The association between lifestyle and maternal vitamin D levels during pregnancy in West Sumatra, Indonesia

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ABSTRACT

Background and Objectives: An adequate level of maternal vitamin D is essential for maternal and fetal health during pregnancy. We examined the relationship between lifestyle, maternal vitamin D intake and the vitamin D status of pregnant women. **Methods and Study designs:** The sample of the cross-sectional study was 203 third trimester pregnant women in September-November 2016 in four different districts of West Sumatra, Indonesia. Questionnaire was used to assess lifestyles, dietary intake, anthropometry, maternal characteristics, demography and socioeconomic data. The Vitamin D serum level was measured by the ELISA method and the data were analyzed using descriptive statistics, chi-squared tests, Pearson's correlation and logistic regression. **Results:** 160 blood serum samples of pregnant women were collected. The means of 25-hydroxyvitamin D and maternal vitamin D intake were 29.06±11.39 ng/mL and 7.92±5.26 µg/day respectively. The prevalence of vitamin D deficiency-insufficiency was 61.25%, and more than 85% of the women had inadequate vitamin D intake. We found that living in mountainous areas (*p*=0.03) and low physical activity (*p*=0.02) were significantly associated with maternal vitamin D levels as a prediction factor. In addition, younger who had lower pre-pregnancy weight had a higher prevalence of vitamin D deficiency. **Conclusions:** Low levels of vitamin D were common among pregnant women in West Sumatra, Indonesia. Additional intake of vitamin D from supplements may be important to meet the recommended dietary level for pregnant women.

Keywords: 25-hydroxyvitamin D, Pregnancy, Lifestyle, Vitamin D Level, West Sumatra

INTRODUCTION

Vitamin D is a derived form of cholesterol from steroid hormones that is unlike other vitamins because it can be synthesized in the human body.[1] In general, vitamin D is divided into two forms: ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Vitamin D_2 is found in plants, yeast, and especially in fungi, while vitamin D3 is found in animal food sources and can be produced in the skin of animals and humans with the help of exposure to ultraviolet light (UV). The main sources of vitamin D are exposure to sunlight, consumption of foods, vitamin D supplements, and food intake with vitamin D fortification. The greatest source of vitamin D in the body is a result of exposure to sunlight, which provides nearly 90% of the serum 25(OH)D levels in the body, while food only contributes 10%.[2]

Vitamin D plays an important role in the body in maintaining the homeostasis of calcium metabolism and bone mineralization. In addition, it has an additional function in many cellular networks through the effect of autocrine and paracrine, playing a role in the anti-inflammatory, anti-infection and regulation work of the proliferation, differentiation and synthesis of insulin cells.[3]This role may influence adverse pregnancy outcomes. Other studies found no association between vitamin D deficiency and preeclampsia.[4,5] In addition, some studies have also shown that an adequate vitamin D level protects against premature birth, low birth weight, gestational diabetes, caesarean birth, and bacterial vaginosis through the modulation effects of the immune system and its anti-inflammatory role.[6–8]

Vitamin D deficiency is prevalentthroughout the world.Conditions of severe hypovitaminosis D are found most commonly in countries in the Middle East and AsiaPacific.[9] Factors associated with deficiencies in vitamin D include socioeconomic factors, seasons, obesity, culture, dress styles, intake of vitamin D supplements, physical activity, use of sunscreens, working status, intake of vitamin D, area of residence, and skin colour. Tropical and subtropical regions have different exposure to sunlight, as well differing altitudes of where people live. However, high rates of vitamin D deficiency are common in individuals who would not normally be considered at risk, such as a fair skinned people, those in high socioeconomic groups, and those living in tropical areas, who are believed to have adequate exposure to sunlight.[10,11] Furthermore, information about maternal vitamin D levels among pregnant women, both at dietary and 25-hydorxyvitamin D levels, is limited in West Sumatra, Indonesia.

This study investigates vitamin D levels in the third trimester, whose functionally enhances calcium absorption in the intestine and represents maternal vitamin D level during pregnancy. It was conducted to test factors associated with a low vitamin D level in the third trimester of pregnancy, and examinesthe relationship between lifestyle, place of residence, maternal vitamin D intake and serum level status of pregnant women in West Sumatra, Indonesia.

PARTICIPANTS AND METHODS

Study design and participants

A cross sectional study of 215 healthy third trimester pregnant women was recruited based at the primary health care facilities in each district. The study was conducted in the West

Sumatra province in Western Indonesia (South latitude 3º 50' and North latitude 1º 20', tropical climate).Two coastal districts (Pariaman and Pasaman Barat) and two mountainous areas (Solok and Tanah Datar) were chosen, and the study was performed from September to November 2016. Participants from the Community Health Centres of each district were recruited. Twelve pregnant women were excluded based on the inclusion criteria. 203 pregnant women met our inclusion criteria: i.e. no history of taking any kind of drug that could interact with vitamin D or calcium metabolism; the absence of chronic disease; >28 weeks of pregnancy; routine attendance at primary health service antenatal reviews; completion of informed consent form; and living in the research area.

