Clinical Research

Vitamin D_3 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 D_3. This study was aimed to compare the vitamin D_3 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 D_3 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 D_3 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 D_3 levels between patients with and without preeclampsia were similar. However, the placental tissue vitamin D_3 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 D_3

Preeclampsia and preterm deliveries play a huge role in maternal morbidities and mortalities, with a global incidence of 3–4%.[1,2] 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%.[3] 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.[4,5] 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 D_3 (25-OH D_3) 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 D_3 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 D_3 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 D_3 in predicting the outcomes of pregnancies and neonates remains unclear, especially its role in preeclampsia. Therefore, 25-OH D_3 supplementation in preeclampsia is controversial. In several studies, 25-OH D_3 plays a role in preeclampsia pathogenesis for placental insufficiencies, abnormal angiogenesis, and hypertension; however, conflicting results show that 25-OH D_3 decrement is not a cause but rather an effect of preeclampsia, considering that the placenta metabolizes the active form of 25-OH D_3.[11,12] For this reason, we aimed to investigate and compare the 25-OH D_3 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

Table 1. Patient demographics

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; †1 missing data in preeclampsia group and non-preeclampsia group

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 D_3 levels by liquid chromatography-mass spectrometry (LC-MS). This method evaluated the 25-OH D_3 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 D_3 levels between healthy patients and patients with preeclampsia are presented on Table 2. The mean value of 25-OH D_3 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 D_3 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 D_3 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 D_3 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 D_3 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 D_3 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 D_3 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 D_3 (ng/ml)	16.30 (6.20–49.00)	13.50 (4.80–29.20)	0.459
Cord blood 25-OH D_3 (ng/ml)	11.80 (3.50–38.60)	11.70 (1.00–28.80)	0.964
Placental tissue 25-OH D_3 (ng/g)	49.00 (22.00–411.00)	43.40 (11.80–153.00)	0.354

*Mann–Whitney *U* test, significant if $p \leq 0.05$

Table 3. Comparison between the 25-OH D_3 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 D_3 (ng/ml)	10.80 (6.20–41.90)	18.00 (7.00–49.00)	0.133
Cord blood 25-OH D_3 (ng/ml)	10.65 (3.50–38.60)	12.65 (6.40–33.20)	0.377
Placental tissue 25-OH D_3 (ng/g)	79.00 (36.00–411.00)	40.00 (22.00–171.00)	**0.006**

GA=gestational age
*Kruskal–Wallis test, significant if $p \leq 0.05$

nmol/l) have no increased risk of preeclampsia among patients with vitamin D deficiency.[13] In our study, the mean 25-OH D_3 levels in both the preeclampsia and non-preeclampsia groups revealed vitamin D deficiency, conforming to that of Wibowo and Irwinda[10] 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 D_3 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[16] found that low 25-OH D_3 levels in early gestational age correlate well with preeclampsia risk. In contrast, Yu et al[17] concluded that patients with 25-OH D_3 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 D_3 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 D_3 in the placental tissue were higher in early-onset preeclampsia, which is inconsistent with that of Robinson et al[18] study that concluded that women with early-onset severe preeclampsia have lower plasma 25-OH D_3 levels. According to Álvarez-Fernández et al,[19] women with vitamin D_3 deficiency had a higher risk of late-onset preeclampsia, but no significant risk increase was found in early-onset preeclampsia. Shifts in placental vitamin D_3 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 D_3 among patients with early-onset preeclampsia. Higher placental 25-OH D_3 levels on early-onset preeclampsia may lead to deficiencies to a certain degree in the transmission of 25-OH D_3 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 D_3 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 D_3 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 D_3 that reached the placental tissue and umbilical cord blood, which possibly affected the 25-OH D_3 levels. Further *in vitro* and *in vivo* studies that could explain these issues should be performed to thoroughly elaborate the impacts of 25-OH D_3. We recommend that more studies investigating the absorption, distribution, and metabolism of 25-OH D_3 should be conducted to further explain the physiologic mechanisms of this nutrient among patients with early- and late-onset preeclampsia. Moreover, 25-OH D_3 supplementation may have beneficial effects on patients with preeclampsia, especially late-onset preeclampsia.

