Soy, Catfish, Anchovy, and Rice Supplementation Increases 25(OH)D Serum Levels in Tuberculosis Patients with Complications

Dina Keumala Sari[1*], Ridha Dharmajaya[2], Mutiara Indah Sari[3], Dewi Masyithah[4]
[1]*Tropical Medicine Study Program, Faculty of Medicine, Universitas Sumatera Utara*
[2]*Neurosurgery Department, Faculty of Medicine, Universitas Sumatera Utara*
[3]*Biochemistry Department, Faculty of Medicine, Universitas Sumatera Utara*
[4]*Parasitology Department, Faculty of Medicine, Universitas Sumatera Utara*
**corresponding author: dina@usu.ac.id; Tel.: +62-813-971-77-693*

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Abstract. Tuberculosis patients that have complications (e.g., diabetes mellitus and human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS)) who live in tropical regions experience vitamin D deficiency, especially in North Sumatra, Indonesia. The presence of vitamin D receptor (VDR) polymorphism genes, *Taq*I and *Fok*I, is one of the predisposing factors, as is high levels of inflammatory markers, also indicating disease progression and malnutrition. This study aims to assess the effect of 50 g of soy-catfish-anchovy-rice (SCAR) porridge per day for 14 days on 25(OH)D, calcium, and biomolecular serum levels in patients with VDR gene polymorphisms (*Taq*I or *Fok*I). The study was a parallel, open, clinical trial. A total of 43 subjects with the VDR gene polymorphisms were selected. The subjects were divided into two groups using block randomization. There were 22 subjects in the intervention group (I) who received 50 g of SCAR porridge once per day, along with dietary counseling, and 21 subjects in the control group (C) who only received dietary counseling. All subjects in both groups completed the study. After 14 days of intervention, there was a significant increase in 25(OH)D serum levels in the I group, and no change was observed in the C group (p = 0.01). There were no significant differences in biomolecular levels, such as albumin, Hs CRP, blood glucose, and calcium. The results show that in patients who have tuberculosis with complications, VDR gene polymorphism, and are supplemented with 50 g of SCAR porridge per day for 14 days experience a significant increase in 25(OH)D serum levels.

1 Introduction

Tuberculosis (TB) is a major health problem around the world, with the prevalence rising each year, and is one of the main causes of mortality in Indonesia. Governments of various countries with the highest prevalence have implemented the directly observed treatment, short-course (DOTS) strategy, as well as the Stop TB strategy; however, new cases continually appear and cases relapse due to emerging resistance [1]. This condition is worsened by factors (e.g., environmental, nutritional, and genetic) that increase a person's susceptibility to *Mycobacterium tuberculosis* infection [2-4]. Previous studies showed that vitamin D receptor gene polymorphism significantly increases the risk of tuberculosis disease and comorbidities such as diabetes mellitus or human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) [2].

Vitamin D receptor gene polymorphism is related to both vitamin D deficiency and an increased risk of tuberculosis disease. Related genes, such as *Fok*I and *Taq*I, are related to low levels of vitamin D, and play a role in the immune regulation in the body [2, 4, 5]. Vitamin D supports the induction of pleiotropic antimicrobial response in tuberculosis patients [6, 7]. Vitamin

D supplements may accelerate sputum conversion and help to improve the clinical condition of tuberculosis patients. Vitamin D promotes an increase in immune cells by activating 25-hydroxyvitamin D receptor [7, 8]. Food sources that contain high levels of vitamin D, such as catfish and mushrooms, could be used as a natural supplementation to address low vitamin D levels. Soy, catfish, anchovy, and rice are food sources that contain high levels of calcium, vitamin D, protein, and carbohydrates. Consuming these ingredients as a porridge could increase vitamin D and calcium levels in the blood.

Vitamin D deficiency is common in tropical areas like Indonesia, occurring even in healthy individuals, despite an abundance of sunlight hours. It is thought that a sun-evading lifestyle, in addition to a lack of vitamin D intake, causes an increase in the prevalence of vitamin D deficiency. Vitamin D deficiency may impact calcium levels, although various studies stated that this is not the case. Calcium plays a role in the regulation of tuberculosis infection via cellular activity.

Vitamin D modulates the body's immune response by increasing vitamin D receptor (VDR) activity, then activating monocytes [7, 9, 10]. Another mechanism is the active form of vitamin D, $1,25(OH)_2D_3$, which hinders the proliferation of T cells and increases the abilities of pathogen-activated macrophages [10, 11].