Data collection

We performed a general pregnancy examination to measure maternal anthropometric data, namelylifestyle, drug history, maternal health history, weight gain pattern, and blood pressure. Demographic information, including maternal age, gravidity, parity, socioeconomic status, and lifestyle, was collected from in-person interviews on enrolment on the study. Weight was measured using scales with a precision of 0.1 kg. Pre-pregnancy body weight was based on antenatal care records from the health care provider. Pre-pregnancy BMI was calculated and classified according to World Health Organization guidelines for Asian populations.Gestational age was determined according to the date of the last menstrual period and confirmed by ultrasound reports in the first trimester. A lifestyle questionnaire was offered to the participants, including questions about duration of sun exposure, working status, physical activity, and sunscreen use.Sun exposure was calculated as an index of the hours per day the pregnant women spent outdoors exposed to sunlight during either their leisure or working time. We then divided the exposure into two groups (≤1 hour or >1 hour). Systolic and diastolic blood pressure was taken before blood sampling.

Dietary data were obtained from validated FFQ by Lipoeto.[12] Mean energy and nutrient intakes were calculated and compared with the Recommended Dietary Allowance (RDA) for pregnant women.[13] Based on calcium and vitamin D intake, the pregnant women were divided into two groups. The RDA for maternal vitamin D intake is 600 IU/day or 15 µg/day, with an Estimated Average Requirement (EAR) of 400 IU and an Upper Level (UL) of 4000 IU, as recommended by the Institute of Medicine (IOM). The level of vitamin D intake was defined by the intake of vitamin D-rich food, as follows: inadequate intake (<15 µg/day or <600 IU/day) and adequate intake (≥15 µg/day or ≥600/day).[14]The level of calcium intake was defined as adequate intake (≥1300 mg/day) and inadequate intake (<1300 mg/day). Vitamin D and calcium intake levels were measured from food and supplement usage and represented by pregnant women's eating habits during gestation.

Blood samples and laboratory analysis

All the third trimester pregnant women enrolled on this study had blood samples of their antecubital vein taken. Non-fasting maternal blood samples were collected and banked by phlebotomistsat the Biomedical Laboratory of Andalas University.160 samples from the 203 participants were taken directly to the biomedical laboratory at the Faculty of Medicine, University of Andalas, for serum 25(OH)D level assay. The serum samples were separated by centrifugation at 3500 rpm at 4˚C for 10 min, then stored in aliquots at -80˚C. The serum 25(OH)D test was assessed using ELISA from Diagnostic Biochemistry Canada (DBC) 25-Hydroxyvitamin D (DBC, London, Ontario Canada). The intra-assay coefficient of variation was 5.8% at 25.32 ng/mL and 1.39% at 17.95 ng/mL. The inter-assay coefficient of variation was 8.4% at 24.6 ng/mL and 5.52% at 24.26 ng/mL. We used the cut-off point suggested by the Endocrine Society and vitamin D level was categorized as sufficient (25(OH)D ≥30 ng/mL), insufficient (25(OH)D=20-29 ng/mL), or deficient (25(OH)D <20 ng/mL). In addition, based on the Endocrine Society Clinical Practice Guidelines, serum 25(OH)D levels of less than 10 ng/mL are considered to represent severe vitamin D deficiency.[15]

Statistical analysis

Data were presented as mean and standard deviation (SD), or median and inter quartile range (IQR), when appropriate, for continuous variables; and, as frequencies and proportions for categorical variables. The unit of measurement of vitamin D concentration was standardized to the S.I. unit, ng/mL for 25-hydroxyvitamin D. Place of residence, maternal characteristics, demographics, socio-economic status, lifestyle choices, and dietary intake data were compared between the groups with deficient-insufficient and sufficient maternal vitamin D levels. The categorical data were analysed using chi-squared tests, and a student's *t* test was used to compare the vitamin D serum levels of the two groups. Logistic regression models were used to estimate the OR's of the dependent variable (vitamin D level) and the independent effects of known risk factors (e.g. sun exposure, physical activity, age).The relevant confounding variables controlled for binary logistic regression analysis included pre-pregnancy BMI, maternal age, physical activity, working status, vitamin D intake, sunscreen use and place of residence, which were considered to be associated with the risk of maternal vitamin D deficiency. Pearson's correlation was used to investigate the correlations between variables. All the data were managed and analyzed descriptively using IBM SPSS, version 20.0, and presented as tables and figures. All reported *p* values were two-sided and considered as significant if <0.05 to determine the relationship.

Ethical approval and consent

The study was approved by the Research Ethics Committee of the Medical Faculty, Andalas University, West Sumatra, Indonesia (No: 108/KEP/FK/2016). Participants provided informed consent and patient anonymity was preserved.

