum stored at -80°C remain stable over extended periods, demonstrating minimal variation.(25,26) All samples underwent a single freeze–thaw cycle and were analyzed in a blinded manner with respect to clinical diagnosis.

Measurement of Serum Cathelicidin

Stored serum samples were thawed, and the level of cathelicidin was examined using the sandwich enzyme-linked immunosorbent assay (ELISA) method. Following the instruction manual of USCNK SEC419Hu ELISA Kit for Cathelicidin (Cloud-Clone Corp, Katy, TX, USA), 100 µL of standards and serum samples were added into microplate wells precoated with a biotin-conjugated antibody specific to cathelicidin and incubated at 37°C for 1 hour. Subsequently, each well was added with avidin-horseradish peroxidase (HRP) conjugate and incubated. After this incubation, 90 µL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution was added and incubated at 37°C in the dark for 10–20 minutes. During this reaction, blue coloration developed in the wells. The reaction was terminated by adding a sulfuric acid-based stop solution, which converted the color from blue to yellow. The absorbance was measured at 450 nm using a spectrophotometer, and cathelicidin levels were calculated by comparing the optical density of the samples with that of the standard curve.

Measurement of Serum Vitamin D

The stored serum samples were thawed, and vitamin D level was measured using a delayed one-step chemiluminescent microparticle immunoassay (CMIA) method. This assay utilized the ARCHITECT 25(OH) D reagent kit and the ARCHITECT i2000SR instrument, following the instruction manual (Abbott, Longford, Ireland). The sample, pre-treatment reagents, assay diluent, and paramagnetic microparticles coated with anti-vitamin D were combined to create a reaction mixture. The vitamin D contained in the sample was released from the vitamin D-binding protein and attached to the microparticles coated with anti-vitamin D, forming antigen-antibody complexes. After incubation, an acridinium-labeled vitamin D conjugate was added to attach to the unoccupied sites on the microparticles coated with anti-vitamin D. Following the incubation and washing steps, pre-trigger and trigger solutions were added to initiate the chemiluminescent reaction. The intensity of this reaction was quantified in relative light units (RLUs). Importantly, there was a direct correlation between the concentration of vitamin D in the sample and the resulting RLUs. The vitamin D level was determined automatically using a calibration curve generated in prior experiments.

Statistical Analysis

Statistical analyses were performed using SPSS software version 20 (IBM Corporation, Armonk, NY, USA). The data distribution was evaluated with the Shapiro-Wilk test. Descriptive statistics were employed to summarize subject characteristics and clinical manifestations. Numerical variables were compared using either the independent t-test or the Mann-Whitney U test, based on data normality, and were expressed as the mean±standard deviation (SD) or median (interquartile range (IQR)), respectively. Categorical variables were evaluated using the chi-square test and presented as frequencies and percentages. Differences in vitamin D status between ITB and non-ITB subjects were examined using Fisher's exact test. The relationship between serum cathelicidin and vitamin D levels was evaluated using Spearman's rank correlation test. Exploratory cut-off values for cathelicidin and vitamin D were assessed using receiver operating characteristic (ROC) curve analysis and the Youden index. Statistical significance was established at $p<0.05$.

Results

Subject Characteristics and Clinical Manifestations

The subject characteristics and clinical manifestations were presented in Table 1. A total of 44 subjects, the median age was 40.50 (26.00–55.00) years, with a female predominance of 54.55%. The median age of the ITB subjects was 35.50 (26.00–57.00) years, with a female-to-male ratio of 1:1. Among the non-ITB subjects, the median age was 44.00 (25.50–52.00) years, with a female predominance of 59.09%. Compared with non-ITB subjects, ITB subjects had a significantly lower BMI ($p=0.001$). The categorized BMI was significantly different between ITB and non-ITB subjects ($p=0.003$), with most of the ITB subjects being underweight to normal BMI (90.90%). Chronic diarrhea, constipation, alternating diarrhea-constipation, weight loss, nonspecific abdominal pain, limp, and appetite loss were the most common symptoms in the ITB subjects. Significant differences in weight loss, limp, and appetite loss were observed between ITB and non-ITB subjects ($p=0.005$, $p=0.003$, and $p=0.033$, respectively). The TB history indicated no statistically significant difference between the subjects with ITB and non-ITB.

