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Vitamin D serum levels and vitamin D receptor *FokI* polymorphisms in children with tuberculosis at Palembang

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Abstract

Background Deficiency vitamin D and vitamin D receptor (VDR) polymorphism are strongly associated with the susceptibility of tuberculosis in four seasons countries. As a country with sufficient sunlight, the incidence of tuberculosis in Indonesia remains high.

Objective To asses the association between vitamin D serum levels and VDR *FokI* polymorphisms with the incidence of tuberculosis in children at Palembang.

Methods A case control study was conducted at Department of Child Health Mohammad Hoesin Hospital Palembang on November 2011 to April 2012. Group samples consisted of children with tuberculosis based on inclusion criteria. Vitamin D ($1.25(OH)_2D_3$/calcitriol) serum level was measured by using IDS 1,25-Dihydroxy Vitamin D EIA kit and VDR *FokI* polymorphism was identified through RFLP analysis.

Results Sixty subjects was divided equally to case and control groups. The mean of calcitriol serum level in case group was lower compared to control which are 105.5 (SD 66.9) pmol/L versus 162.9 (SD 52.9) pmol/L (P=0.001). We found nine subjects with calcitriol deficiency, eight out of thirty in children with tuberculosis and one out of thirty in healthy contacts (OR 10.5; 95% CI 1.2 to 90.7) pmol/L. The incidence of VDR *FokI* polymorphism was 28 out of 30 in case group and 22 out of 30 in control group (OR 5,0; 95% CI 0,9 to 26,4).

Conclusion Vitamin D (calcitriol) deficiency and lower serum level are associated with higher risk of tuberculosis in children at Palembang. Polymorphism *FokI* in VDR gene also contributes to the susceptibility of tuberculosis.

Keywords: *Vitamin D deficiency, VDR FokI polymorphism, tuberculosis, children*

INTRODUCTION

The role of vitamin D on host immunity defense against tuberculosis infection has long been known. The binding between vitamin D's metabolite active form (calctriol) and vitamin D receptor (VDR) at immunity cells modulates macrophage activity to fight against *Mycobacterium* infection.[1] Therefore low level of calcitriol and/or abnormalities of vitamin D receptor may cause impairment to the function of macrophage and increase host's susceptibility to tuberculosis disease.[2,3]

The susceptibility of host in developing active tuberculosis disease can be influenced by several of factors such as *Mycobacterium* virulency, host immuniy, environmental, genetic or interaction among of them.[1] As a country with sufficient sunlight exposure, which is known essential in the vitamin D production process, the incidence of tuberculosis in children at Indonesia remains high. Recent study shows that Indonesia sits on the 5th position in the list of countries with high tuberculosis incidence which is 480,000/year and 10% among them are taken by children.[4] The contribution of low level vitamin D serum and vitamin D receptor polymorphism in tuberculosis development has been investigate in numerous study at four seasons countries with variety population.[2,3] In the present study, we performed a study to asses the association between calcitriol level and vitamin D receptor *FokI* polymorphism with the incidence of tuberculosis in children at Palembang.

METHODS

This was a case control study conducted at Departement of Child Health Mohammad Hoesin Hospital Palembang during November 2011 to April 2012. Consecutive method sampling was used and samples were children under 18 years old diagnosed having active pulmonary or extrapulmonary tuberculosis based on clinical manifestation, laboratorium and radiological finding, and confirmed by a positive tuberculin test. Patients with malabsorption, liver abnormalities, renal dysfunction, immunosuppresed condition, severe malnutrition, and in a long-term anticonvulsant therapy were excluded.

Control group were recruited from our paediatric and adult respirology specialitistic outpatients consisted of children who had household contact with tuberculosis patient proven by a positive result to tuberculin purified protein derivative test and symptom free. All participants' parents gave informed consent for inclusion, and permission to carry out this study was obtained from the local research ethics committee.

Samples of calcitriol were taken from all patients at or shortly after diagnosis. Calcitriol was assayed using IDS® 1,25-Dihydroxy Vitamin D EIA kit and we defined calcitriol deficiency in children to be present at a concentration of less than 67 pmol/L.[5]

Blood was collected in EDTA tubes for vitamin D receptor polymorphism identification and stored at – 20°C until DNA extraction. DNA was purifeid by following Chelex-based DNA purification protocols. DNA was amplified with a standard PCR technique. The sense primer: 5-AGC TGG CCC TGG CAC TGA CTCTGC TCT-3, and the reverse primer: 5-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3 were used. After initial denaturation for 10 minutes at 94°C, samples were subjected to 35 cycles of amplification, consisting of a 60 seconds denaturating phase at 95°C, a 60 second of annealing phase and a another 60 seconds extension phase at 72°C. A 7 minutes 72°C hold was the final step of the program. Following amplification, 0.2 µl of *FokI* restriction enzyme, 1.2 µl buffers, 2.6 µl ddH_2O were added to 8 µl of the PCR product and digested at 37 ˚C. Genotypes were assigned as follows: F/F 265 bp only; F/f 265 bp, 196 bp, and 96 bp; f/f, 169 bp and 96 bp. The *FokI* alleles are designated 'F' as infrequent allele (mutant) and 'f ' as common allele (wild type).