VDR is a member of the nuclear receptor of transcription factors. VDR, along with the activation of $1.25(OH)_2D_3$, binds the DNA response element and forms a heterodimer with the retinoic acid receptor (RXR). This heterodimer links the relationship between vitamin D and vitamin A or carotenoids, including beta carotene.

We performed a randomized clinical trial to study the impact of soy, catfish, anchovy, and rice (SCAR) porridge for 14 days on new patients diagnosed with both tuberculosis and associated complications. We used public health centers as the source of tuberculosis cases in the City of Medan. We tested the levels of 25(OH)D, calcium, and other biomolecular parameters such as albumin, Hs CRP, and blood glucose serum levels before and after treatment.

2 METHODS

We aimed to determine the effect of soy, catfish, anchovy, and rice (SCAR) porridge supplementation for 14 days along with nutritional counseling on vitamin D, calcium, and biomolecular parameters on new patients diagnosed with tuberculosis and associated complications who visited two public health centers: Teladan Public Health Center and Amplas Public Health Center, located in Medan, North Sumatra, Indonesia. The sample was collected on Sumatra Island, at latitude 3.57 N and longitude 98.65 E, with enough sunlight and a temperature of ± 32 °C (90 °F). The study was performed from May to September 2019. This study was a controlled randomized clinical trial. After applying the inclusion and exclusion criteria, 43 patients participated in the study. These patients were divided into two groups: 22 patients were put into the treatment group (I) and 21 into the control group (C).

Research subjects were men or women and met the inclusion criteria, chosen through a purposive sampling technique. The inclusion criteria were: newly diagnosed with lung tuberculosis with an acid fast bacilli (AFB) result (+), aged 18–60 years, with a heterozygote genotype test performed on one of the genes *TaqI* and *FokI*, willing to participate in the study, and provided informed consent. The exclusion criteria were: being pregnant and/or a breastfeeding mother. Research subjects were excluded if they failed to consume the porridge for more than 7 days and if they experienced a worsening of their clinical condition, although they were still included when blood samples were drawn at the end of the study (intention to treat).

Randomization Process

The sample size was determined using a formula based on the tuberculosis prevalence in Medan. To avoid bias in the study, this was a double blind trial. Randomization was performed through block randomization. To further remove selection bias, the names of research subjects were assigned codes, and later, a block randomization process was performed using a block table and divided using the closed envelope method to divide treatment and control groups.

SCAR porridge, based on 100 g of the dry product, contained 60 g of soy powder, 10 g of catfish powder, 5 g of anchovy powder, and 25 g of rice powder, all of which contained high levels of vitamin D and calcium (Table 1).

Treatment

The treatment group was given SCAR porridge supplementation in the form of a soft porridge (a mixture of 50 g dry powder porridge added to 200 mL of hot water) along with nutritional counseling. The control group only received nutritional counseling and no SCAR porridge supplementation. A 50 g portion of SCAR porridge was provided once a day for 14 days. The SCAR porridge was monitored and administered by a researcher once a week to inquire about adherence to the supplementation consumption and perceived side effects. All research subjects went through a clearance period of one week and were requested not to consume any supplements. Afterward, measurements were recorded before and after treatment, including tests for 25(OH)D, calcium, biomolecular parameters (i.e., albumin, Hs CRP, and blood glucose serum levels).

Laboratory Examinations for Vitamin D, Calcium, and Biomolecular Parameters

At the end of the study, samples were stored at –80 °C, and the sample data were analyzed. Laboratory examiners did not know which specimens were case or control samples, as the samples were randomly assigned codes. 25(OH)D serum level was measured using a chemiluminescent immunoassay (CLIA) (Diasorin, Stillwater, MN, USA) device, and the measurement range was between 4.0 and 150 ng/mL. The lowest level was 4.0 ng/mL based on an inter-assay precision of 3.90% CV. Vitamin D levels were categorized as: <20 ng/mL (deficiency), 20–30 ng/mL (insufficiency), and 30–100 ng/mL (sufficiency) [12]. Serum calcium levels were measured using the ADVIA Bayer Assayed Chemistry Controls with a reaction

measurement of 545/658 nm; normal calcium serum levels were considered to be 8.3–10.6 mg/dL. Albumin levels were measured using an Architect c8000; normal albumin serum levels were considered to be 3.4–4.8 g/dL. Hs CRP levels were measured using an Immulite 2000; normal Hs CRP serum levels were considered to be less than 10 mg/dL. Blood glucose was measured using an Architect c8000; normal blood glucose serum levels were considered to be less than 140 mg/dL.

