Int J Clin Exp Pathol 2017;10(11):10841-10851
www.ijcep.com /ISSN:1936-2625/IJCEP0061372

Original Article

Lower maternal and fetal vitamin D status and higher placental and umbilical vitamin D receptor expression in preeclamptic pregnancies

Jian-Ping Xiao[1,2*], Juan-Xiu Lv[1*], Yong-Xiang Yin[3], Ling Jiang[1], Wei-Sheng Li[1], Tao Tao[2], Xiang-Peng Liao[2], Zhi-Ce Xu[1]

[1]Institute for Fetology, First Hospital of Soochow University, Suzhou, China; [2]Prenatal Diagnosis Center, [3]Department of Pathology, Wuxi Maternity and Children Health Hospital Affiliated Nanjing Medical University, Wuxi, China. [*]Equal contributors.

Received July 6, 2017; Accepted September 18, 2017; Epub November 1, 2017; Published November 15, 2017

Abstract: Objective: To explore associations between maternal and fetal vitamin D status in preeclamptic pregnancies. Methods: A case-control experiment was carried out with proportion ratio of 1:1 (controls: n = 60 vs cases: n = 60). Blood collection of both maternal and cord were performed before and during delivery, respectively, and 25(OH)D measurement was conducted. Difference analysis was performed according to returned data. Immunohistochemical analysis, together with semi-quantitative Western blot, was also performed to determine protein expression of vitamin D receptor in placenta and cord tissues of ESPE. Results: Mean ± SD values of maternal 25(OH)D in control and PE group were 38.06 ± 6.28 and 33.05 ± 4.10, respectively, and significant differences with $P < 0.0001$ were found between control and PE in both continuous and categorical variables, especially in ESPE subtype (32.96 ± 4.49). The deficiency category (< 30 nmol/L) showed increased odds of PE (OR, 2.83, 95% CI, 1.32-6.08) in both maternal 25(OH)D and cord 25(OH)D in multivariable logistic regression. Semi-quantitative analysis showed that expression of placenta VDR in the ESPE subgroup was significantly higher than that in control group with $P < 0.001$, while expression of umbilical vein VDR in ESPE subgroup was significantly higher than that in control group with $P < 0.05$. Conclusions: The present study finds that lowest maternal and fetal vitamin D status in ESPE existed in the preeclampsia subsets. The VDR expression in placenta and fetus in ESPE were higher than that of normal pregnancy, which indicated that it might be related to placenta compensatory mechanism and is worthy of further research.

Keywords: Preeclampsia, 25-hydroxy vitamin D, vitamin d receptor, placenta, umbilical vein

Introduction

Preeclampsia (PE) is one of the common pregnancy complications with a worldwide morbidity of 2%~8% [1]. The etiology and pathogenesis of preeclampsia remains unclear and thus makes it even more difficult to prevent and cure this certain disease. Other than varied disease doctrines, factors of dietary structures, such as the intake of vitamin D, have become a new research focus for the pathogenesis and preventing of preeclampsia. It is also believed that preeclampsia is affected by seasons [2]. Studies showed that the morbidity of preeclampsia in winter is twice as much as that in other seasons; it is possible that the lack of sunlight exposure in winter leads to the vitamin D insufficiency and thus causes preeclampsia [3].

Vitamin D refers to a group of lipid-soluble steroidal derivatives which is mainly obtained from food and exposure to ultraviolet light. Vitamin D deficiency is commonly existed, especially in pregnancy women and children, because of insufficient sunlight exposure [4]. Recent studies showed that the metabolism of vitamin D not only involved in calcium homeostasis, bone mineralization, it also plays important role in varied diabetes, cardiovascular diseases, cancers and autoimmune diseases [5]. Moreover, insufficient vitamin D status has been proved to be related to pregnancy complications such as preeclampsia, fetal growth restriction, and ges-

Table 1. Case-control characteristics

Characteristics	Control (n = 60)	PE (n = 60)	*P* value
General characteristics			
Maternal age, year			
Mean ± SD	29.5 ± 4.7	29.2 ± 5.0	0.73
< 30	30 (50.0)	31 (51.7)	0.78
30 - < 35	24 (40.0)	21 (35.0)	
≥ 35	6 (10.0)	8 (13.3)	
Pregnancy Variables			
Pregnancy body mass index, kg/m^2 (BMI)			
Mean ± SD	25.99 ± 2.40	28.53 ± 3.32	< 0.0001*
Normal (< 25.00)	18 (30.0)	6 (10.0)	0.001*
Overweight (25.00 - < 29.99)	40 (66.7)	40 (66.7)	
Obese (≥ 30.00)	2 (3.3)	14 (23.3)	
Parity			0.58
Nulliparous (0)	31 (51.7)	34 (56.7)	
Primiparous/multiparous (> 0)	29 (48.3)	26 (43.3)	
Infant gender			0.06
Female	18 (30.0)	28 (46.7)	
Male	42 (70.0)	32 (53.3)	
Delivery			< 0.0001*
Vaginal	27 (45.0)	2 (3.3)	
Cesarean section	33 (55.0)	58 (96.7)	
Gestational weeks at delivery, week			0.81
Mean ± SD	35.3 ± 2.7	35.1 ± 2.7	
Pregnancy blood pressure			
Systolic blood pressure	123.75 ± 10.12	154.85 ± 17.49	< 0.0001*
Diastolic blood pressure	78.68 ± 7.70	103.02 ± 13.08	< 0.0001*

Data are presented as N. (%) or Mean ± SD. *P-value < 0.05 were calculated and marked, using student t-test for continuous variables and χ^2 or Fisher exact test for categorical variables.

tational diabetes [6, 7]. Intervention studies showed that supplement of vitamin D during pregnancy could lower the risk of getting preeclampsia [8].

Vitamin D is biologically functioned with the help of vitamin D receptor, which exists in both trophoblast cells and tissues of placenta, together with 1-α-hydroxylase, 24-hydroxylase, CYP2R1 and vitamin D binding protein [9]. The metabolism of vitamin D takes place mainly in the placenta during pregnancy. The vitamin D status of pregnant women may have deep influence on the fetal calcium status and thus impact on the growth, intensity and density of skeleton of infants. Vitamin D demanding is increased during pregnancy because of fetal calcium requirements and will also lead to the changes of maternal 25(OH)D biological activities. Therefore, it is notable that whether maternal vitamin D status would influence fetal vitamin D status through VDR expression changes in placenta [10].