In conclusion, the 25-OH D_3 value levels in the maternal serum, cord blood, and placental tissue were similar among patients with and without preeclampsia. The 25-OH D_3 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 D_3 level was higher in the placental tissue of the early-onset preeclampsia group, reflecting the altered metabolism of 25-OH D_3 among patients with late-onset preeclampsia.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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REFERENCES

1. Staff AC, Braekke K, Johnsen GM, Karumanchi SA, Harsem NK. Circulating concentrations of soluble endoglin (CD105) in fetal and maternal serum and in amniotic fluid in preeclampsia. Am J Obstet Gynecol. 2007;197(2):176.e1–6.
2. Wen SW, Guo Y, Rodger M, White RR, Yang Q, Smith GN, et al. Folic acid supplementation in pregnancy and the risk of pre-eclampsia-a cohort study. PLoS One. 2016;11(2):e0149818.
3. Fetomaternal Division, Department of Obstetrics and Gynecology, Faculty of Medicine Universitas Indonesia/ Cipto Mangunkusumo Hospital. 2008 maternal mortality and morbodity data in Cipto Mangunkusumo Hospital. Faculty of Medicine Universitas Indonesia/Cipto Mangunkusumo Hospital; 2008. Indonesian.
4. Rajakumar A, Michael HM, Daftary A, Jeyabalan A, Gilmour C, Conrad KP. Proteasomal activity in placentas from women with preeclampsia and intrauterine growth restriction: implications for expression of HIF-alpha proteins. Placenta. 2008;29(3):290–9.
5. Kimura C, Watanabe K, Iwasaki A, Mori T, Matsushita H, Shinohara K, et al. The severity of hypoxic changes and oxidative DNA damage in the placenta of early-onset preeclamptic women and fetal growth restriction. J Matern Fetal Neonatal Med. 2013;26(5):491–6.
6. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet. 2010;376(9741):631–44.
7. Lewis S, Lucas RM, Halliday J, Ponsonby AL. Vitamin D deficiency and pregnancy: from preconception to birth. Mol Nutr Food Res. 2010;54(8):1092–102.
8. Evans KN, Nguyen L, Chan J, Innes BA, Bulmer JN, Kilby MD, et al. Effects of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 on cytokine production by human decidual cells. Biol Reprod. 2006;75(6):816–22.
9. Nassar N, Halligan GH, Roberts CL, Morris JM, Ashton AW. Systematic review of first-trimester vitamin D normative levels and outcomes of pregnancy. Am J Obstet Gynecol. 2011;205(3):208.e1–7.
10. Wibowo N, Irwinda R. The effect of multi-micronutrient and protein supplementation on iron and micronutrients status in pregnant women. Med J Indones. 2015;24(3):168–75.
11. Aghajafari F, Nagulesapillai T, Ronksley PE, Tough SC, O'Beirne M, Rabi DM. Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. BMJ. 2013;346:f1169.
12. Mistry HD, Gill CA, Kurlak LO, Seed PT, Hesketh JE, Méplan C, et al. Association between maternal micronutrient status, oxidative stress, and common genetic variants in antioxidant enzymes at 15 weeks' gestation in nulliparous women who subsequently develop preeclampsia. Free Radic Biol Med. 2015;78:147–55.
13. Tabesh M, Salehi-Abargouei A, Tabesh M, Esmaillzadeh A. Maternal vitamin D status and risk of pre-eclampsia: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2013;98(8):3165–73.
14. Hyppönen E, Cavadino A, Williams D, Fraser A, Vereczkey A, Fraser WD, et al. Vitamin D and pre-eclampsia: original data, systematic review and meta-analysis. Ann Nutr Metab. 2013;63(4):331–40.
15. Wei SQ, Qi HP, Luo ZC, Fraser WD. Maternal vitamin D status and adverse pregnancy outcomes: a systematic review and meta-analysis. J Matern Fetal Neonatal Med. 2013;26(9):889–99.
16. Achkar M, Dodds L, Giguère Y, Forest JC, Armson BA, Woolcott C, et al. Vitamin D status in early pregnancy and risk of preeclampsia. Am J Obstet Gynecol. 2015;212(4):511 e1–7.
17. Yu CK, Ertl R, Skyfta E, Akolekar R, Nicolaides KH. Maternal serum vitamin D levels at 11–13 weeks of gestation in preeclampsia. J Hum Hypertens. 2013;27(2):115–8.
18. Robinson CJ, Wagner CL, Hollis BW, Baatz JE, Johnson DD. Association of maternal vitamin D and placenta growth factor with the diagnosis of early onset severe preeclampsia. Am J Perinatol. 2013;30(3):167–72.
19. Álvarez-Fernández I, Prieto B, Rodríguez V, Ruano Y, Escudero AI, Álvarez FV. Role of vitamin D and sFlt-1/PlGF ratio in the development of early- and late-onset preeclampsia. Clin Chem Lab Med. 2015;53(7):1033–40.