INTRODUCTION

Tuberculosis is an infection caused by Mtb [1, 2]. The natural history of TB in children shows a variation in susceptibility and a diversity spectrum of clinical manifestations [2]. Vitamin D deficiency may increase the risk of Mtb infection. The active form of vitamin D [1, 25-(OH)-2D3] (1,25-ahydroxyvitamin D3) is involved in the immune system and is important in TB infection. The active form of vitamin D can increase vitamin D receptors (VDR) expression on active monocytes and T and B lymphocytes [1].

VDR gene polymorphism is one of the factors that increases the incidence of tuberculosis infection [3]. Four single nucleotide polymorphisms (SNP) in the gene VDR have been identified, namely the SNP rs228570 (FokI) in exon II, SNP rs731236 (TaqI) in exon IX, SNP rs1544410 (BsmI), and SNP rs7975232 (Apal), which are located in the intron between exons VIII and IX [4]. SNP rs228570 polymorphisms or FokI will generate proteins with different amino acid lengths and affect the physiological roles of vitamin D [5].

Meta analysis of several studies reported that FokI polymorphisms increase the risk of tuberculosis infection in Asians [6].

Mtb infection activates Toll-like receptor (TLR) that triggers vitamin D through active vitamin D (1,25-OH-2D3), then 1,25-OH-2D3 interacts with the vitamin D receptor (VDR) to activate cathelicidin antimicrobial peptide gene (CAMP) and produce the antimicrobial peptide cathelicidin/LL37 [7, 8]. Cathelicidin has two actions against mycobacteria direct antimicrobial against Mtb, and a mediator autophagy by macrophages induced by vitamin D [9, 10]. Vitamin D does not have a direct antimicrobial action against Mtb, but its interaction with VDR is necessary to induce cathelicidin to eliminate Mtb. VDR expression depends on single nucleotide polymorphisms [11].

MATERIALS AND METHODS

We conducted the present cross-sectional study of children aged ≤14 y who had close contact with adult patients who had a sputum smear positive for acid fast bacilli (AFB) indicating pulmonary TB (at least 12 w) in the Public Health Centre, Padang City. Children with a previous history of TB treatment, no BCG scar, had experienced anergy, or had a local or systemic infection were excluded. After obtaining informed consent from parents, blood samples were obtained to determine plasma levels of vitamin D, polymorphisms, and cathelicidin. This study was approved by the Ethics Committee of the Faculty of Medicine, Andalas University.

Statistical analysis

The characteristics of the sample data are described. A chi-square test was used to determine the relationship between sex, nutritional status, AFB source contact, and SNP FokI polymorphism found no association between vitamin D levels and FokI polymorphism and the incidence of TB infection. An independent t-test was used to determine differences in age and the average serum levels of cathelicidin and vitamin D in children with and without TB infection. Regression analysis was used to determine the effect of clinical and demographic characteristics, levels of vitamin D, SNP FokI, and cathelicidin plasma levels on the incidence of TB infection in the children. Results were considered significant when P<0.05 in tests of statistical inference.

RESULTS

A total of 56 children infected with TB and 56 children uninfected participated in this study. Characteristics of the sample are shown in table 1.

Neither age, sex, number of contacts, nor the closeness of contact was significantly associated with the incidence of TB infection. Malnutrition was greater in the group infected with TB and better nutritional status was found in those not infected with TB (P<0.017). The mean levels of vitamin D in children who were infected tended to be lower than those in children not infected, but the difference between the levels was not significant. Most of the children who were infected by tuberculosis had vitamin D deficiency (71.4%). The bivariate analysis of vitamin D levels and FokI polymorphism found no association between the two groups (table 2).
Table 1: Characteristics of the sample

| Characteristics       | Group                  | Uninfected (n = 56) | Infected with TB (n = 56) | p   |
|-----------------------|------------------------|---------------------|---------------------------|-----|
|                       | f                      | %                   | f                         | %   |     |
| Age (months)          |                        |                     |                           |     |
| ≥ 6 mo to < 5 y      | 40                     | 71.4%               | 30                        | 53.6%| 0.051*|
| ≥ 5 y to ≤ 14 y      | 16                     | 28.6%               | 26                        | 46.4%|     |
| Sex                   |                        |                     |                           |     |
| Male                  | 26                     | 46.4%               | 35                        | 62.5%| 0.086*|
| Female                | 30                     | 53.6%               | 21                        | 37.5%|     |
| Nutritional status    |                        |                     |                           |     |
| Wellnourished         | 43                     | 76.8%               | 31                        | 55.4%| 0.017*|
| Undernourished        | 13                     | 23.2%               | 25                        | 44.6%|     |
| AFB contact sources   |                        |                     |                           |     |
| +                     | 13                     | 23.2%               | 10                        | 17.9%| 0.696*|
| ++                    | 18                     | 33.9%               | 18                        | 32.1%|     |
| +++                   | 25                     | 42.9%               | 28                        | 50.0%|     |

*Chi-square test.