Materials and methods

Study designing

This was a case-control study which carried out in Wuxi Maternal and Child Health Care Hospital, Wuxi, Jiangsu Province, China, Asia. Participants were from urban or rural area of the city. Pregnant women who attending their regularly prenatal examinations in the hospital were recruited with a proportion ratio of 1:1 (controls n = 60 vs cases n = 60) from October 2011 to December 2013. Controls and PE patients were selected with average age of 29 and average BMI value of 27. Preeclampsia confirmation was strictly followed the interna-

Table 2. 25-hydroxyvitamin D status and preeclampsia

Characteristics	Controls (n = 60)	PE (n = 60)	*P* value
Maternal plasma 25(OH)D concentration, nmol/L			
Mean ± SD	38.06 ± 6.28	33.05 ± 4.10	< 0.0001*
Median (Minimum-Maximum)	37.46 (28.40~55.30)	32.32 (27.01~44.50)	
Proportion			
< 30	4 (6.7)	13 (21.7)	0.01[a]
30 - < 50	53 (88.3)	47 (78.3)	
≥ 50	3 (5.0)	0 (0.0)	
Cord plasma 25(OH)D concentration, nmol/L			
Mean ± SD	41.49 ± 8.00	37.56 ± 7.44	0.01*
Median (Minimum-Maximum)	40.30 (26.89~57.40)	37.56 (20.95~56.70)	
Proportion			
< 30	2 (3.3)	10 (16.7)	0.01*
30 - < 50	48 (80.0)	47 (78.3)	
≥ 50	10 (16.7)	3 (5.0)	

Data are presented as N. (%) or Mean ± SD. *P-value < 0.05 were calculated and marked, using student t-test for continuous variables and χ^2 or Fisher exact test for categorical variables. [a]Fisher exact test (two-sided).

tional criteria, defined as the presence of hypertension (SBP ≥ 140 mmHg; DBP ≥ 90 mmHg) on 2 occasions, at least 6 hours apart, and in a woman who was normotensive before 20 weeks' gestation and detectable proteinuria ≥ 300 mg/d or > 1 by dipstick. The control group was considered as pregnant women without pregnancy complications after one week of delivery. All had assessments of hypertensive outcomes. PIH (pregnancy induced hypertension) was defined as follows: BP ≥ 140/90 mmHg only occurred during pregnancy with negative urine protein results, and blood pressure became normal in 12 weeks after delivery. Demographic data and venipunctural plasma collection were performed with consent of all participants. The blood samples of maternal and umbilical vein were collected before and during parturients' delivery, respectively. Gestational age was determined according to patients' last menstrual period, together with ultrasound diagnosis. In order to find out the specific relationships between preeclampsia subsets and vitamin D status, we classified four types of preeclampsia subset, including early onset mild preeclampsia (EMPE, n = 5), early onset severe preeclampsia (ESPE, n = 34), late onset mild preeclampsia (LMPE, n = 13), late onset severe preeclampsia (LSPE, n = 8), and ESPE cases must meet the American College of Obstetrics and Gynecology (ACOG) criteria for severe preeclampsia and diagnosed 34 weeks before completed gestation [11, 12].

The study was approved by the ethics committee of Wuxi Maternal and Child Health Care Hospital (No. 137, 2011) with signed informed consent of all participants.

Laboratory measurement of vitamin D concentrations

Blood samples of both maternal and cord were collected before and during operation for delivery. Plasma samples were separated and stored at -80°C immediately. Enzyme-linked immunosorbent assay kit (ELISA kit, Immunodiagnostic System Inc., IDS, batch number 23409) was applied to assess the 25(OH)D concentration according to the standard kit protocol in triplicate for each sample.

Western blot

Placenta and umbilical vein tissues were collected during delivery. The umbilical vein tissues were immediately frozen and preserved at -80°C. The expression of VDR protein was analyzed with western blot. Tissues to be inspected were weighed and cut into pieces and 1 mL of precooled cell lysis buffer was added. Tissues were slowly crushed on ice-bath followed with centrifuged at 12000 rpm/min for 30 min, supernatant was collected and quantified with Bradford method. 60 μg was used for SDS-PAGE electrophoresis isolation and then transferred to PVDF membrane, 6% milk was used to block the isolated protein for 60 min.

Table 3. Statistics of 25-hydroxyvitamin D status and mild and severe preeclampsia (nmol/L)

Continuous	Maternal 25(OH)D	*P* value	Cord 25(OH)D	*P* value	
MPE	33.21 ± 3.12	< 0.0001*	37.29 ± 5.91	0.04*	
Control	38.06 ± 6.28		41.49 ± 8.00		
SPE	32.98 ± 4.48	< 0.0001*	37.77 ± 8.10	0.02*	
Control	38.06 ± 6.28		41.49 ± 8.00		
Characteristics	*Control*	*MPE*	*P value*	*SPE*	*P value*
Maternal 25(OH)D					
Proportion					
< 30	4 (6.7)	3 (21.7)	0.43[a,*]	10 (21.7)	0.01[a,*]
30 - < 50	53 (88.3)	15 (78.3)		32 (78.3)	
≥ 50	3 (5.0)	0 (0.0)		0 (0.0)	
Cord 25(OH)D					
Proportion					
< 30	2 (3.3)	3 (16.7)	0.02[a,*]	7 (16.7)	0.03*
30 - < 50	48 (80.0)	15 (83.3)		32 (76.2)	
≥ 50	10 (16.7)	0 (0.0)		3 (7.1)	

MPE: mild preeclampsia; SPE: severe preeclampsia. Data are presented as N. (%) or Mean ± SD. *P-value < 0.05 were calculated and marked, using student t-test for continuous variables and χ^2 or Fisher exact test for categorical variables. [a]Fisher exact test (two-sided).

VDR primary antibodies of 1:1000 was added and incubated for 1 h at room temperature, second antibodies of 1:5000 was added and incubated for 2 h at room temperature. Then the membrane was taken out and washed with TBST. ECL color developing agent was added and the plate was then analyzed using Kodak in vivo imaging instrument with β-action as internal reference. The relevant expression value of the target protein was calculated with target protein grey value/β-actin grey value, 3 repetitions were carried out and the average value was obtained.

Immunohistochemistry

Placenta and umbilical vein tissues were collected during delivery and fixed in 10% formalin followed by paraffin embedded for further usage. Semi-quantitative analysis of VDR protein expression was carried out using immunohistochemical methods. The detailed processes are as follows: The paraffin sections were removed paraffin, and then immersed in the distilled water following routine methods: antigen retrieval, endogenous peroxidase inactivation, serum blocking, incubation of VDR monoclonal antibodies (1:500), washing, incubation of second antibody, color developing, and results were analyzed with semi-quantitative methods. Instruments applied during immunohistochemical analysis including automatic vacuum dewatering machine (AP3-00S, Leica, Germany), Semiautomatic and heating Microtome (RM2245, Leica, Germany), optical microscope (OLYMPUS BX-45, Japan), immunohistochemistry autostainer (DAKO Link48, Denmark).

Statistical analyses

Demographic characteristics and clinical features chosen to be covariates in the study were estimated according to previous systematic and meta-analysis reviews. Categorical variables, including, parity (nulliparous/primiparous-multiparous), infant gender (female/male), delivery (vaginal/cesarean section), were collected from October 2011 to December 2013. Continuous factors were clustered into certain levels when performing logistic regression analysis, including maternal age (< 30, 30 - < 35, ≥ 35 years), pregnancy body mass index (normal: < 25.00; overweight: 25.00 - ≤ 29.99; obese: ≥ 30, BMI, kg/m2), maternal and cord plasma 25(OH)D concentrations (< 30, 30 - < 50, ≥ 50, nmol/L) [13, 14]. Vitamin D deficiency was 25(OH)D < 30 nmol/L, vitamin D inadequacy was 25(OH)D = 30-50 nmol/L, and adequate levels were 25(OH)D 50 nmol/L. Vitamin D status were also calculated and presented with Mean ± SD, together with other potential cofounders of gestational weeks at delivery and pregnancy blood pressure.

