SNP

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Submission date: 19-Nov-2023 01:24PM (UTC+0700)
Submission ID: 2232639288
File name: Manuskrip_genotip_vit_D_19-11-2023__final.docx (170.06K)
Word count: 6240
Character count: 33868

Supplementary vitamin D3 and vitamin D receptor polymorphisms affect blood vitamin D levels in type-2 diabetes mellitus in Indonesia

Abstract

There are no data on vitamin D receptor (VDR) gene single nucleotide polymorphism (SNP) influence on blood 25-hydroxy-cholecalciferol [25(OH)D] levels after supplementary vitamin D in Indonesian type 2 diabetes mellitus (T2DM) cases.

Objectives

Evaluation of supplementary vitamin D3 and VDR gene SNPs rs1555410 and rs2228570 effect on blood 25(OH)D levels in T2DM cases.

Methods

A randomized double blind placebo-controlled trial (RDPCT) was conducted at one research setting using 85 T2DM subjects divided into vitamin D group (VDG) and control group (CG) and receiving 5,000 IU/day vitamin D3 (cholecalciferol) or placebo once daily for 84 days. Levels of 25(OH)D were determined at start of study and after supplementary vitamin D3 administration for 84 days. Circulatory 25(OH)D was assayed using ELISA. VDR polymorphisms were detected using sequencing.

Results

Post-supplementary blood 25(OH)D rose appreciably from start of study in VDG for VDR rs1544410 genotypes G/G [p=0.001] and G/A [p=0.010], and in VDR rs2228570 genotypes T/T [p=0.012], T/C [p<0.001], and C/C [p=0.001]. Post-supplementary VDG still comtained 30.3% subjects not reaching blood 25(OH)D ≥30 ng/mL.

In attaining blood 25(OH)D ≥30 ng/mL post-supplementation, VDR rs2228570 genotype T/C differed significantly from T/T (52.4% v. 100%; p=0.027), but there were no appreciable differences between genotypes C/C and T/T (78.6% v. 100%; p=0.273), as well as between VDR rs1544410 genotypes G/G and G/A (67.5% v. 100%; p=0.542).

Conclusion

Only 52.4% subjects with VDR rs2228570 genotype T/C achieved sufficiently high blood 25(OH)D level. VDR rs2228570 polymorphisms apparently influence T2DM response to supplementary vitamin D.

Key words: diabetes mellitus, 25(OH)D level, vitamin D receptor polymorphism, Indonesia

INTRODUCTION

Indonesia has 10.7 million type 2 diabetes mellitus (T2DM) patients, thereby occupying the 7th global rank, with predicted T2DM prevalence rising to 16.6 million in 2045.[1] Vitamin D deficiency (VDDEF) occurs at a higher prevalence in persons with T2DM than in persons in the general global population who have prevalences between 63.2 and 83.2%.[2, 3] These prevalences are higher than in Europe where the data show a prevalence of 40.4% of VDDEF (blood 25(OH)D less than 20 ng/mL),[4] while VDDEF prevalence in South Asia is 68%.[5]

At present the utility of providing additional vitamin D to T2DM patients for glucose hemostasis and reduction of tissue resistance to insulin is still debated,[6-8] because T2DM onset and management apparently depend on vitamin D level in the body.[9] Gross VDDEF at

25(OH)D <12 ng/mL (or <30 nmol/L) is considered to be hazardous to health [10] and should be corrected through vitamin D supplementation.[11]

Vitamin D is involved in signal transmission through the vitamin D receptor (VDR).[12] The vitamin D-responsive element (VDRE) gene on chromosome 12q13.1, being in the same group as steroid and thyroid hormone receptors, comprises nine exons and eight introns. [12] The function of VDR is to activate and control gene transcription via the VDRE in the target gene promoter. The VDR gene has more than 470 polymorphisms, [12] the most common being FokI (rs2228570 C to T) and BsmI (rs1544410 A to G).[12] The rs1544410 at intron 8 regulates mRNA stability, thereby affecting gene expression. [13] The rs2228570 lies in the exon 2 start codon and changes the initiation sites.[14] The VDR gene SNPs may influence VDR mRNA and protein stability and activity, causing the individual to not responding optimally to supplementary vitamin D.[15, 16]

