



Vitamin D Receptor Gene Polymorphism Affecting Vitamin D and Beta Carotene Deficiency in Tuberculosis Patients

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Abstract

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BACKGROUND: The working mechanism of Vitamin D in tuberculosis (TB), which is influenced by the work of other vitamins and minerals, remains questionable. This is particularly the case regarding the effect of polymorphism of the Vitamin D receptor (VDR) gene.

AIM: The objective of this research was to examine the differences in serum levels of 25(OH)D, retinol, beta-carotene, and calcium in TB patients compared to healthy people who have VDR gene polymorphisms (Taql, BsmI, and FokI).

METHODS: This research was a case-control study involving 176 men and women with a pair of VDR gene polymorphisms, consisting of 94 TB patients (TB group) and 82 healthy people (control group) in North Sumatera, Indonesia.

RESULTS: There was a significant difference in Vitamin D levels between the TB and control groups ($p = 0.001$), with Vitamin D deficiency of 85.1% in the TB group and 100% in the control group. Significant differences were found in retinol levels and beta-carotene, but there were no significant differences in calcium levels ($p = 0.619$). Based on these results, there was a significant difference between the TBC and normal group in 25(OH)D, retinol, and beta-carotene serum.

CONCLUSION: This study showed that 25(OH)D serum was higher in the TBC group than the control group, but lower in retinol and beta-carotene serum. There is no difference in calcium serum level in both groups.

Introduction

Tuberculosis (TB) is still a health problem, especially in many developing countries, and human genetic variability has been recognized to be relevance in host responses to Mycobacterium TB (MTB) infection [1], [2]. The presence of Vitamin D gene receptor polymorphisms occurs in healthy people and TB patients, occurs in areas of sufficient sun exposure and Vitamin D deficiency can be caused by the presence of the Vitamin D receptor (VDR) gene polymorphism [3], [4]. The VDR gene encodes the transcription of any relationship with the ligand, which is a factor that modulates the various findings reported with Vitamin D, including homeostasis, cell growth, cell differentiation, modulation of the immune response, and macrophage-monocyte activation [1], [3], [5].

The polymorphism of the VDR gene (single-nucleotide polymorphisms [SNPs]) from the Vitamin D gene receptor is specifically revised according to exon 2 (FokI) and 3-end (BsmI and TaqI) associated with TB [6], [7], [8]. The previous studies reported that VDR gene polymorphisms affecting the TB's susceptibility and its affecting the serum levels of 25(OH)D serum levels in the body's circulation, whether this would affecting other vitamin and mineral, still questionable [2], [9], [10].

Low levels of Vitamin D will affect calcium homeostasis in the body. Calcium is needed by the body in bone metabolism and blood pressure in the pathogenesis of TB [11], [12]. When activated, protein-binding (CREB) cAMP responses to proteins that initiate cellular processes regulate macrophage communication associated with apoptotic tracking pathways in macrophages [12], [13].

In addition to calcium, Vitamin A and beta-carotene have also been shown to work with Vitamin D in terms of retinoid X receptors (RXR) [14]. Vitamin A levels are found to be low in TB patients; the action of Vitamin A is through its role to enhance the host's immune response against the pathogenic MTB bacteria [14], [15]. The purpose of Vitamin A through retinoic acid (RA) is by binding to nuclear RA receptor (RAR) and RXR receptors in controlling the transcription expression of various target genes. The target gene includes immunity, and retinol works on T cells, especially T-helper and dendritic cells [1], [14]. The previous studies reported that Vitamin D and Vitamin A levels were low in TB patients [14]. However, the question remains whether Vitamin D, Vitamin A, beta-carotene, and calcium work together in a mechanism of synergy in MTB infection so that these levels can become parameters to be kept within reasonable limits for TB patients. A further outstanding question is whether any connection exists with the occurrence of VDR gene polymorphisms.

Based on the results of the previous studies and the desire to understand the relationships of each micronutrient, research is required that aims to look for differences in serum levels of 25(OH)D, serum retinol, beta-carotene, and calcium in TB patients compared to healthy people with VDR gene polymorphisms (*TaqI*, *BsmI*, and *FokI*). The results of this study are expected to be a step toward further research on how to administer micronutrient administration, including of Vitamin D, Vitamin A, beta-carotene, and calcium, in people with VDR gene polymorphisms.