Dichotomy method was used to valuate VDR color developing results, according to nucleus staining intensity and positive rate in immunohistochemical analysis. Staining intensity included: (1) unstained: 0 score; (2) light yellow: 1 score; (3) yellow: 2 scores; (4) brown: 3 scores. Number of cell percentage in sight: (1) < 25%: 1

Table 4. Statistics of case-control characteristics and 25(OH)D status of preeclampsia subsets

Characteristics	EMPE (n = 5)	ESPE (n = 34)	LMPE (n = 13)	LSPE (n = 8)
Maternal age				
Continuous	NS	NS	NS	NS
Proportion	NS	NS	NS	NS
Residence	NS	NS	NS	NS
BMI				
Continuous	S	S	S	NS
Proportion	S	S	S	NS
Parity	NS	S	NS	NS
Infant gender	NS	NS	NS	NS
Delivery	NS	S	S	S
Gestational weeks at delivery	NS	S	S	S
Pregnancy blood pressure				
SBP	S	S	S	S
DBP	S	S	S	S
Maternal 25(OH)D				
Continuous	S	S	S	S
Proportion	NS	S	NS	NS
Cord 25(OH)D				
Continuous	NS	S	S	NS
Proportion	NS	NS	S	S

S represents for significant; *NS* represents for nonsignificant.

Table 5. Association analysis of maternal and cord 25(OH)D with preeclampsia

	Unadjusted Model	Adjusted Model
	OR, 95% CI	OR, 95% CI
Maternal 25(OH)D (nmol/L)		
< 30	2.83 (1.32-6.08)	3.37 (1.26-9.01)
30 - < 50	1.00 (reference)	1.00 (reference)
≥ 50	0.70 (0.56-0.88)	0.63 (0.47-0.86)
Cord 25(OH)D (nmol/L)		
< 30	3.33 (1.18-9.41)	2.06 (0.61-6.92)
30 - < 50	2.89 (1.13-7.42)▲	1.63 (0.54-4.92)▲
≥ 50	1.00 (reference)	1.00 (reference)

OR. Odds ratio; *CI*, confidence interval; Pregnancy body mass index, maternal age, gestational weeks,residence, parity, infant sex, delivery were used to adjust the model. ▲Odd ratios generated were notsignificantly associated with a *P* > 0.1.

score; (2) 25%-50%: 2 scores; (3) > 50%: 3 scores. Semi-quantitative results were calculated using the product of the two items above [15].

Student t-test was used for continuous variables while χ^2 or Fisher test for categorical variables. Logistic regression was used to test unadjusted and multivariable-adjusted models and described the associations of maternal and cord plasma 25(OH)D status with preeclampsia. The PE risk of confounding factors was assessed with adjusted odds ratios (OR) with 95% confidence intervals (CI). Covariables chosen to adjusted models were maternal age, gestational weeks, residence, pregnancy body mass index, parity, delivery and infant gender.

All analyses were performed using SPSS 19.0 (IBM Company SPSS In., US). Graph Pad Prism 5.0 software was used for the analysis of VDR results of placenta and umbilical vein and Mann-Whitney U test was applied.

Results

Description of demographic characteristics and 25(OH)D status

The proportion of participants who developed preeclampsia and normal pregnancies was 1:1, with n = 60 patients in the PE group and n = 60 in matched controls. The demographic characteristics and clinical features were presented in **Table 1**. Continuous variables were clustered into different levels for logistic regression as well as proportion division. Significant differences were found in terms of pregnancy body mass index (BMI, in both continuous and categorical variables with P < 0.0001 and P = 0.001, respectively), delivery (P < 0.0001), pregnancy blood pressure (both PSBP and PDBP < 0.0001) between normal pregnancies and PE group, while in terms of maternal age and gestational weeks, the control and PE group showed no significant differences.

Maternal plasma 25(OH)D concentrations were measured and presented as mean ± SD and were classified into three levels (< 30 nmol/L, 30 - < 50 nmol/L, ≥ 50 nmol/L). Mean ± SD

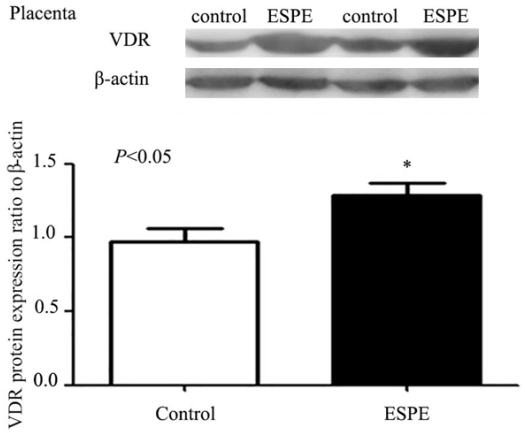

Figure 1. Significant difference of vitamin D receptor (VDR) protein expression between control and early on-set severe preeclampsia groups in placenta (P < 0.05).

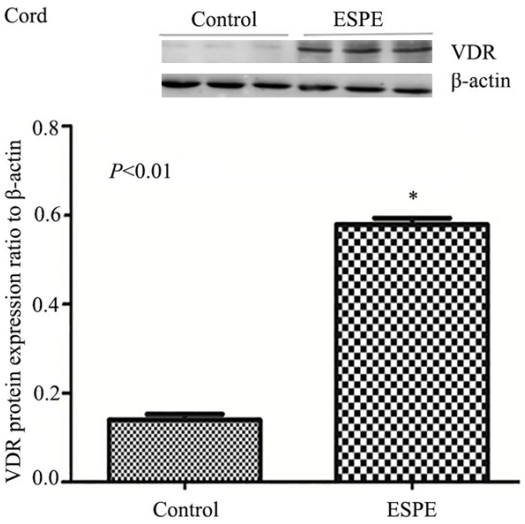

Figure 2. Significant difference of vitamin D receptor (VDR) protein expression between control and early on-set severe preeclampsia groups in umbilical cord (P < 0.01).

values of maternal 25(OH)D in control and PE were 38.06 ± 6.28 and 33.05 ± 4.10, respectively and significant differences with a P < 0.0001 were detected between control and PE in both continuous and categorical variables (**Table 2**).

Umbilical vein plasma 25(OH)D concentrations were analyzed with the same treatment as maternal 25(OH)D. Mean ± SD values of umbilical 25(OH)D in control and PE were 41.49 ± 8.00 and 37.56 ± 7.44, respectively. Although both umbilical and maternal plasma 25-OH-D were significantly decreased at the diagnosis of PE, compared to umbilical plasma 25(OH)D status, maternal plasma 25(OH)D status showed a more significant difference between control and PE group. To clarify the specific preeclampsia subsets' characteristics, we first determined whether mild (n = 18) and severe (n = 42) preeclampsia had certain differences. In our present study, we compared both mild preeclampsia and severe preeclampsia data with controls, the results showed that 25(OH)D concentration of maternal and cord in both MPE and SPE had significant differences (**Table 3**). However, the severe preeclampsia group was more sensitive with a *P*-value of 0.02, compared to mild preeclampsia group (P = 0.04). EMPE, ESPE, LMPE, LSPE were then analyzed between the cases and controls. The detailed significance of each individual subset was presented in **Table 4**.

Results showed that in the four subtypes, in terms of maternal age, infant gender, there were no significant differences, while in terms of pregnancy blood pressure and maternal 25(OH)D, there were significant differences between four subtypes. ESPE subtype was the most significant terms among all subtypes, which was more sensitive. Early onset severe preeclampsia (ESPE) ranked the first in the number of items showed significant differences between control and PE, indicating its importance in diagnosis and prevention of preeclampsia. The detailed information for each subset was presented in Tables S1A, S1B, S1C, S1D and S2A, S2B, S2C, S2D.