A multiplicity of factors exist that possibly affects post-supplementary blood 25(OH)D levels such as sunshine exposure, aging, body mass index, calcium intake, supplementary vitamin D, and genetics.[4, 17] To date it is not known with certainty whether VDR SNPs affect blood vitamin D levels after supplementary vitamin D in T2DM cases.[18] This is because of the difficulty in detecting the possibility of a gene that is associated with the development of T2DM, because of minute differences possibly occurring in the gene in question and its interaction with genetic or non-genetic factors.[19] To determine VDR SNP influence on increases in blood 25(OH)D levels that depict vitamin D condition, RDPCTs are necessary. Currently, there are few RDPCTs aimed at evaluating VDR rs1544410 and rs2228570 relationships with post-supplementary vitamin D therapeutic response. The study of Waterhouse et al.[15] in Australian older persons aged 60 – 80 years who were given vitamin D3 supplementation (30.000 vs. 60.000 IU/month) for 12 months, showed that rs2228570 was not associated with increased changes in 25(OH)D levels after supplementary vitamin D. The RDPCT conducted in China by Yao et al.[16] for 20 weeks on VDDEF cases who were given 2000 IU/day of vitamin D3 or placebo, demonstrated that VDR rs2228570 showed stronger influences on 25(OH)D levels (P<0.04). After vitamin D3 treatment, there was a higher 25(OH)D level in rs2228570-G and its alleles (p = 0.009).

An RDPCT conducted by Cavalcante et al.[20] on elderly females who were 68 ± 6 years old, had vitamin D insufficiency (VDINSUF), and were given 200.000 IU megadose vitamin D3 supplementation for 4 weeks, showed greatly raised blood 25(OH)D concentrations in BsmI VDR gene genotypes BB/Bb (p<0.001). Persons with the BB/Bb genotype showed a greater response to supplementary vitamin D than did those with the bb genotype.

A prospective case-control study involving 125 T2DM patients and 125 controls, revealed that low blood 25(OH)D and VDR gene rs2228570 are connected to T2DM risk.[21] The inconsistent study results on the relationship of VDR SNPs were caused by the varying presupplementary blood vitamin D levels, the study designs used, the small numbers of subjects, vitamin D dose, duration of supplementary vitamin D, level of dietary intake, and ethnicity.

To date there have been no reports on the relationship between VDR SNPs rs1544410 and rs2228570 on the one hand and supplementary vitamin D on the other hand among T2DM patients in Indonesia. To prove a causal relationship in T2DM patients of VDR SNPs rs1544410 and rs2228570 with 25(OH)D levels after supplementary vitamin D, an RDPCT is necessary. Information obtained from such a study may be useful as the basis for vitamin D3 therapeutic dose personalization in T2DM patients, such that it may be expected to decrease the rate of disease and death. The primary outcomes of the study are the responses to supplementary vitamin D administration by comparing post-supplementary blood 25(OH)D and vitamin D levels. Secondary outcomes are the impact of VDR gene polymorphisms (rs1544410 and rs2228570) on changes in blood 25(OH)D and on attainment of blood 25(OH)D of ≥30 ng/mL

PATIENTS AND METHODS

Research design

This study was an RDPCT conducted at one research setting between June – August 2022, namely at the Mampang community medical facility in South Jakarta, Indonesia. Before enrollment, all candidate subjects signed an informed consent form. The protocol of the study was judged to be acceptable by the Research Ethics Committee, Faculty of Medicine, Universitas Trisakti, under ethical clearance No. 001/KER/FK/1/2022.

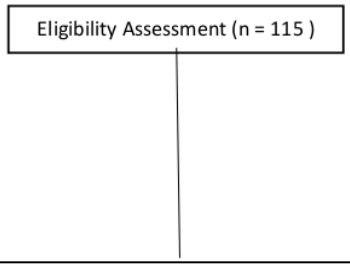
Patients & intervention

The study subjects were recruited from community-resident T2DM patients living in the vicinity of the research setting. The criteria for inclusion in this study were males and females ≥ 18 years old, T2DM, HbA1c 7-8.5%, on single oral antidiabetic drug (monotherapy), and agreed to follow-up controls. The diagnosis of T2DM was made according to the American Diabetes Association guideline criteria, [22] namely upon examination finding fasting blood glucose ≥126 mg/dL, or 2-hour postprandial blood glucose ≥ 200 mg/dL and HbA1c ≥ 6.5%. The exclusion criteria were ever receiving and currently receiving insulin therapy, having

suspect kidney disease (estimated glomerular filtration rate <30 mL/min/1.73m^2), abnormal liver function (3-fold increased SGPT levels above normal upper limit), being pregnant or lactating, having allergies, hypercalcemia (having plasma calcium >2.65 mmol/L), or receiving daily supplementary vitamin D in the last 84 days. Criteria for interrupting the study before completion were blood 25(OH)D >100 ng/mL, hypercalcemia, and cholecalciferol hypersensitivity.