Continuous variables were expressed as continuous variables using means ± SDs. To find the differences in all the parameters between the two groups before and after the treatment, an analysis was performed by using an independent *t*-test. Categorical variables are expressed as percentage proportions and, to show the relationships between vitamin D deficiency between two groups, a Chi-square test was used, and the Fischer test if the data did not meet the criteria. A *p*-value of <0.05 was considered statistically significant. We used the SPSS program (version 11.5; SPSS Inc, Chicago, IL, USA) to perform the analysis. This study was conducted after ethical approval was obtained from the Health Research Ethics Committee of Sumatra Utara University Medical School (No. 135/TGL/KEPK FK USU-RSUP HAM/2019) and all participants provided written informed consent for the study procedures.

3 RESULTS

This study had 43 research subjects after completing the screening process, through inclusion and exclusion criteria, from a total of 57 research subjects. After signing an informed consent form and undergoing block randomization, they were divided into two groups, which were the treatment (22 research subjects) and control groups (21 research subjects). During the study period, one research subject could not participate until the end of the period because they had experienced nausea and did not routinely consume the porridge, although one research subject had problems but still participated in having their blood drawn at the end of the study as part of the research (intention to treat).

Baseline Characteristics

The baseline characteristics of all research subjects showed that most of the tuberculosis prevalence was experienced by those who were: younger, married, male, and normal body weight (Table 2). Statistic testing did not show any differences between the groups and did not indicate any significant differences between the treatment and the control groups. For baseline characteristics, both groups were homogeneous.

Categories and Laboratory Parameter Levels

For the intervention group, vitamin D levels were found to be the most significantly increased of all the studied nutrients (Table 3). Before the study, the percentage of vitamin D deficiency and insufficiency were found in higher numbers in all research subjects (Table 4). No research subjects were categorized as normal; the highest levels were categorized as sufficient. However,

when compared to data collected before the study, vitamin D and calcium levels were homogeneous. There was also no categorical difference in all groups, except for the laboratory parameter vitamin D levels, in which we observed a significant difference when compared to data collected before the study commenced. This indicated that laboratory parameter levels increased, though they were still below normal values (Table 4). Table 4 shows that before the treatment, the data were homogeneous for 25(OH)D, calcium, albumin, Hs CRP, and blood glucose serum levels. There were significant differences between both groups in 25(OH)D serum levels; the average 25(OH)D serum level was low in the treatment group, but high in the control group. Table 4 shows a significant difference in 25(OH)D serum levels before and after treatment. This indicates that the supplementation of SCAR porridge resulted in a significant increase in the intervention group compared to the control group, although other factors resulted in no research subjects being placed in the normal vitamin D category. This is probably caused by VDR gene polymorphism.

4 DISCUSSION

Polymorphism of the VDR gene influences 25(OH)D serum levels in the body. Two widely studied VDR genes are *TaqI* and *FokI*, especially concerning tuberculosis. We included VDR gene polymorphism testing for all research subjects, searching for the heterozygote genotypes on the VDR gene, which are *TaqI* (rs731236) or *FokI* (rs10735810). Hopefully, SCAR porridge supplementation, providing all necessary vitamins and minerals, especially vitamin D and calcium, would result in the improvement of the clinical condition of tuberculosis patients experiencing complications. In previous studies, *FokI* receptor gene polymorphism was related to an increased risk of tuberculosis [2, 4, 5, 13-15]. This study showed that patients with VDR gene polymorphism would exhibit vitamin D deficiency and insufficiency. The administration of SCAR porridge increased vitamin D levels, but still did not significantly increase other vitamin and mineral serum levels compared to the control group. Vitamin D levels in the intervention group were significantly higher than the control group; therefore, the administration of vitamin D supplementation is highly necessary for tuberculosis patients with VDR gene polymorphism. At the end of this study, a significant improvement in sputum conversion was observed.

Other factors to be considered were body fat mass and profession. In this study, patients mostly had low levels of body fat. Although a high level of fat was found in some patients, vitamin D levels were still categorized as either deficient or insufficient. Low vitamin D levels at the start of the study were probably caused by VDR gene polymorphism, aside from other possibilities such as disease progressivity, a sun-evading lifestyle, and lack of high vitamin D and calcium intake [16-19]. A patient's profession also affected vitamin D levels. Professions that involve less sunlight (e.g., indoor jobs) cause more pronounced vitamin D deficiency [20-23].

Other vitamins (e.g., vitamin A) that impact vitamin D levels or function could potentially help with the clinical improvement of tuberculosis patients [24, 25]. Vitamin A acts

through retinoic acid, in which retinoic acid would be converted into 9-*cis* retinoic acid and into ligands, which could activate RXR. RXR would then interact with VDR. The resulting heterodimer then changes protein conformation, which may activate or repress molecules through gene transcription [18].