Logistic regression analysis

Association analysis of both maternal and cord plasma 25(OH)D concentration between normal pregnancies and preeclampsia were performed using multivariable adjusted logistic regression. In both unadjusted and adjusted models of maternal 25(OH)D, we used the mid-dose concentration (30 - < 50 nmol/L) as references [12]. The deficiency category (< 30 nmol/L) showed increased odds of PE (OR, 2.83, 95% CI, 1.32-6.08, **Table 5**). The odds ratio slightly increased after adjusting for a series of covariables (OR, 3.37, 95% CI, 1.26-9.01). On the contrary, category defined as ≥ 50 nmol/L showed decreased odds ratio (OR, 0.70, 95% CI, 0.56-0.88) after adjusting for covariables and both the adjusted and unad-

Table 6. Case-control characteristics of VDR protein expression

Items	Control (n = 23)	PE (n = 34)	*P* value
Placental VDR Mean ± SD	2.67 ± 1.49	4.90 ± 2.50	< 0.0001*
Umbilical cord VDR Mean ± SD	4.43 ± 2.13	5.45 ± 2.35	< 0.0001*

**P*-value < 0.05 were calculated and marked, using student t-test for continuous variables.

justed OR (OR, 0.63, 95% CI, 0.47-0.86) were < 1, indicating the category of ≥ 50 nmol/L was reversely associated between control and PE.

The concentration of ≥ 50 nmol/L was treated as reference when analyzing associations of cord 25(OH)D and PE. The deficiency category (< 30 nmol/L) of cord 25(OH)D (OR, 3.33, 95% CI, 1.18-9.41, **Table 5**) showed consistency with maternal 25(OH)D. After adjusting for covariables, including pregnancy body mass index, maternal age, gestational weeks, parity, infant sex and delivery, the odds ratio decreased (OR, 2.06, 95% CI, 0.61-6.92). When compared concentration of 30 - < 50 nmol/L to reference, the models of both unadjusted and adjusted showed fallibility with both equations' P > 0.1, which thus was not meaningful during logistic regression.

Expression of vitamin D receptor

Western-blot results showed that VDR protein expression of ESPE in both placenta and umbilical cord were higher than that in normal pregnancy with P < 0.05 and P < 0.01, respectively (**Figures 1**, **2**). Moreover, we also performed VDR immunohistochemistry analysis to further determine the relationship between ESPE and normal pregnancy and consistent results were obtained, VDR immunohistochemistry scoring in both syncytiotrophoblasts and umbilical cord were higher than those in normal pregnancy with P < 0.001 and P < 0.05, respectively (**Table 6**; **Figures 3**-**5**).

Discussion

Vitamin D deficiency has been proved to be associated with adverse pregnancy outcomes [6-8], 25(OH)D is the main component of vitamin D in human blood circle system and thus is used to determine a person's vitamin D status.

Our study showed consistent conclusions with previous studies on the relationship between vitamin D deficiency and preeclampsia [16]. Briefly, the association analysis of the odds ratio changes showed that, lower maternal 25(OH)D concentration can cause increased risk of preeclampsia in pregnant women. Covariables were chosen according to the previous studies and we found that pregnancy body mass index showed significantly difference between normal pregnancies and preeclampsia, obese gestation had a larger proportion in PE than that in the control group. It is reasonable and needs further exploration since vitamin D is a lipid-soluble steroidal hormone, obesity may influence the synthesis and absorption of maternal vitamin D and cause the insufficiency during gestation [17].

Previous studies had been focused on the association of vitamin D deficiency and early onset severe preeclampsia (ESPE) [18]. In the present study, we classified preeclampsia into four specific subsets. Consistently, ESPE possessed the most significant characteristics in all subsets. Strong association between 25(OH)D status and the risk of preeclampsia was detected by using logistic regression. Since vitamin D is an unstable molecular, 25(OH)D was used to determine the vitamin D status. Interestingly, the mean ± SD values of both PE (33.05 ± 4.10 nmol/L) and control group (38.06 ± 6.28 nmol/L) in our study were lower than those of the studies in most western countries, we conjectured that this difference could be explained with the following potential reasons such as race, latitude, dietary tradition, average incomes and insufficient vitamin D intakes [19].

Vitamin D also plays important role in placenta trophoblasts development [20]. In syncytiotrophoblasts, vitamin D receptor, 1-α-hydroxylase, 24-hydroxylase and 1-25$(OH)_2D_3$ co-regulate the expression of human HCG, HPL, estradiol and progesterone [21]. 1-25$(OH)_2D_3$ helps the implantation of embryo by regulating variable placental hormones. Studies related to endometrial stromal cells showed that the vitamin D could up-regulates the expression of HOXA10, which mainly expressed in uterus and is vital to the implantation of embryo. Therefore, low vitamin D status may influence the implantation of embryo by regulating other hormones sta-

