Clinical Research

Vitamin D₃ levels in the maternal serum, cord blood, and placenta of preeclamptic pregnant women

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ABSTRACT

BACKGROUND Preeclampsia is affected by oxidative stress, a free-radical produced as a by-product of endothelial damage, and antioxidant imbalance, such as vitamin D₃. This study was aimed to compare the vitamin D₃ levels in the placenta, cord blood, and maternal serum between patients with and without preeclampsia.

METHODS This cross-sectional study included 86 patients from Cipto Mangunkusumo Hospital and Tangerang District Hospital, in which 47 had preeclampsia (13 early-onset and 16 late-onset preeclampsia cases) and 39 had no preeclampsia. The placenta, cord blood, and maternal serum were taken after labor, then were analyzed according to preeclampsia and non-preeclampsia; furthermore, the preeclampsia group was analyzed in a subgroup of early- and late-onset preeclampsia. This is analyzed with either unpaired t-test, Mann–Whitney U test, or Kruskal–Wallis test.

RESULTS The maternal serum, cord blood, and placental tissue vitamin D₃ levels (16.30 [6.20–49.00], 11.80 [3.50–38.60], and 49.00 [22.00–411.00] ng/ml, respectively) of the preeclampsia group were similar to those of the non-preeclampsia group (13.50 [4.80–29.20], 11.70 [1.00–28.80], and 43.40 [11.80–153.00] ng/ml, respectively) (p = 0.459, 0.964, and 0.354, respectively). However, the placental tissue vitamin D₃ levels in early-onset preeclampsia (79.00 [36.00–411.00] ng/ml) were higher than those in late-onset preeclampsia (40.00 [22.00–171.00] ng/ml) (p = 0.006).

CONCLUSIONS The vitamin D₃ levels between patients with and without preeclampsia were similar. However, the placental tissue vitamin D₃ levels in early-onset preeclampsia were higher than those in late-onset preeclampsia, possibly because of the different pathophysiology between early- and late-onset preeclampsia.

KEYWORDS cord blood, serum, placenta, preeclampsia, vitamin D₃

Preeclampsia and preterm deliveries play a huge role in maternal morbidities and mortalities, with a global incidence of 3–4%. In Cipto Mangunkusumo Hospital, the incidence of severe preeclampsia and eclampsia is 16.3%, with maternal mortality rates of 1.9% and perinatal mortality rates of 9.9%. Generally, preeclampsia is caused by uteroplacental and cord ischemia in which placental implantation exhibits defects due to an abnormal trophoblast invasion in the spiral artery. Consequently, the spiral artery manifests vasospasm, causing hypertension, and inducing endothelial dysfunction. Ultimately, necrosis, bleeding, and multiple-organ failure occur, characterizing preeclampsia. The damage caused by preeclampsia is hypothesized to be a two-step process: placental hypoxia and then free-radical production.
caused by oxidative stress. In the second step, antioxidants play an important role to counteract free radicals.⁶

The 25-hydroxyvitamin D₃ (25-OH D₃) is an important micronutrient that is associated with pregnancy starting from the preconception to the perinatal period because of its role in the calcium and bone mineral homeostasis, immune system modulation, anti-inflammatory effect improvement, and endothelial function repair.⁷ Decidual natural-killer cells supplemented with 25-OH D₃ can reduce granulocyte-macrophage colony-stimulating factor 2, tumor necrosis factor, and interleukin-6, which predicts early-onset sepsis in premature neonates.⁷,⁸ Hypovitaminosis of 25-OH D₃ is often found in pregnancies, with a prevalence of 99–100% in Indonesia in 2015, although this country receives an adequate ultraviolet B exposure across the year.⁹,¹⁰

The role of 25-OH D₃ in predicting the outcomes of pregnancies and neonates remains unclear, especially its role in preeclampsia. Therefore, 25-OH D₃ supplementation in preeclampsia is controversial. In several studies, 25-OH D₃ plays a role in preeclampsia pathogenesis for placental insufficiencies, abnormal angiogenesis, and hypertension; however, conflicting results show that 25-OH D₃ decrement is not a cause but rather an effect of preeclampsia, considering that the placenta metabolizes the active form of 25-OH D₃.¹¹,¹² For this reason, we aimed to investigate and compare the 25-OH D₃ levels in pregnancies complicated with preeclampsia versus normal pregnancy and in early- and late-onset preeclampsia as a subgroup analysis.

