Original Article

# Serum 25-hydroxyvitamin D in pregnant women during preterm labor

Nalina Bhupornvivat MD, Vorapong Phupong MD

*Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

**Background and Objectives:** Pregnancy complications hypothesized to be related to vitamin D include preeclampsia, gestational diabetes, low birth weight, preterm delivery, Cesarean section and infectious disease. There have been a few studies which have demonstrated maternal serum vitamin D to be associated with preterm labor. The objective was to evaluate the serum vitamin D concentrations in Thai pregnant women with and without preterm labor and to find the prevalence of vitamin D deficiency and insufficiency in both groups. **Methods and Study Design:** Blood samples were collected from pregnant women with and without preterm labor (matched for gestational age). Serum 25-hydroxyvitamin D (25-OHD) concentrations were measured by chemiluminescence method. **Results:** A total of 60 pregnant women were included into the study, 30 patients in the preterm labor group and another 30 patients in the control group. The serum 25-OHD concentration was 21.0±7.5 ng/mL and the prevalence of vitamin D deficiency was 48.3% in total group of pregnant women. The serum 25-OHD concentrations were not different between the preterm labor and the control groups (20.9±8.4 vs 21.2±6.7 ng/mL, $p$=0.91). The prevalence of vitamin D deficiency and insufficiency were not different between the preterm labor and the control groups (53.3% vs 43.3%, $p$=0.44 and 83.3% vs 90%, $p$=0.45, respectively). **Conclusion:** The serum 25-hydroxyvitamin D concentrations and the prevalence of vitamin D deficiency and insufficiency were not different between the preterm labor and the control groups. The serum 25-OHD could not predict preterm labor in this Thai population.

**Key Words: vitamin D, deficiency, pregnancy, preterm labor, 25-hydroxyvitamin D**

## INTRODUCTION

Vitamin D is a fat-soluble secosteroid hormone. The naturally occurring form of vitamin D in humans is vitamin $D_3$ (cholecalciferol).[1,2] It can be ingested in diet (animal products) or produced in the skin when ultraviolet light interacts with cholesterol derivatives. Vitamin $D_2$ (ergocalciferol) is derived from plant sterols and is the form contained in most vitamin D supplements. The main source (about 95%) is vitamin $D_3$ which is photochemically synthesized in the skin by ultraviolet-B radiation. Both vitamin $D_2$ and $D_3$ circulate in blood bound to vitamin D-binding protein and must be hydroxylated to become active.[3,4] Vitamin D is first hydroxylated in the liver. The resulting metabolite, 25-hydroxyvitamin D (OHD), is very stable. Thus, it is commonly used to measure vitamin D status.[3] Recent studies suggest that the effects of vitamin D deficiency could be much broader than rickets including cardiovascular disease, cancers, diabetes and pregnancy complications. Pregnancy complications hypothesized to be related to vitamin D include preeclampsia, gestational diabetes, low birth weight, preterm delivery, Cesarean section and infectious disease.[3]

Preterm labor, defined as spontaneous labor occurring before 37 weeks gestation, is one common obstetric complication that often leads to preterm birth. Preterm labor precedes 40-50% of preterm births. Preterm birth can be fatal and poses serious health risks for infants.[5] The overall rate of preterm birth is 5-18% of live births.[6] When preterm birth occurs before 34 weeks, neonatal morbidity is high. Neonatal morbidity includes respiratory distress syndrome (RDS), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC) and sepsis.[7,8] The etiology of preterm labor is still inconclusive. Prior preterm birth and short cervical length increase the risk of preterm labor. Recently, there have been a few studies showing low maternal serum vitamin D was associated with preterm labor.[9,10] Although the direct mechanism could not be explained, there is some hypothesis about vitamin D and placental function, calcium homeostasis and inflammatory responses which result in preterm labor.[3] Some studies did not find the association between low maternal serum vitamin D and preterm labor.[11]

Due to the small number of studies between low serum vitamin D concentrations and preterm labor, this study aimed to evaluate the serum vitamin D concentrations in

**Corresponding Author:** Dr Vorapong Phupong, Placental Related Diseases Research Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Rama IV Road, Pathumwan, Bangkok 10330, Thailand.
Tel: 66-2-2564241; Fax: 66-2-256-4257
Email: vorapong.p@chula.ac.th
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Thai pregnant women with and without preterm labor and to find the prevalence of vitamin D deficiency and insufficiency in both groups.

## MATERIAL AND METHODS

This was a cross-sectional study conducted at Department of Obstetrics and Gynecology, King Chulalongkorn Memorial Hospital, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand from July 1, 2014 to May 31, 2015. This study was approved by the Research Ethics Committee of the Faculty of Medicine, Chulalongkorn University. Written informed consent was obtained from all subjects.

