The Association of Maternal Vitamin D Status during Pregnancy and Neonatal Anthropometric Measurements: A Longitudinal Study in Minangkabau Pregnant Women, Indonesia

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(Received June 20, 2019)

Summary

The prevalence of vitamin D deficiency (VDD) appears to be increasing. VDD during pregnancy has been associated with several adverse pregnancy outcomes. This study aimed to investigate the association between VDD and fetal anthropometric measurement. This prospective cohort study consisted of 232 pregnant women in their first trimester who were recruited at the antenatal clinics and they maintained to be subjects of the study until their delivery time. Serum 25-hydroxyvitamin D (25(OH)D) concentration was measured at first and third trimester using enzyme-linked immunosorbent assay. The prevalence of VDD in the first-trimester was 82.8%. Mean of 25(OH)D concentration in the third-trimester was significantly higher than in the first trimester (14.00 (6.98) vs. 21.22 (10.17) ng/mL). After adjusting age, pre-pregnancy BMI, and gestational age at delivery, it was found that VDD during pregnancy was not significantly associated with neonatal anthropometry (p>0.05). It was concluded that VDD was common in a tropical country. Large, well designed, multicentre observational studies are required to determine whether VDD enhances the risk of adverse pregnancy outcomes.

Key Words

pregnancy, 25-hydroxy, vitamin D, vitamin D Status, neonatal anthropometry, West Sumatra

Vitamin D status during pregnancy has an important role for maternal health and foetal growth (1). The foetal’s vitamin D level is totally dependent on their mother’s level of 25-hydroxyvitamin D (25(OH)D). The marker of maternal vitamin D status is the circulating concentration of 25(OH)D (2). Vitamin D status correlates with vitamin D intake and sun exposure of ultraviolet B (UVB) (3).

Deficiency of vitamin D status is common during pregnancy and become the worldwide public health concern (4). Vitamin D deficiency (VDD) may cause low foetal growth and development during pregnancy. Recent studies found that maternal vitamin D status was increasingly associated with adverse pregnancy outcomes such as an increased risk of lower birth weight, type 1 diabetes, foetal miscarriage, preeclampsia, gestational diabetes, bacterial vaginosis, and impaired childhood growth and development in the offspring (5–11). Henceforth, vitamin D status during pregnancy is a main concern to maintain pregnancy health.

Fulfilling the requirements for normal vitamin D level during pregnancy is important and recognizing the risk factors of VDD and ensure primary diagnosis of vitamin D status during pregnancy is essential to prevent maternal complications (1). Therefore, this study was done to investigate the association between maternal vitamin D status during pregnancy and neonatal anthropometry measurements. Other determinants such as maternal characteristics (age, maternal working status, pre-pregnancy BMI), pregnancy profile (parity, gestational age at birth), and lifestyle (occupation status, supplement consumption and length of outdoor activity status) factors were also measured.

MATERIALS AND METHODS

Subjects and study design. Data were taken from Vitamin D on Pregnant Mothers (VDPM) Cohort Study from singleton pregnant women in five cities of Padang, Padang Pariaman, Payakumbuh, Lima Puluh Kota, and Pariaman, West Sumatra Province, Indonesia (12–14). All subjects were from ethnic group indigenous to the Minangkabau highlands of West Sumatra, Indonesia, or also known as Minang.

Subject recruitment was based on registry from the community midwife at public health centres in each city. Subjects who were healthy, had singleton pregnancy and met the inclusion and exclusion criteria were contacted and recruited. The inclusion criteria were 1) resident of the city, 2) willing to comply with the whole study procedure (follow up process) by sign-
ing informed consent, 3) pregnant at less than 13 wk of gestation at recruitment, and 4) have a singleton, alive, normal fetus. The exclusion criteria were women with multiple pregnancies, preeclampsia, miscarriage or still birth, chronic illness like diabetes, hypertension, cardiovascular disease, or hypothyroidism, taking drugs that can interfere with vitamin D metabolism and also fetuses with any congenital abnormalities or congenital.

Data collection. After giving written concern formed, each subject was interviewed for demographic data, lifestyle, and pregnancy profile by research assistant. All records were kept as an individual case report file in data storage for later entry. The selected subjects were followed up from the first trimester (T1), second trimester (T2), third trimester (T3), and at delivery. Blood samples were taken during T1 and T3. Neonatal anthropometry measurements such as birth weight, birth length, head circumferences, were recorded after delivery process.

