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Research Article
Vitamin D Supplementation in Women with Vitamin D Receptor Gene Polymorphisms: A Randomized Controlled Trial

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Abstract

Background and Objective: Women living in the tropical regions, including those from North Sumatera, Indonesia, are shown to experience a vitamin D deficiency. Predisposing factors include Vitamin D Receptor (VDR) gene polymorphisms and low vitamin D intakes. The objective of this study was to assess the effect on women with a VDR gene polymorphism (*Taq*I and *Bsm*I) of 28 days supplementation with 1000 IU vitamin D day^{-1} on serum 25-hydroxyvitamin D (25(OH)D) and calcium. **Methodology:** The study was an open parallel-group randomized clinical trial involving 40 women from North Sumatera with a VDR gene polymorphism, recruited using specific criteria. The subjects were divided into two groups of 20 using a randomized block design. The intervention (D) group received 1000 IU vitamin D day^{-1} and dietary counseling for 28 days and the control (C) group received a placebo and dietary counseling for 28 days. Serum 25(OH) D and calcium were measured on day 0 and 28. SPSS version 11.5 was used for statistical analysis $p<0.05$. **Results:** The study was completed by 19 D group subjects and 17 C-group subjects. The intervention resulted in a significant increase in serum 25(OH) D in the D group ($p = 0.04$) and no change in serum 25(OH) D the C group. At the end of the intervention, the D group had significantly higher serum 25(OH) D than the C group ($p = 0.04$) but no subjects with a vitamin D status of deficient or insufficiency were elevated to a status of sufficient from either group. **Conclusion:** The results revealed that vitamin D deficiency can occur in women with a VDR gene polymorphism even after 28 days supplementation with 1000 IU vitamin D/day.

Key words: *Bsm*I, dietary counseling, inadequate, intervention, placebo, serum 25(OH) D, *Taq*I, vitamin D receptor gene

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Vitamin D deficiency has been associated with skeletal health, increased bone turnover, bone loss and hip fractures[1]. Theoretically, residing in a tropical country with abundant sunlight should reduce susceptibility to vitamin D deficiency, yet vitamin D deficiency has been observed in Indonesian and Malaysian children and adults. Interestingly, a greater prevalence rate has been reported in women than men[2-4]. Certain lifestyle variables, such as avoiding sunlight exposure, consuming foods that are low in vitamin D and maintaining a low level of physical activity, contribute to a higher incidence of vitamin D deficiency[5-7]. Furthermore, total serum 25-hydroxyvitamin D (25(OH) D (levels and Body Mass Index (BMI) are believed to be inversely proportional to one another. Obese adults and children of different ethnicities have been shown to be predisposed to having low levels of 25(OH) D[8-14].

However, the research on the causes and clinical significance of low serum 25(OH) D has so far been inadequate to validate the use of 25(OH) D as a biomarker of vitamin D status. A large number of factors have been shown to contribute to lower 25(OH)D serum levels in women, including avoiding sunlight, suboptimal vitamin D supply, a greater pool of distribution, reduced biological availability, faster clearance and Vitamin D Receptor (VDR) gene polymorphisms[2,3,15-16].

Almost 200 polymorphisms have been found in the VDR gene but their effect on VDR function remains unclear. Most of the polymorphisms are located in the 3' untranslated regions of the gene. This region plays a role in the gene expression, especially in modulating mRNA stability. The VDR gene is located in chromosome 12q13.1, is larger than 100 kbp, contains 14 exons and has a promoter region that is continuously being transcribed in various tissues[17]. A previous study showed that two single nucleotide polymorphisms in the vitamin D receptor genes (VDR) *Taq*I (rs731236) and *Bsm*I (rs1544410) were associated with the susceptibility to inflammatory and metabolic reactions[18]. Previous studies also found vitamin D deficiency in subjects with *Taq*I and *Bsm*I polymorphism with different lifestyle and living areas[19,20].

Previous study reported that the intake of ordinary supplement doses of vitamin D tends to be associated with the decrease in the total mortality rates and increased mean serum 25(OH)D levels[21].The mean daily dose of vitamin D from these trial supplements varied from 300 to 2000 IU but most were between 400 and 833 IU, resulting in a mean dose of 528 IU[22,23]. These trials also indicated a substantial increase in vitamin D from the baseline levels in the intervention groups, while the levels tend to decrease in the control groups. However, none of these trials assessed subjects with VDR gene polymorphisms or any other polymorphisms but the findings of this study would bring us to understand how to manage women with vitamin D receptor gene polymorphism, how it could change lifestyle and higher vitamin D intake to achieve normal levels.

