Are avoiding sunlight exposure and low physical activity resulting micro evolution in tropical country women?

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***Abstract*- Background: Previous studies have shown that low 25(OH)D serum levels may increase mortality and morbidity, especially in women. Low levels often occur during the winter and spring of temperate countries due to low sun exposure but there are many other factors linked with vitamin D deficiency that can occur in tropical countries. Objectives: To assess 25(OH)D serum levels in Indonesian women and determine factors that can influence these levels.**

Method: This cross-sectional study was conducted on 156 healthy Indonesian women during the dry season. This study measured serum 25(OH)D levels, examined two single nucleotide polymorphisms in the vitamin D receptor (TaqI and BsmI), and assessed environmental, lifestyle and physiological factors including occupation, sun exposure, vitamin D intake, and body fat percentage.

Results: The mean serum 25(OH)D level was 18.8 ± 7.0 ng/mL.There were 148 subjects categorized as either deficient and insufficient, and eight were categorized as sufficient. However, none of the subjects achieved normal 25(OH)D values (normal value in sunny countries: 54-90 ng/mL) and there was no significant difference in serum 25(OH)D levels between obese (OG) and non-obese groups (NG). All participants were heterozygous (T>C for TaqI and A>G for BsmI). All subjects were found to have low levels of sun exposure, a high percentage of body fat, and a low intake of vitamin D. There were associations between vitamin D deficiency-insufficiency and sufficiency with indoors occupation (*p*=0.001), less than 1 hour sun ray exposure (*p*=0.007), moderate physical activity (*p*=0.014, OR: 6.25, CI95% 1.26-32.12), low vitamin D intake (*p*=0.012, OR: 5.435, CI95% 1.268-23.29), and high body fat percentage (*p*=0.003, OR: 14.14, CI95% 1.69-118.19).

Conclusion: The results showed that vitamin D deficiency may occur in women with lower body fat percentage and also with high amount of body fat. All participants were heterozygous (T>C for TaqI and A>G for BsmI)TheTaqI and BsmI polymorphisms were present in all women studied. Factors that most influenced vitamin D serum were physical activity, vitamin D intake, and body fat percentage.

***IndexTerms*—vitamin D, polymorphism, lifestyle, body fat, women**

I. Introduction

Vitamin D deficiency and insufficiency occurs in approximately one billion people around the world [1], not only in four season, but also subtropical country with three season. A study in female SouthAsian immigrants in Europe showed vitamin D deficiency and studies in India, found vitamin D deficiency in pregnant women and post menopausal women [2,3]. Vitamin D deficiency isalso found in individuals living in tropical countries with two seasons, a study in Southern India also showed vitamin D deficiency, 52% of 164 post-menopausal women had vitamin D insufficiency, and 30% had a vitamin D deficiency [4]. A study by Rahman*et al.* (2004) found 27% of post-menopausal women had a vitamin D deficiency (based on a serum 25(OH)D level examination), and 71% had a vitamin D insufficiency [5].

The impacts of such deficiency includes decreased bone mineral density in 10-18 year-olds [6], while rickets and osteomalaciais increasing in infants and children and have become one of the causes of high mortality in Southern Asia [7,8]. The risk of osteoporosis has also increased in post-menopausal women living in this area [8].

Vitamin D deficiency is not only affected by season, but also occurs due to lifestyle factors [9,10]. Such factors include occupation, duration of sun exposure, dressing style, the use of sunscreen, physical activity, and vitamin D intake from both regular meals or vitamin D supplements [11].

Another factor found to be able to affect vitamin D level is obesity; studieshave shown anassociation between the amount of body fat and vitamin D levels. Obesity is defined as an excess of fat in adipose tissue that may affect health [12]. Obesity can be quantified by measuring body fat using either the Bioelectrical Impedance (BI) method or, more simply, by calculatingthe Body Mass Index (BMI). Studies have shown that as body fat increases, 25(OH)D level decrease. This is because vitamin D is stored in the adipocytes and the excess fat

makes it is difficult for vitamin D to be released into the bloodstream [13].

