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Suplementation of Vitamin D in Patients with Pulmonary Tuberculosis Ethnic Batak

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***Abstract*— Vitamin D has a complex action on the immune system, among other cathelicidin production stimulator which enhances phagocytosis magrofag. TB is an infectious disease which causes high mortality and morbidity in the world. Vitamin D will give the effect when it binds to its receptor (VDR). Variants VDR gene polymorphism associated with susceptibility and resistance to pulmonary tuberculosis.**

Objective: to determine the effect of vitamin D in patients with pulmonary tuberculosis against ethnic Batak

The study design with RCT, Ninety-two people with pulmonary TB smear positive new cases of ethnic Batak in Medan were randomized to receive 100,000 IU doses administered at 4 doses. Assessment were performed at 14, 28, 42, and 56 days. Levels of vitamin D beginning and end checked with ELISA techniques and *ApaI* polymorphisms of genes RVD checked by PCR RFLP.

After two months of 86 patients who met the inclusion criteria as many as 40 people with vitamin D intervention and 46 to placebo. Sputum conversion times faster intervention group than placebo (p <0.05) with increased levels of vitamin D is much different (95.31% and placebo interventions 6.81%). 5) dengan peningkatan kadar vitamin D jauh berbeda (intervensi 95,31% dan plasebo 6,81%). Sputum conversion based *ApaI* VDR gene polymorphisms in the two groups was not significant (p> 0.05).

Vitamin D proven to accelerate the conversion of sputum in the study population of patients with pulmonary TB ethnic Batak. *ApaI* polymorphism of the VDR gene genotype Aa and aa more conversions at 42 days while the AA genotype at 56 days.

***Keywords*— Vitamin D, pulmonary tuberculosis, sputum conversion, ApaI polymorphism of the VDR gene, ethnic Batak**

I. INTRODUCTION

Tuberculosis (TB) still remains an important cause of morbidity and mortality worldwide, and Indonesia occupies the third place (10%) after India (24%) and China (11%) regarding TB incidence. Environtmental and Genetic factors play important roles in the mechanisms involved in the development of tuberculosis disease. Many studies indicated that in the immune interaction between the host and Mycobacterium tuberculosis, the genetic inherited factors play a key role [2], [12], [13].

The role of vitamin D in prevention of infectious diseases has been very important. Research has stressed the important role of vitamin D for the immune system. Several studies have found that low levels of vitamin D in the body indicates the ability of the body's defense against infection with M. tuberculosis is low as well. It is known that vitamin D and especially its activated metabolite 1,25-dihydroxyvitamin D3 (1,25D3) may increase natural immune regulation to restrict Mycobacterium tuberculosis [3], [14], [15].

The action of vitamin D is mediated through binding to its nuclear receptor (VDR). VDR is a member of the steroid/thyroid hormone receptor family. Variants of VDR gene polymorphisms influenced by ethnic and geography. One of the VDR polymorphisms in the 3' untranslated region (UTR) polymorphisms is Apa I, found in intron 8, results in a T→G change (the T allele is designated 'A' while the G allele is designated 'a') [4], [5], [16], [18].

Ethnic Batak is located in North Sumatra. *ApaI* polymorphism research on ethnic Batak have not been done. Therefore we conduct a clinical trial to investigate the effects of high doses of vitamin D on sputum conversion in ethnic Batak patients receiving intensive-phase antimicrobial treatment for pulmonary tuberculosis and its relationship with *ApaI* polymorphism of the VDR gene.

II. MATERIALS AND METHODS

A. Subject Selection

Case were pulmonary tuberculosis patients were recruited from several health center in Medan city and Deli serdang district, North Sumatera-Indonesia. Inclusion criteria were newly diagnosed pulmonary tuberculosis, positive sputum smeer, Batak ethnic, age≥ 18 years old, and BMI>18,5. Exclusion criteria were HIV positive, consuming immunopressive drugs, renal failure and diabetes mellitus.

The study was approved by medicine faculty of North Sumatera University Research Ethics Committee and written informed consent was obtained from all patients. Patients were randomly assigned to receive four fortnightly doses of 100.000 IU vitamin D3 (bio-tech pharmacal,US) vs placebo (0, 14, 28, and 42 days) [6]. Before the intervention, the blood sample is taken for checking vitamin D pre levels and *ApaI* polymorphism of the VDR gene. The level of vitamin D was analysed with ELISA, while polymorphism was analysed using PCR-RFLP. Patients were followed for two months with sputum smear examination every two weeks (14 days, 28 days, 42 days, and 56 days). Sputum specimens were examined for the presence of acid-fast bacilli by microscopy Blood taken back in 56 days for checking of vitamin D post levels.