Materials and Methods

This study involves a case-control study involving 94 TB patients and 82 healthy people in the city of Medan, North Sumatera, Indonesia, held from July 2019 to September 2020. In the regions of Indonesia, summer ranges from April to October each year and is characterized by a significant amount of sun exposure. Sampling locations were on the island of Sumatera with the same demographic and ethnic diversity. The study area was in the city of Medan, North Sumatera, Indonesia, with a latitude of 3.57 N and longitude 98.65 E, and an average temperature of $\pm 32^{\circ}\text{C}$ (90°F).

This study gathered several parameters: Demographic characteristics; anthropometric parameters including body mass index (BMI) and fat mass; daily food intake using food recall for 2 days of 24 h; laboratory tests for 25(OH)D levels, serum calcium, retinol, and beta-carotene. Intake was assessed in one examination during a meeting with the research subjects. Furthermore, the data obtained were analyzed statistically according to the research

hypothesis that there are differences in levels of 25(OH)D, calcium, retinol, and beta-carotene between the TB and control groups.

The subjects of TB research conducted in four Medan City Public Health Centers were taken as the references for TB. Diagnosis of pulmonary TB in study subjects was based on clinical signs and symptoms, as well as diagnostic work-ups, such as positive sputum smears in acid-fast bacilli staining or positive acid-fast bacilli culture or chest X-ray examinations with features suggestive of pulmonary TB. Inclusion criteria were male or female aged 18–60 years, diagnosed with TB by doctors at the TB Reference Center, who were given rifampicin-based antituberculosis, and had one or both of the VDR gene polymorphisms of the *TaqI* or *FokI* receptor genes. Exclusion criteria included history of Vitamin D, Vitamin A, beta-carotene, and calcium supplement consumption; history of chronic kidney disease or cirrhosis; breastfeeding; or pregnancy. A total of 104 research subjects were initially selected; after screening, 94 research subjects were obtained based on inclusion and exclusion criteria.

Control research subjects were identified based on surveys carried out in offices, schools, hospitals, private banks, and public parks in the city of Medan. Inclusion criteria were male or female aged 18–60 years and had one or both of the polymorphisms of the VDR genes *TaqI* or *BsmI*. Exclusion criteria were routine taking of supplements of Vitamin D, Vitamin A, beta-carotene, or calcium; abnormalities in kidney or liver, or other metabolic disorders; and breastfeeding or pregnancy. Based on the results of the subject collection, 128 research subjects were obtained, and a total of 82 people participated in the study following screening for inclusion and exclusion criteria.

Furthermore, a 1 time data collection check was taken at the time of the meeting with the research subjects. All subjects were analyzed according to their group, that is, the TB group or the healthy (i.e., control) group. All data were compared between the two groups to identify differences and to allow a research conclusion to be drawn.

Detection of SNPs in VDR gene (*TaqI*: rs731236, *FokI*: rs2228570, and *BsmI*: rs1544410) was performed in three steps: (1) DNA isolation through the salting-out method, (2) DNA purity validation, and (3) SNP genotyping using Applied Biosystem StepOne Plus Real-Time polymer chain reaction Systems (Applied Biosystems, Foster City, CA, United States). For genotyping data analysis, individuals were divided into groups based on the genotypes and alleles of the VDR gene. The groups were heterozygotes (Tt, Bb, and Ff), homozygous wild type (TT, BB, and FF), and homozygous mutant (tt, bb, and ff). Research subjects who experienced heterozygotes or homozygous mutants in one or two VDR genes were included in the inclusion criteria in this study after signing consent approval form after

research explanation which probably have lower 25(OH)D serum level.