Serum Cathelicidin Level was Higher in ITB Subjects

A significantly higher level of cathelicidin was found in the ITB subjects than in the non-ITB subjects ($p<0.0001$). The median cathelicidin level in the ITB subjects was 3.67 (2.27–5.95) ng/mL, whereas the median cathelicidin level in the non-ITB subjects was 2.04 (1.66–2.46) ng/mL (Table 1).

Serum Vitamin D Level Tended to be Lower in ITB Subjects

Serum vitamin D level and vitamin D status were presented in Table 1. Both the ITB and non-ITB subjects demonstrated no statistically significant difference in serum vitamin D level ($p=0.091$). However, vitamin D levels tended to be lower in the ITB subjects compared to non-ITB subjects. Median vitamin D level in ITB subjects was 11.05 (6.80–25.25) ng/mL, whereas in non-ITB subjects, it was 17.95 (12.13–23.63) ng/mL.

High Proportion of Vitamin D Deficiency among ITB and Non-ITB Subjects

To evaluate vitamin D status between ITB and non-ITB subjects, this study categorized levels of vitamin D less than 30 ng/mL (<30 ng/mL) as deficient, and levels equal to or above 30 ng/mL (≥30 ng/mL) as sufficient (based on the biological reference value). Vitamin D deficiency was prevalent in both groups, affecting 86.36% of the subjects with ITB and all the subjects (100.00%) without ITB. Our findings revealed no significant difference in vitamin D status between the ITB and non-ITB subjects ($p=0.233$). In addition, a significantly lower level of vitamin D was found in the female subjects than in the male subjects ($p=0.004$), although both are vitamin D deficient. The median level of vitamin D in male subjects was 20.80 (13.90–26.38) ng/mL. The median level of vitamin D in female subjects was 11.05 (7.33–14.30) ng/mL.

Inverse Correlation of Serum Cathelicidin and Vitamin D

We conducted a correlation analysis using Spearman's rank correlation test to determine the relationship between serum cathelicidin and vitamin D in the ITB and non-ITB subjects. Our results revealed a statistically significant moderate negative correlation between cathelicidin and vitamin D in subjects with ITB and non-ITB, with a correlation coefficient (r)=-0.485 and $p=0.001$.

Exploratory Cut-Off Values for Cathelicidin and Vitamin D Levels

Based on the ROC curve and Youden index analysis, the most appropriate cut-off values for cathelicidin and vitamin D levels were identified for descriptive comparison between ITB and non-ITB subjects, as shown in Figure 1, and

Table 1. Baseline characteristics and laboratory data of the ITB and non-ITB subjects.

