

## Case Series

# Vitamin D status in under-five children with a history of close tuberculosis contact in Padang, West Sumatra

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**Background and Objective:** Serum vitamin D influences cellular immunity against *Mycobacterium tuberculosis*, particularly in under-five children. Vitamin D insufficiency and deficiency as well as tuberculosis (TB) are highly prevalent in Indonesia. This study described the association of serum vitamin D in the incidence of latent TB in under-five children with a history of close TB contact. **Methods and Study Design:** This cross-sectional study examined 178 under-five children with a history of close TB contact, with 98 index cases from primary health care facilities in Padang. Latent TB was defined based on a positive tuberculin skin test. Serum vitamin D was determined using ELISA method. General characteristics for age, sex, socioeconomic status, contact score, BCG scars, and nutritional status were also collected. **Results:** Of 168 eligible subjects, 40.5% had latent TB, however no differences between 'latent TB' and 'without latent TB' were observed in participant characteristics. Vitamin D deficiency, insufficiency, and sufficiency were observed in 23.3%, 43.5%, and 33.3% of children, respectively. In general, the 'latent TB' group had lower serum vitamin D than those in the 'without latent TB' group, stratified by age, contact score and nutritional status; however, significant differences were observed in children age <1 years ( $18.0\pm 2.6$  vs  $31.2\pm 10.5$ , respectively,  $p=0.013$ ). Nevertheless, no associations between the two groups were observed using multivariate analysis. **Conclusion:** Vitamin D status was not associated with the incidence of latent TB among under-five children with a history of close TB contact, although this may not obtain in children aged <1 year old.

**Key Words:** vitamin D status, tuberculosis contact, under-five children, latent tuberculosis

## INTRODUCTION

Many studies have reported that serum vitamin D has immunomodulatory properties, such as an antimicrobial.<sup>1</sup> Vitamin D has a considerable effect on the immune system and defense mechanisms against microorganisms, including *Mycobacterium tuberculosis*. In tuberculosis (TB), vitamin D regulates the expression of specific endogenous antimicrobial peptides in macrophages and stimulates the production of cytokines and other inflammatory mediators.<sup>1,2</sup> Vitamin D insufficiency and deficiency are highly prevalent in subtropical and tropical areas.<sup>3</sup> In Indonesia, a wide variation in the prevalence of vitamin D insufficiency and deficiency has been reported, with Prawirohartono et al indicating a rate of 36.1%, the SEANUT study stating 43%, and Soesanti et al stating 75.8%.<sup>4-6</sup>

TB remains a major global problem, with a high incidence of illness and death among adults and children. A WHO global report revealed that at least 500,000 children are infected with TB every year, and up to 74,000 HIV-uninfected children die of TB annually.<sup>7</sup> TB is continuously transmitted from adults to children, particularly in

TB-endemic countries. Household contact is a crucial risk factor for continuous transmission.<sup>8</sup> A study of African children revealed that one-third to two-thirds of children with a history of household TB contact exhibited latent TB, as evidenced by positive tuberculin skin tests (TSTs).<sup>9</sup>

This study investigated the association of serum vitamin D status in the incidence of latent TB in under-five children with a history of close TB contact.

## METHODS

### Study design and setting

This cross-sectional study was approved by the Ethical

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Manuscript received 06 March 2017. Initial review completed 30 March 2017. Revision accepted 13 May 2017.

doi: 10.6133/apjcn.062017.s2

Committee of the Faculty of Medicine with the letter number 263/KEP/FK/2013, Universitas Andalas. The study was conducted from March 2014 to December 2015 in Padang City, West Sumatra, Indonesia. Written informed consent was obtained from the parents of all subjects prior to the commencement of the study. We recruited subjects in close contact with the index patients at 22 primary health care (PHC) facilities in Padang. Index cases were defined as TB cases in adults of the household, who were clinically diagnosed through sputum smear positivity by a PHC physician. The study nurse identified under-five children in close contact with the index patients.

### **Subject recruitment and participation**

Subjects were recruited from the TB contact list at each PHC facility. All under-five children in close contact with the index cases were assessed. Subjects were recruited consecutively. They were excluded if they had received TB treatment or experienced severe infections, such as measles, typhoid, and HIV, in the past 3 months. Furthermore, we obtained data on the age, sex, nutritional status, and socioeconomic status of the subjects as well as details regarding the presence or absence of a BCG scar.

After assessing the eligibility of the subjects, the physician performed tuberculin skin tests (TSTs) and a trained nurse collected blood samples (5 mL) for measuring serum vitamin D.