Anthropometry features included height using a standardized measuring tape in centimeters (Microtoise SH-2A GEA Medical, Indonesia), weight using digital scales (Body Composition Monitor with Scale, HBF-362, Karada Scan-Omron, Omron Healthcare Group, Jepang), and BMI (calculated as kg/m^2). BMI categorization was based on Asian Pacific indexes [16], namely, $< 18.5 \text{ kg/m}^2$, $18.5\text{--}22.9 \text{ kg/m}^2$, $23\text{--}24.9 \text{ kg/m}^2$, and $> 25 \text{ kg/m}^2$ were classified as underweight, normal weight, overweight, and obese, respectively. For the calculation of fat mass, a digital fat mass calculation tool was used which refers to the amount of body fat mass as a proportion of the total body weight (Body Composition Monitor with Scale, HBF-362, Karada Scan-Omron, Omron Healthcare Group, Jepang). Fat mass data were expressed as a percentage according to the categories normal ($\leq 29.9\%$) and high ($> 30.0\%$). Calculation of physical activity used a questionnaire based on the physical activity index, which is the sum of the work activity index, sports activity index, and leisure activity index. Physical activity categories were < 6.2 (low), $6.3\text{--}7.1$ (moderate), and > 7.1 (high).

The assessment of nutrient intake was based on food recall for 2 days (1 day for a weekday and 1 day for the weekend), including energy, carbohydrate, protein, fat, Vitamin D, calcium, Vitamin A, and beta-carotene, further classified according to the percentage of fulfillment. Analysis of food intake was undertaken using the NutriSurvey 2007 computer program, which involved Indonesian dietary foods.

We measured 25(OH)D serum concentration by chemiluminescent immunoassay technology (DiaSorin, Stillwater, Minnesota, United States); measures were between 4.0 and 150 ng/mL. The lowest value was 4.0 ng/mL, which was based on an interassay precision of 3.90% coefficient of variability; reference ranges were categorized as $< 20 \text{ ng/mL}$, deficiency; $20\text{--}32 \text{ ng/mL}$, insufficient; and $32\text{--}54 \text{ ng/mL}$, sufficient [17]. Measurement of retinol and β -carotene serum levels was performed using high-performance liquid chromatography device (HPLC; Waters, Waters Corporation, Milford, United States). The normal range of retinol level was $> 0.7 \mu\text{mol/L}$, while the normal range for beta-carotene serum level was $0.3\text{--}0.6 \mu\text{mol/L}$. Calcium serum was measured by ADVIA Bayer Assayed Chemistry Controls (Siemens Healthineers Global, Erlangen, Germany). The normal concentration of calcium was $8.3\text{--}10.6 \text{ mg/dL}$.

Statistical significance between the study groups was determined using Chi-square and Fisher's tests for categorical variables with normal and non-normal distributions, respectively. In addition, the non-paired t-test and Mann-Whitney U-test for numerical variables were conducted with normal and non-normal distributions, respectively. $p < 0.05$ was considered statistically significant, and SPSS (version 11.5; SPSS

Inc., Chicago, United States) was used to perform the analysis.

This study was carried out after receiving ethical approval from the Health Research Ethics Medical Faculty Committee of Sumatera Utara University (No. 135/TGL/KEPK FK USU-RSUP HAM/2019) and all participants gave written informed consent for the study procedures. This research already registered on Clinical Trials.gov Protocol Registration and Results System with ClinicalTrials.gov Identifier: NCT04593524.

Results

The aim of this study was to look for differences in serum levels of 25(OH) D, serum retinol, beta-carotene, and calcium in TB patients compared to healthy people with VDR gene polymorphisms (*TaqI*, *BsmI*, and *FokI*). This research was conducted on the island of Sumatera, Indonesia, with a high incidence of TB which experiences a tropical climate. Based on these two factors, research into TB and the role of Vitamin D is of interest because of several other relevant factors, particularly the presence of VDR gene polymorphisms.

This study initially included 104 research subjects suffering from TB and 128 healthy research subjects; after applying inclusion and exclusion criteria, 94 research subjects were obtained for the TB group, and 82 study subjects were included in the control group (healthy research subjects). All study subjects experienced VDR gene polymorphisms, including *TaqI*, *BsmI*, or *FokI*, and distribution of VDR gen polymorphism is shown in Table 1. A part of the subject excluded in this research because of exclusion criteria and did not sign the consent approval form after research explanation.