In addition to lifestyle factors and excess body fat, low vitamin D levels are also often associated with polymorphisms in vitamin D receptor genes [13,14]. A study by Al-Daghri*et al.* (2012) showed that two single nucleotide polymorphisms in the vitamin D receptor genes (VDR) TaqI (rs731236) and BsmI (rs1544410) were associated with the development of type-2 diabetesmellitus, in terms of susceptibility to inflammatory and metabolic reactions[16]. A study by Vupputuri*et al*. (2006) found vitamin D deficiency in 94.3% Asian-Indian subjects, with the percentage being higher in the group with the TaqI (T>C genotype) in the VDR gene (TaqI T>C= 82.98%, TT=12.77%, and CC=4.25%). The increasing of vitamin D deficiency was also associated with lower intestinal calcium absorption, inverse relation with serum parathyroid hormone, and affecting the mineral density of the bones [17].

Almost 200 polymorphisms have been found in the VDR gene, but their effect onVDR function remains unclear [18]. Most of the polymorphisms are located in the 3' untranslated regions of the gene. This region plays a role in gene expression, especially in modulating mRNA stability [19]. 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 [20].

As far as the authors are aware, this is the first study to examine serum 25(OH)D in female Indonesians. This study also aimed to assess the relationship oflifestyle factors, body fat, and two single nucleotide polymorphisms in the VDR gene (TaqI and BsmI) withserum 25(OH)D levels in women. The results of this study will help us identify ways of preventing vitamin D deficiency among Indonesian women, which could improve their quality of life.

II. PARTICIPANTS AND METHODS

Study design

This study was a cross sectional study, conducted at participant's work place, in the City of Medan, North Sumatera, Indonesia. This study was carried out after ethical approval was obtained from the Health Research Ethics Committee of Sumatera Utara University Medical School (No. 171/KOMET/FK USU/2012).

Study participants

The subjects of this study consistedof 156 women (78 non-obese and 78 obese). The inclusion criteria were healthy women between 20-50 years old. Exclusion criteria were subjects with history of diabetesmellitus, myocardial infarction, or gastrointestinal, renal or liver dysfunction. In addition to those exclusion criteria, subjects who were pregnant, lactating, or using medications that may alter lipid profile were also excluded. The subjects were then divided into two groups based on their BMIs,the first group included those with a normal BMI <24.9 kg/m^2 (NG; mean age 33.5±8.11 years), and the secondgroup included those with a BMI categorized as obese25 kg/m^2(OG; mean age 37.71±6.64 years). This study was conducted between July and October 2012, during the dry season in Indonesia is between April andOctober.

The participants were classified as belonging to one of three ethnic groups, based on paternal ethnicity. The three main ethnic groups were Javanese, Batakese, and 'other'. All particpants stated how long they were exposed to the sun throughout the day and were asked to choose between one of three options, <1 hour, 1-3 hours, and > 3 hours per day. Dressing style was also included as variable, because women wearing hijabs are less exposed to the sun compared to ones who do not. A woman wearing a hijab usually covers her whole body except her face and hands. Participants were also asked whether they regularly used sun cream (yes/no answer) to gain information as to whether the participant adhered to a sun-avoiding lifestyle (Table 2).

The whole observation was carried out in the participant's workplace. Participants from a variety occupations were included in the study (including teachers, bank employees, doctors, and nurses), most of whom worked indoors. The subjects were included in the study after completing an interview, also all subjects had read and signed an informed consent without any pressure.

Nutrition intake, anthropometric status, and body fat

Nutrition intake was assessed using food recall for two days (one on working day and one on holiday), and included in this assessment was the intake of vitamin D. Calculation was perfomed using Nutrisurvey 2005,including the data of Indonesian cuisine. Assessment of the intake of vitamin D includes vitamin D obtained from meal sources and supplemental vitamin D.

BMI and body fat percentage was assessed using the Body Composition Monitor with Scale (HBF-362, KaradaScan-Omron, Japan). BMI categories were based on Asia-Pacific criteria using the following categories: Underweight (<18.5 kg/m^2), Normal weight (18.5-22.9 kg/m^2), overweight (23-24.9 kg/m^2), obese I (25-29.9 kg/m^2) and obese II (≥ 30 kg/m^2), for subject's grouping : obese (>25kg/m^2) and non-obese (<24.9 kg/m^2) [12].

