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Is Micro Evolution in Tropical Country Women Resulting Low 25(OH)D Level?: A Cross Sectional Study in Indonesia

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Abstract

Background: Low serum 25(OH)D 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, to compare the difference of associated factors, and to find the factors that independently associated with 25(OH)D level.

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 of vitamin D receptor gene (TaqI and BsmI), assessed lifestyle factor, 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), all participants were heterozygous (T>C for TaqI and A>G for BsmI). There were associations between vitamin D deficiency-insufficiency and sufficiency with indoors occupation (p<0.001), low vitamin D intake (p=0.046), less than 1 hour sun ray exposure (p<0.001), and low physical activity (p=0.01). Logistic regression showed that prediction factors that independently associated with the risk of low 25(OH)D level were sun ray exposure, occupation, and vitamin D intake.

Conclusion: The results showed that vitamin D deficiency may occur in women with indoors occupation, low vitamin D intake, less than one hour sun ray exposure, and low physical activity. All participants were heterozygous (T>C for TaqI and A>G for BsmI). Factors that most influenced vitamin D serum were sun ray exposure, occupation, and vitamin D intake.

Keywords: 25(OH)D levels; Micro evolution; Women

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 South Asian 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 is also 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. [5] 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, however, there are no study report in healthy adult women in Indonesia.

The impacts of such deficiency includes decreased bone mineral density in 10-18 year-olds [6], while rickets and osteomalacia is 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 and vitamin D supplements [11]. Another factor found to be able to affect vitamin D level is obesity; studies have shown an association 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 [14,15]. A study by Al-Daghri et al. [16] 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 diabetes mellitus, in terms of susceptibility to inflammatory and metabolic reactions. A study by Vupputuri et al. [17] found vitamin D deficiency in 94.3% Asian-Indian subjects, with the

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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. Almost 200 polymorphisms have been found in the VDR gene, but their effect on VDR 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, to compare the difference of associated factors, and to find the factors that independently associated with 25(OH)D level. The variables were anthropometry, lifestyle, vitamin D intake, and two single nucleotide polymorphisms in the VDR gene (TaqI and BsmI) with serum 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.

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 consisted of 156 women, the inclusion criteria were healthy women between 20-50 years old. Exclusion criteria were subjects with history of diabetes mellitus, 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. This study was conducted between July and October 2012, during the dry season in Indonesia is between April and October.

All participants 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.

Body mass index 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 (>25 kg/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 bioelectrical iimpedance. 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). Physical activity was analysis using Baecke Questionnaire (21), questioning habitual physical activity, classified into three categorized which were low, moderate, and high physical activity.

Biochemical analysis

In this study, we measured serum 25(OH)D levels because of it has a longer half life (2-3 weeks) than 1.25(OH)DE serum, which was 4-6 hours (1). This analysis was using 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 (100-150 ng/mL) and intoxication (>150 ng/mL), in this study, all participants were categorized into: deficiency-insuficiency (<32.9 ng/mL) and sufficiency (>33 ng/mL) [21,22].

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 Biosystem Step One Plus Real-Time PCR Systems.

For DNA isolation, the procedures were: whole blood was collected in an EDTA 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°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 precipitations to the solution and then vortex 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°C for one night, for next day, keep in freezer -20°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*™*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°C for 20 seconds, followed by 40 cycles, and then succeeded by a denaturation process at 95°C for 3 seconds and annealing process at 60°C for 30 seconds. Fluorescence detection occurred at 60°C. The whole assay was operated in a 10 µL 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 at the 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

Sample size was determined based on the confidence interval of 95%, a study power of 90%, and assuming an effect size of 20%. A total of 156 subjects were recruited with consecutive sampling. Numerical variables were used to indicate mean ± standard deviation, while categorical variables were used to indicate the percentage values of proportion. Association between numerical independet variable and 25(OH)D concentration were analized using pearson correlation and expressed as r, a correlation greater than 0.8 is generally described as strong, 0.5-0.8 moderate, and less than 0,5 is generally described as weak; association between dichotomous categorical independet variable and vit D concentration were analized using independent t-test and expressed as geometric mean (95%CI); association between polikotom categorical independet variable and vit D concentration were analized using oneway anova and expressed as geometric mean (95%CI); 25(OH)D concentration was transformed by log10 and was backtransformed for data presentation. Logistic regression analysis was used to look for prediction factors. 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. This study used SPSS program (version 11.5; SPSS Inc, Chicago, IL) for data analysis.