Subjects consisted of 60 singleton pregnant women, gestational age between 24 0/7 - 36 6/7 weeks, receiving care at our hospital with 30 subjects in each of the preterm labor and control groups. Gestational age was calculated from last menstrual period (LMP) and confirmed by ultrasonography. If the date of LMP was uncertain, gestational age was calculated from ultrasonography. The preterm labor group consisted of singleton pregnant women, aged 18-49 years, presented at the labor room with preterm labor diagnosed by American College of Obstetricians and Gynecologists (ACOG) criteria.[5] The control group consisted of singleton pregnant women aged 18-49 years, gestational age matched with preterm labor group, and who had antenatal care without labor pain on the same day as the preterm labor group. Pregnant women with medical complications such as bone disease, renal disease, medical conditions associated with calcium, vitamin D and parathyroid hormone metabolism, obstetric complications such as fetal-maternal hemorrhage, infection, congenital anomalies, fetal demise, multiple pregnancy, prior preterm birth and preterm premature rupture of membrane were excluded. After inclusion into the study, blood samples were collected and demographic details, food consumption, prenatal supplements, sun exposure and sunscreen usage were collected using questionnaires. Obstetric and neonatal outcomes were collected from medical records after delivery. Preterm labor was managed according to ACOG guidelines.[5]

Sample size was calculated by using a formula which compares the two means of the samples. The sample size calculation was based upon serum 25-OHD concentrations in both groups obtained from Shibata et al's study.[10] Mean serum 25-OHD were 11.2 ng/mL and 15.6 ng/mL in the preterm labor group and control group, respectively. Thus, we needed 30 women in each group to detect a statistical difference ($\alpha$=0.05, $\beta$=0.1).

### *Sample collection and serum 25-OHD measurement*

For the preterm labor group, venipuncture was performed and blood was collected into clotted tubes at admission to the labor room. For the control group, venipuncture was performed and blood was collected into clotted tubes from gestational age matched pregnant women at the antenatal care clinic. Blood samples were centrifuged at 2,500 rpm for 10 minutes and and the serum was stored at -80°C until assayed. Maternal serum 25-OHD concentrations were measured using an automated, chemiluminescence method (Diasorin, Liasion, Italy). Interassay and intraassay coefficient of variations for serum 25-OHD concentration were 4% and 6%, respectively. Vitamin D deficiency was defined as serum 25-OHD less than 20 ng/mL and vitamin D insufficiency as serum 25-OHD of 21-29 ng/mL.[12] These thresholds are originally for non-pregnant people.

### *Study outcomes*

The primary outcome was the serum 25-OHD concentrations between the preterm labor and the control groups. Secondary outcomes were the prevalence of vitamin D deficiency and insufficiency in the preterm labor and the control groups.

### *Statistical analysis*

SPSS version 17 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Chi-square test and Fisher-exact test for categorical variables, independent *t*-test for continuous variables and Mann-Whitney U test for nonparametric variables were used when appropriate. A $p<0.05$ was considered statistically significant.

## RESULTS

A total of 60 pregnant women who met inclusion criteria, were included into the study and divided into two groups, 30 patients in the preterm labor group and the other 30 patients in the control group.

Baseline characteristics between the two groups are shown in Table 1. There were no significant differences in maternal age, gravidity, parity, gestational age at blood test, BMI and total weight gain.

**Table 1**. Demographic characteristics

| Characteristics | Preterm labor group (n=30) | Control group (n=30) | *p* value |
|---|---|---|---|
| Age (years) | 28.9±7.6 | 30.5±4.9 | 0.35 |
| Gravidity | | | 0.07 |
| Primigravida | 13 (43.3) | 20 (66.7) | |
| Multigravida | 17 (56.7) | 10 (33.3) | |
| Parity | | | 0.11 |
| Nulliparous | 15 (50) | 22 (73.3) | |
| Multiparous | 15 (50) | 8 (26.7) | |
| GA (weeks) | 32.9±2.7 | 32.4±2.5 | 0.40 |
| BMI (kg/m$^2$) | 20.3±3.5 | 22.2±4.1 | 0.06 |
| Total weight gain (kg) | 10.4±4.3 | 11.5±5.2 | 0.37 |

GA: gestational age; BMI: body mass index.
Data presented as mean±SD or n (%).

Table 2 shows a comparison of food consumption and sun exposure between the two groups. There were no significant differences in food consumption and sun exposure.

The serum 25-OHD concentrations, the percentage of vitamin D deficiency and insufficiency are shown in Table 3. The mean±standard deviation of serum 25-OHD concentrations in total cases was 21.0±7.5 ng/mL. There were no significant differences in the serum 25-OHD concentrations, the percentage of vitamin D deficiency and insufficiency between the preterm labor and the control groups.

Gestational age at delivery, mode of delivery and neonatal outcomes are shown in Table 4. Gestational age at delivery and birth weight in the preterm labor group were significantly lower than in the control group [(34.7±3.1 vs 38.3±1.0 weeks, $p$<0.001) and (2629±620 vs 3100±372 grams, $p$=0.001)]. All cases in the control group were delivered at term. Mode of delivery was significantly different between groups with vaginal delivery occurring more in the preterm labor group. There were no statistical differences in neonatal sex and Apgar scores.

## DISCUSSION

In the present study, the serum 25-OHD concentrations, the prevalence of vitamin D deficiency and insufficiency were not different between the preterm labor and the control groups.