The number of subjects meeting the eligibility criteria comprised 115 patients who were enrolled in this study and were allocated by simple randomization to VDG (intervention) and CG (placebo) at 1:1 ratio. The randomization was conducted by personnel who were blinded to the intervention to be given. The VDG was given daily vitamin D3 tablets containing 5,000 IU cholecalciferol, whereas the CG was given a placebo tablet (calcium 120 mg) once daily, all tablets being taken for 84 days. The vitamin D tablets were produced by PT. Imedco Djaja (Banten, Indonesia) and repackaged by PT. Ikapharmindo Putramas (East Jakarta, Indonesia) such that the supplementary vitamin D tablets appeared identical to the placebo tablets, which were also produced by the latter company. The vitamin D and placebo tablets had identical visual, olfactory, and gustatory qualities, and were contained in dark-colored glass bottles that were coded A and B. Participants and the statistician were all blinded to the origin of the tablets, whether from the VDG or the CG. Compliance to the administered intervention was determined by weekly checks on the number of the remaining tablets.

During the study there were 6 persons in the VDG who did not complete the study by reason of not following the protocol, returning to the village of origin, and due to diarrhea, while in the CG there were 7 persons who did not complete the study, by reason of not willing to participate, not following the protocol, and due to nausea and vomiting. A total of 85 study subjects completed the study, consisting of 43 in the VDG and 42 in the CG **(Fig. 1).**



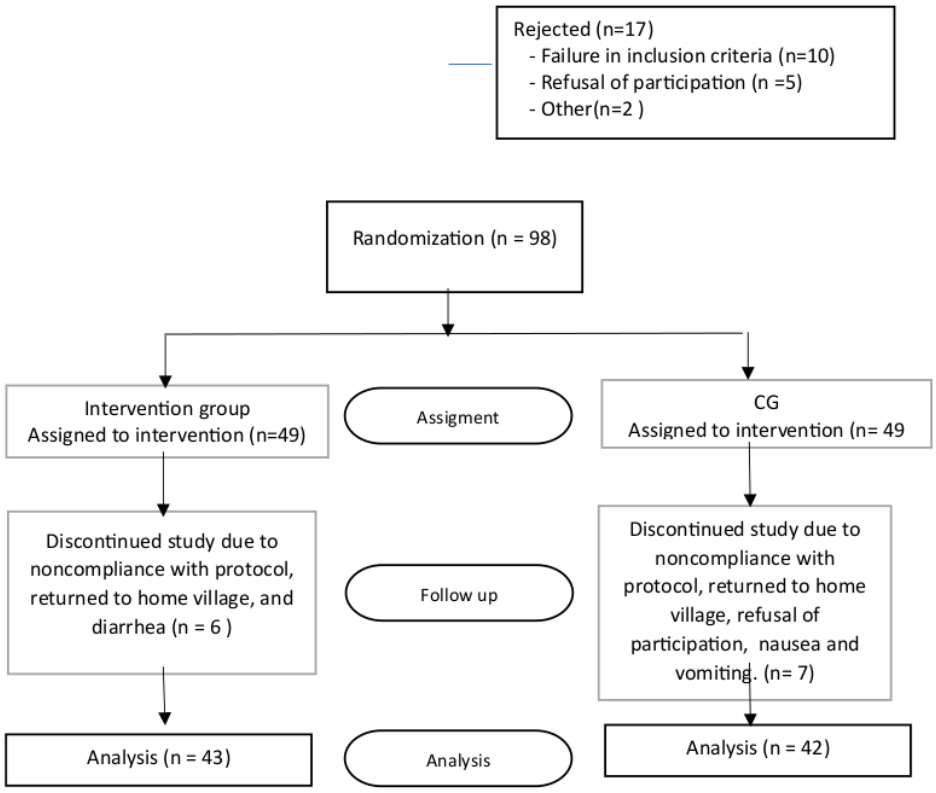


Fig. 1. Flow of participants throughout trial

The researchers were blinded to subject allocation in all phases of the study (recruitment, enrollment, data collection, and assignment to study groups). For purposes of verification and improvement of subject compliance, all study subjects were directed to return the empty medication bottles every month to the cadres who at the completion of the study had to evaluate subject compliance to supplementation. During the study all complaints reported by the subjects (potential adverse events) were written down for recording purposes. The principal investigator leading the research evaluated the importance of the complaints and their relationship with the trial supplements. The development of all reported symptoms was monitored until the completion of the study. The allocation codes were in possession of a third party not directly involved in the study and were opened after the completion of the study.

Measurements

The study subjects meeting the recruitment criteria were interviewed on the day of admission (day zero) before the start of the supplementary vitamin D administration, the interviews being conducted to collect subject data on age (years), gender, and duration of diabetes mellitus (months), followed by drawing of blood samples at 08.00 and 9.00 a.m. local time.

Biochemical measurements

From each of the study participants, a venous whole blood sample was collected, of which 3 mL was stored for determination of blood 25(OH)D level and 2 mL was stored in tubes containing ethylenediaminetetraacetic acid (EDTA) for VDR SNP genotyping.