Results

All participant were based on general characteristic (age), anthropometry (BMI, waist, and body fat percentage), vitamin D intake, sun ray exposure, dressing style, sunscreen application, physical activity, and serum calcium level 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.

Table 2, a mean serum 25(OH)D level of 18.7 ± 7.0 ng/mL. The result of this study show that serum 25(OH)D levels did not reach normal category for a sunny country (normal serum 25(OH)D level is 54-90 ng/mL). 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. that geometric mean of 25(OH)D levels were 17.71 ng/mL with CI95% 16.22-19.34, it also showed that 94.9% were categorized vitamin D deficiency-insufficiency and 5.1% categorized in vitamin D sufficiency, none of the study

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 ± 10.03	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)	1413.12 ± 543.19	1462.86 ± 549	1363.37 ± 535.21	0.062
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	23836±222.71	0.554
Fiber	5.8±6.9	6.59±9.29	5.03±3.14	0.076
Biochemical biomarkers				
Calcium serum (mg/dL)	9.11±0.49	9.05±0.58	9.169±0.37	0.603
25-hydroxy vitamin D serum 9ng/mL)	18.75±7.01	18.34±5.99	19.16±7.92	0.917

Table 1: Comparison of study parameters between obese and non obese group.

Characteristics[1]	Obese Group n=78 n(%)	Non-obese Group n=78 n(%)	*p*-value	Vitamin D deficiency-insuffiency n=148 n(%)	Vitamin D ufficiency n=8 n(%)	*p*-value
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*
	22(28.2)	27(34.6)		41(27.7)	8(100)	
Sun exposure per day(mean±SD)						
=60 minutes (38.2±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±2.80mg/day)	68(87.2)	61(78.2)	0.204	125(84.5)	4(50.0)	0.012*
Moderate (17.12±8.9mg/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			
25(OH)D serum categorized (mean±SD)						
Deficiency-insufficiency (17.78±5.68ng/mL)	77(98.7)	72(9.3)	0.152			
Sufficiency (37.37±4.79ng/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 2: Associations in obese and vitamin d deficiency-insuficiency groups.

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 3: Correlation Analysis Among 25(OH)D Levels With Other Variables.

participant reached normal categorized in sunny area. Percentile 50 of 25(OH)D value was 18.4 ng/mL, with minimum percentile was 7.1 ng/mL and maximum percentile was 42.5 ng/mL.

Table 3 showed associations between demographic, anthropometry, lifestyle, intake, and calcium serum with 25(OH)D serum concentration. Association showed that indoor occupation [$p<0.001$, 14.81 ng/mL (CI95% 13.98-15.70)], less vitamin D intake [$p=0.046$, 17.03 ng/mL (CI95% 15.98-18.16)], ≤ 1 hour sun ray exposure per day [$p<0.001$, 14.29 ng/mL(CI95% 13.28-15.39)], low physical activity [$p<0.01$, 15.97 ng/mL(CI95% 14.90-17.11)] were significantly association with vitamin D deficiency. Table 4 showed the results from multivariate analyses, after adjusting for all related factors, the multivariate model revealed that less than 1 hour sun ray exposure per day, indoor occupation, and low vitamin D intake were all significant independent correlates of

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

Table 4: Frequency of vdr gene polymorphism TaqI DAN BsmI.

vitamin D deficiency-insufficiency.

Table 4 showed the frequency of alleles and genotype of single nucleotide polymorphisms of VDR gene. 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 *Taq*I, and the whole group were found to be heterozygous (AG). Based on there was only one cluster, so to analysis association between single nucleotide polymorphism of vitamin D receptor gene TaqI and BsmI with 25(OH) D serum, using abductive analysis; a form of logical inference that goes from observation to a hypothesis that accounts for the reliable data (observation) and seeks to explain relevant evidence. So, based on this analysis, there was an association between single nucleotide polymorphism of vitamin D receptor gene TaqI and BsmI with low level of 25(OH)D serum.