The serum 25-OHD concentrations in both preterm labor and control groups did not differ in this study (20.9±8.4 vs 21.2±6.7 ng/mL). This finding was different from the Shibata et al study which found that mothers with threatened premature delivery had a significantly lower 25-OHD concentrations (11.2±3.2 ng/mL) than those in mothers with normal delivery (15.6±5.1 ng/mL).[10] However, all participants in their study eventually delivered at term. This finding was similar to a study by Baker et al that found no difference in the third trimester 25-OHD concentrations between mothers delivering preterm versus normal term babies.[11] It might be explained that maternal vitamin D concentrations in Thai pregnant women are not significantly related to preterm labor. Thota et al found that 25-OHD concentrations in Caucasian and African American pregnant women between term and preterm deliveries were not significantly different. Only serum 1,25 hydroxyvitamin D concentra-

**Table 2**. Food consumption and sun exposure

| | Preterm labor group (n=30) | Control group (n=30) | $p$ value |
|---|---|---|---|
| Eggs consumption (per week) | 6.6±4.7 | 5.4±3.0 | 0.27 |
| Milk consumption (glasses per week) | 10.1±8.0 | 11.7±5.9 | 0.40 |
| Grains consumption (meals per week) | 0 (0, 0) | 0 (0, 2) | 0.19 |
| Sun exposure duration (minutes per week) | 65 (20, 210) | 70 (0, 125) | 0.36 |
| Sunscreen use | 8 (26.7) | 15 (50) | 0.06 |

Data presented as median (interquartile), mean±SD or n (%).

**Table 3.** Serum 25-OHD concentration, vitamin D deficiency and vitamin D insufficiency

| | Total (n=60) | Preterm labor group (n=30) | Control group (n=30) | $p$ value |
|---|---|---|---|---|
| 25-OHD concentration (ng/mL) | 21.0±7.5 | 20.9±8.4 | 21.2±6.7 | 0.91 |
| Vitamin D deficiency | 29 (48.3) | 16 (53.3) | 13 (43.3) | 0.44 |
| Vitamin D insufficiency | 23 (38.3) | 9 (30.0) | 14 (46.7) | 0.18 |

25-OHD: 25-hydroxyvitamin D.
Data presented as mean±SD or n (%)

**Table 4**. Gestational age at delivery, mode of delivery and neonatal outcomes

| | Preterm labor group (n=30) | control group (n=30) | $p$ value |
|---|---|---|---|
| GA at delivery (weeks) | 34.7±3.1 | 38.3±1.0 | <0.001 |
| Range | 24-39 | 37-41 | |
| Mode of delivery | | | 0.002 |
| Vaginal delivery | 24 (80.0) | 11 (36.7) | |
| Cesarean section | 6 (20.0) | 19 (63.3) | |
| Sex | | | 0.60 |
| Men | 15 (50.0) | 17 (56.7) | |
| Women | 15 (50.0) | 13 (43.3) | |
| Birth weight (grams) | 2629±620 | 3100±372 | 0.001 |
| Apgar scores | | | |
| At 1 minute <7 | 3 (10.0) | 0 | 0.24 |
| At 5 minutes <7 | 0 | 0 | NA |

GA: gestational age.
Data presented as mean±SD or n (%).

tions were significantly lower in participants delivering at preterm compared to those delivering at term.[9]

The prevalence of vitamin D deficiency in pregnant women in the present study was 48.3%. This prevalence was less than in a Japanese study (89.5%).[10] The mean serum 25-OHD concentration in total pregnant women in this study was 21.0±7.5 ng/mL. This concentration was higher than the Shibata et al study (14.5±5.0 ng/mL).[10] The difference of these findings might be from the difference between geography and populations. Bangkok, Thailand is at the latitude 13° N while Tokai, Japan is at the latitude 35.5° N meaning that Thai pregnant women have more sun exposure than the Japanese. This prevalence was also different from a British study.[13] where the prevalence of vitamin D deficiency was 36%.

The role of vitamin D in preterm labor can be explained by its importance in regulating cell-mediated immune responses. Thus, vitamin D insufficiency or deficiency during pregnancy may enhance inflammation in pregnant women and increase the risk of preterm birth.[3,4] However, in this study, the prevalence of vitamin D deficiency and insufficiency were not different between both preterm labor and control groups (53.3% vs 43.3%, and 30% vs 46.7%, respectively). This finding may confirm that vitamin D is not associated with preterm labor in our population.

The strength of this study was that we compared serum 25-OHD concentrations between preterm labor cases and control cases with the same gestational age without labor pain. Gestational age between the preterm labor and the control groups was matched and blood samples were collected in the same period. All cases in the control group delivered at term. Limitations of the study were questionnaires regarding food consumption and sun exposure that derived from recall memory of patients. This may have led to recall bias. However, there were no statistical differences in the issue of food consumption and sun exposure between groups in this study.

In conclusion, the serum 25-OHD concentration and the prevalence of vitamin D deficiency and insufficiency were not different between the preterm labor and the control groups**.** The serum 25-OHD could not predict preterm labor in this Thai population.

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**AUTHOR DISCLOSURES**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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