Blood 25(OH)D level was determined by chemiluminescent microparticle immunoassay (ARCHITECT 25-OH Vitamin D assay, Abbott), with measuring interval of 8.0 - 160.0 ng/mL (20.0 - 400.0 nmol/L), with limit of detection (LoD) ≤ 10.0 ng/mL, limit of quantitation (LoQ) ≤ 20 ng/mL, and imprecision ≤ 10% within total CV.

The blood 25(OH)D data were presented as median ± SD, and categorized on the basis of the clinical practice guideline of the Endocrine Society, i.e. < 20 ng/mL was defined as VDDEF, 20 – 30 ng/mL as VDINSUFF, 30 – 100 ng/mL as vitamin D sufficiency (VDSUFF).[11]

Vitamin D receptor single nucleotide polymorphisms

Extraction of genomic DNA was by means of the QIAamp DNA Blood Mini Kit (QIAGEN) from 200 µL whole blood samples containing EDTA, and its purity and level were determined by a NanoDrop 2000 spectrophotometer (ThermoScientific). VDR SNPs were detected by PCR followed by sequencing.

PCR was performed using MyTaq HS Red Mix Kit (Bioline) to amplify the target sequence using specific primers for detecting VDR variant-SNP ID rs1544410 (forward (F) primer 5'-GGG AGT ATG AAG GAC AAA GAC C -3' and reverse (R) primer 5'- CCC GCA AGA AAC CTC AAA TAA C -3') and SNP ID rs2228570 (forward (F) primer 5'-GCA CTG ACT CTG GCT CTG -3' and reverse (R) primer 5'-TGG ACA TTG TAA GGA AGG AGA TG-3'). PCR amplification was performed with 2 µl of DNA, 8.5 µL of nuclease-free water, 2x 12.5µl of My Taq HS Red Mix, and 1.0 µl each of forward and reverse primers. The PCR reaction conditions used were the following: PCR initial activation step at 95°C for 5 minutes, denaturation cycle at 95°C for 15 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 30 seconds for a total of 40 cycles, and final extension at 72°C for 3 minutes. PCR products on 2% agarose gel were visualized by electrophoresis. Purified PCR products were sequenced

using BigDye Terminator Kit (Thermo Fisher Scientific) and ABI 3500 sequencer (Applied Biosystems). Sequence analysis using BioEdit software was performed to confirm mutations, which were compared to the NCBI BLAST database as reference sequence with accession number NG_008731.1.

Sample size

In each group, a calculated number of 40 subjects was required to detect a difference in treatment at a two-sided significance level of 5% (0.05), a study power of 90%. $\mu 1 - \mu 2$ = predicted difference between two group means, estimated at 4, $\alpha 2$ = expected population variance from preliminary study, estimated at 1.02. In anticipation of dropouts, the number of study subjects was increased to 95 to obtain adequate power to detect differences in outcome measures.

Statistical analysis

Continuous data of normal distribution were shown as mean and standard deviation (SD), while continuous data of skewed distribution were shown as median (minimum – maximum). Categorical data were expressed in percentages. All of the data were checked for normal distribution using the Kolmogorov-Smirnov test. Unpaired Student's t test, Mann-Whitney U test, Chi-square test, and Fisher exact test were used for comparisons at start of study between VDG and CG, based on type of variable and distribution of data. Comparison of blood 25(OH)D in VDG and CG at start of study and after supplementation was done with the Mann-Whitney test. To determine differences in vitamin D status at start of study and after supplementation, we performed Fisher's exact test and Chi-square test. To compare the blood 25(OH)D levels in VDR rs1544410 between genotypes in VDG at start of study and after supplementation, the Mann-Whitney test was used. For comparison of VDG blood 25(OH)D levels between VDR rs2228570 genotypes at start of study and after supplementation, we performed the Kruskal-Wallis test, Wilcoxon signed rank test, and the paired t-test at level of statistical significance $p < 0.05$. Statistical analysis used SPSS for Windows version 23.

RESULTS

Subject characteristics at start of study

This study involved 85 T2DM participants, with mean age of 55.8 ± 0.6 years. The majority of the subjects were female, totalling 68 (80%), median duration of diabetes was 12 (1 – 36) months, and blood 23(OH)D levels of the subjects was 11.6 (2.4 – 30.3) ng/mL. The greatest proportion of VDR gene rs1555410 was of genotype G/G, comprising 79 subjects (92.9%),

followed by genotype G/A with 6 subjects (7.1%), but no genotype T/T was found. The greatest proportion of VDR gene rs2228570 had genotype T/C, with 38 subjects (44.7%), followed by genotype C/C with 32 subjects (37.6%), and genotype T/T with 15 subjects (17.6%). Subjects' vitamin D status was mostly deficiency with 74 subjects (84.1%), followed by insufficiency, with 9 subjects (10.6%), and sufficiency, with 2 subjects (2.4%). After randomization based on intervention group, no significant differences were observed in age, gender, duration of T2DM, and VDR genotype between vitamin D and CGs (Table 1).