Table 2: Bivariate analysis of factors that affect the incidence of tuberculosis infection in children

| Variable              | Group                  | Uninfected (n = 56) | Infected with TB (n = 56) | OR crude (95% CI) | p   |
|-----------------------|------------------------|---------------------|---------------------------|--------------------|-----|
|                       | f                      | %                   | f                         | %                 |     |
| SNP VDR gene rs2228570 (FokI) |                        |                     |                           |                   |     |
| FF                    | 18                     | 32.1%               | 24                        | 42.9%             | 0.374*|
| Ff                    | 24                     | 42.9%               | 23                        | 41.1%             |     |
| ff                    | 14                     | 25%                 | 9                         | 16.1%             |     |
| Allele (n = 224)      |                        |                     |                           |                   |     |
| f                     | 60                     | 53.6%               | 71                        | 63.4%             | 0.666|
| f                     | 52                     | 46.4%               | 41                        | 36.6%             | 0.136*|
| Polymorphism FokI     |                        |                     |                           |                   |     |
| Mutant homozygote (FF) and heterozygote (Ff) | 42                  | 75.0%               | 47                        | 83.9%             | 0.574|
| Normal                | 14                     | 25.0%               | 9                         | 16.1%             | 0.242*|
| Vitamin D (25-OH-D) level |                        |                     |                           |                   |     |
| X ± SD (ng/ml)        | 56                     | 28.2±9.6            | 56                        | 9-26.0±8.12       | 0.192***|
| Normal                | 23                     | 41.1%               | 16                        | 28.6%             | 0.165*|
| Deficient             | 33                     | 58.9%               | 40                        | 71.4%             |     |

Description: if the value of P<0.25 then followed by multivariate analysis, *Chi-squared test, **Independent t test, ***Independent t test.

There were differences between the mean plasma levels of cathelicidin in the infected and uninfected groups. The average level of cathelicidin in the infected group was lower than that in the group that was not infected (Table 3).

Table 3: Relationship of mean cathelicidin levels and the incidence of tuberculosis infection

| Levels of cathelicidin | Group                  | Uninfected n = 56 | Infected n = 56 | p   |
|------------------------|------------------------|-------------------|-----------------|-----|
| X±SD                   |                        | 153.42±77.81      | 119.37±81.20    | 0.025*|
| Min-max                |                        | 12.36 to 321.92   | 11.03 to 308.63 |     |
| Range                  |                        | 309.56            | 297.60          |     |

*Independent t test.

In the malnourished group, there was a significant difference in the mean level of cathelicidin between those who were infected and those who were not, whereas in the wellnourished group there was no difference in the mean level of cathelicidin between those who were infected and those who were not (Table 4).

In the age group of 6 mo to < 5 y, mean levels of cathelicidin differed between those who were infected and those who were not, whereas in the group aged 5–14 y there was no significant difference between the mean levels of cathelicidin (Table 5).

Modeling with multiple logistic regression showed that the factors associated with the incidence of TB infection in children were nutritional status, levels of cathelicidin that interact with nutritional status, and levels of cathelicidin that interact with age categories (Table 6). Of these factors, nutritional status was most strongly associated with the incidence of TB infection in children (β coefficient 1.846 and P = 0.019). The OR for nutritional status indicates that malnutrition increases the risk of TB infection in children by 6.332 times compared with children with good nutrition.
DISCUSSION

Vitamin D level was not associated with tuberculosis infection, although most of the children infected with TB had an insufficient level of vitamin D. The geographic area covered by the two groups was Padang City, which has full sunlight. Setiabudiawan found normal vitamin D levels in both infected and uninfected participants [1]. In studies of adults, no differences in vitamin D levels were found between TB patients and uninfected controls, even though levels were insufficient in both groups [12].

There was no significant difference in the proportion of genotype polymorphism VDR between children with and without TB infection (P>0.05). In the group of children with TB infection, the highest proportion of genotype FokI was FF (42.9%). Setiabudiawan found that the FF genotypewas highest in children between TB infection (57.2%) [1]. We found no significant difference between the proportion of alleles in infected and uninfected groups. The F allele was most common in the infected group (63.4%). Setiabudiawan found that the proportion with the F allele was significantly different between case and control TB, with the proportion of allele being highest at 61.9% [1]. FokI polymorphisms were not significantly different between infected and uninfected individuals. FokI polymorphisms were found in 83.9% of infected patients and 75.0% of uninfected patients. This is in contrast with the findings of Setiabudiawan and Karmila, who found FokI polymorphism was most common in the infected group (63.4%). Setiabudiawan found factors that were associated with tuberculosis of children were sex, FokI and Apol polymorphisms, and deficient serum levels of 1,25-(OH)-D. Of those four factors, vitamin D deficiency had the strongest association [1]. Karmila found factors related to the incidence of TB in children were vitamin D deficiency and FokI polymorphism [13].

We found no association of vitamin D level and SNP FokI with the incidence of TB infection in children in Padang. There is a possibility that other gene polymorphisms may play a role, so further research on the role of BsmI, Apal, and TaqI polymorphisms on TB infection in children in Padang is warranted. Cathelicidin, nutritional status, and age<5 y appear to have an important roles in tuberculosis infection, suggesting that future studies should focus on children<5 y old.

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AUTHORS CONTRIBUTIONS
All the author have contributed equally

CONFLICT OF INTERESTS
The authors affirm no conflict of interest in this study

LIMITATION OF STUDY
Limitation of this study due to age of participant was not distribute properly between under and above five years old, but from statistical analysis was not significantly different as basic characteristic.

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