The lifestyle questionnaire included questions about occupation status, supplement consumption, and the duration of sun exposure. Sun exposure was calculated as an index hours per week the women spent outdoors exposed to sunlight. We then divided the exposure into two groups (≤1 h inadequately or >1 h adequately). Supplement consumption was recorded as a category of taking supplement before pregnancy routinely or not. The occupation status was determined from workplace in outdoor or indoor area.

Maternal and neonatal anthropometric measurements. We recruited nutritionists for interviewing and measuring subjects using standardized protocols performing anthropometric measurements of mother-infant pairs. In this study, gestational age at birth was calculated from estimated gestational age examined by obstetricians or midwives using transabdominal ultrasound performed or date of last menstrual period in the obstetricians or midwives using transabdominal ultrasound performed or date of last menstrual period in the absence of ultrasound at the Maternal Clinic or Hospital. Maternal and neonatal weight were measured to the nearest 100 grams using an electronic scale (Seca 803, Seca GmbH, Co. kg, Hamburg, Germany). Maternal and neonatal height were measured to the nearest one millimeter using a stadiometer (OneMed-Medicom stature meter, YE05.05.VA.1022, Jakarta, Indonesia) and mid-upper arm circumference (MUAC) were measured by using a meter line and rounded to the nearest one millimeter (Medline-OneMed Medicom, Jakarta, Indonesia). Post partum BMI was calculated and classified according to World Health Organization guidelines for Asian populations (<18.5 kg/m² underweight; normal, 18.5–23.49 kg/m²; overweight, 23.5–24.99 kg/m²; Pre-obese, 25–29.99 kg/m²; Obese, ≥30 kg/m²) (15).

Serum 25(OH)D concentration measurements. Maternal blood was collected two times at <13 and >27 wk of gestation. For measuring vitamin D levels, 5 ml of venous blood was obtained and stored as blood serum. Serum samples were stored at −70°C until they were analyzed for 25(OH)D. Serum 25(OH)D was assayed by using xMark Microplate Spectrophotometer (Bio-Rad Laboratories Inc, Hercules, California, USA). Serum concentration of 25(OH)D was assessed using ELISA from Diagnostic Biochemistry Canada (DBC) 25-Hydroxyvitamin D ELISA kit (DBC, London, Ontario Canada). The assay has a sensitivity of 5.5 ng/mL, an intra- and inter-assay coefficient of variation of 5% and 8.1%, respectively. The serum concentration of 25(OH)D was considered in the analyses: ≤12 ng/mL (vitamin D deficient), 12–19 ng/mL (vitamin D insufficient), ≥20 ng/mL (vitamin D sufficient) according to Institute of Medicine (IOM) guideline of vitamin D status (16).

Ethic statements. This study protocol was conducted in accordance with the declaration of Helsinki and approved by the Ethics Committees of Medical Faculty, Andalas University (No. 262/KEP/FK/2016). All women gave written informed consent form for themselves prior to data collection in this study.

Statistical analysis. We used the SPSS statistical package (version 23; SPSS Inc., Chicago, IL, USA) for the statistical analysis. Results from the descriptive analyses were presented as means and standard deviations (SD) for continuous variables and as percentages for categorical variables. Linear regression model was used to assess the association of 25(OH)D concentration and neonatal anthropometry measurements. We included maternal age, working status, pre-pregnancy BMI, parity status, and sun exposure status as confounding variables (12, 17). Results were presented as linear regression mean (SD) and logistic regression Odds Ratio (OR) and 95% CI. OR was determined to measure if vitamin D insufficiency could predict the probability of low weight, length, and head circumference at birth. p-value <0.05 was considered as significant.

RESULTS

Characteristics of the study subjects

From June 2017 until April 2018, a total of 239 singleton Minangkabau pregnant women had been screened and recruited. Since the parents follow up process is still ongoing, only 195 subjects were followed up until they delivered and thus included in this report (Fig. 1). Pregnant women with healthy singleton pregnancy in the first trimester of pregnancy who met the inclusion and exclusion criteria were 279 subjects. Out of 276, 44 subjects rejected to comply and follow this study procedures. Furthermore, 232 subjects attended follow up during the gestation. However, the final sample size of this study was 184 subjects (mother and infant pairs) who successfully complete this study procedures.

In total, 186 women had blood samples collected in both T1 and T3. The maternal characteristics of our study were shown in Table 1. The mean of birth weight was 3.204.87 (495) g, birth length 48.56 (2.875) cm, head circumference 33.89 (2.52) cm, and mean of gestational age at delivery was 38.88 (1.91) wk.

