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PLASMA VITAMIN D LEVEL AND VITAMIN D RECEPTOR GENETIC POLYMORPHISM OF SPINAL TUBERCULOSIS SUSCEPTIBILITY IN WEST JAVA POPULATION, INDONESIA: DOES IT CORRELATED?

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ABSTRACT To explores the potential role of plasma vitamin D level and VDR gene polymorphisms in susceptibility to tuberculosis (TB) in the West Java population, Indonesia. A case-control study was performed on 53 Spinal TB patients, 53 Pulmonary TB patients and 53 healthy controls. Plasma Vitamin D levels were analyzed by ELISA, genetic polymorphisms of VDR gene (TaqI, FokI, BsmI, ApaI) were analyzed using PCR-RFLP and sequencing, There was a significant difference ($p<0.01$) of plasma vitamin D level among all groups. Genotype analysis of VDR gene polymorphism Fok1, BsmI, ApaI and TaqI has shown no significant difference in all group ($p>0.01$). The combination of vitamin D deficiency and VDR gene polymorphism Fok1 in FF genotype were contributing factors to the susceptibility of spinal tuberculosis in the West Java population, Indonesia.

KEYWORDS : Spinal tuberculosis, Vitamin D, VDR gene polymorphism

INTRODUCTION

M. tuberculosis infection of the musculoskeletal system, especially the spine, is caused by differences in the immune response to tuberculosis. Many studies suggest that the occurrence of TB is not only determined by bacterial factors alone, but also by the body immune system mechanisms that suppress *M. tuberculosis* virulence. [1,2] Lack of vitamin D (1,25-*dihydroxycholecalciferol*) is associated with the risk of TB infection. People with vitamin D deficiency have a greater susceptibility to infections of tuberculosis.[3-5]

Previous studies showed that abnormalities of vitamin D receptor structure, in this case polymorphism, has a role in immune system activity against TB infection. Some types of vitamin D receptor polymorphisms gene such as *Bsm1*, *Apa1*, *Taq1* and *Fok1* are thought to correlate with the incidence of TB.[6-12]

There have been no studies done in Indonesia about the correlation of plasma vitamin D level and the role of vitamin D receptor gene *Bsm1*, *Apa1*, *Taq1* and *Fok1*. This encourage researcher to analyze the role of vitamin D levels in plasma and vitamin D receptor polymorphism gene *Bsm1*, *Apa1*, *Taq1* and *Fok1* against immunopathology that affect the vulnerability and resilience of spinal TB infection.

METHOD

This is case control study. The research subjects are spinal tuberculosis that met the inclusion criteria, namely: spinal tuberculosis patients that had been operated, pulmonary tuberculosis patients and healthy patients with age $\geq$ 15 years old. Cases group consist of 53 spinal TB patients, 53 pulmonary tuberculosis patients and control groups consist of 53 healthy patients. Total plasma vitamin D measured for all sample by ELISA. Polymorphism of vitamin D receptor (VDR) done by sequencing method and PCR-RFLP technique. All statistical analysis was processed using SPSS version 16. The Chi-square test was used to compare the frequency of variable, $p< 0,05$ was considered statistically significant.

RESULTS

Based on Table 1, there were significant differences of plasma vitamin D level in all three groups ($p <0.01$). In further comparison test, it was showed that plasma vitamin D concentrations and tuberculosis was closely related (mean differences of normal and spinal TB patients = 4, 35 and mean differences of normal and pulmonary TB patients = 4, 19; p = 0,000). PCR products restricted with Fok1 reveals genotypes (after treatment with enzyme) denoted FF (265 bp), Ff (265,196 and 69 bp), or ff (196 and 69 bp); Bsm1 genotypes denoted BB (825 bp), Bb (825, 650 and 175 bp), bb (650 and 175 bp); Apa1 genotypes denoted AA (740 bp), Aa (740, 530 and 210 bp), aa (530 and 210 bp) and Taq1 genotypes denoted TT (495 and 245 bp), Tt (495, 290, 245 And 205 bp), tt (290, 245 and 205 bp).

TABLE 1- Analysis and Further Comparison Test Plasma Vitamin D Level

Vitamin D	Normal (n=53)	Spinal TB (n=53)	Pulmonary (n=53)	p value
Plasma Vitamin D Level				
Mean (SD)	11,90 (6,83)	7,55 (6,32)	7,71 (8,06)	0,000**a)
Median (Range)	10,82 (3 -33,88)	5,53 (3 -28,21)	4 (3 - 41,77)	

Comparison	p-value
Normal vs Spinal TB	0,000b
Normal vs Pulmonary TB	0,000b
Spinal TB vs Pulmonary TB	0,445

p value was measured from a) Kruskal Wallis(data not normally distributed), b), P value was measured from Mann Whitney test, difference consideredc significantif $p < 0,05$ and ccvery significantif $p < 0,01$.