The aim of this study was to assess the effect of supplementation with 1000 IU vitamin D/day for 28 consecutive days on serum 25(OH) D and calcium levels in women from North Sumatera with polymorphism in the VDR *Taq*I and *Bsm*I genes. The vitamin D supplement in this study was bioavailable 1000 IU vitamin D3 active-form soft gels, given to women along with their habitual diet.

MATERIALS AND METHODS

Subjects and sampling: This study was a parallel-group randomized clinical trial with a pre/post-test design. Present study was conducted between May and October 2016 during the dry season when there was abundant sunlight. The subjects were recruited from Sumatera Island, North Sumatera (Sumatera Utara, Medan), which has a latitude of 3.57 N, a longitude of 98.65 E and an average temperature of 32°C (90°F)[24].

Inclusion criteria were women volunteers with a polymorphism in the VDR genes *Taq*I and *Bsm*I, who were aged 20-50 years, work outdoors as farmers and have no medical history of the metabolic disease (diabetes mellitus, hypo or hyperparathyroidism). Exclusion criteria were routine ingestion of a vitamin D supplement in the previous two months and pregnant or nursing mothers. The subjects who did not complete the daily vitamin D supplementation schedule were excluded from the analysis.

Forty subjects meeting these criteria were enrolled in this study and 36 completed the study. Subjects were divided into two groups of 20 by randomized blocking. The vitamin D group (D group) received one soft gel capsule containing 1000 IU vitamin D3/day along with counseling for 28 consecutive days. The placebo group (C group) received one hard capsule containing 65 mg milk powder day^{-1} (containing less vitamin D and calcium fortification than the vitamin D capsule) along with counseling group for 28 consecutive days. Nineteen subjects from the D group and 17 from the C group completed the study (Fig. 1).

All the measurements were taken at the subjects' place of work. Subjects were recruited following the compilation of a

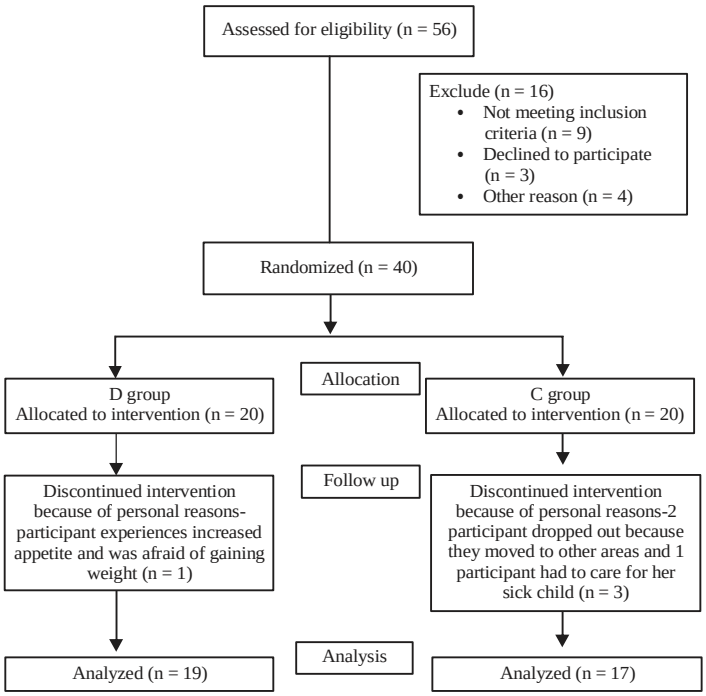

Fig. 1: Consort flow diagram of this study

detailed medical history, a physical examination and baseline investigations including sunlight exposure, physical activity and daily food intake. The measurements and blood sampling took place at the baseline (19-21 April, 2016) and endpoint (19-21 May, 2016). All the participants gave a written informed consent for the study, which was approved by the Health Research Ethical Committee of North Sumatera Utara/RSUP H Adam Malik c/o Medical School, University of Sumatera Utara No. 120/KOMET/FK USU/2015.

Intervention: Participants were advised to maintain their usual diets, follow the counseling instructions and avoid taking additional vitamin D or calcium supplements for the two months prior to the study and during the study. Subjects were supplied with one bottle of 28 capsules and food recall sheets (including a capsule recall sheet) to fill out. They were instructed to take one capsule each day with breakfast or lunch but preferably breakfast. The vitamin D and placebo capsules differed in taste and appearance, vitamin D a yellow soft gel capsule and the placebo a pink hard capsule. During the trial, three participants discontinued treatment and one dropped out for personal reasons.