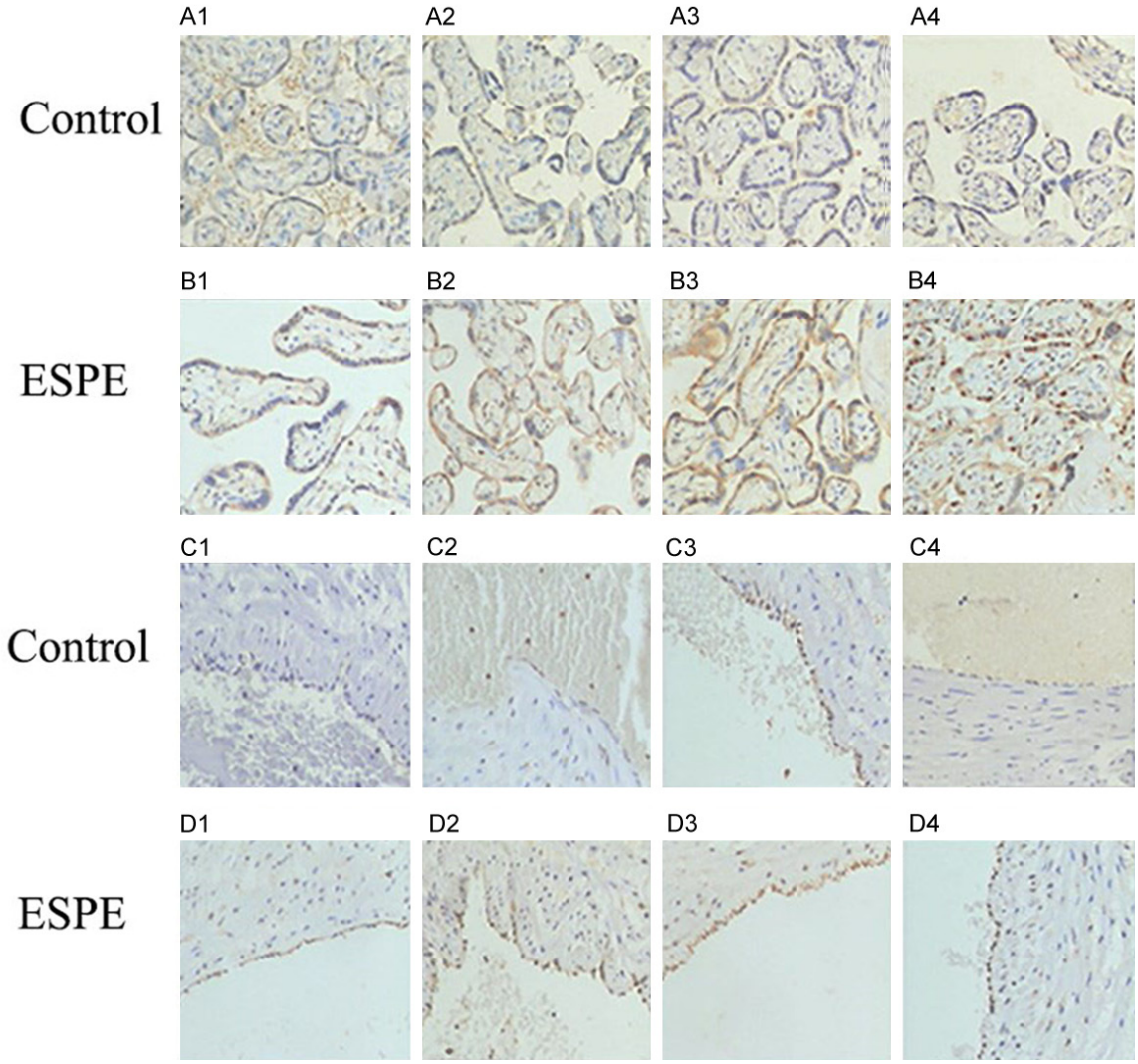

Figure 3. VDR expression and distribution of placenta and umbilical cord between control and PE. A1-A4. Are the control samples from placenta tissues after immunohistochemical treatment. B1-B4. Are the preeclampsia samples (ESPE) from placenta tissues after immunohistochemical treatment. C1-C4. Are the control samples from umbilical cord tissues after immunohistochemical treatment. D1-D4. Are the preeclampsia samples (ESPE) from umbilical cord tissues after immunohistochemical treatment. Placenta VDR: Control figures: 7, 10, 14, 16 & ESPE figures: 1, 2, 16, 26; (Figure number). Umbilical cord VDR: Control figures: 2, 3, 4, 6 & ESPE figures: 7, 8, 16, 27; (Figure number).

tus and certain genes functions on endometrium, and thus lead to the formation failure of uterine spiral artery and preeclampsia [13]. Both maternal and umbilical results showed significant differences between PE and the control group and the associations were detected between the risk of PE and vitamin D insufficiency.

In the present study, we found out that in the four classifications of preeclampsia, both the maternal and cord $25(OH)_2D_3$ concentrations of ESPE were the most lowest in subtypes and the deficiency of vitamin D of ESPE was thus more serious although the model of umbilical vein plasma group showed no significant category (30 - < 50 nmol/L). We blamed the small size of samples for the unpredicted outcome. In most studies, the blood collection step was done around 20 to 30 weeks of gestation, while we did blood sample collection right before delivery. It needs further exploration of the relevance between gestational weeks and the maternal vitamin D status [22]. To our knowl-

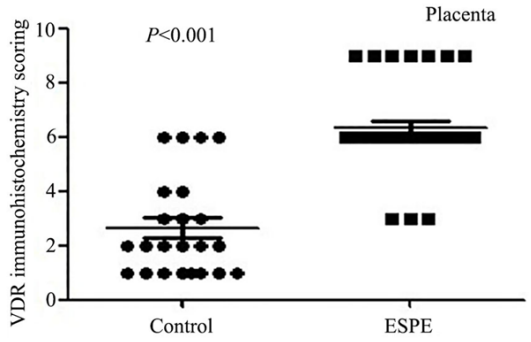

Figure 4. Significant difference of VDR immunohistochemistry scoring results between control and early on-set severe preeclampsia groups in placenta (P < 0.001).

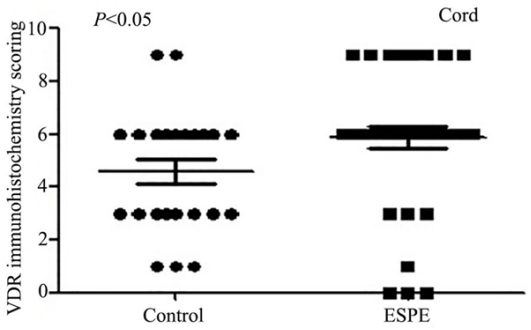

Figure 5. Significant difference of VDR immunohistochemistry scoring results between control and early on-set severe preeclampsia groups in umbilical cord (P < 0.05).

edge, this is the first study that contains both maternal and umbilical vein plasma 25(OH)D concentrations for association analysis between PE and normotensive pregnant women. The biological effects of 1, $25(OH)_2D_3$ are mediated by vitamin D receptor, therefore, we focused on the VDR expression and serological statistics of ESPE in both placenta and umbilical cord tissues.

During pregnancy, vitamin D may play a role in implantation and placental function potentially due to angiogenic, immunomodulatory and anti-inflammatory effects [23, 24]. Studies showed that 1, $25(OH)_2D_3$ mainly up-regulates the VDR in protein level, while the regulation of mRNA are more complex with both up-regulation and down regulation exist. Both the immunohistochemical and Western blot results in our study showed that VDR protein was highly expressed in placenta and umbilical vein tissues during ESPE pregnancy, which was discordant with other researches [25]. We presumed that it might be related to the transportation of vitamin D in placenta and the compensatory mechanism of VDR might be contributed to this certain results [26]. In the last trimester of pregnancy, mesenchymal chorionic villi developed from immature intermediate villi to mature intermediate villi. At the age of 32-34 weeks, mature intermediate villi were further developed into terminal villi [27]. The dysplasia of chorionic villi may be related to the anoxia of placenta. Front placenta anoxia is thought to be related to maternal anemia, plateau pregnancy, maternal cyanosis and other situations with pregnancy women going through anoxia. Typical uterine placenta anoxia is gestational hypertension and preeclampsia with placenta going through anoxia [28]. Afterwards placenta anoxia is commonly seen in the cases that fetal growth was restricted with deficiency of end-diastolic umbilical cord blood flow and end-diastolic reversed umbilical cord blood flow, with or without preeclampsia. Secondly, the classification and degree of placental vascular development is controlled by oxygen levels of placenta, and in return influences the volume and shape of terminal chorionic villi [29, 30]. During front placenta anoxia, low oxygen level induces the formation of highly branched, reticular vascular beds, providing less vascular resistance [31]. Therefore, even if the vitamin D status in both maternal and umbilical vein are lower in preeclampsia group than control group, the differences between maternal vitamin D status happens to be even bigger, thus we conjectured that preeclampsia women might going through front placenta anoxia compensatory mechanism during placenta anoxia period, which leaded to the instant high expression of VDR protein in order to meet the priority needs of fetal vitamin D demand to avoid the developmental risk of infant skeleton [32].

In conclusion, the vitamin D status in maternal blood of late on-set preeclampsia are lower than normal pregnancy, especially in severe preeclampsia, We did find out that the vitamin D receptor protein were highly expressed both in placental and umbilical cord vein tissues in early on-set severe preeclampsia pregnancy, and we believed that it had something to do with compensatory mechanism, since it could preferentially meet the fetal need of vitamin

D and ensure the healthy intrauterine growth, the detailed mechanism needs further exploration.

There were also certain limitations of the present study. First, the VDR expression analysis was did only to the ESPE group due to the lack of subjects in other subgroup and incomplete of experiment conditions, thus we could not make comparisons between different subgroups. Second, the differences of cord vitamin D between normal pregnancies and PE was smaller than that of maternal vitamin D, we don't yet understand how the vitamin D differences in early life influence the bone growth and bone mineral density and further explorations are needed.