VDR Genotyping

The DNA was extracted (Promega, USA) and stored at minus 20ºC. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) was used to identify ApaI polymorphism of Vitamin D Receptor gene. The primer sequences used in this study were as follows: Forward Primer: 5'- AGA GCA TGG ACA GGG AGC AAG -3' and Reverse Primer: 5'-GCA ACT CCT CAT GGC TGA GGT CTC A . PCR conditions were as follows: denaturation at 94ºC for 5 min, followed by 40 cycles of PCR at 94ºC (30 sec), annealing at 60ºC (30 sec), and 72ºC (30 second). Final extension was continued at 72ºC for 5 min. Following PCR, the amplified PCR products was digested with ApaI restriction enzyme(Thermo Scientific) at 37ºC for 2 hours. Digested products were analyzed using electrophoresis in 2% agarose gel and ethidium bromide stains. The bands were visualyzed by Gel Documentation System.

Depending on the digestion pattern of ApaI polymorphism, individuals were scored as aa when homozygous for the presence of the Apa I site (531 bp and 214 bp), AA when homozygous for the absence of the ApaI site (745 bp), or Aa in case of heterozygosity (745 bp, 532 bp and 214 bp).

B. Statistical Analysis

Analyses were done with SPSS (version 17.0), Significance was tested at the 5% level. Hardy-Weinberg equilibrium test was done in case and control groups for ApaI and BsmI polymorphisms using the web tool HWE Testing calculator, available on line.

III. RESULT

We have 92 patients, 46 received anti-tuberculosis drug with vitamin D and 46 received anti-tuberculosis drugs with placebo. Anti-tuberculosis drugs given was in accordance with the DOTS program, first category: 2RHZE/4RH. Six patients (placebo groups) had no follow-up sputum culture of data, 86 patients formed the intention-to-treat population. Clinical and demographic characteristics of Patients were comparable for intervention and control groups at baseline in Table1. The result of vitamin D levels in table 2.

TABLE I
CLINICAL AND DEMOGRAPHIC CHARACTERISTICS OF PATIENTS

Karakteristik	**Vitamin D (n=40)**		**Plasebo (n=46)**	
	N	**%**	**N**	**%**
Age				
18 – 27	12	30	6	13,04
28 – 37	6	15	8	17,40
38 – 47	16	40	14	30,43
≥ 48	6	15	18	39,13
Gender				
Male	26	65	30	65,21
Female	14	35	16	34,79
Baseline acid fast bacilli				
+3	10	25	13	28,26
+2	9	22,5	25	54,35
+1	21	52,5	8	17,39
25(OH)D pre				
< 20 ng/ml	6	15	9	19,57
20-30 ng/ml	11	27,5	19	41,30
> 30 ng/ml	23	57,5	18	39,13
25(OH)D post				
< 20 ng/ml	0	0	7	15,22
20-30 ng/ml	1	2,5	18	39,13
> 30 ng/ml	39	97,5	21	45,65

Statistical analysis showed there are differences in vitamin D levels were significantly in the intervention group with a mean value of vitamin D levels was 31.77 ± 9.45 and 62.05 ± 22.07 for each. Levels of vitamin D in this group increased very high at 95.31%. The average value of vitamin D levels in the placebo group before and after was 26.86 ± 7.87 and 28.69 ± 7.20 for each with the rising levels of vitamin D in this group amounted to 6.81%.

TABLE II
COMPARISON of VITAMIN D LEVELS BEFORE and AFTER the INTERVENTION and PLACEBO GROUPS

Groups		**Levels of vitamin D**		
		Mean	**SD**	**p**
Pre	VitaminD	31,77	9,45	0,01
	Placebo	26,86	7,87	
Post	VitaminD	62,05	22,07	0,00
	Placebo	28,69	7,20	

Sputum conversion in the intervention group were significantly faster ($p < 0.05$) with a mean of 3.95 ± 1.39 weeks than placebo group 5.52 ± 2.11 weeks.

TABLE III
SPUTUM CONVERSION TIME INTERVENTION and PLACEBO GROUPS

Groups	**N**	**Mean ± SD**	**p**
Vitamin D	40	3,95 ± 1,39	0,000*
Placebo	46	5,52 ± 2,11	

Here are the results of electrophoresis ApaI polymorphism pulmonary TB patients ethnic Batak on 2% agarose gel.

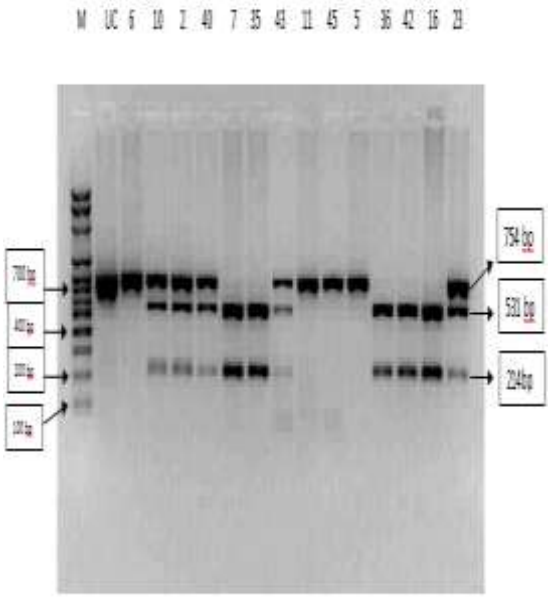

Fig.1 Result of RFLP VDR ApaI polymorphism pulmonary TB patients ethnic Batak

ApaI polymorphism genotypes AA, Aa and aa in the vitamin D group all sputum conversion to 28 days, in the placebo group sputum conversion up to 56 days.