Body fat percentage based on body fat mass represents the amount of body fat mass to total body weight. This calculation was based on simple formula of bioelectricalimpedance:.Categories for body fat percentage are: normal (<29.9%) and high (>30%).

Waist circumference was measured using a non-elastic measuring tape, and the results were categorized into: normal (<80 cm) and high (>80 cm) for Asian [12]. Participant height was measured using a free standing stadiometer with nonstretchable tape (microtoise) . Systolic and diastolic blood pressures were measured using Automatic Blood Pressure Monitor (Omron, Japan).

Biochemical Analysis

In this study, we measured serum 25(OH)Dlevelsusing chemilluminescent immunoassay (CLIA) technology (Diasorin, Stillwater, MN). This measurement can detect levels ranging from 4.0 to 150 ng/mL, based on 3.90% CV inter-assay precision. Serum 25(OH)D levels were categorized into: deficiency (<20 ng/mL), insufficiency (20-32 ng/mL), sufficient (33-53 ng/mL), normal for tropical countries (54-90 ng/mL), excessive (100-150ng/mL) and intoxication (>150 ng/mL), in this study, were categorized into: deficiency-insuficiency (<32.9 ng/mL) and sufficiency (>33 ng/mL) [21].

Serum calcium levels were measured using ADVIA (Bayer Assayed Chemistry Controls). The reaction was measured on 545/658 nm. The normal level of serum calcium is 8.3 – 10.6 mg/dL, we categorized normal (>8.3 mg/dL) and low (<8.29 mg/dL).

Analysis of single nucleotide polymorphisms in VDR genes

Analysis of single nucteotide polymorphisms (SNP) in VDR gene through three steps: first step was DNA isolation using 'salting out method', second step was checking for purity of DNA isolation, and the third step was SNP genotyping with Applied BiosystemStepOnePlus Real-Time PCR Systems.

For DNA isolation, the procedure were: whole blood was collected in anEDTA collection tube (BD Vacutainer, USA). The protocol were (1) Add 3 mL whole blood in EDTA tube and centrifuge 3000 rpm for 10-15 minutes to get leucocyte sediments. (2) Add 300 microliters leucocyte sediments + 900 microliters Eritrosit Lysis Solution in 1.5 Eppendorf Tube. (3) Invert 2-3 times. Incubate 10 mins, in 4^{o}C (refrigerator temperature). (4) Centrifuge 13000 rpm for 3 minutes to get leucocyte pellet. Remove all the supernatan, this protocol can be repeated for 5 times until solution homegenous. (5) Vortex to disperse pellet. (6) Add 300 µl of Nuclei Lysis Solution (Reagent: Promega). Invert 2-3 times. (7) Add 100 µl protein precipitation to the solution and thenvortex for 20 seconds. (8) Centrifuge for 13000 rpm for 3 minutes in room temperature. (9) Add the supernatant into 1.5 Eppendorf Steril Tube filled with isopropanolol 300 µl and then vortex for 3 seconds. (10) Centrifuge 13000 rpm for 1 minute, observe for DNA pellet. Carefully remove supernatant. (11) After remove supernatant, add 70 ethanol 300 µl, centrifuge 13000 rpm for 1 minute. (12) Remove supernatant, air dry inside laminar hood for one night. Dilute with 100 µl DNA rehydration solution, and keep in 4^{O}C for one night, for next day, keep in freezer -20^{o}C.

The second step was checking for DNA purity, using nanophotometer (IMPLEN; P360). Ratio 260/280 in the range 1.8-2.2, showed good purity of DNA extraction from the first step. The third step was SNP genotyping using 1-10 ng DNA. Both VDR gene polymorphism (TaqI and BsmI) were tested by allele discrimination using *StepOnePlus*^{TM}*Real Time* PCR device (Applied Biosystems, Foster City, CA, USA), with added TaqMan probes (Applied Biosystems, Foster City, CA, USA). 'Fast' method was used to operate the PCR, beginning with the activation of DNA polymerase at 95^{o}C for 20 seconds, followed by 40 cycles, and then succeeded by a denaturation process at 95^{o}C for 3 seconds and annealing process at 60^{o}C for 30 seconds. Fluorescence detection occurred at 60^{o}C. The whole assay was operated in a 10µL reaction, using *Taq*Man genotyping Master Mix with 96-Well Reaction plates, and using MicroAmp Fast Optical 96-Well Reaction plate covered with MicroAmp Optical Adhesive Film (Applied Biosystem, Foster City, CA, USA).