**METHODS**

This cross-sectional study consecutively recruited 86 patients between January 2017 and March 2018 in the Labor and Delivery Service of Cipto Mangunkusumo Hospital and Tangerang General Hospital. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No: 0189/UN2.F1/ETIK/2018) and all participants provided an informed consent for their participation. The inclusion criteria were pregnant women diagnosed with preeclampsia, gestational age of at least 20 weeks, singleton pregnancy, and agreed to participate in this research. The sub-group analysis was done among early-onset preeclampsia, defined as onset of preeclampsia between 20 to 34 weeks and late-onset preeclampsia, defined as onset of preeclampsia above 34 weeks. Conversely, the exclusion criteria were pregnancies

| Variable                                      | Preeclampsia, mean (SD) (N = 47) | Non-preeclampsia, mean (SD) (N = 39) | P     |
|-----------------------------------------------|----------------------------------|--------------------------------------|-------|
| Age (years)                                   | 32.52 (7.17)                     | 29.95 (6.00)                        | 0.091*|
| Gestational age (weeks), median (min–max)     | 36.0 (28–40)                     | 38.5 (31–41)                       | <0.005*|
| Types of delivery, n (%)                      |                                  |                                      | 0.25* |
| Vacuum extraction                             | 0 (0)                            | 4 (10)                              |       |
| Cesarean section                              | 37 (79)                          | 16 (41)                             |       |
| Spontaneous delivery                          | 10 (21)                          | 19 (49)                             |       |
| BMI (kg/m²)                                   | 26.38 (4.75)                     | 26.73 (4.81)                       | 0.757*|
| BMI category, n (%)                           |                                  |                                      | 0.025*|
| Underweight                                   | 3 (7)                            | 1 (3)                               |       |
| Normal                                        | 8 (17)                           | 8 (21)                              |       |
| Overweight                                    | 7 (15)                           | 7 (18)                              |       |
| Obesity                                       | 28 (61)                          | 22 (58)                             |       |
| Maternal upper arm circumference (cm)         | 27.73 (2.70)                     | 26.71 (3.49)                       | 0.911*|
| Birth weight (g)                              | 2,262 (857.90)                   | 2,909 (604.21)                     | <0.05*|
| Neonatal abdominal circumference (cm), median (min–max) | 28.0 (19.0–46.0) | 30.0 (23.0–51.0) | 0.027*|
| Neonatal head circumference (cm), median (min–max) | 31.5 (23.0–36.5) | 32.0 (27.0–36.0) | 0.039*|
| Placental weight (g), median (min–max)        | 447.5 (218–820)                  | 500 (270–800)                      | 0.039*|

SD=standard deviation; BMI=body mass index
*Mann–Whitney U test; † missing data in preeclampsia group and non-preeclampsia group

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with major congenital deformation; pregnancies complicated with intrauterine infection, diabetes mellitus, human immunodeficiency virus infection, or other autoimmune diseases; and pregnancy with intrauterine fetal death.

Venous blood samples were extracted after labor and placed into a serum separator tube within 30 min of drawing. Placental tissue samples were also collected after labor, each in two parts from placental margins and two parts from the placental parenchyma's full thickness. One part of the samples from each location was stored in 4% formaldehyde solutions and made into a paraffin block for measuring 25-OH D₃ levels by liquid chromatography-mass spectrometry (LC-MS). This method evaluated the 25-OH D₃ levels according to the physical separation capabilities of LC with the mass analysis capabilities of MS. Data were analyzed using the SPSS version 21 (IBM Corp., USA) and either unpaired t-test, Mann–Whitney U test, or Kruskal–Wallis test depending on the data distribution.