TABLE IV
SPUTUM CONVERSION TIME RELATIONSHIP WITH APAI POLYMORPHISM VDR GENES.

Geno type	**Groups**	**Conversion time**					**p**
		14 days	**28 days**	**42 days**	**56 days**	**No conversion**	
AA	Vitamin D	0	1 (50%)	1 (50%)	0	0	0,978
	Placebo	0	2 (25%)	3 (37,5%)	2 (25%)	1 (12,5%)	
Aa	Vitamin D	6 (33,3%)	9 (50%)	3 (16,7%)	0	0	0,491
	Placebo	3 (16,7%)	7 (38,9%)	4 (22,2%)	3 (16,7%)	1 (5,6)	
aa	Vitamin D	4 (20%)	11 (55%)	5 (25%)	0	0	0,560
	Placebo	0	10 (50%)	7 (35%)	1(5%)	2(10%)	

IV. DISCUSSION

Clinical and demographic characteristics of patients were comparable for intervention and control groups at baseline (table 1). Our study show that median age did not differ significantly in two groups. This is in line with the WHO in 2012 and the Ministry of Health in 2014, the age of patients with pulmonary tuberculosis in Indonesia at most 15-44 year amounted to 58.45%. Number of TB patients was higher in certain age groups probably caused by different physiological processes at all ages, such as the role of the interaction of hormones to TB infection. Research Donald et al, 2010 stated the interaction between dehydroepiandrosterone (DHEA) and glucocorticoids that affects multiple lymphocyte function [8].

Percentage of males were higher than females TB patients.TB is more common in patients with male sex than women due to risk factors such as smoking and high alcohol consumption. Sinaga et al have shown that smoking and alcohol consumption increases the risk of pulmonary tuberculosis [9].

Vitamin D levels before and after the intervention in the two groups were increased. The mean levels of vitamin D before and after intervention in the intervention group was 31.77 ± 9.45 ng / ml and 62.05 ± 22.07 ng / ml, whereas the placebo group 26.86 ± 7.87 ng / ml and 28 69 ± 7.20 ng / ml. Differences between the mean levels of vitamin D improved considerably by 95.31% while in the placebo group only 6.81%.

The results of this study showed that the group of patients who were given vitamin D showed increased levels of vitamin D are very much different than the group who were given a placebo. Parikh, 2012 in a study stated the number of bacilli TB germs more on acid fast bacilli (+3) have a higher risk of developing into a mutant drug-resistant acid fast bacilli than smaller ones (+2 and +1) [17].

The results of this study showed the average value of sputum conversion time of study subjects in the intervention group was 3.95 ± 1.39 weeks, while the control group 5.52 ± 2.11 weeks. Sputum conversion intervention group significantly faster than placebo. Median sputum conversion time for the intervention group was 4 weeks (28 days), and placebo group of 6 weeks (42 days). RCT double blind study in Indonesia Nursyam et al, and in the UK Coosens et al, 2012 intervention of vitamin D results of sputum conversion is significantly higher in the treatment group compared with placebo [10], [11], [19].

The results of this study proved that vitamin D significantly accelerate the conversion of sputum of TB patients ethnic Batak. Giving vitamin D in patients with TB is highly recommended as one way to break the chain of transmission of infectious germs TB. Suplementasi vitamin D may increase the patient's immune system by inducing the cathelicidin production that play a role in fighting TB germs [2], [3], [13].

Micronutrients as potential adjunctive immunotherapy are a growing field of medical attention since scientific evidence of a specific antimycobacterial activity of vitamin D3 in macrophages has increased. These things need to be considered as one adjunctive therapy in TB cases [13].

Sputum conversion rate associated with ApaI polymorphism of the VDR gene in this study gives results that the VDR gene polymorphism Batak ApaI genotype Aa and aa in both groups were the most conversions at 28 days and at 42 days genotype AA. These results showed no difference in sputum conversion rate in both groups. Statistical analysis using the Kolmogorov-Smirnov also give the same result value of $p > 0.05$ for all three genotypes which means there is no relation between sputum conversion speed with *ApaI* polymorphisms of VDR genes.

ApaI polymorphism of the VDR gene occur in areas that affect intron splicing process and result in disruption of the transcript. Splicing errors that occurred resulted in the formation of protein cathelicidins errors are important in the fight against TB germs.

V. CONCLUSIONS

Giving vitamin D on therapy Pulmonary TB in the study population of patients with pulmonary TB ethnic Batak have proven showed a significant difference in sputum conversion compared with placebo . ApaI polymorphism of the VDR gene

genotype Aa and aa more conversions at week-6 while the AA genotype at week 8. Vitamin D should be considered as additional nutrients for each patient with pulmonary tuberculosis.

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