In assay genotyping using two alleles with probes for each allele, one of the tip of the probe were labelled with fluorescence staining FAM and VIC. The label was used with the help of a reporter located atthe 5' end of the molecule in the form of high energy fluorescence staining (FAM and VIC) and a quencher located atthe 3' end of the probe

Statistical Analysis

Numerical variables were expressed as mean values ± standard deviation, while categorical variables were expressed as percentages. A Chi-square test was used to assess significance between the OG and NG, also deficiency-insuficiency group and sufficiency group with $p<0.05$. A unpaired t-test was used to compare numerical data in all comparison groups (OG and NG; deficiency-insuficiency and suficiency) with p-<0.05. This study used SPSS program (version 11.5; SPSS Inc, Chicago, IL) for data analysis.

For genotyping data analysis, individuals were divided into groups based on the genotypes and alleles of VDR gene. The groups were: heterozygotes, homozygous wildtypes, and homozygous mutants. Subsequent analysis was conducted to look for associations with serum 25(OH)D level.

III. RESULTS

The participant groups were based on general characteristic (age and blood pressure), anthropometry (BMI, waist, and body fat percentage), vitamin D intake, serum calcium level, and serum 25(OH)D level, and were listed in **Table 1**. The mean age of the subjects was 35.60 ± 7.68 years with a mean BMI of 25.49±4.70 kg/m^2, and a mean serum 25(OH)D level of 18.75±7.01 ng/mL. The result of this study show that, in all groups, the serum 25(OH)D levels did not reach normal category for a sunny country (according to Holik, 2004; normal serum 25(OH)D level is 54-90ng/mL). Table 1 showed that there were significant differences in systolic blood pressure, BMI, abdominal circumference, and body fat amount, but serum 25(OH)D and calcium level did not show any significant difference between OG and NG. It was apparent that the low level of serum 25(OH)D was not affected by body fat percentage . In, based on the comparison of groups, there were three factors that were related to development of obesity: age, waist circumference and body fat percentage.

Table 2, showed that in 25(OH)D serum categories, there were no differences between obese and non-obese group in terms of the development of vitamin D deficiency-insufficiency. Only 8 out of the 156 participants showed a sufficient serum level of 25(OH)D. There were no subjects with a normal or excessive level of 25(OH)D found in this study. There were associations between deficiency-insufficiency vitamin D with occupation (p=0.001), sun exposure (p=0.007), physical activity (p=0.014, OR: 6.25, CI95% 1.216-32.120), vitamin D intake (p=0.012, OR: 5.43, CI 95% 1.268-23.29), and body fat percentage (p=0.003, OR: 14.14, CI 95% 1.69-118.19). **Table 3** showed that there were two correlation found, which were sun ray exposure and physical activity. Correlation between serum 25(OH)D level with sun ray exposure showed strong correlation and weak correlation between serum 25(OH)D level with physical activity.

The frequency of alleles and genotype of single nucleotide polymorphisms of VDR gene were shown in **Table 4**. On examining single nucleotide polymorphism of vitamin D receptor gene *Taq*I, it was apparent that the whole group were heterozygous (CT). Thus, only one cluster was visible. Examination of single nucleotide polymorphism of vitamin D receptor gene BsmI showed similar results with TaqI, and the whole group were found to be heterozygous (AG).