Table 1: VDR gene receptor polymorphisms in patients with tuberculosis and healthy control

VDR gene receptor polymorphisms	Tuberculosis group n (%)	Control group n (%)
rs731236 (<i>TaqI</i>)		
Allele frequency		
T	113 (54.59)	170 (66.41)
t	94 (45.41)	86 (33.59)
Genotype		
TT	10 (9.62)	46 (35.93)
Tt	93 (89.42)	78 (60.94)
tt	1 (0.96)	4 (3.14)
Total	104 (100)	128 (100)
rs1544410 (<i>BsmI</i>)		
Allele frequency		
B	207 (99.52)	172 (58.90)
b	1 (0.48)	120 (41.1)
Genotype		
BB	103 (99.03)	46 (35.93)
Bb	1 (0.97)	80 (62.50)
bb	0 (0)	2 (1.57)
Total	104 (100)	128 (100)
rs2228570 (<i>FokI</i>)		
Allele frequency		
F	104 (50)	226 (88.28)
f	104 (50)	30 (11.72)
Genotype		
FF	1 (0.96)	101 (78.91)
Ff	102 (98.08)	24 (18.75)
ff	1 (0.96)	3 (2.34)
Total	104 (100)	128 (100)

The characteristics of the TB group, diagnosis was based on established criteria, based on severity class or TB score reported that 85 research subjects had TB score in WHO Class II (90.4%) and nine research subjects were in Class III (9.6%). Research subjects also showed the bacillus Calmette-Guérin (BCG) scar whereas the most precisely with a clear scar were 69 subjects (73.4%), no scar were 6 subjects (6.4%), and dubious were 19 subjects (20.2%).

Sociodemographic characteristics

The sociodemographic characteristics of the two groups in this study are presented in Table 2. The age of study subjects diagnosed with active TB was higher than in the control group ($p = 0.002$): The median age in the TB group was 37.6 years, with the youngest aged 19 years and the oldest aged 59 years; the control group had a younger median age of 32.5 years, with the youngest aged 21 years and the oldest 48 years. Table 2 also shows that in the TB group, 2 times the incidence of TB was found in men compared to women (67% vs. 33%), while the control group contained more women than men, but showed no meaningful difference in both groups.

Table 2: Sociodemographic characteristics

Characteristics and lifestyle variables	TBC group n = 94	Control group n = 82	p-value
Age	40.12 ± 13.54	33.23 ± 8.01	0.002*
Age classification			
18–30	30 (31.9)	38 (46.3)	0.001*
1–40	18 (19.1)	26 (31.7)	
41–50	14 (14.9)	18 (22)	
51–60	32 (34)	0 (0)	
Gender			
Male	63 (67)	17 (20.7)	0.315
Female	31 (33)	65 (79.3)	
Marital status			
Married	71 (75.5)	56 (68.3)	0.001*
Not married/single	23 (24.5)	26 (31.7)	
Ethnic			
Batakese	57 (60.6)	29 (35.3)	0.001*
Javanese	23 (24.5)	35 (42.7)	
Malay	5 (5.3)	3 (3.7)	
Others	9 (9.6)	15 (18.3)	
Occupation			
Government employee	38 (40.4)	46 (56.1)	0.001*
Entrepreneur	30 (31.9)	0 (0)	
Doctor and nurse	0 (0)	36 (43.9)	
Housewife	10 (10.6)	0 (0)	
Non-employee	6 (6.4)	0 (0)	
Student	10 (10.6)	0 (0)	

Data are given as mean ± SD or number (%); P values determined using Chi-square test and Fisher's exact test (significance $P < 0.05$); *: Significant value.

There was a relationship between the two groups in terms of marital and ethnic status; it was found that marital status had a relationship with the incidence of TB compared to the control group. Ethnic Batak was more common in the TB group (60.6%) than in the control group. In the control group, there were more Javanese ethnics compared to other tribes.

The most common occupation in the TB group was civil servant, and the lowest prevalence was unemployed. In the control group, there were more research subjects with jobs as civil servants, doctors, and nurses. The two groups show differences in terms of job distribution.