All subjects were asked whether they had noticed any potential adverse events related to the supplied supplements. All symptoms (former as well as ongoing) were recorded. The physician responsible for the study assessed the recorded symptoms for severity and relevance to the trial supplements. Unresolved symptoms reported at the midpoint were specifically followed-up at the endpoint.

Anthropometry and nutrient intake: Anthropometry measurements include height (to the nearest 0.5 cm), weight (to the nearest 0.1 kg) and BMI (kg m^{-2}). Body Mass Index (BMI) categories were based on Asia Pacific guidelines, with <18.5 kg m^{-2} classified as underweight, 18.5-22.9 kg m^{-2} classified as normal weight, 23-24.9 kg m^{-2} classified as overweight/at risk, 25-29.9 kg m^{-2} classified as obese I and >30 kg m^{-2} classified as obese II[25].

The assessment of nutrient intake was based on food recall for 2 days (1 weekday and 1 weekend day, 24 h each) per week and include the energy, protein, fat, carbohydrate, calcium, phosphorus, cholesterol and vitamin D. Calculations were made using Nutrisurvey 2005[26], which includes Indonesian foods. The total vitamin D intake for each individual was calculated as the sum of dietary and supplement vitamin D.

Analysis of VDR Single Nucleotide Polymorphisms (SNPs): Detection of an SNP in the VDR genes was performed in three steps: (1) DNA isolation through the sorting out

method, (2) DNA purity validation and (3) SNP genotyping. The SNP genotyping was initiated using 1-10 ng of DNA. Both the VDR gene polymorphisms, *Taq*I and *Bsm*I were tested by allele discrimination using *Taq*Man probes with a StepOnePlus Real-Time PCR instrument (Applied Biosystems, Foster City, CA, USA) run on 'Fast' mode. The protocol involved 40 cycles of the DNA polymerase activation at 95°C for 20 sec, denaturation at 95°C for 3 sec and annealing at 60°C for 30 sec. Strand fluorescence was detected at 60°C. The assay was performed for a 10 µL reaction solution using *Taq*Man Genotyping Master Mix in MicroAmp Fast Optical 96-Well Reaction Plates covered with MicroAmp Optical Adhesive Film (Applied Biosystems). Genotyping was conducted using probes for two alleles. Probe tips were labelled with a high-energy fluorescent stain on one side only using a reporter located at the 5′ end and a quencher located at the 3′ end of the probe[3].

Laboratory analyses: This present study measured 25(OH)D concentrations in serum by chemiluminescent immunoassay (Diasorin, Stillwater, MN). Concentrations were measured between 4.0 and 150 ng mL^{-1}, with the lower limit based on an inter-assay precision of 3.90% CV. Concentrations <20 ng mL^{-1} were categorized as vitamin D deficient, 20-30 ng mL^{-1} as insufficient, 30-100 ng mL^{-1} as sufficient, >100 ng mL^{-1} as excessive and >150 ng mL^{-1} as intoxicated[1].

Serum calcium concentrations were measured by ADVIA bayer assayed chemistry controls (Enzyme control, Class I, K031644) using a procedure based on the calcium ions forming a violet complex with o-cresolphthalein complexone in an alkaline medium. The normal range for serum calcium was defined as 8.3-10.6 mg dL^{-1} [27].

Statistical analysis: Categorical variables are expressed as percentage proportions and chi-square tests were used for these variables to compare the two groups[28]. Continuous variables are expressed as Mean±Standard Deviation (SD). To compare continuous variables between the two groups, unpaired t-tests were used for normally distributed data and Mann Whitney tests for non-normally distributed data[28]. Values of $p<0.05$ were considered statistically significant. SPSS version 11.5 (SPSS Inc, Chicago, IL) was used for all analyses[28].

RESULTS

The baseline characteristics of the study on the participants by the treatment group are shown in Table 1. There were no significant differences between the two groups in any of the characteristics. Present study measured including the age, serum 25(OH) D and calcium, sunlight expose and physical activity and most subjects were categorized by BMI as overweight.

Likewise, there were no significant differences in the baseline nutrient intakes between the two groups (Table 2). Dietary intakes did not change during the study intervention period for either group, with the exception of vitamin D which increased significantly in the D group ($p = 0.01$).

There were no significant differences between the two groups in the vitamin D categorization of subjects at the baseline or endpoint (Table 3). Despite taking a daily supplement for 28 days, only one subject from the D group was categorized as vitamin D sufficient at the endpoint. However, in the D group, six subjects were categorized as vitamin D deficient at the baseline and then improved to vitamin D insufficient at the endpoint, while there was no change in the C group. For calcium level classification, there were no differences between the two groups at the baseline or endpoint, with all subjects were classified into the normal group at both time points.