In the TSTs, 0.1 mL (2 tuberculin units) of the purified protein derivative RT 23 from Biofarma Institute, Bandung, Indonesia, was intradermally administered at the volar forearm of the subjects. The TST results were evaluated after 72 h, and the transverse diameter of the induration was measured. The results were considered positive for an induration diameter of  $\geq 10$  mm.

We assessed 178 under-five children exposed to 98 index cases from March 2014 to December 2015. Ten subjects were excluded from this study, and the TSTs and serum vitamin D status measurements were performed in 168 eligible subjects (participation rate, 94.3%).

### **Determination of index cases, demographic data, contact score, nutritional status and latent TB**

#### **Index cases**

According to the WHO criteria, TB was diagnosed in index cases with at least two initial sputum smears positive for acid-fast bacilli observed through direct microscopy.

#### **Demographic data**

We classified certain variables as follows: age groups as 0 to <1 year (infant), >1–3 years, and 3–5 years; sex as boys and girls; and BCG scars as positive and negative. Parents were classified as low educated if their educational level was lower than the junior high school level, and parents' occupation was classified as working and not working.

#### **Contact score**

Contact scores were categorized as <2 if the index patient lived in the same house but in different room from the participant and >2 if the index patient lived in the same house and same room, regardless of work status.

#### **Nutritional status**

The nutritional status of the subjects was classified as malnourished and well-nourished as per their weight-for-height z-scores, which were determined according to the WHO growth chart.

#### **Latent TB**

Latent TB was diagnosed in subjects with positive TSTs.

#### **Laboratory analyses**

Serum 25(OH)D was measured by using ELISA kit from DiaSorin Inc (DiaSorin, USA) in Prodia Clinical Laboratory located in Jakarta, Indonesia. Prodia is Clinical Laboratory has been granted International Standard Operation (ISO) accreditation 15189 2016. Serum vitamin D were classified as sufficient (>30 ng/mL), insufficient (20–29 ng/mL), and deficient (0–19 ng/mL).

#### **Statistical analysis**

Data analysis was done using SPSS 23.0 statistical analysis software for Windows (SPSS Inc., Chicago, IL, USA). The dependent and independent variables were latent TB and serum vitamin D status. Differences in the incidence of latent TB based on vitamin D status between the two groups were compared using the chi-squared test. Differences of serum vitamin D between two groups stratified by age groups, contact score and nutritional status were compared using independent t test when the data were normally distributed, and Mann Whitney test when the data were abnormally distributed. A *p* value of <0.05 was considered statistically significant, and confidence intervals were calculated for the prevalence of outcomes. We used multivariate analysis to control the confounding variables.

## **RESULTS**

### **Subject characteristics**

We observed that 40.5% and 59.5% of the subjects were with and without latent TB, respectively. Table 1 summarizes the characteristics of the study subjects according to the proportion of latent TB. The overall proportions of subjects in the categories listed in the table were higher in those without latent TB than those in the latent TB group, except for the proportion of malnourished subjects, which was higher in the latent TB group (52.5% vs 47.5%); however, the differences between the groups were not significant (*p*=0.08). Furthermore, latent TB was particularly prevalent in the 3–5 years age group (43.4%), boys (46.1%), parents with a low education level (41.3%), subjects with a contact score >2 (44.2%), subjects with a BCG scar (41.4%), malnourished subjects (52.5%), and subjects from rural areas (42.1%).

### **Incidence of latent TB with regard to serum vitamin D**

Vitamin D deficiency, insufficiency, and sufficiency were observed in 23.2%, 43.5%, and 33.3% of the study subjects, respectively. Table 2 shows the association of vitamin D status with the incidence of latent TB. Overall, with or without controlling for the confounding variables, compared with vitamin D sufficiency, the status of vitamin D insufficiency and deficiency had no significant influence on the incidence of latent TB. However, with-

**Table 1.** Characteristics of children, according to latent TB incidence

Variable	Latent TB		Without Latent TB		<i>p</i> value
	n	%	n	%	
Age group (y)					
0–1	5	22.7	17	77.3	0.078
>1–3	27	42.9	36	57.1	0.950
3–5	36	43.4	47	56.6	
Sex					
Girls	27	34.2	52	65.8	0.117
Boys	41	46.1	48	53.9	
Parent's education					
Low educated	62	41.3	88	58.7	0.514
High educated	6	33.3	12	66.7	
Parent's occupation					
Working	60	42.9	80	57.1	0.160
Not-working	8	28.6	20	71.4	
Contact score					
≤2	34	37.4	57	62.6	0.371
>2	34	44.2	43	55.8	
BCG scar					
Present	46	41.4	65	58.6	0.722
Not present	22	38.6	35	61.4	
Nutritional status					
Malnourished	21	52.5	19	47.5	0.076
Well-nourished	47	36.7	81	63.3	
Primary health care location					
Rural	56	42.1	77	57.9	0.402
Urban	12	34.3	23	65.7	

TB: tuberculosis.