Parameters	ITB	Non-ITB	Total	*p*-value
Subject Characteristics				
Age (years), median (IQR)	35.50 (26.00–57.00)	44.00 (25.50–52.00)	40.50 (26.00–55.00)	0.842[a]
Sex, n (%)				
Female	11 (50.00)	13 (59.09)	24 (54.55)	0.545[b]
Male	11 (50.00)	9 (40.91)	20 (45.45)	
BMI (kg/m^2), mean±SD	19.82±4.20	24.78±5.33	22.30±5.36	0.001[c]*
Categorized BMI, n (%)				
Underweight–Normal (<25.00 kg/m^2)	20 (90.90)	11 (50.00)	31 (70.45)	0.003[b]*
Overweight–Obese (≥25.00 kg/m^2)	2 (9.10)	11 (50.00)	13 (29.55)	
Clinical Manifestations				
Nonspecific abdominal pain, n (%)	17 (77.27)	18 (81.81)	35 (79.55)	0.709[b]
Chronic diarrhea, n (%)	18 (81.81)	15 (68.18)	33 (75.00)	0.296[b]
Constipation, n (%)	18 (81.81)	14 (63.63)	32 (72.73)	0.176[b]
Alternating diarrhea-constipation, n (%)	18 (81.81)	14 (63.63)	32 (72.73)	0.176[b]
Blood in stool, n (%)	10 (45.45)	11 (50.00)	21 (47.73)	0.763[b]
Mucus in stool, n (%)	10 (45.45)	12 (54.54)	22 (50.00)	0.546[b]
Blood and mucus in stool, n (%)	10 (45.45)	11 (50.00)	21 (47.73)	0.763[b]
Weight loss, n (%)	18 (81.81)	9 (40.91)	27 (61.36)	0.005[b]*
Fever, n (%)	6 (27.27)	2 (9.09)	8 (18.18)	0.118[b]
Night sweat, n (%)	2 (9.09)	1 (4.54)	3 (6.81)	0.550[b]
Limp, n (%)	16 (72.72)	6 (27.27)	22 (50.00)	0.003[b]*
Appetite loss, n (%)	13 (59.09)	6 (27.27)	19 (43.18)	0.033[b]*
Cough, n (%)	3 (13.63)	1 (4.54)	4 (9.09)	0.294[b]
TB History				
Contact history with TB subject, n (%)	1 (4.54)	1 (4.54)	2 (4.55)	1.000[b]
TB history (PTB or extrapulmonary TB), n (%)	3 (13.63)	1 (4.54)	4 (9.09)	0.294[b]
Blood Examination				
Cathelicidin levels (ng/mL), median (IQR)	3.67 (2.27–5.95)	2.04 (1.66–2.46)	2.40 (1.88–3.74)	<0.0001[a]*
Vitamin D levels (ng/mL), median (IQR)	11.05 (6.80–25.25)	17.95 (12.13–23.63)	13.55 (8.78–24.53)	0.091[a]
Male			20.80 (13.90–26.38)	0.004[a]*
Female			11.05 (7.33–14.30)	
Vitamin D status				
Deficiency (<30 ng/mL), n (%)	19 (86.36)	22 (100.00)	41 (93.18)	0.233[d]
Sufficiency (≥30 ng/mL), n (%)	3 (13.64)	0 (0.00)	3 (6.82)	

Tested with [a]Mann-Whitney U test, [b]Chi-squared test, [c]Independent t-test, [d]Fisher's exact test. *Statistically significant at $p<0.05$.

validated by the Chi-square test (Table 2). A cathelicidin level of ≥3.21 ng/mL was significantly associated with ITB status ($p<0.0001$), yielding a sensitivity of 59.09%, specificity of 95.45%, and an AUC of 0.811 (95% CI: 0.681–0.940). Similarly, a vitamin D level of <11.75 ng/mL was significantly associated with ITB status ($p=0.027$), with a sensitivity of 54.55%, specificity of 81.82%, and an AUC of 0.649 (95% CI: 0.478–0.820). These findings are not intended to establish diagnostic cut-off values.

Discussion

The current study identified higher levels of cathelicidin in the ITB subjects than in the non-ITB subjects. This finding aligns with previous studies reporting that TB patients had higher cathelicidin levels than their controls did.(15,18) The higher level of cathelicidin in ITB subjects could be attributed to the elevated bacterial load during *M.*

