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Relationship between vitamin D status in pregnancy and the risk for preeclampsia: A nested case-control study

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Abstract

Background &Aims. Vitamin D is thought to be involved in the pathogenesis of preeclampsia. To evaluate the relationship between vitamin D insufficiency in the first trimester of pregnancy and preeclampsia.

Methods. Nested case-control study (FEPED study) in type 3 obstetrical units. Pregnant women from 10 to 15 WA. For each patient with preeclampsia, 4 controls were selected from the cohort and matched by parity, skin color, maternal age, season and BMI. The main outcome measure was serum 25(OH)D status in the first trimester

Results: 83 cases of preeclampsia were matched with 319 controls. Mean 25(OH)D levels in the first trimester were 20.1 ± 9.3 ng/mL in cases and 22.3 ± 11.1 ng/mL in controls (p=0.09). The risk for preeclampsia with 25(OH)D level $\geq$30 ng/mL in the first trimester was decreased, but did not achieve statistical significance (OR, 0.57; 95% CI, 0.30–1.01; p=0.09). High 25(OH)D during the 3rd trimester was associated with a significantly decreased risk of preeclampsia (OR, 0.43; 95%CI, 0.23-0.80; p=0.008). When women with 25(OH)D levels <30 ng/mL both in the first and 3rd trimesters ("low-low") were taken as references, OR for preeclampsia was 0.59 (95% CI, 0.31–1.14; p=0.12) for "low-high" or "high-low" women and 0.34 (95% CI, 0.13–0.86; p = 0.02) for "high-high" women.

Conclusions: No significant association between preeclampsia and vitamin D insufficiency in the first trimester was evidenced. However, women with vitamin D sufficiency during the 3rd trimester and both in the first and 3rd trimesters had a significantly lower risk of preeclampsia.

Introduction

Preeclampsia is defined as *de novo* high blood pressure and proteinuria after 20 weeks of amenorrhea (WA) (1). This syndrome is observed in 1.5–5% of pregnancies (2). The maternal and neonatal mortality associated with preeclampsia is high in low-income and middle-income countries (3,4). Abnormal implantation and development of the placenta associated with poor utero-placental perfusions are considered as the major causes of preeclampsia (5). Abnormal implantation may originate from the response of the maternal immune system to the placenta after disruption of immunological tolerance during pregnancy (6). The only curative treatment of preeclampsia is delivery (7). Only low-dose aspirin proved effective for the prevention of preeclampsia in women at risk in a recent trial (8). Vitamin D supplementation has been suggested to be beneficial during pregnancy beyond its classical actions on calcium balance and bone metabolism (9). Meta-analyses and systematic literature reviews have concluded that a low maternal serum 25-hydroxyvitamin D [25(OH)D] concentration is associated with an increased risk of preeclampsia (10-14). These results on the relationship between vitamin D deficiency/insufficiency and preeclampsia suggest that vitamin D supplementation could prevent preeclampsia.

Interventional studies have assessed the effects of vitamin D supplementation on maternal and fetal outcomes. A systematic review and meta-analysis of randomized clinical trials concluded that vitamin D supplementation was associated with increased circulating 25(OH)D levels, birth weight and birth length, but the incidence of preeclampsia was not significantly changed (14). However, only three randomized studies, which were heterogeneous, were included in assessment of preeclampsia rates. A recent Cochrane review including two randomized trials for a total of 219

women concluded that vitamin D supplementation may reduce the risk of preeclampsia, low birth weight and preterm birth (15). The quality of evidence was, however, considered low and the authors of these reviews underscored that further randomized trials with rigorous designs are needed to confirm the benefit of vitamin D supplementation in pregnant women.

These meta-analyses and systematic reviews included trials with vitamin D administered after 20 WA. Yet, the initial study of Bodnar et al. showed that vitamin D deficiency in early pregnancy was an independent risk factor of preeclampsia (16). Further studies confirmed that high vitamin D status in early pregnancy is protective against preeclampsia (17-19), while other studies found this protective association in mid or late pregnancy (20-23).