RESULTS

We collected 86 subjects comprising 47 subjects with preeclampsia and 39 non-preeclampsia subjects. Table 1 summarizes their demographic data.

The maternal serum, umbilical, and placental tissue 25-OH D₃ levels between healthy patients and patients with preeclampsia are presented on Table 2. The mean value of 25-OH D₃ levels between the two groups had no difference in a manner of which they could be explained other than by chance. Thus, the mean values between the two groups were similar.

The 25-OH D₃ levels between patients with early-onset preeclampsia and those with late-onset preeclampsia were compared in subgroup analysis which are presented in Table 3. The mean values of the maternal serum, cord blood, and placental tissue 25-OH D₃ levels in the preeclampsia group were similar to those in the non-preeclampsia group (p = 0.459, 0.964, and 0.354, respectively). Meanwhile, the placental tissue vitamin D₃ levels on early-onset preeclampsia (79.00 [36.00–411.00] ng/ml) were higher than those on late-onset preeclampsia (40.00 [22.00–171.00] ng/ml) (p = 0.006).

DISCUSSION

The maternal serum, umbilical cord blood, and placental tissue levels of 25-OH D₃ in pregnant women with and without preeclampsia were similar, which is inconsistent with several previous studies. Many meta-analyses by Tabesh et al.¹³ Hyppönen et al.¹⁴ and Wei et al.¹⁵ showed that the 25-OH D₃ level is well associated with preeclampsia risk. Tabesh et al.¹³ demonstrated that the lower cut-off points of vitamin D levels (e.g., 38

### Table 2. Comparison of the 25-OH D₃ levels in the maternal serum, cord blood, and placental tissue between patients with and without preeclampsia

| Variable               | Preeclampsia, median (min–max) | Non-preeclampsia, median (min–max) | p*  |
|------------------------|---------------------------------|-----------------------------------|-----|
| Maternal serum 25-OH D₃ (ng/ml) | 16.30 (6.20–49.00) | 13.50 (4.80–29.20) | 0.459 |
| Cord blood 25-OH D₃ (ng/ml)      | 11.80 (3.50–38.60) | 11.70 (1.00–28.80) | 0.964 |
| Placental tissue 25-OH D₃ (ng/g)   | 49.00 (22.00–411.00) | 43.40 (11.80–153.00) | 0.354 |

*Mann–Whitney U test, significant if p ≤ 0.05

### Table 3. Comparison between the 25-OH D₃ level in the maternal serum, cord blood, and placental tissue between early-and late-onset preeclampsia

| Variable               | Early-onset preeclampsia (24–34 weeks GA), median (min–max) | Late-onset preeclampsia (34–42 weeks GA), median (min–max) | p*  |
|------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-----|
| Maternal serum 25-OH D₃ (ng/ml) | 10.80 (6.20–41.90) | 18.00 (7.00–49.00) | 0.133 |
| Cord blood 25-OH D₃ (ng/ml)      | 10.65 (3.50–38.60) | 12.65 (6.40–33.20) | 0.377 |
| Placental tissue 25-OH D₃ (ng/g)   | 79.00 (36.00–411.00) | 40.00 (22.00–171.00) | 0.006 |

GA=gestational age
*Kruskal–Wallis test, significant if p ≤ 0.05
nmol/l) have no increased risk of preeclampsia among patients with vitamin D deficiency. In our study, the mean 25-OH D₃ levels in both the preeclampsia and non-preeclampsia groups revealed vitamin D deficiency, conforming to that of Wibowo and Irwinda study, which showed vitamin D deficiency across all three trimesters in pregnant women in Indonesia. This widespread micronutrient deficiency may be a factor leading to our results of having no higher nor lower mean value levels of 25-OH D₃ in both the preeclampsia and non-preeclampsia groups, considering that vitamin D deficiency is immensely prevalent among Indonesian pregnant women. Although vitamin D deficiency is merely theoretically associated with preeclampsia, both conditions have been found among Indonesian patients with and without preeclampsia. Currently, this association has remained to be inadequately studied; thus, such association may be the basis of future studies.