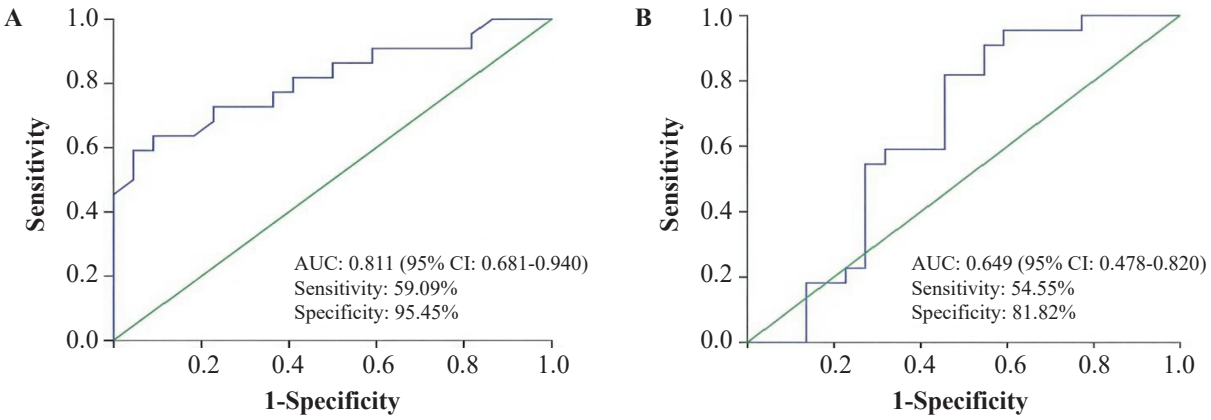

Figure 1. ROC curve analysis for cathelicidin (A) and vitamin D (B) in identifying appropriate cut-off values between ITB and non-ITB subjects. AUC: area under the curve; CI: confidence interval.

tuberculosis replication, extensive tissue damage observed in active TB patients, and differences in cathelicidin expression pathways.(17,27,28) In ITB subjects, *M. tuberculosis* bacterial cell wall components, such as triacyl or diacyl lipopeptides and lipoteichoic acid, are recognized by TLR-2, which initiates a vitamin D-dependent signaling that upmodulates cathelicidin synthesis.(17,27,29) However, these mechanisms were not directly assessed in the present study and are only discussed as a biological framework to contextualize our findings. In contrast, non-ITB subjects are not stimulated by *M. tuberculosis*, so the circulating cathelicidin level may reflect baseline innate immune activity rather than pathogen-driven induction.

The present study revealed that vitamin D levels were lower in subjects with ITB compared to those without ITB, although the difference did not show statistical significance. This finding is consistent with previous studies that reported lower vitamin D levels in TB patients.(15,30) In individuals with ITB, macrophages activated through TLR-2 may increase local conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D, a form that is immediately utilized for immune response.(17,27,31) In addition, the vitamin D measured is vitamin D in the inactive form (25-hydroxyvitamin D). Consequently, circulating (serum) vitamin D levels may appear lower in individuals with ITB relative to non-ITB subjects.

Table 2. Exploratory cut-off values for cathelicidin and vitamin D levels.

Parameters	ITB	Non-ITB	*p*-value
Cathelicidin, n (%)			
≥3.21 ng/mL, n (%)	13 (59.09)	1 (4.55)	<0.0001[a]*
<3.21 ng/mL, n (%)	9 (40.91)	21 (95.45)	
Vitamin D			
<11.75 ng/mL, n (%)	12 (54.55)	4 (18.18)	0.027[a]*
≥11.75 ng/mL, n (%)	10 (45.45)	18 (81.82)	

[a]Tested with Chi-squared test. *Statistically significant at $p<0.05$.

In the present study, both ITB and non-ITB subjects showed a high proportion of vitamin D deficiency. However, three subjects with ITB had sufficient levels of vitamin D. Upon reviewing their treatment history or any supplementation, it was found that these three individuals had not consumed anything related to vitamin D metabolism. This finding is similar to a recent study that reported 4 of 8 patients with abdominal TB presented a pattern of involvement in the intestine had sufficient vitamin D.(32) However, several studies have shown that the adult population in Indonesia also has vitamin D deficiency.(33-35) These findings showed that vitamin D deficiency is common in both ITB and non-ITB populations in Indonesia and may reflect population-level and disease-related factors rather than ITB-specific effects alone. In addition, the female subjects who dominated this study showed lower vitamin D levels than males, even though both groups had vitamin D deficiency. Factors that influence vitamin D status in this study include differences in sex and the presence of chronic diseases.(36-38)

This study revealed a significantly moderate negative correlation between cathelicidin and vitamin D. These results indicate higher cathelicidin levels and lower vitamin D levels among the ITB subjects. This finding is comparable to a previous study that reported a significantly weak negative correlation between cathelicidin and vitamin D, as measured in individuals with active TB, latent TB, and those without TB.(18) Moreover, lower levels of vitamin

D and higher levels of cathelicidin were reported among TB patients compared with non-TB individuals.(28) This negative correlation may be explained by the use of serum samples to measure cathelicidin and vitamin D levels, which are representative of systemic immune responses and may not fully represent local immune responses at sites of infection. Local immune responses and a positive correlation were reported between local cathelicidin expression in granulomatous lesions of lymph nodes affected by TB and plasma vitamin D level.(21) This inverse relationship may reflect the complex regulation of cathelicidin expression by vitamin D in chronic *M. tuberculosis* infection. However, the exact mechanisms cannot be inferred from a serum-based measurement stand-alone test.