There was a significant increase ($p = 0.04$) in the concentration of the serum 25(OH) D in the D group from the baseline to the endpoint (Table 4). Serum calcium levels at the

Table 1: Demographic and lifestyle characteristics of subjects before intervention

Characteristics	Intervention (D) group (n = 19)	Control (C) group (n = 17)	p-values
Age (years)*	30.4±5.5	31.5±5.5	0.5
Body mass index (kg m^{-2})*	23.2±2.5	23.8±3.6	0.5
Serum 25(OH)D (ng mL^{-1})*	20.4±5.4	19.9±4.1	0.8
Serum calcium (mg dL^{-1})*	9.6±0.3	9.8±0.5	0.3
Sunlight exposure#			
<30 min	11 (57.9)	10 (58.8)	0.4
≥30 min	8 (42.1)	7 (41.2)	
Physical activity#			
High	10 (52.6)	12 (70.5)	0.6
Moderate	5 (26.3)	2 (11.8)	
Low	4 (21.1)	3 (17.7)	

Data are shown in Means±Standard deviation or number of subjects (percentage), *Analyzed using an independent t-test, #Analyzed using a chi-square (x^2) test, 25(OH)D: 25-hydroxyvitamin D

Table 2: Energy and nutrient intake of subjects before and after intervention*

	Intervention (D) group (n = 19)			Control (C) group (n = 17)		
Nutrient intake	Before	After	p-values	Before	After	p-values
Energy (kcal)	702.1	751.9	0.25	768.1	726.8	0.63
Protein (g)	22.4	27.2	0.10	25.1	24.5	0.85
Fat (g)	19.1	20.5	0.61	18.8	18.3	0.85
Carbohydrate (g)	110.2	115.9	0.39	124.5	116.1	0.55
Cholesterol (mg)	95.1	139.3	0.22	83.9	83.8	0.99
Fibre (g)	5.2	6.8	0.03	4.9	4.8	0.94
Vitamin A (μg)	485.9	769.4	0.07	582.6	448.8	0.44
Carotene (mg)	0.1	0.1	0.55	1.2	0.1	0.17
Calcium (mg)	229.1	195.4	0.47	272.4	181.5	0.14
Vitamin D (μg)	3.4	30.2	0.01	3.6	2.9	0.12

Data are shown in Means±Standard deviation, *Analyezed using a dependent t-test

Table 3: Categorization of serum vitamin D and calcium levels before and after intervention*

Categories	Intervention (D) group (n = 19)	Control (C) group (n = 17)	p-values
Baseline			
Vitamin D category			
Sufficient	1 (5.3)	0	0.6
Insufficient	8 (42.1)	8 (47.1)	
Deficient	10 (52.6)	9 (52.9)	-
Calcium category			
High	-	-	-
Normal	19 (100)	17 (100)	
Low	-	-	
Endpoint			
Vitamin D category			
Sufficient	1 (5.3)	0	0.1
Insufficient	14 (73.7)	8 (47.1)	-
Deficient	4 (21.0)	9 (52.9)	
Calcium category			
High	-	-	-
Normal	19 (100)	17 (100)	
Low	-	-	

Data are shown in number of subjects (percentage), *Analyzed using a chi-square (x^2) test

Table 4: Mean serum 25(OH)D and calcium levels before and after intervention

Variable	Baseline	Endpoint	Mean change (%)	#p-values
Serum 25(OH)D (ng mL^{-1})				
Intervention (D) group	20.4±5.4	22.3±4.4	+165	0.04
Control (C) group	19.9±4.1	19.4±3.9	-47	0.01
*p-value		0.04		
Serum calcium (mg dL^{-1})				
Intervention (D) group	9.6±0.3	8.9±0.3	-76	0.34
Control (C) group	9.8±0.5	8.9±0.4	-82	0.01
*p-value		0.05		

Data are shown in Means±Standard deviation, D group; n = 19; C group; n = 17, *Analyzed using an independent t-test, #Analyzed using a dependent t-test, 25(OH)D: 25-hydroxyvitamin D

endpoint were not elevated in any of the study subjects (Table 4). Serum calcium showed no change during the intervention in the D group but significantly decreased ($p = 0.01$) from the baseline to the endpoint in the C group. There was no significant difference in serum calcium between the two groups at the endpoint.

No adverse events or adverse effects related to the trial supplements (vitamin D or placebo) were reported by the subjects at midpoint or endpoint. The most commonly reported side effect during the intervention was increased appetite in the D group.