Regarding the role of vitamin D deficiency as a risk factor for preeclampsia, the maternal status of vitamin D during prenatal and early pregnancy might predict preeclampsia incidence. The correlation between vitamin D levels in early pregnancy and the risk of preeclampsia itself has been controversial. Achkar et al found that low 25-OH D₃ levels in early gestational age correlate well with preeclampsia risk. In contrast, Yu et al concluded that patients with 25-OH D₃ deficiency have no increased risk of preeclampsia on the early trimesters of pregnancy, which is similar to our findings in which no higher nor lower mean value levels of 25-OH D₃ in maternal serum and cord blood were noted among patients with early- and late-onset preeclampsia.

Our subgroup analysis found that in early- and late-onset preeclampsia, which were generally thought to differ in pathogenesis, the mean value levels of 25-OH D₃ in the placental tissue were higher in early-onset preeclampsia, which is inconsistent with that of Robinson et al study that concluded that women with early-onset severe preeclampsia have lower plasma 25-OH D₃ levels. According to Álvarez-Fernández et al, women with vitamin D deficiency had a higher risk of late-onset preeclampsia, but no significant risk increase was found in early-onset preeclampsia. Shifts in placental vitamin D₃ metabolism in preeclampsia have been hypothesized in previous studies, and this altered metabolism may be the key to the higher levels of placental 25-OH D₃ among patients with early-onset preeclampsia. Higher placental 25-OH D₃ levels on early-onset preeclampsia may lead to deficiencies to a certain degree in the transmission of 25-OH D₃ levels from the maternal serum and cord blood to the placenta on late-onset preeclampsia; however, this insight has not been able to deduce from our findings. Nonetheless, it could be a further point of research for explaining the impact of micronutrient deficiency on preeclampsia pathophysiology.

The clinical implication of this study is that the impact of the differing 25-OH D₃ levels in the maternal serum, cord blood, and placental tissue on the risk of having preeclampsia has remained unobserved. Hence, multiple pathophysiological mechanisms could trigger preeclampsia, and 25-OH D₃ supplementation for preventing preeclampsia still needs to be studied in vivo. Regarding the limitation of this study, we had not further investigated on our patients’ confounding factors (e.g., the levels of other antioxidants and free radicals), history of supplementation before sample collection, or metabolisms of 25-OH D₃ that reached the placental tissue and umbilical cord blood, which possibly affected the 25-OH D₃ levels. Further in vitro and in vivo studies that could explain these issues should be performed to thoroughly elaborate the impacts of 25-OH D₃. We recommend that more studies investigating the absorption, distribution, and metabolism of 25-OH D₃ should be conducted to further explain the physiologic mechanisms of this nutrient among patients with early- and late-onset preeclampsia. Moreover, 25-OH D₃ supplementation may have beneficial effects on patients with preeclampsia, especially late-onset preeclampsia.

In conclusion, the 25-OH D₃ value levels in the maternal serum, cord blood, and placental tissue were similar among patients with and without preeclampsia. The 25-OH D₃ mean value levels in the maternal serum and umbilical cord levels between patients with early- and late-onset preeclampsia were also similar. However, the 25-OH D₃ level was higher in the placental tissue of the early-onset preeclampsia group, reflecting the altered metabolism of 25-OH D₃ among patients with late-onset preeclampsia.

Conflict of Interest
The authors affirm no conflict of interest in this study.

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