Table 1. COMPARISON OF STUDY PARAMETERS BETWEEN OBESE AND NON OBESE GROUP

Parameters	All n=156	OG n=78	NG n=78	*p*-value
Age (years)	35.60±7.68	37.71±6.64	33.5±8.11	0.001*
Blood pressure				
Systolic blood pressure (mmHg)	123.22±15.42	127.45±14.56	118.99±15.18	0.01*
Diastolic blood pressure (mmHg)	78.74±10.03	81.13±9.23	76.35±10.29	0.742
Anthropometry				
BMI (kg/m^2)	25.49±4.70	29.17±3.70	21.82±1.85	0.001*
Waist circumference (cm)	83.35±11.04	91.27±8.77	75.42±6.42	0.007*
Body fat percentage (%)	31.99±5.42	35.91±3.34	28.06±4.11	0.001*
Nutrient intake per day				
Energy (kcal)	1413.12±543.19	1462.86±549.97	1363.37±535.21	0.062
Vitamin D intake (µg)	5.24±6.94	4.61±5.53	5.87±8.09	0.073
Carbohydrate (g)	188.29±85.06	188.68±82.46	187.91±88.12	0.703
Protein (g)	44.52±18.37	45.52±19.89	43.52±16.78	0.459
Fat (g)	46.23±38.65	51.47±42.44	40.99±33.92	0.041*
Cholesterol	238.73±210.68	239.09±199.37	238.36±222.71	0.554
Fiber	5.8±6.9	6.59±9.29	5.03±3.14	0.076
Biochemical biomarkers				
Calsium serum (mg/dL)	9.11±0.49	9.05±0.58	9.169±0.37	0.603
25-hydroxyvitamin D serum (ng/mL)	18.75±7.01	18.34±5.99	19.16±7.92	0.917

Data presented in mean±standard deviation, * = *p* value with significance at $p<0.05$

Table 2. ASSOCIATIONS IN OBESE AND VITAMIN D DEFICIENCY-INSUFICIENCY GROUPS

Characterisitics[1]	Obese Group	Non-obese Group	*p*-value	Vitamin D deficiency-insuficiency	Vitamin D suficiency	*p*-value
	n=78	n=78		n=148	n=8	
	n(%)	n(%)		n(%)	n(%)	
Ethnicity[2]						
Javanese	24 (30.8)	48 (61.5)	0.334	61 (41)	2 (25)	0.591
Bataknese	39 (50)	23 (29.5)		59 (40)	4 (50)	
Others	19 (19.2)	7 (9)		36 (19)	2 (25)	
Occupation						
Indoors	56 (71.8)	51 (65.4)	0.388	107 (72.3)	0	0.001*
Outdoors	22 (28.2)	27 (34.6)		41 (27.7)	8 (100)	
Sun exposure per day (mean±SD)						
=60 minutes (38.65±10.12 minutes)	39 (50)	34 (43.6)	0.422	75 (50.7)	0	0.007*
>60 minutes (218.33±99.34 minutes)	39 (50)	44 (56.4)		73 (49.3)	8 (100)	
Dressing style						
Wearing hijab	27 (34.6)	33 (42.3)	0.323	91 (61,.5)	5 (62.5)	1.00
Not wearing hijab	51 (65.4)	45 (57.7)		57 (38.3)	3 (37.5)	
Physical activity (mean±SD)						
Low (4.70±0.59)	52 (66.7)	50 (64.1)	0.860	100 (67.6)	2 (25)	0.014*
Moderate (6.94±0.16)	26 (33.3)	28 (35.9)		48 (32.4)	6 (75)	OR:6.25
						CI 95%
						1.21-32.12
Vitamin D intake (mean±SD)						
Less (2.81±2.80 mg/day)	68 (87.2)	61 (78.2)	0.204	125 (84.5)	4 (50.0)	0.012*
Moderate (17.12±8.9 mg/day)	10 (12.8)	17 (21.8)		23 (15.5)	4 (50.0)	OR:5.43
						CI 95%
						1.26-23.29
Body fat percentage (mean±SD)						
Normal (26.21±3.34%)	2 (2.6)	54 (69.2)	0.001*	49 (33.1)	1 (12.5)	0.003*
High (35.19±3.33%)	76 (97.4)	24 (30.8)	OR:0.012	99 (66.9)	7 (87.5)	OR:14.14
			CI 95%			CI 95%
			0.17-0.35			1.69-118.19
25(OH)D serum categorized (mean±SD)						
Deficiency-insuficiency (17.78±5.68 ng/mL)	77 (98.7)	72 (92.3)	0.152			
Sufficiency (37.37±4.79 ng/mL)	1 (1.3)	6 (7.7)				