We analyzed the difference between calcitriol level using independent t test. Differences in proportions between 2 groups were analyzed by using Fisher's exact and chi square test. Odd's ratios (OR) with 95% confidence interval were calculated with P value of < 0.05 was considered significant. Logistic regression analysis was used to asses significant association between tuberculosis, vitamin D deficiency, and VDR *FokI* polymorphism with other factors.

RESULTS

Thirty patients with active tuberculosis and 30 healthy contacts as controls were enrolled in this study. The mean of age in all subjects was 4.7 (SD 3.1) years ranging from 5 months to 11.4 years. The characterisitcs of the subjects are shown in Table 1.

Table 1. Characteristics of subjects

Characteristics	Tuberculosis patients N = 30	Healthy contact N = 30
Age		
≤ 5 years	17	17
> 5 years	13	13
Sex		
Male	16	18
Female	14	12
Nutritional status		
Undernourished	12	11
Wellnourished	18	19
Family income		
Above Regional Minimum Wage	25	22
Below Regional Minimum Wage	5	8
Father's education		
High school or below	10	7
Above high school	20	23
Mother's education		
High school or below	8	9
Above high school	22	21

The mean level of calcitriol in children with active tuberculosis was 105.5 (SD 66.9) pmol/L and 162.9 (SD 52.9) in healthy contacts. This mean difference was statistically significant with P=0.001. We also found that eight children with calcitriol deficiency in active disease group and one child in the healthy contacts group. Statisitcal analysis showed a significant association between calcitriol deficiency and active tuberculosis in children (P=0.026, OR 10.5, 95% CI 1.2 to 90.7) pmol/L.

The result of the PCR genotyping of VDR *FokI* polymorphisms is presented in figure 1.

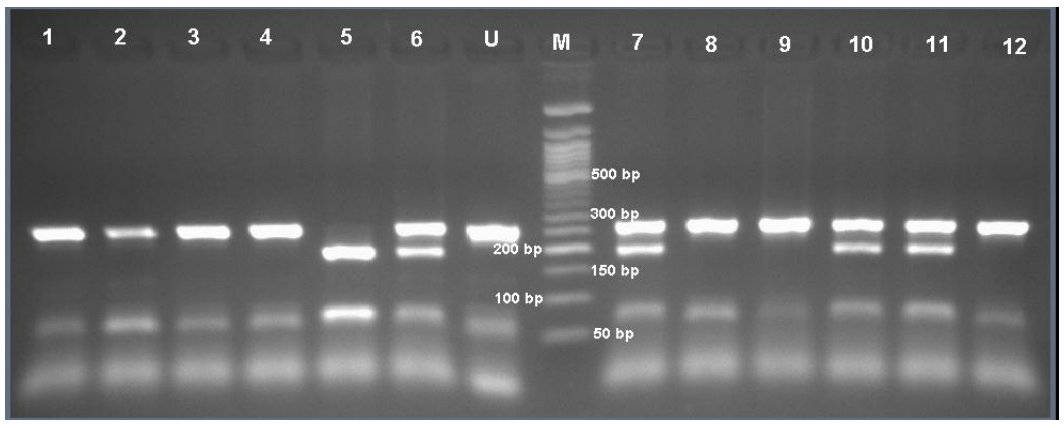

Figure 1. Genotyping of VDR *FokI* polymorphism by PCR-RFLP.

Lane 1,2,3,4,8,9, and 12: FF genotype; lane 5: 196 ff genotype;

lane 6,7,10 and 1: Ff genotype

Table 2 showed the proportion of genotype and allele vitamin D receptor *FokI* polymorphism in subjects.

Table 2. Distribution of genotype and allele vitamin D receptor *FokI* in subjects

	Tuberculosis patients N = 30	Healthy contact N = 30
Genotype		
FF (mutant)	15	5
Ff (heterozygote)	13	17
ff (wild type)	2	8
Total	30	30
Allele		
F	43	27
f	17	33
Total	60	60

Vitamin D receptor *Foki* polymorphisms (FF and Ff) were found in 28 out of 30 tuberculosis patients and in 22 out of 30 healthy contacts. We found a statistically significant association between tuberculosis disease and VDR *FokI* polymorphisms (P=0.038, OR=5, CI 95% 0.9 to 26.4). The F alleles frequency were significantly higher among tuberculosis patients compared to healthy contacts (P=0.003, OR=3.1, Ci 95% 1,4 to 6,5). Logistic regression indicated that the role of vitamin D deficiency and VDR *FokI* polymorphisms to tuberculosis disease is 24.3%.