In this study, 59 subjects (35.40%) were in the high-risk age group (<20 y old or ≥35 y old). Maternal nutritional status as defined by pre-pregnancy BMI was

| Maternal Characteristics | Mean (SD) |
|-------------------------|----------|
| Age (y)                 | 27.3 (3.7) |
| BMI (kg/m²)             | 23.5 (3.2) |
| Pre-pregnancy BMI       | 23.5 (3.2) |
| Parity                   | 1.1 (1.2)  |
| Occupation status       | Full-time |
| Sun exposure status     | ≥1 h/week |
within normal range. Maternal nutritional status based on WHO classification were underweight (12.50%), normal weight (43.50%), overweight (11.60%), pre-obese (19.80%), and obese (12.50%). The mean of total weight gain in this study was 8.64 (3.01) kg. None of the study subjects followed a special diet.

Maternal 25(OH)D serum concentration

In the first trimester, the maternal serum vitamin D values ranged from 3.00 to 49.29 ng/mL with the average 14.00 (6.98) ng/mL. Maternal vitamin D status in the first trimester showed that 88 (47.30%) were vitamin D deficient, 66 (35.50%) were insufficient and 32 (17.20%) were sufficient.

In the third trimester, the maternal serum vitamin D values ranged from 4.44 to 60.84 ng/mL with the average 21.22 (10.17) ng/mL. Maternal vitamin D status in the third trimester showed that 35 (18.80%) were vitamin D deficient, 64 (34.40%) were insufficient, and 87 (46.80%) sufficient. A total of 186 pregnant women had complete this study. There was a significant different proportion between maternal vitamin D status at first and third trimester during pregnancy ($p<0.001$) (Fig. 2).

The association between maternal vitamin D status and neonatal anthropometric measurements

We tested the relationship between maternal vitamin D status during pregnancy and neonatal anthropometric outcomes in terms of birth weight, birth length, and head circumference. Table 2 and Table 3 showed that maternal vitamin D status both at T1 and T3 had no significant association with neonatal anthropometry and with maternal weight gain and pre-pregnancy BMI ($p>0.05$). Similarly, maternal vitamin D status has no significant association with the outcomes low birth weight, short birth length, and small head circumference (Table 4).

Pregnancy loss

As shown in Fig. 1, the total subjects lost during follow-up were 18.82%; of which 0.34% were due to miscarriage and 8.48% because the subjects did not comply with the research procedure. The rate of late pregnancy loss was 10.34%. There were 3 cases of preterm birth, 8 cases of stillbirth, and 14 cases of miscarriage.

DISCUSSION

The aimed of our study was to examine the relationship between maternal vitamin D status during pregnancy and neonatal anthropometric measurement. We were also measuring other determinants as confounding factors such as subject characteristics, pregnancy profile, and lifestyle during pregnancy. The main finding of this study was that there was no association between maternal vitamin D status, either in the first or third trimester, and neonatal anthropometry measurements. To our knowledge, ours is one of the first studies to investigate the associations between vitamin D status and
pregnancy outcomes.

The present study was conducted in West Sumatra province, located a latitude 0.7399˚ S, 100.8000˚ E. Whereas there is abundance of sun exposure almost in a whole year in Indonesia, VDD is highly prevalent in pregnant Indonesia women, particularly in the first trimester of pregnancy.

As located in the tropical region, VDD is common, not only in Indonesia, but also in other South East Asia countries (18–23). More than 80% of our study population had insufficient-deficient vitamin D status. This result showed that maternal VDD status in early pregnancy was similar to the previous study in Indonesia. Bardosono et al., and Judistiani et al., found that 99.6% and 96% first trimester of pregnant women had insufficient-deficient vitamin D status (17, 24).

This finding demonstrates that VDD is likely a major concern of public health problem among pregnant women in Indonesia. Previous finding from Vitamin D on Pregnant Mothers (VDPM) cohort study showed that factors associated with low vitamin D status in early pregnancy were no working status, nulliparous parity status, length of outdoor activity of less than an hour and no taking supplements before pregnancy (12–14). Lifestyle factors had high consideration to meet the requirement of sufficient maternal vitamin D status as sunlight exposure is the major sources of vitamin D (25, 26).

We had no association between maternal weight gain during pregnancy and pre-pregnancy BMI to maternal vitamin D status. Although previous studies suggested that higher pre-pregnancy BMI and gestational weight gain were associated with lower vitamin D status (16). Higher level of BMI or gestational obesity involved in reduced placental transfer of vitamin D to fetus, bioavailability, mother and child vitamin D serum concentration through adipose tissue which is blocking the 25(OH)D concentration and resulting inverse relationship between BMI and vitamin D (19, 27).