RESULTS

Study population

In total, 203 pregnant women over 28 weeks of pregnancy were recruited, 112 subjects from coastal areas and 91 from mountainous areas. All the women were healthy and not taking any medication at the time. Table 1 shows the characteristics of the study population. The subjects were divided into two groups, deficient-insufficient and sufficient maternal vitamin D status, by measuring serum 25(OH)D levels. The mean age was 29.96±6.13 years (ranging from 16 to 45 years old), of which the age range of 20-35 years is that recommended for safe pregnancy. The self-reported ethnicities of the participants were Minangkabau (93.1%), Javanese (6.3%) and Batak (0.6%). Most of the subjects were high school graduates (64.6%), while 35.4% had not graduated from high school. Median of monthly household income was higher than standard minimum wages in West Sumatra (2.400.000 (2.000.000) vs 1.800.000 IDR). Women with a monthly household income less than and above of the minimum wage were 70% and 30%, respectively. Sixty-five (32%) of women were primiparous women and 138 (68%) were multiparous. The average week of pregnancy in the insufficient-deficient and sufficientof vitamin D status categories was 32.0±63.5 and 31.37±3.4 weeks respectively.

The maternal characteristics that significantly differed between the groups were mean household income (p=0.014), age group (p=0.030), and place of residence (p=0.009). The groups were not significantly different when stratified by ethnic group and education level ($p \geq 0.05$) (Table 1).

Prevalence of vitamin D levels

Figure 1 shows that the frequency distribution of the 25(OH)D level was skewed to the left, and most of the results were <30 ng/mL. Vitamin D deficiency-insufficiency was present in 61.25% of the women. Only 38.75% of the study population had 25(OH)D concentrations of ≥30 ng/mL. The total 25(OH)D serum level was 29.06±11.4 ng/mL. The mean values of the 25(OH)D serum level were 22.4±5.2 ng/mL for the deficient-insufficient group and 39.5±10.5 ng/mL for the sufficient group. This level differed significantly between the vitamin D deficient-insufficient and sufficient groups (p=0.001).

Anthropometric characteristics and blood pressure of the pregnant women

The anthropometric characteristic data of the pregnant women are presented in Table 1. Their mean±SD values were 152.74±5.37 cm for height, 61.05±10.11 kg for weight, 21.85±3.72 kg/m^2 for pre-pregnancy BMI, 10.01±5.26 kg for weight gain during pregnancy, 26.93±3.46cm for MUAC, and 115.60±12.04 mmHg and 74.52±8.69 mmHg for systolic and diastolic blood pressure respectively.

The mean pre-pregnancy body weights of the deficient-insufficient and sufficient groups of maternal vitamin D level pregnant women were 49.6±9.07 kg and 53.4±9.3 kg (p=0.010) respectively; with corresponding figures of 21.18±3.5 kg/m2 and 22.9±3.5 kg/m2 for pre-pregnancy BMI (p=0.003); 10.5±4.6 kg and 9.5±6.6 kg for weight gain (p=0.266); 26.27±3.39 cm and 27.7±3.25 for MUAC (p=0.007);115.28±12.7 mmHg and 116.11±11 mmHg for systolic blood pressure (p=0.669); and 74.12±9 mmHg and 75.16±8.2 mmHg for diastolic blood pressure (p=0.463).In total, 18.5% of the pregnant women were underweight, and more than 60% of them had MUAC >25 cm (obese) during pregnancy. Most of the subjects had normal blood pressure (86.25%), while 13.75% had hypertension.

Those women with deficient-insufficient of vitamin D status showed a negative association with weight gain, height and blood pressure. However, women who have deficient-sufficient of vitamin D status had significantly lower pre-pregnancy weight (p=0.01), pre-pregnancy BMI (p=0.003) and MUAC (p=0.007).

Pregnant women's dietary intake

Table 2 shows an analysis of the subjects' average daily nutrition intake and the comparison of macronutrients intake as daily percentage of energy. The dietary assessment of the pregnant women was compared with the RDA for calcium and vitamin D intake

(1300 mg/day and 15 μg/day).Average macronutrients intake compares to calories percentage were 44% for carbohydrate intake, 17% for protein intake, and 39% for fat intake. This study showed that higher percentage of calories from fat intake, slightly higher percentage of calories from protein intake, and lower percentage of calories from carbohydrate intake than the standard macronutrient proportion of energy in our daily requirement (45-65% for carbohydrate, 10-35% for protein, and 20-35% for fat).[14] Mean of calcium and vitamin D were well below the RDA, at 60% and 52.8% respectively. There is no women took vitamin D supplement during the third trimester of pregnancy.

The relationship between maternal vitamin D intake and maternal vitamin D level can be seen in Figure 2. There was no significant difference between dietary vitamin D (p=0.514) and calcium (p=1.00) levels and maternal vitamin D levels.60-70% of the pregnant women were in the vitamin D deficient-insufficient category and had inadequate vitamin D and calcium intakes. Only 30-40% of the pregnant women were in the sufficient category. With regard to adequate maternal vitamin D intake, 10% of the women had vitamin D deficiency-insufficiency and 4.38% had vitamin D sufficiency.