Lifestyle, anthropometry, fat mass, and nutrient intake

Lifestyle and anthropometry are presented in Table 3. The category of work (indoor or outdoor) shows that indoor work was more often found in the control group (56.1%), whereas in the TB group, it was more varied, including students, unemployed, housewives, and traders. This was also related to sun exposure, which is higher in the control group, where the percentage of sun exposure was 96.3%.

Table 3: Lifestyle, anthropometric, and fat mass characteristics

Characteristics and lifestyle variables	TBC group n = 94	Control group n = 82	p-value
Type occupation			
Indoor	63 (67)	79 (96.3)	0.001
Outdoor	31 (33)	3 (3.7)	
Daily sun ray exposure, n (%)			
≤ 1 h	67 (71.3)	78 (95.1)	0.001
> 1 h	27 (28.7)	4 (4.9)	
BMI (kg/m ²)	21.91 ± 3.39	23.87 ± 3.54	0.001
BMI classification			
Underweight	21 (22.3)	2 (2.4)	0.001
Normal weight	47 (50)	39 (47.6)	
Overweight	18 (19.1)	10 (12.2)	
Obese	8 (8.5)	31 (37.8)	
Fat mass (%)	19.82 ± 6.73	31.21 ± 4.79	0.001
Fat mass:			
Normal	88 (93.6)	27 (32.9)	0.001
High	6 (6.4)	55 (67.1)	
Physical activity, n (%)			
Low	59 (62.8)	68 (82.9)	0.008
Medium	17 (18.1)	9 (11)	
High	18 (19.1)	5 (6.1)	

Data are given as mean ± SD or number (%); P values determined using Chi-square test and Fisher's exact test (significance $P < 0.05$); *: Significant value.

From the anthropometric analysis presented in Table 3, BMI showed significant differences between the two groups. In the TB group, a median value of 21.9 kg/m² was found; the lowest BMI was 12.98 kg/m² and the highest was 30.97 kg/m². In the control group, the median BMI value was 22.9 kg/m²; the lowest BMI value was 16.8 kg/m², and the highest was 31.5 kg/m². Fat mass in the two groups also showed significant differences; fat mass in the TB group was lower than the fat mass in the control group. Likewise, in physical activity, a significant difference was seen; it appeared that the TB group had higher physical activity compared to the control group ($p = 0.003$). Being underweight increases the risk of TB, which, in turn, can lead to malnutrition. This is revealed to not only be a risk factor for the progression of latent TB infection to active disease but also increases the risk of drug toxicity. Relapse and death, once TB develops, is also affected by inadequate nutrition intake.

Based on food recall analysis, a lower food intake was found, especially for Vitamin D, Vitamin A, beta-carotene, and calcium intake, and this result was similar to normal subjects. The previous studies reported that women mainly had lower intake of food sources, as presented in Table 4.

Vitamin D, Vitamin A, Beta-carotene, and Calcium

This study showed a significant difference in the levels of Vitamin D, retinol, and beta-carotene in

the TB and control groups, but not in calcium levels. Calcium levels showed no significant differences and relationships between the TB and control groups (Table 5). Serum 25(OH)D levels were higher in the TB group than in the control group ($p = 0.001$). In the TB group, the median serum 25(OH)D level was 23.8 ng/mL, with a lowest level of 7.3 ng/mL and a highest level of 41.2 ng/mL. These levels were much higher and included normal serum 25(OH)D levels compared to the control group. In the control group, the median value was 14.75 ng/mL, with the lowest value being 7.9 ng/mL and the highest being 27.7 ng/mL. These levels in the control group were probably caused by the presence of VDR gene polymorphisms and a lifestyle that avoids sun exposure.