[1]Lifestyles: occupation, sunlight exposure per day, dressing style, sunscreen application, physical activity;[2] Ethnicity: Bataknese: Batak, Mandailing, and Karo; Others: Aceh, Minangkabau, Malay, Chinese, Nias; * = *p* value with significance at $p<0.05$

Table 3. CORRELATION ANALYSIS AMONG 25(OH)D LEVELS WITH OTHER VARIABLES

Variables	r	*p*
Age	0,087	0,278
Sun ray exposure	**0,739**	**0,001***
Physical activity	**0,338**	**0,001***
Vitamin D intake	0,044	0,587
Calcium serum level	-0,005	0,950

Analysis using Pearson's correlation; significant value:$p<0.05$
Correlations: weak (r<0.4), moderate (r=0.4-0.6), strong (r>0.6).

Table 4. FREQUENCY OF VDR GENE POLYMORPHISM TaqI DAN BsmI

SNPs Alelel/genotype	n	%
rs731236 (TaqI)		
C	156	50
T	156	50
CC	-	-
TT	-	-
CT	156	100
rs1544410 (BsmI)		
A	156	50
G	156	50
AA	-	-
GG	-	-
AG	156	100
Total	156	100

IV. DISCUSSION

This study was conducted in the Sumatera Island (North Sumatera, Medan), in the 3.57°N latitude and 98.65°E longitude. Average temperature was ±32°C (90°F) with efficient UV B exposure for absorption and producing sufficient amount of vitamin D3, [22,23] in contrast to areas with high latitudes (more than 37°) and a very slant sun ray angle that results in low production of vitamin D3 [24, 25]. It was shown that in some areas with latitudes higher than 51°, there was no vitamin D production in the skin [25]. A study was conducted using an angle greater than 70°, and it was found that dermal vitamin D synthesis can be absent from 5th October through 10th March five months [25].

In our study, we found a very different result compared to other studies. We found that the average level of serum 25(OH)D was lower, and was not affected by obesity. The findings of this study were interesting, because the low level of serum 25(OH)D may occur in tropical countries apart from whether the women (20-50 years) are obese or not.

A study by Khor*et al.* (2011) also found showed a different result, the subjects were school children, which was a contrasting association between serum 25(OHD) level and BMI in accordance to age, especially in boys [26]. The association between serum 25(OH)D level and BMI produced a risk factor of 5.958 times more likely of developing vitamin D deficiency with appropriate BMI for older age [26]. Another factor associated with this condition was low intake of vitamin D. Vitamin D-fortified meal was said to be consumed in very low amounst. Other factors that could lowering 25(OH)D serum level in children were sun exposure, indoor activities, and dressing style[26]. In our research, factors that associated with deficiency-insuficiency were occupation, sun exposure, physical activity, body fat percentage, and vitamin D intake.

This occurred because high body fat mass causes low levels of 25(OH)D in circulation, by trapping vitamin D inside fat cells.

The study also stated that high amount of body fat decreases the bioavailability of vitamin D and suggest a direct examination of adipocyte cells, and not the examination of serum 25(OH)D level in blood serum, because examination of adipocyte cell is the appropriate indicator for subjects with high amount of body fat.

A study by Ferrarezi*et al*. (2012) aimed to see the correlation between the variation of vitamin D receptor genes and the height of children (pre-pubertal and pubertal children). The study reported an inversed correlation between the level of serum 25(OH)D and the BMI in pubertal children. In this research reported that BsmI and TaqI genotypes were signigicantly associated with the height in pubertal children, but the association did not reach statistical significance in prepubertal children. The study concluded that the polymorphism affected the function of vitamin D receptor gene, and also affected body height by affecting bone growth in puberty [27].