Table 1. Subject characteristics at start of study in VDG and CG

Characteristic	Treatment group		P value
	VDG (n=43)	CG (n=42)	
Age (years)	56 (35 – 80)	56 (35 – 69)	0.396[a]
Gender			
Male	8 (47.1)	9 (52.9)	0.745[b]
Female	35 (51.5)	33 (48.5)	
Duration of DM (months)	12 (1 – 36)	12 (1 – 36)	0.967[a]
VDR genotype (n,%)			
rs1544410			
G/G	40 (50.6)	39 (49.4)	1.000[c]
G/A	3 (50)	3 (50)	
A/A	0 (0)	0 (0)	
rs 2228570			
T/T	8 (53.3)	7 (46.7)	0.614[b]
T/C	21 (55.3)	17 (44.7)	
C/C	14 (43.8)	18 (56.2)	

Values presented as median (min-max) or n(%)
Statistical analysis: [a] Mann-Whitney test; [b] Chi-square test; [c] Fisher's exact test; $p<0.05$ = statistically significant.
VDG = vitamin D group; CG = control group

Blood 25(OH)D and vitamin D status at start of study and after supplementation

In Table 2 can be seen the proportions of blood 25(OH)D and vitamin D status at start of study and after supplementary vitamin D3 administration for 84 days in the vitamin D and CGs.

Table 2. Blood 25 (OH)D level and vitamin D status in VDG and CG at start of study versus 84 days after supplementation with vitamin D3.

Group	Start of study		p value	After supplementation		p value
	VDG (n=43)	CG (n=42)		VDG (n=43)	CG (n=42)	
Blood 25(OH)D level (ng/mL)	10.5 (4.7 – 30.3)	13.05 (2.4 – 26.9)	0.264[a]	46 (9.4 – 79.4)	14.4 (6.9 – 38.3)	0.001[a]*

Vitamin D status						
Deficiency	40 (50.6)	34 (45.9)		3 (8.6)	32 (91.4)	
Insufficiency	2 (22.2)	7 (77.8)	1.000[c]	10 (62.5)	6 (37.5)	0.001[b]*
Sufficiency	1 (50)	1 (50)		30 (88.2)	4 (11.8)	

Groups: VDG= vitamin D 5.000IU/day, CG = placebo
Vitamin D status (blood 25(OH)D level): deficiency (< 20 ng/mL); insufficiency (20 – 30ng/mL); sufficiency (30 – 100 ng/mL). Statistical analysis: [a]Mann Whitney test; [b]Chi-square test; [c]Fisher's exact test; *p<0.05 =statistically significant
VDG = vitamin D group; CG = control group

In the present study, after vitamin D3 supplementation for 84 days, there was a much greater increase in blood 25(OH)D in VDG as compared to the CG (46(9.4-79.4) v. 14.4(6.9-38.3); (p<0.001) (Table 2).

Comparison of blood 25(OH)D levels by VDR genotype after supplementary vitamin D3 administration in VDG

To determine if VDR genotype affected blood 25(OH)D after supplementary vitamin D3 administration, the 25(OH)D blood levels were compared between genotypes in the VDG. In Table 3 may be seen the comparison of blood 25 (OH)D in VDG by VDR genotype after supplementary vitamin D administration. As compared to start of study values, blood 25(OH)D levels increased significantly after supplementary vitamin D3 administration in VDR rs1544410 genotypes G/G [10.5 (4.7 – 30.5) v. 46.5 (9.4 – 79.4) ng/mL; p=0.001] and G/A [10.8 ± 1.3 v. 45.7 ± 7.2 ng/mL; p =0.010]. When compared between genotypes after supplementation, no great differences were found in 25(OH)D levels between genotypes G/G and G/A [46.5 (9.4 – 79.4) ng/mL v. 45.7 ± 7.2 ng/mL; p=0.924].