Table 4: Calorie and nutrient intake

Calorie and nutrient intake (daily)	TBC group, n = 94	Control group, n = 82	p-value
Energy (Cal/day)	1178.23 ± 174.58	1745.02 ± 401.89	0.001*
Energy intake classification:			
Low	94 (100)	76 (92.7)	0.028*
Normal	0 (0)	4 (4.9)	
High	0 (0)	2 (2.4)	
Amount of protein (g/day)	28.77 ± 4.26	51.61 ± 19.14	0.001*
Protein intake classification:			
Less	94 (100)	62 (75.6)	0.001*
Normal	0 (0)	20 (24.4)	
Amount of Vitamin D (mcg/day)	5.53 ± 0.90	4.89 ± 5.51	0.011*
Vitamin D intake classification:			
Less	94 (100)	78 (95.1)	0.045*
Normal	0 (0)	4 (4.9)	
Amount of Vitamin A (mcg/day)	458.62 ± 55.85	459.91 ± 55.19	0.935
Vitamin A intake classification:			
Less	94 (100)	82 (100)	-
Normal	0 (0)	0 (0)	
Amount of beta-carotene (mcg/day)	0.35 ± 0.12	4.67 ± 1.99	0.001*
Beta-carotene intake classification:			
Low	94 (100)	19 (23.2)	0.001*
Normal	0 (0)	38 (46.3)	
High	0 (0)	25 (30.5)	
Amount of calcium (mg/day)	105.04 ± 33.82	135.07 ± 37.31	0.001*
Calcium intake classification:			
Less	94 (100)	82 (100)	-
Normal	0 (0)	0 (0)	

Data are given as mean ± SD or number (%); P values determined using Chi-square test and Fisher's exact test (significance $P < 0.05$); *: Significant value.

Retinol levels in this study showed significantly lower levels in the TB group compared to the control group (Table 5). The median level of retinol in the TB group was 1.54 $\mu\text{mol/L}$, with the lowest level being 0.02 $\mu\text{mol/L}$ and the highest being 3.95 $\mu\text{mol/L}$. In the control group, retinol levels were higher: The

Table 5: Laboratory parameters 25(OH) D, retinol, beta-carotene, and calcium serum

Laboratory parameters	TBC group n = 94	Control group n = 82	p-value
25(OH) D serum levels (ng/mL)	24.09 ± 7.48	15.61 ± 4.68	0.001*
Vitamin D classification; n (%)			
Deficiency	28 (29.8)	67 (81.7)	0.001*
Insufficiency	52 (55.3)	15 (18.3)	
Sufficiency	14 (14.9)	0 (0)	
Retinol serum ($\mu\text{mol/L}$)	1.51 ± 0.79	3.23 ± 0.38	0.001*
Vitamin A classification; n (%)			
Deficiency	15 (16)	0 (0)	0.001*
Normal	79 (84)	82 (100)	
Beta-carotene serum ($\mu\text{mol/L}$)	3.07 ± 2.12	0.57 ± 0.40	0.001*
Beta-carotene classification; n (%)			
Deficiency	6 (6.4)	22 (26.8)	0.001*
Normal	10 (10.6)	34 (41.5)	
High	78 (83)	26 (31.7)	
Serum calcium (mg/dL)	9.16 ± 0.48	9.21 ± 0.39	0.619
Calcium classification; n (%)			
Deficiency	4 (4.3)	0 (0)	0.162
Normal	86 (91.5)	79 (96.3)	
High	4 (4.3)	3 (3.7)	

Data are given as mean ± SD or number (%); P values determined using Chi-square test and Fisher's exact test (significance $P < 0.05$); *: Significant value.

median was 3.2 $\mu\text{mol/L}$, with the lowest content being 2.76 $\mu\text{mol/L}$ and the highest being 3.9 $\mu\text{mol/L}$.

Beta-carotene levels also showed significant differences between the two groups: Beta-carotene levels were more elevated in the TB group than in the control group (Table 5). The median level of beta-carotene in the TB group was 3.2 $\mu\text{mol/L}$, with the lowest level being 0.09 $\mu\text{mol/L}$ and the highest level being 12.42 $\mu\text{mol/L}$. In the control group, beta-carotene levels were found to be much lower, with a median value of 0.49 $\mu\text{mol/L}$, a lowest value of 0.08 $\mu\text{mol/L}$, and a highest value of 1.95 $\mu\text{mol/L}$.

This study examined four parameters that influence each other; however, one that is not related to the other three is the calcium level. Serum calcium levels did not show significant differences between two groups and, in the case, where Vitamin D deficiency occurred, a decrease in serum calcium levels was not shown.