**Table 2.** Association of serum vitamin D with latent TB incidence

Variable	Latent TB		Unadjusted			Adjusted <sup>†</sup>		
	with n (%)	without n (%)	<i>p</i>	OR	CI 95%	<i>p</i>	OR	CI 95%
Vitamin D status								
Deficient	18 (46.2)	21 (53.8)	0.32	1.67	0.72-3.86	0.37	1.48	0.63-3.51
Insufficient	31 (42.5)	42 (57.5)	0.42	1.44	0.70-2.96	0.41	1.37	0.66-2.85
Sufficient	19 (33.9)	37 (66.1)						

TB: tuberculosis; OR: odds ratio.

<sup>†</sup>Adjusted OR value is the OR value of vitamin D for the incidence of latent TB, after controlling for the confounding factors of age, sex, parents' occupation, contact scores, and nutritional status

out considering some variables (age group, sex, socioeconomic status, contact score, and nutritional status), the incidence of latent TB among subjects with vitamin D insufficiency and deficiency was 1.44 and 1.67 fold higher, respectively, than that among subjects with sufficient vitamin D. Moreover, when we used logistic regression to control for confounding factors, the results revealed a similar trend, but the values were lower than those with unadjusted variables.

Differences in serum vitamin D between latent TB and without latent TB groups stratified by age, contact score and nutritional status is presented in Table 3. Significant difference in serum vitamin D between groups was observed in children aged < 1 year old ( $p=0.013$ ), not in children aged >1-3 yo and 3-5 yo. No differences were found between groups stratified by contact score and nutritional status. Subjects with low serum vitamin D and malnourished too, tend to had more higher incidence latent TB than subject with normal serum vitamin D and malnourished (32.7% vs 26.3), however no significant differences.

## DISCUSSION

Previous study indicates that serum vitamin D associated with TB. Arnedo-Pena et al. reported that vitamin D sufficiency protects adults with a history of close TB contact against TB.<sup>10</sup> Furthermore, a study conducted by Talat in Pakistan investigated the role of vitamin D in TB progression and revealed that low serum vitamin D were associated with a 5-fold increased risk for progression to TB.<sup>11</sup> Ho-Pham reported that vitamin D insufficiency is a risk factor for TB in adult men.<sup>12</sup> We hypothesised that the same condition will found in children under-five. Therefore we conduct this study.

The present findings indicate that overall serum vitamin D status is not significantly associated with latent TB in under-five children with a history of close TB contact. Nevertheless, latent TB was highly prevalent among the 3–5 years age group, boys, and malnourished subjects. However, the present findings differed from those of other studies. We could not find another study of under-five children with a history of close TB contact with which our study could be directly compared.

**Table 3.** Serum vitamin D (ng/mL) in 'latent TB' and 'without latent TB' stratified by age, contact scores, and nutritional status

Variable	Latent TB	Without latent TB	<i>p</i>
Age group (yo)			
0–<1 <sup>†</sup>	18.0±2.6	31.2±10.5	0.013
>1–3 <sup>†</sup>	25.6±7.5	27.5±9.5	0.407
>3–5 <sup>‡</sup>	26.7 (13.2–53.8)	25.1 (12.9–50.7)	0.872
Contact score <sup>‡</sup>			
≤2	24.1 (13.2–40.5)	26.0 (11.7–50.7)	0.093
>2	24.4 (12.8–53.8)	26.1 (9.0–51.7)	0.682
Nutritional status <sup>‡</sup>			
Malnourished	22.4 (12.8–53.8)	21.30 (9.0–45.6)	0.903
Well-nourished	24.6 (13.2–47.2)	26.1 (11.7–51.7)	0.160

TB: tuberculosis.

<sup>†</sup>Mean±SD, independent t test.

<sup>‡</sup>Median (range), Mann-Whitney test.