The dimer forms of VDR–RXR would then bind to a specific sequence in the target promoter region called vitamin D response elements (VDRE). Several genes involved in the regulation of calcium, phosphor homeostasis, and vitamin D metabolism are related to VDRE. This transcription process affects enzyme production related to vitamin D metabolism, such as enzymes involved in vitamin D synthesis. Increased vitamin D levels in the circulation would affect VDR activities in T lymphocytes, macrophages, and thymus tissues.

Counseling regarding the increase of vitamin D food sources was also provided. Vitamin D food sources are often expensive food products, such as codfish oil, salmon, or mushrooms. Fortification of vitamin D in milk is a good choice for increasing vitamin D intake. Sunlight is a renewable source in tropical areas, although jobs in which the workers are mostly covered from sunlight might result in inadequate sunlight exposure for vitamin D production. Vitamin D supplementation in tuberculosis patients may provide more optimal synergistic effects by increasing β carotene food sources[12, 26-28].

We did not find any relationship between calcium and vitamin D. Calcium intake also did not increase in this study. Dietary sources of calcium such as milk, sour milk, or cheese are food products not commonly a part of daily meals for tuberculosis patients. This lack of calcium may be caused by the lack of consumption of such products by tuberculosis patients. This may be caused by the high prices of food, or milk not being a commonly consumed food product. Other calcium food sources include fish consumed along with their bones, such as anchovies[29, 30]. This form is a kind of food product that may be provided to tuberculosis patients to fulfill calcium requirements.

We did not find low serum calcium levels in the blood, even though the mean of 25(OH)D serum levels was low. In tuberculosis patients, low levels of 25(OH)D were found, which should have an interaction between vitamin D and calcium. If the level of vitamin D in the blood is high, calcium absorption will increase; however, this mechanism did not occur in this study. Although 1000 IU of vitamin D was administered per day for 28 days and vitamin D levels increased (though not increasing enough for any patient to be categorized as sufficient), this did not affect calcium levels in the blood. Calcium plays a vital role in bone remodeling and is supported by vitamin D, which is converted to calcitriol and would help in the absorption of calcium in the intestine.

About 300–500 mg of calcium is sourced from 900 mg of extracellular calcium to aid the process of bone remodeling. This increases the required calcium intake from food sources, so calcium intake requirements range from 1000 to 1500 mg [31-33]. This puts the serum calcium into a homeostatic (balanced) state. Phosphorus also plays a role in the balance of calcium levels in

the blood and the rate of calcium storage in the intestine. The process of calcium absorption with the support of vitamin D is significant for the metabolism of calcium and phosphorus[34]. Calcium absorption by the digestive tract increases as calcium and phosphorus from the bone mobilizes and vitamin D controls the expenditure and balance of minerals in the blood [33]. Although calcium and vitamin D metabolism appear to be altered in tuberculosis patients, we found no association between vitamin D and calcium categorization in this study. Previous studies confirmed that although serum calcium is raised in tuberculosis patients, the effect may be reduced by a low calcium intake [35].

With the length of this study, the administration of vitamin D along with nutritional counseling, including a high intake of β carotene food, would show improved sputum conversion in both groups. A significant increase in vitamin D levels, despite the lack of difference between the treatment and control group observed at the end of this study, still showed clinical improvement of tuberculosis patients.

This study has several limitations, such as not measuring parathyroid hormone levels and other clinical parameters of tuberculosis improvement, such as chest X-ray and blood inflammation parameters.

5 CONCLUSION

Based on the results of this study, we concluded that tuberculosis patients with the VDR polymorphism genes, *FokI* and *TaqI*, may experience an increase in vitamin D levels from the supplementation of SCAR porridge along with nutritional counseling for 14 days.

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COMPETING INTERESTS

There are no funding or other conflicts of interest to declare for this research.

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Table 1. Nutrition facts of soy, catfish, anchovy, and rice (SCAR) porridge.

Nutrient per 100 grams	Nutrient analysis	
	Results	Unit
Total calories	396,60	Kcal/100 g
Carbohydrates	62,49	%
Protein	18,12	%
Total fat	8,24	%
Saturated fatty acids	1,64	%
Monounsaturated fatty acids	2,12	%
Polyunsaturated fatty acids	4,48	%
Sugar	5,62	%
Fiber	7,72	%
Vitamin D	137,02	mcg/100 g
Calcium	367,32	mg/100 g
Natrium	180,35	mg/100 g
Kalium	366,84	mg/100 g
Vitamin E	0,89	mg/100 g
Vitamin A	13,84	mcg/100 g
Vitamin B6	10,35	mg/100 g
Cholesterol	10,35	mg/100 g

Table 2. Demographic characteristics of all subjects.