We also performed the ROC curve and Youden index analyses to identify the exploratory cut-off values of serum cathelicidin and vitamin D levels for descriptive comparison between ITB and non-ITB subjects. A cathelicidin cut-off value of ≥3.21 ng/mL was associated with ITB status, yielding an AUC of 0.811, with moderate sensitivity and high specificity. Notably, our cut-off value is significantly lower than in the previous study, which established a cut-off value of >214.8 ng/mL in the population with latent PTB and active PTB.(39) The discrepancy in cut-off values originates from differences in the study populations and measurement methods used. In our study, we analyzed stored serum samples from individuals diagnosed with ITB and those with non-ITB conditions, including those with hemorrhoids, nonspecific ileocolitis, inflammatory bowel diseases, and malignancy. Conversely, the previous study assessed stored serum samples exclusively from individuals with latent PTB and active PTB.(39) Additionally, we measured cathelicidin level using the sandwich ELISA method, which has a detection range of 0.78–50 ng/mL. Meanwhile, previous study utilized the competitive ELISA method (39), which has a significantly broader detection range of 123.5–10,000 ng/mL.

In addition, the exploratory vitamin D cut-off value of <11.75 ng/mL was associated with ITB status, demonstrated limited discriminatory ability (AUC of 0.649), moderate sensitivity, and high specificity. This cut-off value was comparable to another study that used a cut-off value of <12.05 ng/mL in the population with latent PTB and active PTB, with an AUC of 0.684, low sensitivity, and high specificity.(40)

The findings of elevated serum cathelicidin levels, lower tendency of vitamin D levels, and inverse correlation between serum cathelicidin and vitamin D levels suggest a potential dysregulation of the cathelicidin-vitamin D immune axis in ITB. In the future, these findings may contribute to the development of immunological biomarkers and monitoring of ITB, although further longitudinal and mechanistic studies are still required. Cathelicidin has the potential to be a candidate biomarker for ITB laboratory examination, while vitamin D can be a supporting test for high cathelicidin expression in ITB, as vitamin D is a precursor in cathelicidin expression.

It is crucial to acknowledge that our study has several limitations that may affect the interpretability of our findings. This cross-sectional study was performed in a single center, potentially limiting the applicability of the findings to larger populations. Additionally, we used serum samples to measure cathelicidin level, which reflects the systemic innate immune response rather than the local immune response. Therefore, we suggest performing a multicenter study using serum samples and granulomatous lesions from biopsy materials to compare systemic innate immune responses and local innate immune responses in individuals with ITB.

Conclusion

The present study revealed that the ITB is associated with elevated cathelicidin levels and a tendency toward lower vitamin D levels. The inverse correlation suggests a complex relationship between vitamin D status and cathelicidin expression, highlighting immunological mechanisms involved in ITB pathogenesis.

Acknowledgments

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Authors Contribution

NDI was involved in conception and planning the research. NDI, NA, PMSDP, and FA performed the data acquisition/collection. NA, PMSDP, and FA calculated the experimental data and performed the analysis. NDI and NA drafted the manuscript and designed the figures and tables. NDI, AG, NS, DW, and DHD aided in supervising, validating, and interpreting the results. All authors took part in giving a critical revision of the manuscript.

Ethical Statement

We conducted this study in compliance with the principles of the Declaration of Helsinki. The study's protocol was reviewed and approved by the institutional review boards at the Ethical Committee of Faculty of Medicine, Universitas Indonesia–Dr. Cipto Mangunkusumo Central General Hospital (Approval No. KET-844/UN2.F1/ETIK/PPM.00.02/2024 for cathelicidin assay and KET-1711/UN2.F1/ETIK/PPM.00.02/2024 for vitamin D assay). Written informed consent was obtained.

Conflict of Interest

The authors have no conflicts of interest to declare.

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