Acknowledgements

The present study was supported by grants: BL2014025 from Science and Technology Department of Jiangsu Province, F201305 and BL2014025 from the Department of Health, Jiangsu Province. The funders had no role in study design, data collection and analysis, decision for publish, or preparation of the manuscript. The authors thank the obstetricians and the pregnant women who agreed to participate in this study.

Disclosure of conflict of interest

None.

Address correspondence to: Zhi-Ce Xu, Institute for Fetology, First Hospital of Soochow University, 708 Renmin Road, Xiangcheng Dirstrict, Suzhou 215000, China. Tel: +86 512 67781951; Fax: +86 512 67-781951; E-mail: zhicexu_123@163.com

References

[1] Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet 2005; 365: 785-799.

[2] Rylander A, Lindqvist PG. Eclampsia is more prevalent during the winter season in Sweden. Acta Obstet Gynecol Scand 2011; 90: 114-117.

[3] Diaz L, Arranz C, Avila E, Halhali A, Vilchis F, Larrea F. Expression and activity of 25-hydroxyvitamin D-1 alpha-hydroxylase are restricted in cultures of human syncytiotrophoblasts from preeclamptic pregnancies. J Clin Endocrinol Metab 2002; 87: 3876-82.

[4] Sheryl L, Rifas-Shiman SL, Janet W, Rich-Edwards JW, Willett C. Changes in dietary intake from the first to the second trimester of pregnancy. Paediatr Perinat Epidemiol 2006; 20: 35-42.

[5] Dawodu A, Wagner CL. Mother-child vitamin D deficiency: an international perspective. Arch Dis Child 2007; 92: 737-740.

[6] Halicioglu O, Aksit S, Koc F, Akman SA, Albudak E, Yaprak I, Coker I, Colak A, Ozturk C, Gulec ES. Vitamin D deficiency in pregnant women and their neonates in spring time in western Turkey. Paediatr Perinat Epidemiol 2012; 26: 53-60.

[7] Brannon PM. Vitamin D and adverse pregnancy outcomes: beyond bone health and growth. Proc Nutr Soc 2012; 71: 205-212.

[8] Fleet JC, DeSmet M, Johnson R, Li Y. Vitamin D and cancer: a review of molecular mechanisms. Biochem J 2012; 441: 61-76.

[9] Laqishetty V, Liu NQ, Hewison M. Vitamin D metabolism and innate immunity. Mol Cell Endocrinol 2011; 347: 97-105.

[10] Burris HH, Rifas-Shiman SL, Kleinman K, Litonjua AA, Huh SY, Rich-Edwards JW, Camargo CA Jr, Gillman MW. Vitamin D deficiency in pregnancy and gestational diabetes mellitus. Am J Obstet Gynecol 2012; 207: 182, e1-8.

[11] Burris HH, Rifas-Shiman SL, Camargo CA Jr, Lition AA, Huh SY, Rich-Edwards JW. Plasma 25-hydroxyvitamin D d uring pregnancy and small for-gestational age in black and white infants. Ann Epidemiol 2012; 22: 581-586.

[12] American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American college of obstetricians and gynecologists' task force on hypertension in pregnancy. Obstet Gynecol 2013; 122: 1122-31.

[13] Francis R, Aspray T, Fraser W, Gittoes N, Javaid K, Macdonald H, Patel S, Selby PL, Tanna N. Vitamin D and bone health: a practical clinical guideline for patient management. National Osteoposis Society: 2013.

[14] Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium; Ross AC, Taylor CL, Yaktine AL, Del Valle HB, editors. Washington (DC): National Academies Press (US); 2011.

[15] Soslow RA, Dannenberg AJ, Rush D, Woerner BM, Khan KN, Masferrer J, Koki AT. COX-2 is expressed in human pulmonary, colonic, and mammary tumors. Cancer 2011; 89: 2637-2645.

[16] Oken E, Ning Y, Rifas-Shiman SL, RichEdwards JW, Olsen SF, Gillman MW. Diet during pregnancy and risk of preeclampsia or gestational

hypertension. Ann Epidemiol 2007; 17: 663-668.
[17] Johnson JA, Grande JP, Roche PC, Kumar R. Immunohistochemical detection and distribution of the 1, 25-dihydroxyvitamin D3 receptor in rat reproductive tissues. Histochem Cell Biol 1996; 105: 7-15.
[18] Brunhild MH, Jennifer FL, Pagano I, Cooney W, Reni AS, Adrian AF. Vitamin D deficiency in cord plasma from multiethnic subjects living in the tropics. J Am Coll Nutr 2013; 32: 215-223.
[19] Wei SQ, Audibert F, Luo ZC, Nuyt AM, Masse B, Julien P, Fraser WD. Maternal plasma 25-hydroxyvitamin D levels, angiogenic factors, and preeclampsia. Am J Obstet Gynecol 2013; 208: 390.
[20] ACOG Committee on Practice Bulletins-Obstetrics. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Obstet Gynecol 2002; 99: 159-167.
[21] Heather HB, Sheryl LR, Susanna YH, Kleinman K, Augusto AL, Oken E. Vitamin D status and hypertensive disorders in pregnancy. Ann Epidemiol 2014; 24: 399-403.
[22] Tim JG, Li WY, Susan IB, Jahani M, Gwen EC. Vitamin D supplementation is associated with higher serum 25(OH)D in Asian and White infants living in Vancouver, Canada. Matern Child Nutr 2012; 11: 253-259.
[23] Joong SS, Mee YC, Mark SL, Nelson DM. Vitamin D effects on pregnancy and the Placenta. Placenta 2010; 31: 1027-1034.
[24] Pratumvinit B, Wongkrajang P, Wataganara T, Hanyongyuth S, Nimmannit A, Chatsiricharoenkul S, Manonukul K, Reesukumal K. Maternal vitamin D status and its related factors in pregnant women in Bangkok, Thailand. PLoS One 2015; 10: e0131126.
[25] Evans KN, Bulmer JN, Kilby MD, Hewison M. Vitamin D and placental-decidual function. J Soc Gynecol Investig 2004; 11: 263-271.
[26] Carroll KN, Gebretsadik T, Larkin EK, Dupont WD, Liu Z, Van Driest S, Hartert TV. Relationship of maternal vitamin D level with maternal and infant respiratory disease. Am J Obstet Gynecol 2011; 205: 215.
[27] Nandal R, Chhabra R, Sharma D, Lallar M, Rathee U, Maheshwari P. Comparison of cord blood vitamin D levels in newborns of vitamin D supplemented and unsupplemented pregnant women: a prospective, comparative study. J Matern Fetal Neonatal Med 2015; 29: 1-19.
[28] Wei SQ. Vitamin D and pregnancy outcomes. Curr Opin Obstet Gynecol 2014; 26: 438-447.
[29] Stelmach I, Majak P, Jerzynska J, Podlecka D, Stelmach W, Polanska K, Gromadzińska J, Wąsowicz W, Hanke W. Cord serum 25-hydroxy-vitmian D correlates with early childhood viral-induced wheezing. Respir Med 2015; 109: 38-43.
[30] Gernand AD, Simhan HN, Klebanoff MA, Bodnar LM. Maternal serum 25-hydroxyvitamin D and measures of newborn and placental weight in a U.S. multicenter cohort study. J Clin Endocrinol Metab 2013; 981: 398-404.
[31] Carolin L, Berthold PH, Ralf H, Michael MS, Alexandra G, Maekus S, Grasemann C. Maternal vitamin D status in preeclampsia: seasonal changes are note influenced by placenta gene expression of vitamin D metabolizing enzymes. PLoS One 2014; 9: e105558.
[32] Christopher JR, Carol LW, Bruce WH, John EB, Donna DJ. Maternal vitamin D and fetal growth in early-onset severe preeclampsia. Am J Obstet Gynecol 2011; 204: 1-4.