In this study we found that body fat percentage and vitamin D intake are predicting factors of vitamin D deficiency-insuficiency. We found that the body fat percentage are evenly distributed in all participants (obese or non-obese group) because of most results showed that the participants had greater amount of body fat percentage. Most studies used BMI to describe body fat, but this study used a more accurate measure, bioelectrical impedance. This measured showed that the majority of subjects in our study could be categorized as having high or very high body fat percentages. This study showed contrasting results with a previous study [28,29], where we found that subjects with high body fat percentages have low serum 25(OH)D levels, but similar result were also found in subjects with normal and low of body fat percentage.

The probability of someone with high body fat percentage to develop a deficiency of vitamin D is 14 times greater than people with normal body fat percentage. However, despite the fact that low level of vitamin D is associated with body fat, in this study, most of the subjects with low and normal body fat percentage (n=56; 35.89%) also developed vitamin D deficiency. It means that there are other factors beside the amount of body fat causing vitamin D deficiency in women with low or normal body fat percentage. This result showed that the average level of serum 25(OH)Dfell into the deficiency-insufficiency category. Serum 25(OH)D levels falling into sufficient category were considered outliers, or in other words, the normal value was lyingoutside the average level of serum 25(OH)D of the overall study subjects.

Brock et al (2010) mentionedthat vitamin D intake was a predictivefactor of the development of vitamin D deficiency. Main food source for this vitamin is fortified milk. Another factor that was found to be strongly correlated with the level of vitamin D was continuous physical activities. It was said that, compared to the sun exposure, a continuous physical activity can maintain the body status of vitamin D [30].

The underlying cause of low level of serum 25(OH)D in OG and NG groups is perhaps the single nucleotide polymorphism of vitamin D receptor genes. This result was shown in all subjects who were heterozygous, meaning that the receptor gene carried the genotype TC for the polymorphism of vitamin D receptor gene TaqI, and the genotype AG for BsmI[15]. Even though the mutation only occurred in one base (silent mutation), it seems that this mutation affected the level of serum 25(OH)D. These results were apparent in categories of deficiency, insufficiency, and sufficiency.

Level of $1,25(OH)_2D$ is an indicator for the level of vitamin D, and an active form of vitamin D. The level of $1,25(OH)_2D$ was maintained by human body until a deficiency in vitamin D occurred, but it is not a good indicator for an early-stage vitamin D deficiency [30, 31]. The examination of 25(OH)Dlevel in the circulation is recommended in assessing the normal function of vitamin D. The disturbed cellular pathways of vitamin D may affect the level of vitamin D with serum 25(OH)D level as a parameter [32].

The activity of $1,25(OH)_2D$ form is homologous to steroid hormones, and the activity with thetarget cell is achieved through VDR, which is a nuclear transcription factor [33,34]. Additionally, VDR is a transcriptional factor that will form a ligand and will bind to vitamin D through a carboxy-terminal bond. VDR is also a nuclear receptor, along with steroid, thyroid, and retinoic acid receptors [35].

When $1,25(OH)_2D$ diffuses into a target cell, it bindstoVDR and increases the heterodimer formation with RXR. Dimers form activates or suppresses transcription of target genes by binding the stimulating (co-activator) or inhibiting (co-suppressor) coregulators. This activity is different in each tissue,andeventually represents the activity of $1,25(OH)_2D$ and its production. The co-activator complex contains histone acetylate, and subsequently transcription occurs on the exposed DNA, whichforms a bridge between the initiation complex and the dimer, increasing the RNA polymerase II [36]. The VDR-RXR dimer then bonds to specific sequence in the target promoter region called vitamin D response elements (VDRE). Some genes involved in the regulations of calcium, phosphor homeostasis and vitamin D metabolisms found present with VDRE. [35,36](Figure 1).

This study showed that the frequency of minor allele found for T and C was 0.5%, and this is similar to the result foundbyVupputuri*et al*. (2006) [17]. A study by Jain (2010) on Southern Asian women living in New Zealand showed 0% frequency genotype for homozygous wildtype and 156 (65.3%) for heterozygous, while the frequency for heterozygous mutant was 69 (28.9%). These results were obtained using the same device on 225 female samples. For BsmI, of the 238 samples, only 1 sample (0,4%) showed homozygous wildtype form, while 194 (81.5%) showed heterozygous form and 43 (18.1%) showed homozygous mutant form [37].

