The Role of Vitamin D, Vitamin D Receptor Gene Foki Polymorphism, and Cathelicidine in Tuberculosis Infection in Children

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Original Article

THE ROLE OF VITAMIN D, VITAMIN D RECEPTOR GENE FOKI POLYMORPHISM, AND CATHELICIDIN IN TUBERCULOSIS INFECTION IN CHILDREN

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ABSTRACT

Objective: Recent studies have reported that vitamin D has an important role in tuberculosis. Polymorphism of single nucleotide polymorphism (SNP) rs2228570 or *FokI* will generate proteins with various amino acid lengths, and affect the molecular physiological roles of vitamin D. Cathelicidin acts as an antimicrobial directly against *Mycobacterium tuberculosis* (Mtb) and mediator autophagy by macrophages induced by vitamin D. This study aims to determine the role of vitamin D, vitamin D receptor gene SNP rs2228570 (*FokI*), and plasma levels of cathelicidin in children with tuberculosis infection.

Methods: This cross-sectional was conducted on children aged ≤14 y who had close contacts with an adult who had sputum smear positive for acid-fast bacilli indicating pulmonary TB. The children were divided into two groups: those with and those without TB infection. Demographic data and blood samples were taken from children participating in this study to examine polymorphisms and cathelicidin plasma levels, and to determine the association between these levels in children with and without TB infection.

Results: There were no differences in the proportion of genotype and allele of SNP rs2228570 vitamin D receptor gene (*FokI*), polymorphisms of VDR, and cathelicidin plasma levels between children with and without TB infection (p>0.05). However, nutritional status and levels of cathelicidin by age category increased the risk of TB infection (p<0.05).

Conclusion: Cathelicidin level in children with TB infection lower than without. Vitamin D and *FokI* polymorphism did not play role in TB infection.

Keywords: Tuberculosis infection, FokI polymorphism, Vitamin D, Vitamin D receptors, Children

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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 Mth infection. The active form of vitamin D [1, 25-(OH)-2D3] has been known to affect the mononuclear phagocytic ability to suppress the growth of Mth intracellularly, especially in the early days of infection and latent infection. The phagocytic response is mediated by vitamin D receptor (VDR) expressed 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 rs2228570 (*FokI*) in exon II, SNP rs731236 (*TaqI*) in exon IX,SNP rs1544410 (*BsmI*), and SNP rs7975232 (*ApaI*), which are located in the intron between exons VIII and IX [4]. SNP rs2228570 polymorphisms or *FokI* will generate proteins with different amino acid lengths and affectthe physiological roles of vitamin D [5]. Meta analysis of several studies reported that *FokI* polymorphisms increase the risk of tuberculosis infection in Asians [6].

Mth 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 Mth, and a mediator of autophagy by macrophages induced by vitamin D [9, 10] Vitamin D does not have a direct antimicrobial action against Mth, but its interaction with VDR is necessary to induce cathelicidin to eliminate Mth. 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* 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 inchildren 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 BTA 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				p
	Uninfected (n = 56)		Infected with TB (n = 56)		
	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%	0.088*
• 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	%				
SNPVDR 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	0.136*
• f	52	46.4%	41	36.6%	(0.390 to 1.137)	
Polymorphisms FokI						
• Mutant homozigot (FF) and heterozigot (Ff)	42	75.0%	47	83.9%	0.574	0.242*
• Normal	14	25.0%	9	16.1%	(0.226 to 1.463)	
Vitamin D (25-OH-D) level						
X ± SD (ng/ml)	56	28.26+9.6	56	9-26.06+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		p
	Uninfected n = 56	Infected n = 56	
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 this 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 incidence in children by 6.332 times compared with children with good nutrition.

Table 4: Average levels of cathelicidin andincidence of tuberculosis infection based on the nutritional status

	Levels of cathelicidin	Group		p
		Uninfected	Infected	
Wellnourished		(n = 43)	(n = 31)	
	X±SD	145.86±71.83	123.90±81.44	0.224*
Malnourished		(N = 13)	(N = 25)	
	X±SD	178.46±93.82	113.75±82.23	0.035*

*Independent *t* test.

Table 5: Mean levels of cathelicidin and the incidence of tuberculosis infection by age group

	Levels of cathelicidin	Group		p
		Uninfected	Infected	
6 mo to<5 y		(n = 40)	(n = 30)	0.034*
	X±SD	151.95±69.29	113.93±77.12	
5 y to≤14 y		(n = 16)	(n = 26)	
	X±SD	157.10±98.45	125.65±86.78	0.285*

*Independent *t* test.

Table 6: Multivariate analysis of risk factors relationship with the incidence of TB infection

Variables	β coefficient	SE	p	OR (95% CI)	
Nutritional status	1.846	0.786	0.019	6.332	(1.356-29.572)
Cathelicidin nutritional status	-0.007	0.004	0.092	0.993	(0.985-1.001)
Cathelicidin by age categories	-0.005	0.002	0.037	0.995	(0.990-1.000)
Constants	.150	.326	0.645	1.162	

DISCUSSION

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

There was no significant difference inthe 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 *Fok*I was FF (42.9%). Setiabudiawan found that the Ff genotypewas highest in children with 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 *Fok*I allele was significantly different between case and control TB, with the proportion of allele fbeing highest at 61.9% [1]. *Fok*I polymorphisms were not significantly different between infected and uninfected individuals. *Fok*I 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 *Fok*I polymorphism was significantly different between case and control TB [1, 13].

In the present study, the serum level of cathelicidin in infected children was lower than it was in children without infection and to our knowledge, is the first study of cathelicidin in children. Sovira found the serum level of cathelicidin in adult patients with TB cavity was lower than without cavity [14]. Another study found that the level of cathelicidin was low in dialysis patients and that low levels increased the risk of death[15]. Nutritional status and cathelicidin levels increase the risk of the incidence of tuberculosis infection. Cathelicidin levels that increase the risk interact with undernutrition and age<5 y *(modifier effect)*. Young children are still developing immunity and are at high risk of tuberculosis infection[16]. Nutrition also contributes to the immune response in tuberculosis infection, starting from adequate breastfeeding until appropriate complementary food [17].

Multivariate analysis of vitamin D level, *Fok*I polymorphism, and cathelicidin found no association with the incidence of tuberculosis infection. Setiabudiawan found factors that were associated with tuberculosis of children were sex, *Fok*I and *Apa*I 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 *Fok*I polymorphism [13].

We found no association of vitamin D level and SNP *Fok*I 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 *Bsm*I, *Apa*I, and *Taq*I 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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