Factors associated with vitamin D serum levels

Table 3shows the variables, such as age (r=0.22), duration of sun exposure (r=0.20), pre-pregnancy weight (r=0.20), and pre-pregnancy BMI (r=0.26), associated with vitamin D level. Other variables, such as vitamin D intake (r=0.03), weight gain (r=0.03), systolic blood pressure (r=0.005) and diastolic blood pressure (r=-0.12), were not significantly related to 25(OH)D levels. In the multivariate analysis, place of residence and physical activity were significantly associated with 25(OH)D serum level (Table 4).

Association between lifestyle, place of residence and serum 25(OH)D levels

The relationships between place of residence, vitamin D and calcium, and 25(OH)D are shown in Table 5. Intakeof vitamin D status was better in thecoastal areas with 18.8% adequate than in mountainous area that only 6.6% adequate. Intake of calcium status also showed the same results that women who live in coastal areas was better than in mountainous areas with 12.5% vs 7.7% of adequate intake status. The association between vitamin D intake status and place of residence were significantly associated (p=0.02), however calcium intake status showed not significantly associated (p=0.37). Women who live in coastal areas had a higher calcium and vitamin D intake status compare to those living in mountainous area.

More than half of the pregnant women subjects had inadequate vitamin D intake (86.7%) and calcium intake (89.7%). One in seven of them (13.3%) had adequate intake of vitamin D, and about one in ten (10.3%) had adequate calcium intake from dietary sources,based on the current RDA for pregnant women. The means of vitamin D and calcium intake were 7.92±5.26 µg/day and 784.88±409.77 mg/day respectively. Table 5 shows the differences between dietary intake of vitamin D, calcium and 25(OH)D serum levels in the women in relation to place of residence. The average intake of vitamin D of the pregnant women in relation to place of residence shows a significant difference (p=0.001); that of those who live in coastal areas is higher than for those who live in mountainous areas(9.04±0.56 µg/day vs 6.55±0.41 µg/day). The average calcium intake of the pregnant women with respect to place of residence does not show a significant difference (p=0.09). The average intake of calcium in the coastal areas is higher than in the mountainous areas (812.385±434.840 vs 751.043±376.263 mg/day).

The association between lifestyle choices and 25(OH)D serum levels is shown in Table 1. Lifestyle variables include sun exposure, physical activity, working status and sunscreen use. In this study, pregnant women with less than 1 hour/day sun exposure had significantly different maternal vitamin D levels compare to those had >1 hour/day (p=0.003). On the other hand, the categorical of sun exposure and vitamin D status had no significant association (p=0.164). This finding was similar result with the association between physical activity and maternal vitamin D level (OR=0.4, 95%CI (0.17-1.2), p=1.00). However, women who had indoor working areas (p=0.028) and using sin screen application (p=0.042 had significantly associated with low vitamin D status.

DISCUSSION

Our data are quite similar to a prior study by Bardosono et al (2016), who reported that 90% of pregnant women in Jakarta, Indonesia had vitamin D levels <30 ng/mL in their first trimester.[16]Other studies have reported a high prevalence of vitamin D deficiency among first trimester pregnant women. In North Sumatra, Indonesia, 95% had a vitamin D deficient-insufficient status, with the remaining 5% having a sufficient status. In Jakarta and Kuala Lumpur, 60% had insufficient 25-hydroxyvitamin D serum levels, or less than 50 nmol/L.[17,18] Other studies have demonstrated that vitamin D deficiency is also common in pregnant women in several South and Southeast Asian countries, such as India, Malaysia, Thailand, Singapore and Vietnam. Most had less than 30 ng/mL of 25-hydroxyvitamin D serum levels (deficient and insufficient status).[19–21] Vitamin D deficiency was prevalent in sunny countries such as Indonesia, where residents tend to avoid exposure to sunlight, spending most of their time indoors, and have a low dietary intake of vitamin D.[17]

There is no definite recommendation for an adequate duration of sun exposure. One report mentions that15 minutes of whole-body exposure to sun enhances vitamin D levels up to 20,000 IU, depending on skin pigmentation, geographic latitude, season, time of day, and on the extent of body attainable UVB.[22]The body surface area required for adequate 25(OH)D synthesis is not entirely known. However, the recommended body surface area required for maintaining adequate 25(OH)D levels is >27%, and the body surface area exposed to the sun is the main source related to 25(OH)D level.[23]

Dietary vitamin D intake could be one of the factors associated with vitamin D levels. Our study suggests that there is no significant difference between vitamin D intake and vitamin D serum levels.[24] Gurr et al obtained similar findings, that there was no significant relation between dietary vitamin D intake and serum 25(OH)D level.[25] In fact, dietary consumption of vitamin D, as well as vitamin D levels, were low in both groups. Even though average vitamin D intake differed significantly based on place of residence, the average vitamin D of those from either coastal or mountainous regions was under Indonesia RDA (<15 µg/day). The total mean of vitamin D intake in the coastal and mountainous areas was 9.04±5.92 µg/day and 6.55±3.92 µg/day respectively. Besides sun exposure, vitamin D can be obtained from food, such as liver, beef, eggs, milk and various types of fish.[26] It appears that the pregnant women who lived in coastal areas had easier access to food, but availability of fish does not guarantee adequate vitamin D intake.