Allele and genotype frequencies of VDR gene polymorphisms in the spinal TB, pulmonary TB and normal group were analyzed by SPSS 16 program as shown in Table 2. Table 3 showed, all genotype frequencies of VDR gene were not a significant association with plasma Vitamin D level concentration except FokI-FF genotype (p=0.030).

TABLE 2- Proportional Analysis of Genotype dan AlleleVDR gene polymorphismin Spinal TB group, Pulmonary TB group and case control group

Genotypes	Frequency (%)			p Value
	Spinal TB (n=53)	Pulmonary TB (n=53)	control (n=53)	
Bsm1				
BB	0 (0)	2 (3,77)	3 (5,66)	0,562
Bb	6 (11,32)	6 (11,32)	5 (9,43)	
bb	47 (88,68)	45 (84,91)	45 (84,91)	
Alleles				
B	6 (11,32)	8 (15,09)	8 (15,09)	0,810
b	47 (88,68)	45 (84,91)	45 (84,91)	
Apa1				
AA	3 (5,66)	4 (7,55)	9 (16,98)	0,305
Aa	24 (45,28)	23 (43,4)	18 (33,96)	
aa	26 (49,06)	26 (49,06)	26 (49,06)	
Alleles				
A	27 (50,94)	27 (50,94)	27 (50,94)	1,000
a	26 (49,06)	26 (49,06)	26 (49,06)	
Taq1				
TT	48 (90,57)	49 (92,45)	47 (88,68)	0,702
Tt	5 (9,43)	4 (7,55)	5 (9,43)	
tt	0 (0)	0 (0)	1 (1,89)	
Alleles				
T	53 (100)	53 (100)	52 (98,11)	0,366
t	0 (0)	0 (0)	1 (1,89)	
Fok1				
FF	14 (26,42)	22 (41,51)	20 (37,74)	0,351
Ff	27 (50,94)	23 (43,4)	27 (50,94)	
ff	12 (22,64)	8 (15,09)	6 (11,32)	
Alleles				
F	41 (77,36)	45 (84,91)	47 (88,68)	0,276
f	12 (22,64)	8 (15,09)	6 (11,32)	

P value was measured from Chi Square test, difference considered *significant if $p < 0,05$ and **very significant if $p < 0,01$

TABLE 3- Correlation Analysis Between Genotype VDR Gene Polymorphism with Mean Plasma Vitamin D Level in All Group

	Genotype	Plasma Vitamin D Level	
		Mean (SD)	P value
Bsm1	BB	14,24 (14,13)	0,197
	Bb	8,17 (5,13)	
	bb	8,93 (7,15)	
Apa1	AA	12,44 (11,21)	0,079
	Aa	7,96 (5,64)	
	aa	9,21 (7,41)	
Taq1	TT	9,2 (7,59)	0,578
	Tt	7,16 (3,84)	
	tt	12,24 (-)	
Fok1	FF	9,73 (7,41)	0,030*
	Ff	9,70 (7,99)	
	ff	5,50 (3,13)	

DISCUSSION

In human, mycobacterial pathogenicity varies from one mycobacterial species to another. There is much variability among individuals in the response to mycobacterial infections, but it is not known why certain people develop disease when challenged with mycobacteria and others remain healthy.[13] Plasma vitamin D levels and the polymorphisms in VDR gene have been studied in relation to tuberculosis in many populations. Epidemiological evidence suggests that there is a link between vitamin D deficiency and susceptibility to leprosy and TB. Recently genetic variation has been shown to be associated with single nucleotide polymorphisms (SNP) in the vitamin D receptor gene in many populations of leprosy and TB.[13-14]

Vitamin D works in the immune system by lowering the levels of inflammatory proteins and increase the antimicrobial protein that can destroy bacteria such as tuberculosis. Vitamin D metabolism leads to activation of macrophages and restricts the intracellular growth of mycobacteria. This effect of vitamin D may be influenced by polymorphisms at three sites (*Taq1*, *Bsm1* and *Fok1*) in the vitamin D receptor gene.[13] Previous studies show that people with low levels of vitamin D are more susceptible to TB than people with high vitamin D levels. Therefore it is clear that on our study (table 1) there are significant differences between the vitamin D levels in the normal group and the case group (spinal TB and pulmonary TB).Vitamin D deficiency causes secondary hyperparathyroidism and bone matrix and mineral loss and increased risk of osteoporosis and bone damage. In infections of the spine, bone destruction occurs which will aggravate the state of vitamin D deficiency.[14] This causes plasma vitamin D level in a group of spinal TB group (7,55) was inferior to pulmonary TB group (7,71) for the event of TB infection in the bone of vitamin D (table 1).