This study proved that the level of vitamin D was affected by the genetic mutation, based on the fact that the examination of single nucleotide polymorphism of VDR genes TaqI and BsmI showed that all subjects carried the genotype TC and AG. The similarities found were associated with linkage disequillibrium between both genes that commonly occurred through polymorphism in TaqI as well as BsmI[16]. The genetic variance of VDR genes plays a role in metabolic disturbances. Single nucleotide polymorphism occurring only in one base is called missense, but a silent mutation may cause a change in the coded protein. Ogunkulade *et al.* (2002) have suggested that single nucleotide polymorphisms (SNPs) within the VDR gene may influence the stability, quantity, and activity of VDR protein and the rate of VDR gene transcription [38, 39, 40].

The limitation of this study lies in the fact that it does not have anydata about the level of phosphorus and the concentration of the parathyroid hormones, no association can be made regarding those parameters. In addition to that, the design of

this study limitsthe capability of concluding a causal association. To show associations, a randomized controlled trial is recommended on a larger sample size, and with more between-ethnic variations especially in Indonesia, with abundant sun exposure (tropical area). This study found that from all varieties of single nucleotide polymorphism, only one heterozygous cluster was found. There were no homozygous wildtype or homozygous mutant gene found in associationwith the low level of 25(OH)D. This silent mutation form eventually may cause an evolution in human,with affects the susceptibility against diseases.

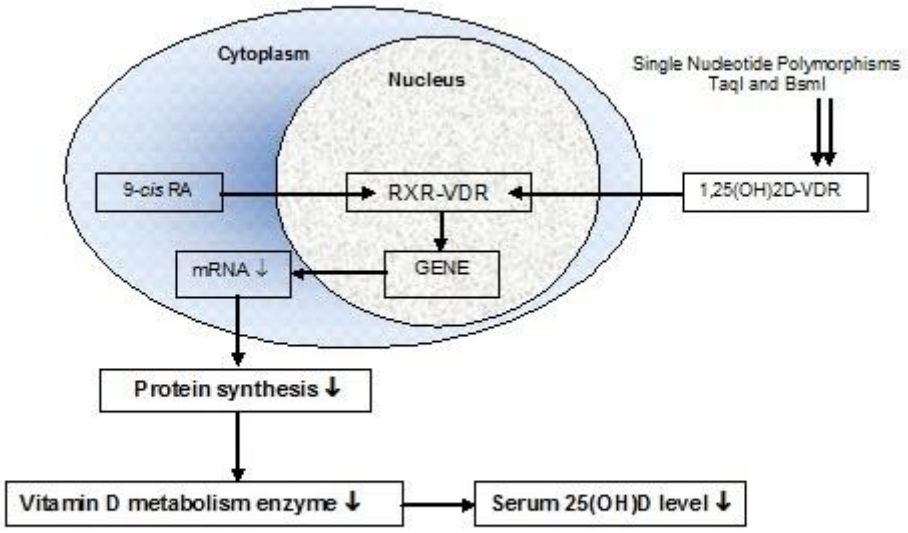

FIGURE 1. VDR-RXR PATHWAY IN INFLUENCING SERUM 25(OH)D LEVEL

V. CONCLUSION

The result of this study showed that there was an association between allele frequency of the genotype T>C of TaqI and A>G of BsmI with the serum 25(OH)D level in the subjects. No associations that can be analyzed in this study were found, because we only found one heterozygous cluster, both for TaqI (TC) and for BsmI (AG). However, based on theexisting theory,the presence of heterozygous form may preventthe serum 25(OH)D level from reaching normal values. We can only found levels categorized as deficiency, insufficiency and sufficiency. There were associations between deficiency-insufficiency vitamin D with occupation, sun ray exposure, physical activity, vitamin D intake, and body fat percentage in women with single nucleotide polymorphism TaqI and BsmI in this area.

VI. COMPETING INTEREST

There is no funding sources or conflict of interest in this research

VII. AUTHOR'S CONTRIBUTION

DKS carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript, HAR carried out the immunoassays and sequence alignment, NIL participated in the design of the study and performed the statistical analysis, ZL conceived of the study and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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