The immunomodulatory activities of serum vitamin D strongly influence the progression to latent TB and increase macrophage activity against *M. tuberculosis*.<sup>1</sup> Accordingly, this study demonstrated that the mean serum vitamin D in the latent TB group were lower than those in the no latent TB group; however, the difference was not significant. Furthermore, 66.8% of the study subjects reported vitamin D insufficiency and deficiency, particularly age group 0–<1 years old in the latent TB group had a mean vitamin D level of <20 (18.0±2.6) ng/mL, which increases the risk of TB. It has been indicated by several studies that infants are more susceptible to TB,<sup>8</sup> however, low serum vitamin D should be considered as a risk factor. With the older children, there were more with higher serum vitamin D than for children <1 year, but they still had latent TB. However, the association is not clearly defined. A cross-sectional, case-controlled study of 42 patients with a TB contact history reported that vitamin D sufficiency protects patients against latent TB, and that the mean serum vitamin D [25(OH)D] in the positive TST cases was lower than that in the control cases.<sup>13</sup> A systematic review by Sutaria indicated that patients with TB have a lower serum vitamin D than healthy controls.<sup>14</sup>

The risk of progression to TB is associated with close contact with patients with sputum smear-positive TB and malnourishment. Our findings revealed that the prevalence of latent TB was higher in subjects with a contact score of >2 than in those with a contact score of ≤2 (44.2% vs 37.4%); however, the difference was not significant. A study on tuberculin conversion in adults reported that the subjects who developed TB had greater exposure to TB, as evidenced by the higher prevalence in those with high (>6) contact scores than in those with low (<1) scores (48.3% vs 14.8%); these subjects also had lower vitamin D.<sup>15</sup> Therefore, malnourishment can increase the risk of TB. Our study revealed that TB is most prevalent in malnourished children, which is consistent with the findings of another study describing that severely malnourished individuals had a 3.97-fold increased risk of TB.<sup>16</sup>

Although several studies of adults and teenagers have been conducted, this study demonstrates a possible association of vitamin D status in under-five children with a history of close TB contact. However, our study has some

limitations. We did not assess some risk factors for TB (e.g. passive smoking) and factors influencing serum vitamin D (e.g. diet composition, food security, sunlight exposure, and vitamin D receptor [VDR] polymorphism). Passive smoking affects the progression of TB, especially in children, because damage to the airway epithelium results in a failure to protect against *M. tuberculosis* infection.<sup>17</sup> In Indonesia, the highest prevalence (approximately 69.5%) of passive smoking was found to be among under-five children, leading to an increased risk of progression to TB.<sup>18</sup>

Moreover, micronutrient deficiencies in the diets of under-five children affect the prevalence of vitamin D deficiency. A study in Yogyakarta suggested that imbalance in the dietary intake of preschool children caused micronutrient deficiencies (iron, 2%; zinc, 0.4%; iodine, 54.3%; and vitamin D, 63.5%).<sup>4</sup> The SEANUT study in Indonesia reported a vitamin D deficiency rate of 43%,<sup>5</sup> which was similar to that in Malaysia (47.5%)<sup>19</sup> and Thailand (24.5%–31.3%),<sup>20</sup> all of which are tropical countries. A higher incidence of latent TB with low vitamin D and malnourishment, if seen, might reflect a decrease in body fat in malnourished children.<sup>21</sup>

The main food sources of vitamin D are meat, fish, and liver, as well as exposure to sunlight. In Northeast Asia, increased atmospheric pollution leads to ecosystem degradation and disturbances in the food system, which does not derive UV irradiation, thereby causing a decrease in vitamin D from food sources.<sup>22</sup>

Sunlight exposure is the most important factor for activating provitamin D in the skin. There are many factors that contribute to a lack of exposure to sunlight among under-five children in Indonesia, including more indoor activity, less outdoor activity, and full-time school day programs.<sup>1,2</sup> In addition, VDR polymorphism contributes to the immune system modulatory action of vitamin D, especially the FokI genotype, which has a role in the risk of TB disease that varies according to race and place.

In conclusion, we did not find any evidence that serum vitamin D had association with the incidence of latent TB in under-five children with a history of close TB contact. Nevertheless, it may be considered in children <1 year old, albeit the fact that the potential causes of the incidence of latent TB are multifactorial. Studies with a

larger sample size are required for providing additional evidence regarding the effect of serum vitamin D status on TB, particularly in under-five children.

#### AUTHOR DISCLOSURES

The authors declare that they have no conflicts of interest. This paper is a part of the doctoral dissertation of FY at the Post Graduate Program Biomedical Medicine Faculty of Medicine Universitas Andalas, Padang Indonesia. The abstract was presented as a poster presentation at the Third Asia Pacific Pediatric Pulmonology Society Congress in November 2016.

#### Funding disclosure

This study was conducted as part of a research project investigating the effect of vitamin D supplementation in TB prevention, which was funded by the Indonesian Danone Grant start from January 1, 2014 until June, 30 2016.

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