Discussion

This research was conducted on the island of Sumatera, Indonesia, specifically, in the area of North Sumatera, an area with a tropical climate and significant sunshine. This situation should not lead to inhabitants having Vitamin D deficiency, however, many Vitamin D deficiencies are found in healthy people [4], [18], [19]. Various causes include the lack of intake of food sources with Vitamin D and lifestyles that avoid sunlight. In addition to Vitamin D deficiency occurring in healthy people, it is also found in the cases of other infections, such as TB.

The previous studies have shown an association between *TaqI* VDR gene polymorphisms and susceptibility to TB in certain ethnic groups [1]. This susceptibility is related to low serum 25 (OH) D levels, as an illustration of Vitamin D levels in the body's circulation [20], [21]. Low levels of Vitamin D will cause low levels of calcium in the body, which is associated with low bone density. This study showed that all TB and control patients experienced VDR gene polymorphisms, including heterozygote forms of *TaqI*, *FokI*, or *BsmI*. The presence of polymorphisms in two of these receptor genes indicates greater Vitamin D deficiency in both groups. However, it was seen that serum 25(OH)D levels were even greater in the TB group. Probably, patients with TB had higher serum 25(OH)D levels because of they had higher multivitamin intakes, whereas healthy people (control group) did not maintain Vitamin D intake or lifestyle such as avoiding sunlight exposure.

Many studies have found Vitamin D deficiencies in countries with four seasons [22], [23], [24]; however,

this study showed that Vitamin D deficiency was found in healthy subjects and those suffering from TB. This was an interesting finding because Vitamin D affects TB progressivity; however, this research found that healthy subjects that were obese or malnourished had low 25(OH)D serum levels. This study showed that even with abundant sun exposure, such as in a tropical country, Vitamin D deficiency could be found in healthy subjects and those suffering from TB not only in higher adiposity (obese) subjects but also in normal adiposity (overweight and obese) subjects, as reported in other studies [4], [21].

Latitude has an influence on Vitamin D deficiency. The number of solar UV B photons (280–320 nm) reaching the earth depends on zenith angle of the sun; above about 35° north latitude, little or no Vitamin D3 can be produced. The location of recruitment for this study was Sumatera Island (Sumatera Utara, Medan) with latitude 3.57 N and longitude 98.65 E, and average temperature of 32°C (90°F).

The age of those taking part in this study was higher than that of most TB patients in the area. Moreover, they worked and all had to leave their house to travel to their workplace; however, TB patients spent all day exposed to sunlight. There was a significant difference between the TB group and control group: Those in the control group spent all day in a building, which led to less sunlight exposure and less physical activity than for TB patients.

Adiposity assessed from BMI measures showed higher means of BMI and body fat percentage in the control group compared to the TB group. This was in conjunction with lower 25-hydroxyvitamin D serum concentrations. Despite higher adiposity, however, Vitamin D deficiency was found in normal, overweight, and non-obese participants in both groups. This study showed that although there were significant differences in adiposity measures, such as BMI and body fat percentage, there is no significant difference in 25-hydroxyvitamin D concentration between the groups.

Vitamin D intake was shown to be below the recommended dietary allowance in all subjects. Vitamin D3 has 5 times the activity of Vitamin D2, and dietary food sources may not supply enough for adequate health. Cholecalciferol (D3) is found mainly in salmon, sardines, mackerel, tuna, and codfish oil, and is also found in limited quantities in milk, egg yolk, butter, and margarine [17], [25]. Supplements commonly contain ergocalciferol (D2) extracted from mushroom or D3 extracted from lanolin. Ordinary dietary sources of Vitamin D3 evidently do not supply enough for adequate health (around 250–300 IU/day in the USA). Individuals with low Vitamin D intake are advised to take supplements that are safe and reliable sources of Vitamin D3 [26].

However, according to this study, neither Vitamin D supplements nor food sources of Vitamin D

are consumed on daily basis. Alarmingly, this study revealed that healthy subjects consumed very limited amounts of Vitamin D food sources (egg yolk, fish, meat, and mushroom) and also seldom consumed Vitamin D supplements. Low dietary intake of Vitamin D and low sunlight exposure can have detrimental effects on health, especially in calcium homeostasis; however, this study reported that calcium status had a normal range in both TB and healthy subjects.