Table 3. Blood 25(OH)D levels in VDG by VDR genotype after supplementary vitamin D3 administration

	Blood 25(OH)D level (ng/mL)		
VDG	**Start of study (n=43)**	**84 days (n=42)**	**P value**
VDR genotypes			
rs1544410			
G/G (n= 40)	10.5 (4.7 – 30.5)	46.5 (9.4 – 79.4)	0.001[c]*
G/A (n= 3)	10.8 ± 1.3	45.7 ± 7.2	0.010[d]*
P value	0.924[a]	0.924[a]	
rs2228570			
T/T (n=8)	11.4 (6.2 – 17.9)	61.2 (32.1 – 79.4)	0.012[c]*
T/C (n=21)	10.5 (7.6 – 30.3)	34.2 (9.4 – 67.4)	<0.001[c]*
C/C (n=14)	11.0 ± 4.4	43.7 ± 12.4	0.001[d]*
P value	0.373[b]	0.024[b]*	

Statistical analysis: [a]Mann-Whitney test; [b]Kruskal-Wallis test;[c] Wilcoxon signed rank test; [d]paired t-test; *p value <0.05 = statistically significant
VDG = vitamin D group; CG = control group

As compared to start of study, blood 25(OH)D levels in VDR rs2228570 increased significantly after supplementary vitamin D3 administration for the three genotypes, namely T/T [11.4 (6.2 – 17.9) v. 61.2 (32.1 – 79.4); p=0.012], T/C [10.5 (7.6 – 30.3) v. 34.2 (9.4 – 67.4); p<0.001], and C/C [11.0 ± 4.4 v. 43.7 ± 12.4; p=0.001] (Table 3).

In the comparison between genotypes after supplementary vitamin D3 administration, there were significant differences in 25(OH)D levels of genotypes T/T, T/C, and C/C [61.2 (32.1 – 79.4) ng/mL v. 34.2 (9.4 – 67.4) ng/mL v. 43.7 ± 12.4 ng/mL; p=0.024] (Table 3).

To determine which of the genotypes resulted in the differences in VDR rs2228570 after supplementary vitamin D3 administration, a post-hoc analysis was conducted, the results of which showed significant differences between genotypes T/T and T/C (p=0.015) and between genotypes T/T and C/C (p=0.017), but no differences between genotypes T/C and C/C (p=0.391).

In Table 4 may be seen the responses to supplementary vitamin D administration regarding blood 25(OH)D between VDR genotypes. From the 43 subjects in the vitamin D group, only 30 subjects (69.7%) attained blood 25 (OH)D levels ≥ 30 ng/mL.

There were no prominent differences between VDR rs1544410 genotypes G/G and G/A in attaining blood 25(OH)D ≥ 30 ng/mL (67.5% v. 100%; p=0.542). In VDR rs2228570, significant differences occurred in attainment of blood 25(OH)D levels in genotype T/C versus genotype T/T (52.4% v. 100%; p=0.027).

Table 4. Attainment of blood 25(OH)D levels in VDG by VDR genotype after supplementary vitamin D administration

VDR genotype	Blood 25(OH)D level		p value
	< 30 ng/mL (n,%)	≥30 ng/mL (n,%)	
VDG			
rs 1544410			
G/G (n=40)	13 (32.5)	27 (67.5)	
G/A (n=3)	0 (0)	3 (100)	0.542
rs 2228570			
T/T (n=8)	0 (0)	8 (100)	
T/C (n= 21)	10 (47.6)	11 (52.4)	0.027*
C/C (n=14)	3 (21.4)	11 (78.6)	0.273

Statistical analysis: logistic regression test; *p value <0.05 = statistically significant
VDG = vitamin D group

DISCUSSION

VDDEF was found in 84.1% of subjects, having a median blood 23(OH)D level of 11.6 (2.4 – 30.3) ng/mL.

VDDEF prevalence in the present study was even greater than that shown by European data where 25(OH)D levels lower than 20 ng/mL and12 ng/mL are observed in 40.4% and 13.0%, respectively, of the general population. [4] In comparison, VDDEF prevalence in adults of 5 tropical South Asian countries was 68% [95% CI: 64 to 72%].[5] The results of our study agree with the results of earlier studies demonstrating that VDDEF is frequently found in T2DM cases with a prevalence of around 63.2 – 83.2%.[2, 3] Our study results confirm that VDDEF is also found in tropical countries with 2 seasons, such as Indonesia that has abundant sunshine for induction of cutaneous vitamin D synthesis.

Vitamin D as a prohormone is available in 2 forms, namely vitamin D2 (ergoalciferol) found in foods and vitamin D3 (cholecalciferol) found in the skin and formed after UV irradiation. [23] Generally, VDDEF is caused by low dietary intake and reduced cutaneous vitamin D synthesis due to inadequate sunlight exposure as a result of geographic location, genetically determined skin color, age, life style (physical activity), and cultural or religious practices.[24, 25] The greater VDDEF prevalence in the present study showed that foods and sunlight alone do not suffice to preserve best vitamin D status, therefore necessitating supplementary vitamin D administration.