DISCUSSION

Our study found that lower calcitriol serum level, calcitriol deficiency, and vitamin D receptor *FokI* polymorphism have a significant association with active tuberculosis disease in children. Children with vitamin D deficiency have a greater risk in developing tuberculosis (10 fold higher), while those with vitamin D receptor *FokI* polymorphism have a 5 fold higher risk in having active tuberculosis compared to healthy contacts. Our results supports previous study results that showed calcitriol together with vitamin D receptor may have an important role in modulating immune response to tuberculosis infection. Lower serum level and genetic variation, in this case VDR *FokI* polymorphism, has increased the risk of developing active tuberculosis disease. [3]

Even in a region that is considered with sufficient sunlight exposure which is essential in vitamin D production, the finding of vitamin D deficiency in these population should drawn more attention. Low vitamin D level in children could be cause by higher requirement due to children's growth, certain lifestyles or cultural practices that decreases the time spending outdoors or increase the amount of body surface area covered by clothing, darker skin pigmentation, use of sunscreens, and even prolong breastfeed without supplementation nor adequate sunlight exposure.[6,7]

Polymorphism defines as a variation of genetic sequence that occurs in certain population that might effects host susceptibility in certain disease or therapy responds. Polymorphism starts as a mutation and makes allele frequency difference between ethnic groups as a result of evolutionary processes and population genetic behaviour. There are several VDR polymorphism that has been known such as *FokI, BsmI, ApaI* and *TaqI*. In VDR *FokI* polymorphism we will find two protein variants that corresponds to two available start sites: a long version 427 aa and a short veriosn 424 aa. This variation somewhat makes a difference on each transactivation capacity as a transcription factor. Due to this reason some promoter areas of vitamin D targets gene might be more sensitive to this genotype-dependent difference activity compared to others.[8]

The mechanism of how the interaction between calcitriol and vitamin D receptor modulates immune response against tuberculosis infection is still under study, however there are two possible mechanisms which are emerging lately. Calcitriol enhances the fusion of phagosome and lysosome in infected macrophages and reduce the viability of *M. Tuberculosis.* Calcitriol also enhances the production of LL-37 which is known as an antimicrobial peptide from the cathelicidin family that appears involved as one of the first line defense in tuberculosis prevention by it's direct bactericidal activity and by attracting monocytes, T cells, and neutrophils to the site of infection. [1]

An association between low vitamin D serum or vitamin D deficiency and tuberculosis has been demonstrated in several other studies. Talat et al found 79% of tuberculosis patients are in the state of vitamin D deficiency in Paksitan, a study from India reported significantly lower vitamin D levels in tuberculosis patients compared to control.[9,10] Others like Gibney *et al* in Australia, Ustianowski and Wilkinson in London, and Syafii in Cimahi, West Java, Indonesia have also found similiar finding.[11-14] But previously another study by Grange et al at Indonesia on 1985 failed to demonstrate the association,[15] this condition might be related to the time difference and age of subjects of study that regards to the difference of lifestyle and host immunity system maturation.

Previous studies also suggested a higher risk of tuberculosis in patients with VDR *FokI* polymorphism. In West India, Selvaraj et al found 47% spinal tuberculosis patients have VDR *FokI* polymorphism, later Liu *et al* in China found 23% tuberculosis patients have *FokI* polymorphism.[16,17] Similiar result was also found by Setiabudiawan in West Java Indonesia where mutant allele were found in 66.7% children with tuberculosis.[18] In the other hand, Wilbur et al in Paraguay revealed that VDR *FokI* poymorphism gave a protective effect to host against tuberculosis infection.[19] This result supports that genetic variation may gives different responds in different ethnic population.

This present study has several limitation. We did not measure calcidiol level which is the most common parameter in determinig vitamin D nutrition status, so we coudn't evaluate the relationship between calcidiol and calcitriol serum level regarding it's role in the immunity system. We also did not identify other VDR polymorphisms such as *BsmI, ApaI* and *TaqI* that might also have a contribution in the susceptibility of host to tuberculosis infection in Palembang.

CONCLUSION

Low serum level or calcitriol deficiency may account for a proportion of the acquired susceptibility of children against tuberculosis active disease and *FokI* polymorphisms in VDR gene also contributes to higher risk of tuberculosis disease in Palembang.

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