DISCUSSION

This study was the first author's knowledge to assess the effect of supplementation with 1000 IU vitamin D day^{-1} for

28 days on serum 25(OH)D and calcium levels in women with a polymorphism in the VDR genes *Taq*I and *Bsm*I in North Sumatera, Indonesia. Despite no significant improvement in vitamin D status, several subjects supplemented with vitamin D moved from a vitamin D deficient status to an insufficient status during the study intervention period.

Vitamin D supplementation can decrease all-cause mortality, but the mechanisms are not clear. The physiologically active form of vitamin D (1.25-dihydroxyvitamin D) acts as a hormone that has a pleiotropic skeletal and other skeletal effects on other things, calcium homeostasis, bone formation, cellular proliferation and differentiation, the immune system, bile acid transport, rennin production, endothelium and vascular walls and the endocrine system[21]. Some effects mediated by the activation of VDRs, such as inhibition of cellular proliferation and activation of cellular differentiation, can reduce the aggressiveness of cancerous processes and expansions of atheromatous lesions[21]. In this study, however, while most subjects had low serum 25(OH)D levels, serum calcium was in the normal range. Thus, vitamin D deficient/insufficient status was not associated with serum calcium.

Vitamin D regiments used in previous trials have ranged from daily doses of 300-833 IU and most publicly available vitamin D supplements involve daily doses of 400-600 IU that entail no toxic effects. Serum 25(OH)D concentration is considered to be a good reflection of skin synthesis and food intakes of vitamin D[25]. Data from previous trials showed that the use of vitamin D supplements resulted in increases in serum 25(OH)D levels. The 1000 IU vitamin D supplement used in this study, equivalent to 25 mg vitamin D was chosen based on the dietary allowance for Indonesian women (aged 20-50 years) of 15 mg per day[29].

Vitamin D intake was shown to be below this recommended dietary allowance in all the subjects except in the D group subjects during the study intervention. Cholecalciferol (vitamin D3) is found mainly in salmon, sardine, mackerel, tuna and cod fish oil. Minute quantities are available in milk, egg yolk, butter and margarine[30,31]. Typical dietary sources of vitamin D3 are evidently not consumed regularly enough in Indonesia to support adequate health. Individuals with low vitamin D intake are advised to take vitamin D3 supplements that are safe and reliable, a few of which are extracted from lanolin[1]. Commercial supplements commonly contain ergocalciferol (vitamin D2) extracted from mushrooms. Vitamin D2, however, is five times less active than vitamin D3.

Studies have shown that neither vitamin D supplements nor vitamin D food sources are consumed on a daily basis globally[32]. Alarmingly, this study revealed that working women in Indonesia seldom consume adequate portions of foods rich in vitamin D (egg yolk, fish, meat and mushrooms). Moreover, present study's subjects rarely consumed vitamin D supplements, instead of favouring the supplements rich in vitamins C and E because of their reported antioxidant effects on the skin. However, low dietary intakes of vitamin D and limited sunlight exposure can have detrimental effects on health.

A previous study has shown that UV-B exposure from sunlight for 25 min, three times a week for six weeks can improve the vitamin D status of elderly Indonesian women[7]. Hypovitaminosis vitamin D researchers identified VDR gene polymorphisms as the cause of poor vitamin D status. The prevalence of SNPs in different VDR genes, such as *Taq*1, *Bsm*1, *Fok*1 and *Apa*1, differs between populations[2,19,20]. In North Sumatera women, in agreement with previous studies, all subjects were heterozygous (TC for *Taq*I and AG for *Bsm*I)[2,3], promoting vitamin D deficiency and insufficiency.

A limitation of this study was that present study did not assess the relationship between the serum 25(OH)D levels, non-skeletal effects and other bone skeletal parameters such as parathyroid hormone and bone density. Also, the short time of this study could be the cause of inadequate vitamin D status.

CONCLUSION

It is believed that the low serum 25(OH)D levels of women with SNPs of VDR genes reflect vitamin D deficiency and insufficiency. A daily supplement of 1000 IU vitamin D given to these women for 28 days increased their serum 25(OH)D levels but not enough to achieve adequate vitamin D status.

SIGNIFICANCE STATEMENT

This study discovered the inadequate vitamin D status after supplementation in women with vitamin D receptor gene polymorphism. The findings revealed the potential limits and even in adequate dose 1000 IU vitamin D day^{-1}, women with vitamin D receptor gene polymorphism still need to change lifestyle and increased vitamin D resources.

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