Signal transmission in the body occurs through the binding of vitamin D to the vitamin D receptor (VDR), [12] which is also expressed by a number of tissues that are sensitive to insulin, such as hepatic, striated muscle, or adipose tissue. Apparently vitamin D may have a direct influence by raising tissue insulin sensitivity and insulin receptor expression, thereby enhancing glucose transport stimulation by insulin. On the other hand, vitamin D may have an indirect influence by reducing inflammatory responses and thus tissue resistance to insulin, because inflammatory responses are one of the causes of tissue resistance to insulin.[9]

The results of a systematic review-based meta-analysis showed vitamin D supplements to be able to raise blood 25(OH)D and reduce tissue insulin resistance in T2DM patients.[6] By contrast, systematic reviews of T2DM RCTs failed to find adequate evidence for the efficacy of vitamin D supplements in glucose hemostasis as well as in decreasing tissue insulin resistance. [7, 8] Apart from the remaining inconsistencies on the impact of vitamin D supplements in T2DM patients in reducing tissue insulin resistance, VDDEF should be

corrected because vitamin D serves an essential function in calcium hemostasis and bone metabolism. [24]

Our study results showed that the largest proportion of the VDR gene rs1555410 had genotype G/G, namely in 79 (92.9%) subjects, while no subjects were found with genotype A/A. The proportion of VDR genotypes in our study differed from the study of Hu et. al.[18] that was conducted on Chinese T2DM patients, showing that the most numerous VDR rs1544410 genotype was G/A (93.75%), and consistent with our study, no subjects were found with genotype A/A. Our study results showed that in VDR rs2228570 the greatest proportion was of genotype T/C in 44.7% of subjects, followed with C/C in 37.6% of subjects, and T/T in 17.6% of subjects. The study of Hu et al.[18] showed that in subjects with VDR rs2228570 the largest proportion was for genotype C/C (59.8%), followed by T/T (23.2%) and C/T (16.9%). Our study and that of Hu et. al.[18] were similarly conducted among Asian ethnic groups, but there were variations in the proportions of the VDR gene rs1544410 and rs2228570. The proportions of the VDR genotypes in our study were also different from the study of Sari et al.[26] that was conducted in healthy Indonesian women in North Sumatera, Indonesia, the results of which showed that all study subjects had the heterozygous genotype A>G for BsmI (rs1544410), and T>C for TaqI (rs2228570). Their study showed that even among Indonesians there are differences in the proportions of the VDR genotypes, which according to these results lends more support to the personalization of supplementary vitamin D doses.

This study showed that after supplementary vitamin D administration at 5000 IU/day to the vitamin D group, a 3.2-fold significant rise occurred in blood 25(OH)D as compared to controls [46 (9.4 – 79.4) ng/mL v. 14.4(6.9 – 38.3) ng/mL; p=0.001] (Table 2). Blood 25(OH)D level may rise around 1 ng/mL (2.5 nmol/L) per 100 IU daily vitamin D3 supplements given for 56 - 84 days,[27] although the connection of supplementary dose with increased blood 25(OH)D may not be linear.[28] There are many factors that influence the post-supplementary vitamin D response, such as VDR polymorphisms.

After supplementary vitamin D administration at 5000 IU/day for 84 days, of the total of 43 subjects, there were still 13 (30.2%) subjects who did not attain blood 25 (OH)D levels ≥ 30 ng/mL (Table 4.) Our findings agree with those of the study of Yao et al.,[16] in the shape of a 140-day RDPCT, in which 448 persons from China having inadequate vitamin D were given 2000 IU/day vitamin D3 or placebo. The investigators found that vitamin D supplements increased blood 25(OH)D levels, but could not overcome VDDEF in 25% of the Chinese subjects. Al-Daghari et al.[29] in their study on T2DM patients who were given supplementary

vitamin D at 2000 IU for 12 months showed that 42% of subjects still could not reach the target blood 25(OH)D concentrations. In the study of Hu et al.[18] on T2DM subjects with daily supplementary vitamin D at 800 IU of 12-month duration, it was also found that 44.6% of subjects did not attain sufficient post-supplementary vitamin D levels, which may have been partially the result of genetic modification.

To date there are still inconsistencies as to whether supplementary vitamin D3 administration is influenced by the VDR gene SNPs. Our study demonstrated a strong rise above start of study values in post-supplementary blood 25(OH)D levels in VDR rs1544410 genotypes G/G (p=0.001) and G/A (p=0.010). These results agree with the study of Cavalcante et al.[20] who showed that supplementary vitamin D strongly raised blood 25(OH)D levels in subjects with genotype BB/Bb (p=0.009), but not in those with genotype bb. In contrast with the study of Cavalcante et al.[20] in our study no genotype C/C was found.