Vitamin D in food is rare, but a variety of fish and dairy products, such as vitamin D-fortified milk, are recommended as good sources of vitamin D.[27] In 2015, total fish consumption in Indonesia rose from 37-38 kg per capita per year, to 41 kg per capita per year. Nevertheless, the level of fish consumption in Indonesia is still far behind neighbouring countries such as Malaysia (70 kg/capita/year), Singapore (80 kg/capita/year), and Japan (approximately 100 kg/capita/year).[28] Indonesia's level of milk consumption also remains very low compared with other ASEAN countries, at an annual average of just 14.3 litres per person, while Filipinos consume22.1 litres, Malaysians 50.9 litres and Thais 33.7 litres).[29] Based on a study by Charatcharoenwitthaya et al, consumption of milk fortified with vitamin D affects serum 25(OH)D levels.[11]In rural Southern Thailand, dairy products are under-consumed, with a mean intake of only 5% of the recommended level.

This appears to be because of insufficient money to buy milk, rather than food preference.[30]

Lack of calcium and vitamin D-rich food consumption may be overcome with a good dietary pattern. Vitamin D occurs naturally in fish (particularly salmon, sardines and tuna), liver, in some plants, and in fortified foods such as milk, yogurt, cheese and breakfast cereal, but it is not present in significant amounts in meat, poultry, dairy products, fruit or vegetables.[31] In this study, the prevalence of vitamin D insufficiency was 61.25% and more than 85% had inadequate vitamin D intake. Increasing of the fortification of food may be one of the solutions to meet daily vitamin D requirements and to address population-wide nutrient deficiencies.[32] Vitamin D was not taken by any of the participants as a single nutrient supplement. Therefore, we suggest that pregnant women should be encouraged to consume more vitamin D-fortified food and vitamin D supplements, and to have more exposure to sunlight.

The predictive factors for vitamin D level in this study were based on multivariate logistic regression analysis. The results for women living in mountainous areas (p=0.01, OR: 0.4, 95% CI 0.17-0.93), working indoors (p=0.16, OR: 3.98, 95% CI 0.58-28.2), and engaged in low-middle physical activity (p=0.02, OR: 0.26, 95% CI 0.08-0.85) were similar to those found by Sari et al. The correlation between vitamin D and the independent variables tends to be weak, as each of these correlations has r value <0.4. Age, duration of sun exposure, pre-pregnancy weight and BMI were associated with maternal vitamin D levels.[33]This study has found that in general subjects who were younger, had lower sun exposure, and lower pre-pregnancy BMI and body weight, were significantly associated with maternal vitamin D levels. Our study is similar to that of Pratumvinit et al, who found that the prevalence of vitamin D deficiency increased in women who had a lower pre-pregnancy BMI.[19]However, Bodnar et al reported that pregravid obese women (BMI ≥30 kg/m^2) at 4-22 week had lower mean serum 25(OH)D (56.5vs. 62.7nmol/L; p<0.05) compared to lean women (BMI <25 kg/m^2) and higher prevalence of vitamin D deficiency (61% vs 36%; p <0.01).[34] Pregnant women with low vitamin D levels may have a lower pre-pregnancy BMI (Underweight vs Obese, 25.59±9.99 vs 32.67±13.62 ng/mL; p=0.038) and weight (r=0.20; p=0.04), but gained more weight during pregnancy compared to those with a sufficient vitamin D level (10.5±4.6 vs 9.5±6.6 kg; p=0.266).This higher weight gain may be contributed to lower circulating levels of 25(OH)D.