Table S1A. Characteristics of Preeclampsia subsets (EMPE)

Characteristics	Control (n = 60)	Early onset mild preeclampsia (EMPE, n = 5)	*P* value
General characteristics			
Maternal age, year			
Mean ± SD	29.5 ± 4.7	31.4 ± 6.1	.40
< 30	30 (50.0)	2 (40.0)	.19[a]
30 - < 35	24 (40.0)	1 (20.0)	
≥ 35	6 (10.0)	2 (40.0)	
Residence			.37[a]
Urban	38 (63.3)	2 (40.0)	
Rural	22 (36.7)	3 (60.0)	
Pregnancy Variables			
Pregnancy body mass index, kg/m^2 (BMI)			
Mean ± SD	25.99 ± 2.40	28.95 ± 3.40	.01*
Normal (< 25.00)	18 (30.0)	1 (20.0)	.03[a,*]
Overweight (25.00 - < 29.99)	40 (66.7)	2 (40.0)	
Obese (≥ 30.00)	2 (3.3)	2 (40.0)	
Parity			.67[a]
Nulliparous (0)	31 (51.7)	2 (40.0)	
Primiparous/multiparous (> 0)	29 (48.3)	3 (60.0)	
Infant gender			1.00[a]
Female	18 (30.0)	1 (20.0)	
Male	42 (70.0)	4 (80.0)	
Delivery			.07[a]
Vaginal	27 (45.0)	0 (0.0)	
Cesarean section	33 (55.0)	5 (100.0)	
Gestational weeks at delivery, week			.50
Mean ± SD	35.3 ± 2.7	34.9 ± 0.8	
Pregnancy blood pressure			
Systolic blood pressure	123.75 ± 10.12	142.40 ± 16.56	< .0001*
Diastolic blood pressure	78.68 ± 7.70	90.60 ± 8.62	.002*

Data are presented as N. (%) or Mean ± SD. *P-value < 0.05 were calculated and marked, using student t-test for continuous variables and χ^2 or Fisher exact test for categorical variables. [a]Fisher exact test (two-sided).

Table S1B. Characteristics of preeclampsia subsets (ESPE)

Characteristics	Control (n = 60)	Early onset severe preeclampsia (ESPE, n = 34)	*P* value
General characteristics			
Maternal age, year			
Mean ± SD	29.5 ± 4.7	30.1 ± 4.5	.56
< 30	30 (50.0)	14 (41.2)	.62[a]
30 - < 35	24 (40.0)	15 (44.1)	
≥ 35	6 (10.0)	5 (14.7)	
Residence			
Urban	38 (63.3)	23 (67.6)	.67
Rural	22 (36.7)	11 (32.4)	
Pregnancy Variables			
Pregnancy body mass index, kg/m^2 (BMI)			
Mean ± SD	25.99 ± 2.40	27.87 ± 2.87	.001*
Normal (< 25.00)	18 (30.0)	3 (8.8)	.007[a,*]
Overweight (25.00 - < 29.99)	40 (66.7)	25 (73.5)	
Obese (≥ 30.00)	2 (3.3)	6 (17.6)	
Parity			
Nulliparous (0)	31 (51.7)	26 (76.5)	.02*
Primiparous/multiparous (> 0)	29 (48.3)	8 (23.5)	
Infant gender			
Female	18 (30.0)	17 (50.0)	.05
Male	42 (70.0)	17 (50.0)	
Delivery			
Vaginal	27 (45.0)	0 (0.0)	< .0001*
Cesarean section	33 (55.0)	34 (100.0)	
Gestational weeks at delivery, week			
Mean ± SD	35.3 ± 2.7	33.8 ± 2.6	.01*
Pregnancy blood pressure			
Systolic blood pressure	123.75 ± 10.12	157.59 ± 17.98	< .0001*
Diastolic blood pressure	78.68 ± 7.70	107.41 ± 12.54	< .0001*

Data are presented as N. (%) or Mean ± SD. *P-value < 0.05 were calculated and marked, using student t-test for continuous variables and χ^2 or Fisher exact test for categorical variables. [a]Fisher exact test (two-sided).

Table S1C. Characteristics of preeclampsia subsets (LMPE)

Characteristics	Control (n = 60)	Late onset mild preeclampsia (LMPE, n = 13)	*P* value
General characteristics			
Maternal age, year			
Mean ± SD	29.5 ± 4.7	28.0 ± 5.0	.30
< 30	30 (50.0)	8 (61.5)	.82[a]
30 - < 35	24 (40.0)	4 (30.8)	
≥ 35	6 (10.0)	1 (7.7)	
Residence			
Urban	38 (63.3)	6 (46.2)	.25
Rural	22 (36.7)	7 (53.8)	
Pregnancy Variables			
Pregnancy body mass index, kg/m^2 (BMI)			
Mean ± SD	25.99 ± 2.40	30.98 ± 4.00	.001*
Normal (< 25.00)	18 (30.0)	1 (7.7)	< .0001[a]
Overweight (25.00 - < 29.99)	40 (66.7)	6 (46.2)	
Obese (≥ 30.00)	2 (3.3)	6 (46.1)	
Parity			
Nulliparous (0)	31 (51.7)	3 (32.1)	.06
Primiparous/multiparous (> 0)	29 (48.3)	10 (76.9)	
Infant gender			
Female	18 (30.0)	5 (38.5)	.53[a]
Male	42 (70.0)	8 (61.5)	
Delivery			
Vaginal	27 (45.0)	2 (15.4)	.048*
Cesarean section	33 (55.0)	11 (84.6)	
Gestational weeks at delivery, week			
Mean ± SD	35.3 ± 2.7	37.5 ± 1.7	.006*
Pregnancy blood pressure			
Systolic blood pressure	123.75 ± 10.12	145.15 ± 9.85	< .0001*
Diastolic blood pressure	78.68 ± 7.70	93.30 ± 6.51	< .0001*

Data are presented as N. (%) or Mean ± SD. *P-value < 0.05 were calculated and marked, using student t-test for continuous variables and χ^2 or Fisher exact test for categorical variables. [a]Fisher exact test (two-sided).