In VDG members with VDR rs2228570, large post-supplementary differences were found in blood concentrations of 25(OH)D of genotypes T/T, T/C, and C/C (p= 0.024) (Table 3.). Post-hoc analysis showed visible differences between genotypes T/T and T/C (p=0.015) and between genotypes T/T and C/C (p=0.017), but there were no differences between genotypes T/C and C/C (p=0.391). Our study results showed that VDR polymorphism rs2228570 apparently influenced supplementary vitamin D response in T2DM subjects. In subjects with genotype T/C only 52.4% attained a sufficiently large 25(OH)D level, that was far lower than in the other genotypes (Table 4). Our study results confirm that the doses should be adapted ("personalized") in subjects with VDR SNP rs2228570 of genotype T/C to obtain optimal benefits from the supplementary vitamin D.

The results of our study differ from the study of Al-Daghari et al.[29] on subjects with T2DM who showed genotype-related differences in post-supplementary blood 25(OH)D, in that genotype T/T subjects evidenced better therapeutic responses than the other genotypes. The study of Hu et al.[18] on T2DM subjects showed in VDR rs2228570 no remarkable differences in blood 25(OH)D in genotypes T/C versus T/T (p=0.964). Apart from differences in the proportion of VDR genotypes rs2228570 of the latter study with ours, there were also differences in subject characteristics from the standpoint of age, start of study blood 25(OH)D levels, and the supplementary vitamin D dose used. The study of Hu et al.[18] involved older persons who were 66.3 ± 9.1 years old, whereas our study subjects were 55.8 ± 0.6 years old. In addition, start of study blood concentration of 25(OH)D exceeded that in the study of Hu et. al.[18] namely 22.7 ± 1.9 ng/mL whereas in our study it was 11.6 (2.4 – 30.3) ng/mL, presumably

because of the lower vitamin D3 supplementation dose (800 IU) in the study of Hu et al.[18] Several studies that showed a lower start of study level was associated with significantly higher blood 25(OH)D responses.[16]

There are still diverse opinions regarding optimal blood 25(OH)D levels in humans. There is also no uniform definition of VDDEF and insufficiency in the different guidelines. The IOM recommend that the minimum blood 25(OH)D concentration should be 20 ng/mL (50 nmol/L), that has is connected with bone health.[30]
According to the Endocrine Society, 25(OH)D levels must exceed 30 ng/mL (or 75 nmol/L) for preventing infections and for obtaining other non-calcemic benefits of vitamin D.[11] The present study showed that by using supplementary vitamin D3 at 5000 IU/day for 84 days, there were still 32.5% of subjects who could not attain vitamin D sufficiency with blood 25(OH)D ≥ 30 ng/mL. The Endocrine Society clinical practice guideline[11] recommends supplementary vitamin D3 administration to increase vitamin D levels and to determine the blood 25(OH)D concentrations, because 25(OH)D is the most frequent form of circulatory vitamin D, with a half-life of 14 – 21 days, which is extremely useful for monitoring vitamin D status in persons at high risk of VDDEF. Our study results confirm the need for the personalization of supplementary vitamin D dose and determination of blood 25(OH)D in high risk patients in relation to VDR.

Our study results confirm the need for personalization of vitamin D supplement dose as well as measurement of blood 25(OH)D in high-risk patients in relation to SNP VDR, apart from the contradictory relationship of VDDEF in glucose hemostasis and insulin resistance reduction. [7, 8, 21, 31] There is a need for dosage adjustment ("personalization") in subjects with VDR gene SNP rs2228570 genotype T/C to obtain better gains from supplementary vitamin D. The results of this study may provide inputs on policies for management of T2DM patients who are susceptible to vitamin D deficiency, particularly in Indonesia.

In some populations, the interplay of genes and lifestyle may well obscure the genetic component, therefore studies on interactions of genes with diet and physical activity are mandatory to confirm the relationship. Other longer-term RCTs with larger sample size are also necessary to better utilize the results of vitamin D supplements in patients with type 1 and type 2 diabetes.

Strengths and limitations

Our study used an RDPCT design that is the best design for measuring any cause-and-effect relationships. An identical therapeutic procedure and dosage of supplementary vitamin D3 was used to minimize variation among the subjects.

Admittedly, this study has several limitations. Because the study subjects were Indonesian T2DM patients, their genetic background may or may not influence vitamin D status of T2DM patients in the ethnic groups of other nations. Other confounders such as physical activity, diet, exposure to sunlight, BMI, and parathyroid hormone, were not accounted for.

CONCLUSION

After supplementary vitamin D administration, blood 25(OH)D level rose perceptibly, but there still was 30.3% of subjects who failed to attain blood 25(OH)D levels ≥ 30 ng/mL. VDR rs2228570 genotype T/C had only 52.4% of its subjects attaining a sufficiently large 25(OH)D level, that was perceptibly lower than in genotypes T/T and C/C. The VDR rs2228570 polymorphisms apparently influence T2DM response to supplementary vitamin D.

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