Our result indicates that maternal vitamin D status was increased from T1 to T3 of gestation. Insufficient-deficient vitamin D status declined from 82.8% at the T1 to 53.2% at T3. This present study had similar results with Charatcharoenwitthaya et al., who conducted a study in Thailand that the prevalences of VDD were 83.3% and 27.4% in first and third trimesters respectively (18). It was found that increases of macro-

| Description                  | n (%) | Mean (SD) |
|------------------------------|-------|-----------|
| Maternal age, y              | 29.77 | (5.69)    |
| Maternal age groups          |       |           |
| a. <20 y old                 | 7 (3.00) |
| b. 21–30 y old               | 120 (51.70) |
| c. 31–35 y old               | 53 (22.80) |
| d. ≥35 y old                 | 52 (22.40) |
| Maternal education levels    |       |           |
| a. Primary                   | 67 (28.90) |
| b. Secondary                 | 94 (40.50) |
| c. Tertiary                  | 71 (30.60) |
| Maternal working status      |       |           |
| a. Working                   | 74 (31.90) |
| b. Not working               | 158 (68.10) |
| Pre-pregnancy BMI, kg/m²     | 23.45 | (4.56)    |
| Pre-pregnancy BMI            |       |           |
| a. Underweight               | 29 (12.50) |
| b. Normal                    | 101 (43.50) |
| c. Overweight                | 27 (11.60) |
| d. Pre-obese                 | 46 (19.80) |
| e. Obese                     | 29 (12.50) |
| Parity                       |       |           |
| a. Nulliparous               | 59 (25.40) |
| b. Multiparous               | 173 (74.60) |
| Total weight gain, kg        | 8.64  | (3.01)    |
| Duration of sun exposure (min/d) | 60 (53.75) |
| Length of outdoor activity   |       |           |
| a. <60 min/wk                | 110 (47.80) |
| b. ≥60 min/wk                | 121 (52.20) |
| Occupation status            |       |           |
| a. Indoor                    | 175 (75.40) |
| b. Outdoor                   | 57 (24.60) |
| Taking supplement before pregnancy |       |           |
| a. Yes                       | 30 (12.90) |
| b. No                        | 202 (87.10) |
| 25(OH)D at T1, ng/mL         | 14.00 | (6.98)    |
| 25(OH)D at T3, ng/mL         | 21.22 | (10.17)   |
| Gestational age at birth, wk | 38.88 (1.91) |
| Birth weight, gram           | 3,204.87 (495) |
| Birth length, cm             | 48.56 (2.875) |
| Head circumference, cm       | 33.89 (2.52) |

T1, first trimester; T3, third trimester; 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index. Data presented as mean (SD) and n (%).

Fig. 2. Maternal vitamin D status in the first and third trimester (n=186).
Maternal Vitamin D Status and Pregnancy Outcomes

Maternal vitamin D status and pregnancy outcomes may be influenced by nutrient and micronutrient intake, such as vitamin D and calcium intake during pregnancy, which may contribute to the decrease prevalence of vitamin D insufficiency-deficiency status (18, 23). The increase of 25(OH)D concentration in the third trimester might be caused by less sunlight exposure and lower physical activity (13, 32).

We had a negative finding between maternal vitamin D status and neonatal anthropometry outcomes. Neither maternal vitamin D status at T1 nor T3 had a significant association with neonatal anthropometric measurements. Our results had similar findings with a study that measured maternal vitamin D status in multiple times (first and third trimester) and found no association with neonatal birthweight and birth length (33). Other studies also reported the same results (34–36). In contrast, several other studies found an increased risk of poor neonatal anthropometry measurements such as birthweight and birth length (15, 16, 37, 38).

Table 2. The differences between maternal weight gain, pre-pregnancy BMI, and neonatal anthropometric measurement at birth among vitamin D T1 status.

| Maternal vitamin D Status T1 | p-value |
|------------------------------|---------|
| Sufficient | Insufficient | Deficient |
| Neonate anthropometric measurements\(^a\) | | | |
| Birth weight, gram | 3.203.03 (516.29) | 3.272.79 (473.510) | 3.156.38 (502.10) | 0.331 |
| Birth length, cm | 48.21 (2.83) | 48.82 (1.82) | 48.49 (1.47) | 0.588 |
| Head circumference, cm | 34.15 (2.03) | 34.04 (2.00) | 33.69 (2.97) | 0.575 |
| Maternal weight gain\(^b\) | | | |
| Total weight gain, kg | 8.52 (2.95) | 8.63 (2.85) | 8.65 (3.18) | 0.996 |
| Maternal pre-pregnancy BMI\(^c\) | | | |
| Pre-pregnancy BMI, kg/m² | 22.60 (4.60) | 23.41 (3.76) | 23.60 (4.39) | 0.670 |