Pregnant women in coastal and mountainous areas are advised to modify their lifestyle and consume food containing a high amount of vitamin D. Beneficial lifestyle choices include staying outdoors, modifying clothing style and engaging in physical exercise, which are determinants of sun exposure. A LASA study conducted on 1255 community-dwelling older men and women showed that total daily physical activities, both indoors and outdoors, revealed in kcal/day were positively related to 25-hydroxyvitamin D levels. Some types of outdoor physical activities with a high intensity, such as gardening and cycling, were associated with 25(OH)D. Exposure to sunlight during outdoor physical activity can explain the relationship between physical activities as determinants of vitamin D level.[35]

Our study has several strengths. First, it represents dietary habits and serum levels. In addition, it has also examined the factors associated with vitamin D level, such as the lifestyles of pregnant women, length of sun exposure, working status, physical activities status, and sunscreen use. Our study may be the first to evaluate the 25-hydroxyvitamin D levels of third trimester pregnant women living in West Sumatra. However, some limitations should be addressed in future investigations. One limitation of our study was the lack of a sample size that could accurately represent the vitamin D levels of the entire West Sumatran population. The sample size of this study is small and more studies need to be conducted with larger sample sizes. Another limitation was the diagnostic criteria we used to assess vitamin D levels. We used Endocrine Society Clinical Practice Guidelines, although different results may be derived if different guidelines were adopted. 25-hydroxyvitamin D serum level was not longitudinally measured throughout gestation.

Conclusion

Low vitamin D levels were common among pregnant women in West Sumatra, Indonesia. Additional intake of vitamin D from supplements may be important to meet their recommended dietary allowance.

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AUTHOR DISCLOSURES

The authors declare that they have no conflicts of interest.

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Table 1. Study variables of the pregnant women according to maternal vitamin D status (n=160)

Variables[†]	Deficiency-insufficiency status[‡]		Sufficiency status[‡]		*p* value[*]
	Value (%)	Mean (SD)	Value (%)	Mean (SD)	OR (95% CI)
N (%)	98 (61.2)		62 (38.2)		
Age, years (n, %)		28.5 (5.6)		31.1 (6.3)	0.007[**]
Age group					0.015[**]
<20	3.1		3.2		
20-29	57.1		33.9		
≥30	39.8		62.9		
Ethnic group (%)					0.531
Non-Minangkabau	91.8		95.2		0.57 (0.14-2.2)
Minangkabau	8.2		4.8		
Education level (%)					0.582
Low-middle level	34.7		40.3		0.78 (0.4-1.5)
High level	65.3		59.7		
Place of residence (%)					0.165
Coastal areas	52		64.5		0.59 (0.3-1)
Mountainous areas	48		35.5		
Household income, IDR		3523469 (4559549)		2512903 (1296209)	0.014[**]
Category of Income per month					1.00
<minimum wages (IDR 1800)	32.7		32.3		1.1 (0.5-2)
≥minimum wages (IDR 1800)	67.3		67.7		
25OHD serum levels, ng/mL		22.4 (5.2)		39.5 (10.5)	0.001[**]
Vitamin D intake, µg/day		8.43 (5.9)		7.94 (4.5)	0.584
Status of vitamin D intake (%)					0.514
Adequate	16.3		11.3		0.6 (0.25-1.6)
Inadequate	83.7		88.7		
Calcium intake, mg/day		828.7 (427.2)		794.6 (440.3)	0.628
Status of calcium intake (%)					1.00
Adequate	12.2		11.3		0.9 (0.33-2.45)
Inadequate	87.8		88.7		
Week of pregnancy, weeks		32.06 (3.5)		31.37 (3.4)	0.225
Parity status (%)					0.077
Nulliparous	40.8		25.8		1.9 (0.9-3.9)
Multiparous	59.2		74.2		
Pre-pregnancy weight, kg		49.6 (9.07)		53.4 (9.3)	0.01[**]
Pre-pregnancy BMI, kg/m^2		21.18 (3.5)		22.9 (3.5)	0.003[**]
Weight gain, kg		10.5 (4.6)		9.5 (6.6)	0.228
Height, cm		152.9 (4.9)		152.6 (5.7)	0.79
Category of maternal height (%)					1.00
Normal (≥145)	96.9		96.8		1.05 (0.17-6.5)
Stunted (<145)	3.1		3.2		
MUAC, cm		26.27 (3.39)		27.7 (3.25)	0.007*
Category of MUAC (%)					0.132
Underweight	15.3		9.7		
Normal	22.4		12.9		
Overweight	62.2		77.4		
Blood pressure, mmHg					
Systolic		115.28(12.7)		116.11 (11)	0.669
Diastolic		74.12 (9)		75.16 (8.2)	0.463
Blood pressure status (%)					0.34
Normal	83.7		90.3		0.5 (0.2-1.4)
Hypertension	16.3		9.7		
Sun exposure, hours		54.65 (31.6)		78.95 (70.2)	0.003[**]
Sun exposure status (%)					0.164
<1 hour	67.3		56.5		1.59 (0.82-3.06)
≥1 hour	32.7		43.5		

BMI: body mass index; SD: standard deviation.

[†]Categorical and continuous variables were expressed as the numbers (%) and as the mean (SD).

[*]*p* values are based on chi-square test or Fischer's exact for categorical variables and independent sample t test for continuous variables. Differences were considered statistically significant at $p < 0.05$ level.

[‡]Maternal vitamin D status defined as 25(OH)D less than 30 ng/mL and 25(OH)D levels >30 ng/mL;

[**]*p* value significant at $p < 0.05$ level.