Characteristic	n (%)	Mean ± standard deviation
Age (year)		41.4 ± 16.1
20–30	18 (40,9)	
30–40	4 (11,4)	
40–50	8 (18,2)	
50–60	6 (13,6)	
>60	7 (15,9)	
Gender		
Female	20 (46.5)	
Male	23 (53.5)	
BMI (kg/m^2)		21.0 ± 3.9
BMI categorized		
Underweight	10 (22,7)	
Normal	22 (50,0)	
Overweight	1 (4,5)	
Obese 1	8 (18,2)	
Obese 2	2 (4,5)	
Marital status		
Married	26 (59,1)	
Single	17 (40,9)	
Occupation		
Employeed	22 (50,0)	
Businessman	9 (20,5)	
Housewife	4 (9,1)	
Unemployed	1 (4,5)	
Student	7 (15,9)	
Smoking status		
Smokers	24 (54,5)	
Non-smokers	19 (45,5)	
Sunlight exposure		
<30 minutes	11 (57.9)	
≥30 minutes	7 (42.1)	
Physical activity		
High	-	
Moderate	-	
Low	32 (72,7)	
Medium	11 (27,3)	
Scar BCG		
Clear	32 (72,7)	
Dubious	5 (13,6)	
None	6 (13,6)	

Data are shown in mean ± SD or number of subjects (percentage).

Table 3. Energy and nutrient intake of subjects before and after intervention period [1].

Nutrient intake	Intervention group (n = 22)			Control group (n = 21)		
	Before	After	*p*-value	Before	After	*p*-value
Energy (kcal)	702.1±73.4	751.9±85.4	0.25	768.1±76.2	726.8±69.7	0.63
Protein (g)	22.4±5.6	27.2±8.3	0.10	25.1±6.8	24.5±9.7	0.85
Fat (g)	19.1±3.2	20.5±6.7	0.61	18.8±5.7	18.3±5.4	0.85
Carbohydrate (g)	110.2±34.7	115.9±23.5	0.39	124.5±54.3	116.1±55.6	0.55
Cholesterol (mg)	95.1±15.6	139.3±21.1	0.22	83.9±14.3	83.8±18.5	0.99
Fiber (g)	5.2±1.2	6.8±1.4	0.03	4.9±2.3	4.8±1.6	0.94
Vitamin A (μg)	485.9±155.8	769.2±212.2	0.07	582.6±221.2	448.8±201.4	0.44
Carotene (mg)	0.1±0.01	0.1±0.02	0.55	1.2±0.4	0.1±0.04	0.17
Calcium (mg)	229.1±78.3	195.4±55.2	0.47	272.4±65.4	181.5±66.7	0.14
Vitamin D (μg)	**3.4**±0.6	**30.2**±8.7	**0.01**	3.6±0.8	2.9±1.1	0.12

Data are shown in mean ± SD.
[1]Analysis using dependent *t*-test.
Significance value: $p < 0.05$.

Table 4. Vitamin D, calcium, and biomolecular serum levels before and after intervention**.**

Variable	Baseline Day 0	Endpoint Day 14	*p*-value (dependent *t*-test)
	Mean (SD)		
25(OH)D serum (ng/mL)			
D group (n = 22)	24.6±9.4	32.5±5.9	**0.01**
C group (n = 22)	25.6±9.7	27.5±8.8	**0.01**
p-value (independent *t*-test)	0.74	**0.03**	
Calcium serum (mg/dL)			
D group (n = 22)	9.6±0.5	9.8±0.7	0.13
C group (n = 22)	9.4±0.5	9.6±0.8	0.27
p-value (independent *t*-test)	0.17	0.05	
Albumin serum (mg/dL)			
D group (n = 22)	3.91±0.4	3.86±0.3	0.57
C group (n = 22)	3.86±0.3	3.81±0.4	0.71
p-value (independent *t*-test)	0.09	0.54	
Hs CRP serum (mg/dL)			
D group (n = 22)	24.0±7.7	12.1±2.7	**0.02**
C group (n = 22)	17.0±4.3	11.0±9.4	0.27
p-value (independent *t*-test)	0.21	0.32	
Blood Glucose serum (mg/dL)			
D group (n = 22)	184.6±88.7	182±90.3	0.93
C group (n = 22)	139.05±28.1	139.7±23.9	0.93
p-value (independent *t*-test)	0.22	0.15	

Data are shown in mean ± SD.
Significance value: $p < 0.05$.