\(^a\) Live born infants only; \(^b\) Maternal total weight gain was defined as bodyweight T3–pre-pregnancy bodyweight; \(^c\) pre-pregnancy BMI was categorized by WHO classification (15); Data presented as mean (SD); Maternal vitamin D status was defined as <12 ng/mL (vitamin D deficient), 12–19 ng/mL (vitamin D insufficient), >20 ng/mL (vitamin D sufficient) according to Institute of Medicine (IOM) guideline of vitamin D status (16); All models were adjusted for maternal characteristics (age, maternal working status, pre-pregnancy BMI), pregnancy profile (parity, gestational age at birth), and lifestyle (occupation status, supplement consumption and length of outdoor activity status) factors; T1, first trimester; BMI, body mass index.

Table 3. The differences between maternal weight gain, pre-pregnancy BMI, and neonatal anthropometric measurement at birth among vitamin D T3 status.

| Maternal vitamin D Status T3 | p-value |
|------------------------------|---------|
| Sufficient | Insufficient | Deficient |
| Neonate anthropometric measurements\(^a\) | | | |
| Birth weight, gram | 3.147.09 (458.73) | 3.246.04 (403.14) | 3.242.86 (576.65) | 0.386 |
| Birth length, cm | 48.53 (2.06) | 48.86 (1.89) | 48.11 (5.17) | 0.300 |
| Head circumference, cm | 33.55 (1.89) | 34.21 (1.97) | 33.91 (4.25) | 0.320 |
| Maternal weight gain\(^b\) | | | |
| Total weight gain, kg | 7.02 (2.71) | 7.97 (3.17) | 8.40 (3.020) | 0.413 |
| Maternal pre-pregnancy BMI\(^c\) | | | |
| Pre-pregnancy BMI, kg/m² | 23.61 (4.36) | 23.91 (4.99) | 21.71 (2.86) | 0.101 |

\(^a\) Live born infants only; \(^b\) Maternal total weight gain was defined as bodyweight at T3–pre-pregnancy bodyweight; \(^c\) pre-pregnancy BMI was categorized by WHO classification (15); Data presented as mean (SD); Maternal vitamin D status was defined as <12 ng/mL (vitamin D deficient), 12–19 ng/mL (vitamin D insufficient), >20 ng/mL (vitamin D sufficient) according to Institute of Medicine (IOM) guideline of vitamin D status (16); All models were adjusted for maternal characteristics (age, maternal working status, pre-pregnancy BMI), pregnancy profile (parity, gestational age at birth), and lifestyle (occupation status, supplement consumption and length of outdoor activity status) factors; T1, first trimester; BMI, body mass index.
SGA, lower birthweight, small head circumference, and shorter birth length with low maternal vitamin D status (7, 37–39). These inconsistent findings may be related to sample size, ethnic disparities, study design, and different vitamin D status definition. The important roles of vitamin D during pregnancy are also linked to maternal health that lead to adverse pregnancy outcomes such as gestational blood pressure, pre-eclampsia, cesarean section rate, and preterm labour (11, 40–42).

The strength of this study lies on its population-based design and multiple locations that represented several geographical areas of West Sumatra, the first study in West Sumatra, and its prospective design. The present study provides important evidence that maternal VDD status was common in the early pregnancy and improving awareness during pre-conception to take vitamin D supplement should be considered to prevent VDD in pregnant Minangkabau women populations.

In conclusion, our findings demonstrate the high prevalence of maternal VDD status in healthy pregnant Minangkabau women, West Sumatra. However, VDD during pregnancy was not associated with poor neonatal anthropometry measurements. Future research on maternal vitamin D status during pregnancy and its influence on neonatal anthropometry measurements need to be done with larger sample size to confirm our findings.

Disclosure of state of COI
No conflicts of interest to be declared.

Acknowledgments
ASA is grateful for the six months stay program from PKPI scholarship program and thanks to Dr. Vimal Karani for the guiding during PKPI scholarship program at the Department of Food and Nutrition Science, University of Reading, England, UK. We are grateful to Britannia Proofreading Service for looking English language by a native British proof-reader of Britannia Proofreading Service. This research was funded by the Ministry of Research, Technology and Higher Education of the Republic of Indonesia (Menristekdikti) with project name The Research of Master Program Leading to Doctoral Degree for Excellent Students (PMDSU Batch-2) in the year of 2018 and Indonesian Danone Institute Foundation (Grant No: 007/ROG-D/IDIF/X2016). The views expressed herein are those of the individual authors and do not necessarily reflect those of Indonesian Danone Institute Foundation.

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