Table 1. Study variables of the pregnant women according to maternal vitamin D status (n=160) (cont.)

Variables[†]	Deficiency-insufficiency status[‡]		Sufficiency status[‡]		*p* value[*]
	Value (%)	Mean (SD)	Value (%)	Mean (SD)	OR (95% CI)
Physical activity (%)					1.00
Low-middle	8.2		16.1		0.4 (0.17-1.2)
High	91.8		83.9		
Working status (%)					0.028[**]
Indoor	98		88.7		6.1 (1.2-3)
Outdoor	2		11.3		
Sunscreen application (%)					0.042[**]
Yes	81.6		66.1		2.2 (1.09-4.74)
No	18.4		33.9		

BMI: body mass index; SD: standard deviation.
[†]Categorical and continuous variables were expressed as the numbers (%) and as the mean (SD).
[*]*p* values are based on chi-square test or Fischer's exact for categorical variables and independent sample t test for continuous variables. Differences were considered statistically significant at $p< 0.05$ level.
[‡]Maternal vitamin D status defined as 25(OH)D less than 30 ng/mL and 25(OH)D levels >30 ng/mL;
[**]*p* value significant at $p< 0.05$ level.

Table 2. Dietary intake of subjects (n=203)

Variables	Min-Max	Mean±SD
Energy (kcal)	1577.97-3484.23	2443.77±479.99
Total Carbohydrate (g)	137.07-440.42	266.57±57.98
Total Protein (g)	54.37-198.52	106.33±30.79
Total Fat (g)	56.08-180.29	109.56±26.09
Calcium (mg)	181.73-2993.79	784.88±409.77
Vitamin D (μg)	0.39-29.28	7.92±5.26

SD: Standard deviation.

Table 3. Correlation Analysis among 25(OH)D Levels with other Variables

Variables	R[†]	*p* value[*]
Age	0.22	0.004[*]
Duration of sun exposure	0.20	0.017[*]
Vitamin D intake	0.03	0.68
Weight gain	0.03	0.73
Pre-pregnancy weight	0.20	0.04[*]
Pre-pregnancy BMI	0.26	0.001[*]
Systolic blood pressure	0.005	0.52
Diastolic blood pressure	-0.12	0.88

BMI: Body mass index.
[†]Data was analyzed by Pearson's Correlation with significant value $p<0.05$. Correlation: weak ($r<0.4$), moderate ($r=0.4-0.6$), strong ($r>0.6$).
[*]*p* value significant at $p<0.05$ level.

Table 4. Multivariate analysis of predictive factors with vitamin D deficiency in 160 pregnant women

Variables[†]	B	OR (95% CI)	*p* value
Age group (<20 years versus >20 years)	0.6	1.13 (1.1-2.8)	0.13
Place of residence (coastal versus mountainous areas)	-0.89	0.4 (0.17-0.93)	0.03[*]
Physical activity (low-middle versus high)	-1.32	0.26 (0.08-0.85)	0.02[*]
Working status (indoor versus outdoor)	1.38	3.98 (0.56-28.2)	0.16
Pre-pregnancy BMI	0.11	1.11 (0.98-1.27)	0.08
Vitamin D intake (inadequacy versus adequacy)	0.013	1.01 (1-1.03)	0.12
Sunscreen use (yes versus no)	0.45	1.6 (0.65-3.13)	0.31

[†]Multivariate logistic regression was made to analyze predictive factors associated with vitamin D deficiency by exploring variables with P<0.025 without multicollinearity from univariate analysis.
[*]*p* value significant at *p*<0.05 level.

Table 5. Association between 25(OH) D levels, vitamin D and calcium intake status and place of residence

Variables[†]	Place of residence (n=203)				*p* value[*]	*p* value[**]
	Coastal		Mountainous			
	%	Mean (SD)	%	Mean (SD)		
Vitamin D intake (μg/day)		9.04 (5.92)		6.55 (3.92)		0.001
Adequate	18.8		6.6		0.02	
Inadequate	81.2		93.4		(OR: 0.3; 0.1-0.8)	
Calcium intake (mg/day)		812.385 (434.840)		751.043 (376.263)		0.09
Adequate	12.5		7.7		0.37	
Inadequate	87.5		92.3		(OR: 0.6; 0.2-1.5)	
25(OH)D levels (ng/mL)		31.47 (11.71)		25.87 (10.20)		0.002
Deficiency-insufficiency	52		64.5		0.16	
Sufficient	48		35.5		(OR: 0.6; 0.3-1.1)	

SD: standard deviation; 25(OH)D: 25-hidroxyvitamin D.
[†]Categorical variables were expressed as numbers and percentages and analyzed using a chi-square test; Continuous variables were performed as mean (SD) and analyzed using independent sample t test; 25(OH)D levels from 160 subjects.
[*]Chi-squared test with *p* value significant at *p*<0.05 level.
[**]Independent sample *t* test with *p* value significant at *p*<0.05 level.