This study showed low levels of Vitamin D in TB patients, but these levels were nonetheless higher than in healthy people. Food intake and the lifestyle of TB patients are perhaps healthier due to socialization or counseling about the importance of Vitamin D; conversely, healthy people may not be aware of Vitamin D deficiency [19]. Interestingly, healthy people who work in confined spaces have lower sun exposure than TB patients. Counseling or outreach about the importance of Vitamin D should be undertaken as often as possible. While the role of retinol in supporting the clinical improvement of TB was seen with low levels of retinol in TB group, in the control group they were normal.

Vitamin A provides support to the body's immune system, and the role of this vitamin is closely related to the pathogenesis of TB [1], [14]. Much of this is due to the role of RA, which binds to nuclear receptors RAR and RXR to control the transcription expression of various target genes [14]. The target gene includes the function of T cells that play a role in the immune response in TB. The role of Vitamin A is also related to Vitamin D, and the link between Vitamins D and A is through the action of the VDR gene that binds to transcription factors with RXR in modulating other biological processes, including calcium hemostasis and immune function. Genetic variations involving the VDR gene cause increased vulnerabilities, such as due to TB and autoimmunity. Linkages of sunlight and cod liver oil are stated to help reduce the risk of TB [27], [28], [29].

This study also looked at differences in levels of beta-carotene, as provitamin A, in TB infection, and seen in the role of fat-soluble vitamins as antioxidants, such as Vitamin A, Vitamin E, and beta-carotene, which affect the oxidase status in the body. Antioxidant levels in the body of TB patients are low compared to those of healthy people [30], [31], [32]. However, this study showed that serum beta-carotene levels were higher in TB patients compared to healthy people, different from the results of the previous studies. Oxidative stress is higher in healthy people compared to TB patients, so beta-carotene levels are higher in TB cases. Another possibility is the better intake in TB patients compared with healthy patients.

The previous studies have shown a large role for calcium in the worsening of TB, but this study found different results [4], [5], [33], [34], [35]. This study showed that calcium levels in TB patients are not different from healthy patients. It was seen that there is no influence of low serum 25(OH)D levels and serum

calcium levels in the TB or healthy groups; this is most likely due to high calcium intake in TB patients or the influence of the parathyroid hormone.

The previous studies have also shown normal levels of calcium, although low Vitamin D levels were found [6], [18], whereas, in this study, normal calcium levels in both groups showed a homeostatic mechanism of calcium to maintain normal calcium levels in circulation. This situation is not influenced by low levels of 25(OH)D serum or the presence of VDR gene polymorphisms [18]. However, the results of this study can be extended by examining the link with bone density. The next focus of the proposal is giving Vitamin D and Vitamin A as supplements to TB patients to reduce the progression of TB. In healthy people, higher Vitamin D intake and lifestyle changes can be recommended to ensure Vitamin D status returns to normal.

Weaknesses in this study included not examining patients' parathyroid hormone levels and the presence of a bias when conducting food intake interviews. TB or malnutrition patients often exaggerate food intake during interviews, and biases can also be caused by interviewer error in describing the portions when analyzing food intake.

Conclusions

Based on the results of the study, there was a significant difference between TBC and normal group in 25(OH)D, retinol, and beta-carotene serum. This study showed that 25(OH)D serum was higher in the TBC group than the control group, but lower in retinol and beta-carotene serum. However, serum calcium levels did not show differences. The difference in 25(OH)D serum may be due to the polymorphism of the VDR gene and differences in sunlight exposure due to occupation or lifestyle. However, retinol was lower in the TB group, perhaps showing the role of retinol in TB. Beta-carotene levels were found to be lower in the control group, with higher antioxidant activity and lower intake in healthy people. Although calcium levels can be maintained in both TB and healthy patients, low Vitamin D levels can affect serum calcium levels in later life. The results of this study can be a reference in the provision of vitamin supplementation, particularly for both TB patients and healthy people who have polymorphisms of the VDR gene.

Authors' Contributions

DKS designed the study, coordinated the study, collected data, drafted the manuscript, and the

statistical analysis. NKA conducted the laboratory analyses. All authors have read and approved the final manuscript.

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