In our study, there was no statistically meaningful relationship between the allele and genotype frequencies of *Fok1*, *Bsm1*, *Apa1* and *Taq1* polymorphisms in the VDR gene and susceptibility to tuberculosis (table 2), but when they were analyzed with respect to plasma vitamin D concentrations, a significant association was seen (table 3). There was significant relationship in plasma vitamin D level with *Fok1*-FF genotype (p= 0,030).

The findings in our study indicated that vitamin D deficiency (≤ 10 ng/ml) (especially in Fok1-FF individuals) can carry a higher risk of tuberculosis; therefore moderate supplementation may be useful.Although several investigators reported and suggested that the VDR polymorphism may be of immunoregulatory importance for many disease processes, it is not clear that the polymorphism determine susceptibility to the development of clinical disease or susceptibility to infection. Further studies will be required to investigate how VDR polymorphism may influence susceptibility to infectious disease or development of clinical disease.[13]

CONCLUSION

Deficiency of vitamin D plasma levels and receptor gene polymorphisms FokI vitamin D (RVD) is a risk factor for spinal tuberculosis.The combination of plasma levels of vitamin D deficiency and homozygous genotypes of polymorphism FokI FF RVD has the great chance of susceptibility of spinal tuberculosis in The West Java population, Indonesia.

REFERENCES:

1. Tuli, M. (2013), "Historical aspects of pott's disease (spinal tuberculosis) management."Euro Spine J. 22(22).529-538.
2. Millet J.P., Moreno A., Fina L., Bano L.D., Orcau A., Olalla P.G., et al. (2013). "Factors that influence current tuberculosis epidemiology."Euro Spine J. 22(4).539-548.
3. Bianci, C.D. (2012). "The potential role of vitamin D for prevention and treatment of tuberculosis and infectious diseases." Ann 1st Super Sanita. 48(3).319-327.
4. Holick M.F. (2008). "Vitamin D-deficiency :Aworldwide problem with health consequences."The American Journal of Clinical Nutrition. 87.1080S-1086S.
5. Bedoya P. (2009). "Vitamin D and tuberculosis."Emerging Science. 67(5).289-293.
6. Neport M.J., and Nejentsev S. (2004). "Genetics of susceptibility to tuberculosis in humans."61(2).102-111.
7. Wit E.D. (2009). 'Analysis of host determining factors in susceptibility to tuberculosis in South African coloured population."Stellenbosch University.
8. Alexandra S.G., Nicoleta C., Daniela P.M., Traian S., Veronica S.(2012). "Apal and Taql polymorphisms of VDR (Vitamin D Receptor) gene in association with susceptibility to tuberculosis in Romanian population." 18(1).
9. Champman S.J., and Adrian V.S.H. (2012). "Human genetic susceptibility to infectious disease. Natural Review Genetic." 13.175-188.
10. Wilbr A.K., Hurtado A.H., Hill K.R., Stone A.C. (2007). "Vitamin D receptor gene polymorphisms and susceptibility M.Tuberculosis in native Paraguayans." Elsevier Tuberculosis. 87.329-37.
11. Selvaraj P., Harishankar M., Vidyarani M. (2008). "Regulatory region polymorphism of vitamin D receptor gene in pulmonary tuberculosis patients and normal healthy subjects of South India."35(3).251-254.
12. Vetten M. (2009). "Pilymorphisms in the regulatory regio of the vitamin D receptor gene (VDR): In silico analysis, tuberculosis association and functional impact analytic. South Africa."University of Johannesburg.
13. Kang T.J., Jin S.H., Yeum C.E., Lee S.B., Kim C.H., Lee S.H., et al. (2011). "Vitamin D receptor gene Taq1, Bsm1, and Fok1 polymorphisms in Korean patients with tuberculosis."Immune Network. 11(5).253-257.
14. Rashedi J., Asgharzadeh M., Moaddah S.R., Sahebi L., Khalili M., Mazani M., et al. (2014). "Vitamin D receptor gene polymorphisms and vitamin D plasma concentration : correlation with susceptibility to tuberculosis." Adv Pharm Bull. 4(Suppl 2).607-611.
15. Lappe J.M. (2011). "The role of vitamin D in human health: a paradigm shift. " Journal of Evidence-based Complementary & Alternative Medicine. 16(1). 58-72.