Table S1D. Characteristics of preeclampsia subsets (LSPE)

Characteristics	Control (n = 60)	Late onset severe preeclampsia (LSPE, n = 8)	*P* value
General characteristics			
Maternal age, year			
Mean ± SD	29.5 ± 4.7	26.1 ± 5.0	.06
< 30	30 (50.0)	6 (75.0)	.44[a]
30 - < 35	24 (40.0)	2 (25.0)	
≥ 35	6 (10.0)	0 (0.0)	
Residence			
Urban	38 (63.3)	5 (62.5)	1.00[a]
Rural	22 (36.7)	3 (37.5)	
Pregnancy Variables			
Pregnancy body mass index, kg/m^2 (BMI)			
Mean ± SD	25.99 ± 2.40	27.11 ± 2.08	.21
Normal (< 25.00)	18 (30.0)	1 (12.5)	.55[a]
Overweight (25.00 - < 29.99)	40 (66.7)	7 (87.5)	
Obese (≥ 30.00)	2 (3.3)	0 (0.0)	
Parity			
Nulliparous (0)	31 (51.7)	3 (37.5)	.71[a]
Primiparous/multiparous (> 0)	29 (48.3)	5 (62.5)	
Infant gender			
Female	18 (30.0)	5 (62.5)	.11[a]
Male	42 (70.0)	3 (37.5)	
Delivery			
Vaginal	27 (45.0)	0 (0.0)	.02[a,*]
Cesarean section	33 (55.0)	8 (100.0)	
Gestational weeks at delivery, week			
Mean ± SD	35.3 ± 2.7	37.1 ± 1.1	.002[*]
Pregnancy blood pressure			
Systolic blood pressure	123.75 ± 10.12	166.75 ± 15.75	< .0001[*]
Diastolic blood pressure	78.68 ± 7.70	107.88 ± 13.44	< .0001[*]

Data are presented as N. (%) or Mean ± SD. [*]*P*-value < 0.05 were calculated and marked, using student *t*-test for continuous variables and χ^2 or Fisher exact test for categorical variables. [a]Fisher exact test (two-sided).

Table S2A. 25-hydroxyvitamin D status and preeclampsia subsets (EMPE)

Characteristics	Controls (n = 60)	Early onset mild preeclampsia (EMPE, n = 5)	*P* value
Maternal plasma 25(OH)D concentration, nmol/L			
Mean ± SD	27.99 ± 6.16	21.76 ± 2.88	.03*
Median (Minimum-Maximum)	27.46 (18.30~45.30)	21.46 (17.89~25.64)	
Proportion			.22[a]
< 25	21 (35.0)	4 (80.0)	
25 - < 35	32 (53.3)	1 (20.0)	
≥ 35	7 (11.7)	0 (0.0)	
Cord plasma 25(OH)D concentration, nmol/L			
Mean ± SD	31.49 ± 8.00	30.34 ± 7.18	.77
Median (Minimum-Maximum)	30.30 (16.89~47.40)	28.11 (23.50~40.57)	
Proportion			
< 25	14 (23.3)	2 (40.0)	.72[a]
25 - < 35	25 (41.7)	2 (40.0)	
≥ 35	21 (35.0)	1 (20.0)	
Seasons of last menstrual period			
Spring	10 (16.7)	2 (40.0)	.41[a]
Summer	40 (66.7)	3 (60.0)	
Autumn	1 (1.7)	0 (0.0)	
Winter	9 (15.0)	0 (0.0)	

Data are presented as N. (%) or Mean ± SD. *P-value < 0.05 were calculated and marked, using student t-test for continuous variables and χ^2 or Fisher exact test for categorical variables. [a]Fisher exact test (two-sided).

Table S2B. 25-hydroxyvitamin D status and preeclampsia subsets (ESPE)

Characteristics	Controls (n = 60)	Early onset severe preeclampsia (ESPE, n = 34)	*P* value
Maternal plasma 25(OH)D concentration, nmol/L			
Mean ± SD	27.99 ± 6.16	22.96 ± 4.49	< .0001*
Median (Minimum-Maximum)	27.46 (18.30~45.30)	21.54 (17.24~34.50)	
Proportion			
< 25	21 (35.0)	23 (67.6)	.003[a,*]
25 - < 35	32 (53.3)	11 (32.4)	
≥ 35	7 (11.7)	0 (0.0)	
Cord plasma 25(OH)D concentration, nmol/L			
Mean ± SD	31.49 ± 8.00	27.67 ± 7.93	.03*
Median (Minimum-Maximum)	30.30 (16.89~47.40)	26.36 (10.95~46.70)	
Proportion			
< 25	14 (23.3)	11 (32.4)	.19
25 - < 35	25 (41.7)	17 (50.0)	
≥ 35	21 (35.0)	6 (17.6)	
Seasons of last menstrual period			
Spring	10 (16.7)	4 (11.8)	.93[a]
Summer	40 (66.7)	23 (67.6)	
Autumn	1 (1.7)	1 (3.0)	
Winter	9 (15.0)	6 (17.6)	

Data are presented as N. (%) or Mean ± SD. *P-value < 0.05 were calculated and marked, using student t-test for continuous variables and χ^2 or Fisher exact test for categorical variables. [a]Fisher exact test (two-sided).

Table S2C. 25-hydroxyvitamin D status and preeclampsia subsets (LMPE)

Characteristics	Controls (n = 60)	Late onset mild preeclampsia (LMPE, n = 13)	*P* value
Maternal plasma 25(OH)D concentration, nmol/L			
Mean ± SD	27.99 ± 6.16	23.77 ± 3.14	.001*
Median (Minimum-Maximum)	27.46 (18.30~45.30)	23.50 (17.89~27.27)	
Proportion			
< 25	21 (35.0)	7 (53.8)	.28[a]
25 - < 35	32 (53.3)	6 (46.2)	
≥ 35	7 (11.7)	0 (0.0)	
Cord plasma 25(OH)D concentration, nmol/L			
Mean ± SD	31.49 ± 8.00	26.12 ± 5.20	.005*
Median (Minimum-Maximum)	30.30 (16.89~47.40)	25.43 (17.40~35.89)	
Proportion			
< 25	14 (23.3)	4 (30.8)	.12[a]
25 - < 35	25 (41.7)	8 (61.5)	
≥ 35	21 (35.0)	1 (7.7)	
Seasons of last menstrual period			
Spring	10 (16.7)	4 (30.8)	.61[a]
Summer	40 (66.7)	8 (61.5)	
Autumn	1 (1.7)	0 (0.0)	
Winter	9 (15.0)	1 (7.7)	

Data are presented as N. (%) or Mean ± SD. *P-value < 0.05 were calculated and marked, using student t-test for continuous variables and χ^2 or Fisher exact test for categorical variables. [a]Fisher exact test (two-sided).

Table S2D. 25-hydroxyvitamin D status and preeclampsia subsets (LSPE)

Characteristics	Controls (n = 60)	Late onset severe preeclampsia (LSPE, n = 8)	*P* value
Maternal plasma 25(OH)D concentration, nmol/L			
Mean ± SD	27.99 ± 6.16	23.04 ± 4.77	.03*
Median (Minimum-Maximum)	27.46 (18.30~45.30)	22.53 (17.01~31.60)	
Proportion			
< 25	21 (35.0)	5 (62.5)	.39[a]
25 - < 35	32 (53.3)	3 (37.5)	
≥ 35	7 (11.7)	0 (0.0)	
Cord plasma 25(OH)D concentration, nmol/L			
Mean ± SD	31.49 ± 8.00	28.21 ± 9.35	.29
Median (Minimum-Maximum)	30.30 (16.89~47.40)	28.54 (17.52~43.83)	
Proportion			
< 25	14 (23.3)	3 (32.4)	.40[a]
25 - < 35	25 (41.7)	4 (50.0)	
≥ 35	21 (35.0)	1 (17.6)	
Seasons of last menstrual period			
Spring	10 (16.7)	1 (11.8)	1.00[a]
Summer	40 (66.7)	6 (67.6)	
Autumn	1 (1.7)	0 (3.0)	
Winter	9 (15.0)	1 (17.6)	

Data are presented as N. (%) or Mean ± SD. *P-value < 0.05 were calculated and marked, using student t-test for continuous variables and χ^2 or Fisher exact test for categorical variables. [a]Fisher exact test (two-sided).