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 ± 32C (90°F) with efficient UV B exposure for absorption and producing sufficient amount of vitamin D3 [23,24], in contrast to areas with high latitudes (more than 37°C) and a very slant sun ray angle that results in low production of vitamin D3 [25,26]. It was shown that in some areas with latitudes higher than 51°, there was no vitamin D production in the skin [26]. 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 [26].

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 body mass index or body fat percentage. 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) have high BMI or body fat percentage. A study by Khor et al. [27] 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. 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 [27]. Another factor associated with this condition was low intake of vitamin D. Vitamin D-fortified meal was said to be consumed in very low amounts. Other factors that could lowering 25(OH)D serum level in children were sun exposure, indoor activities, and dressing style [27]. In our research, factors that associated with deficiency-insufficiency were occupation, sun exposure, physical activity, and vitamin D intake.

In previous study [27], low 25(OH)D level because of 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. [28] 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 inverse 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 significantly 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.

In this study, we found that the body fat percentage is evenly distributed in all participants 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 [29,30], 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.

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 also developed vitamin D deficiency. It means that there are other factors causing vitamin D deficiency in women with low or normal body fat percentage. This result showed that the average level of serum 25(OH)D fell 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 lying outside the average level of serum 25(OH)D of the overall study subjects.

Brock et al. mentioned that vitamin D intake was a predictive factor of the development of vitamin D deficiency [31]. 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 [31]. In this study resulting an equation to predict the probability of deficiency and insufficiency vitamin D, and finding that factors that independently associated with the risk of low 25(OH)D level are sun ray exposure per day, occupation, and classification of vitamin D intake.

The underlying cause of low level of serum 25(OH)D 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

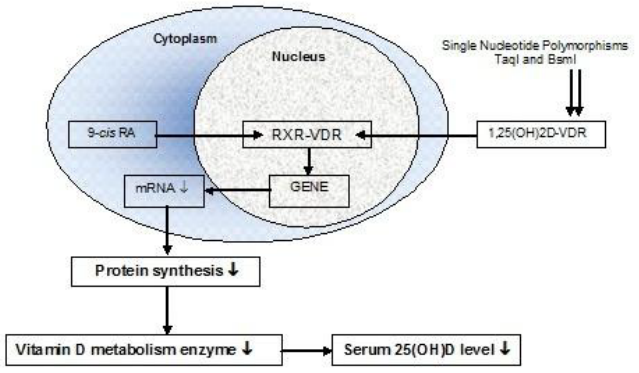

Figure 1: VDR-RXR pathway in influencing serum 25(OH)D level.

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, instead of 25(OH)D level [31,32]. The examination of 25(OH)D level 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 [33].

The activity of $1,25(OH)_2D$ form is homologous to steroid hormones, and the activity with the target cell is achieved through VDR, which is a nuclear transcription factor [34,35]. 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 [36].

When $1,25(OH)_2D$ diffuses into a target cell, it binds toVDR 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,and eventually 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, which forms a bridge between the initiation complex and the dimer, increasing the RNA polymerase II [37]. 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 [36,37] (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 found by Vupputuri et al. [17]. A study by Jain (2010) on Southern Asian women living in New Zealand showed 0% frequency genotype for homozygous wild type 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 wild type form, while 194 (81.5%) showed heterozygous form and 43 (18.1%) showed homozygous mutant form [38].

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 disequilibrium 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. [39] 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.

The limitation of this study lies in the fact that it does not have any data 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 limits the 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 wild type or homozygous mutant gene found in associaton with the low level of 25(OH)D. This silent mutation form eventually may cause a micro evolution in human, with affects the susceptibility against diseases.

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. In this study we found only one heterozygous cluster, both for TaqI (TC) and for BsmI (AG), so it can assumed that lower level of 25(OH)D serum. However, based on the existing theory, the presence of heterozygous form may prevent the 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.

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