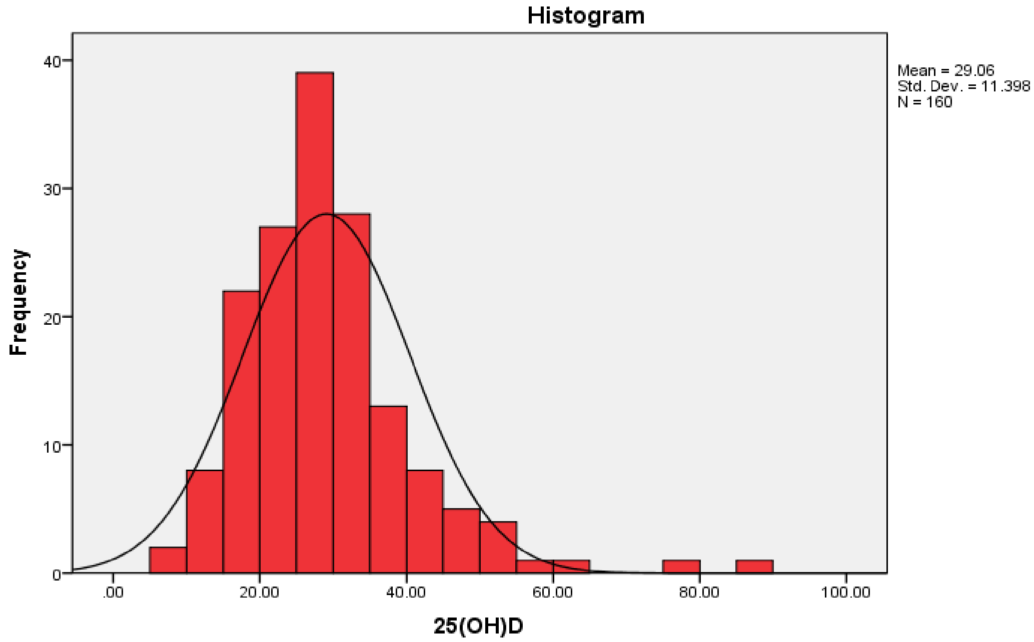

Figure 1. Frequency distribution of the 25(OH)D measurements. 25(OH)D concentration is shown as a skewed distribution, and most of the values are less than 30 ng/mL.

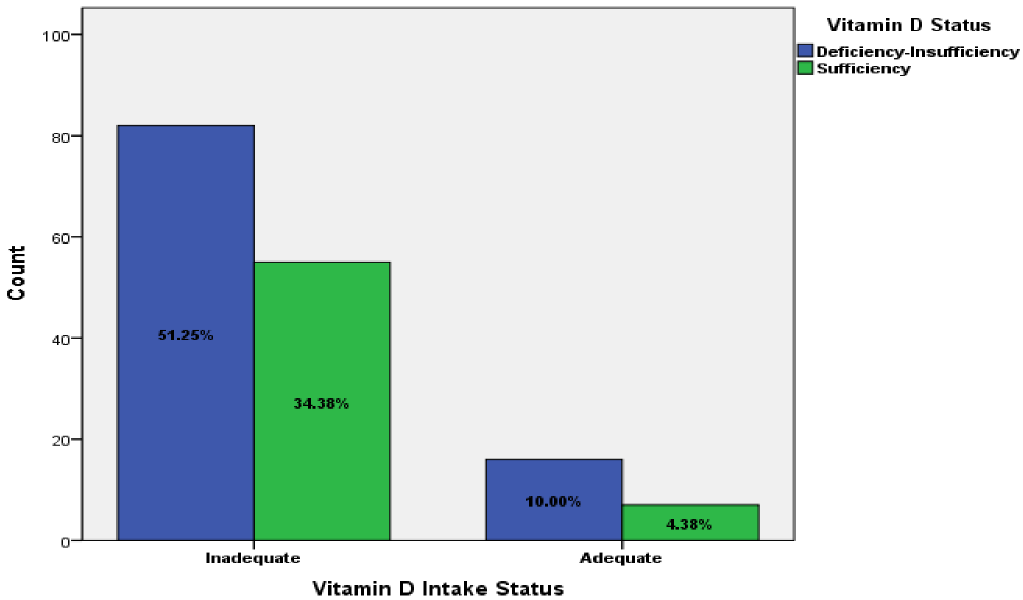

Figure 2. Vitamin D status among pregnant women, according to maternal vitamin D intake status. Vitamin D status was indicated as the mean value of the serum 25-hydroxyvitamin D (25(OH)D) level as deficiency-insufficiency (≤30 ng/mL) and sufficiency (>30 ng/mL). Maternal vitamin D intake was classified as Inadequate (<15 µ/day) (n=137) and adequate (≥15 µ/day) (n=23). There was no statistically association between vitamin D intake status and serum levels (*p*>0.05).