Because of these conflicting results, there is currently no consensus for vitamin D supplementation during pregnancy for the prevention of preeclampsia.

The present case-control study in a French-Belgian cohort explored the relationship between vitamin D insufficiency (defined by a 25(OH)D level <30 ng/mL) in the first trimester and the occurrence of preeclampsia later in pregnancy. Secondary objectives were to evaluate this potential relationship between preeclampsia and 25(OH)D during the third trimester of pregnancy.

Materials and Methods

Study design

We performed a nested case-control study within a prospective observational cohort (FEPED cohort) including pregnant women in six centers: one in Belgium (latitude 50.83°N) and five in France. Four of the five French centers were located in Paris or its suburbs (Béclère, Bicêtre, Cochin and Trousseau university hospitals, latitude 48.86°N) and the last one was located in Nantes (latitude 47.22°N).

The primary objective was to assess the risk of preeclampsia according to vitamin D status in the first trimester (< 15 WA). A secondary objective was to assess this risk according to vitamin D status in the third trimester. Vitamin D insufficiency was defined as a serum 25(OH)D level below 30 ng/mL (i.e. 75 nmol/L).

Women were included in the cohort if they were at the first trimester (from 10 to <15 WA) of a singleton pregnancy. Exclusion criteria were the following: hypercalcemia (> 2.65 mmol/L) or any other calcium-phosphorus imbalance, hypertension (>140/90 mmHg) from the first trimester, renal insufficiency (creatinine > 120 µmol/L), bone disease, lithium therapy, bowel malabsorption or kidney stone disease.

A bolus vitamin D dose (100 000 IU of cholecalciferol) was prescribed to the patients at the 7th month of pregnancy according to current French recommendations.

Written informed consent was obtained from each patient before inclusion in the study. The protocol was conducted in accordance with the Declaration of Helsinki and was approved by a local independent Ethics Committee (2011/13NICB). The study was sponsored by the Assistance Publique-Hôpitaux de Paris (AP-HP) and was funded by a grant from the Programme Hospitalier de Recherche Publique – PHRC national 2010 (Ministry of Health – AOM10113). This grant includes external

peer review for scientific quality and only accepts 10% of applications. It is registered with the ClinicalTrials.gov identifier NCT01648842. Samples were stored in the Perinat Collection (ANR-10-EQPX-0010).

Selection of cases and controls

We matched cases of preeclampsia to eligible controls randomly selected in a 1:4 ratio. Preeclampsia was defined as a blood pressure > 140 mmHg or a diastolic blood pressure > 90 mmHg and proteinuria > 0.3 g/24 h. This definition is the one currently used in France and it was also used internationally at the time of the trial set up. Matching factors were parity (primiparous or not), season at conception, skin color (< 5 or ≥ 5 according to Fitzpatrick scale), body mass index (< 25 or ≥ 25 kg/m^2) and maternal age (< 35 or ≥ 35 years). In addition, cases were matched to controls with the closest age whenever possible. Skin color was preferred instead of the more commonly used variable ethnicity, because its use seemed more reliable.

Patients were excluded from selection of controls if we could not be sure whether preeclampsia occurred or not (patients not followed up to delivery or no data on blood pressure or proteinuria), if pregnancy was interrupted (abortion, intrauterine fetal death) or if there were no data on delivery. The sample of eligible controls was obtained from controls without preterm delivery (≥ 37 WA), whose newborn was alive in the delivery room and presented no intrauterine growth restriction (< 5th percentile) at birth, with vitamin D measurement available in both the first and third trimesters and with no missing data on any matching factors.

Assessment of vitamin D status

25(OH)D was measured in maternal blood samples obtained during the first (11 to <15 WA) and third (28–40 WA) trimesters. The patients did not need to be fasting

before blood sample collection. All blood samples were centrifuged and stored locally at -20°C and subsequently transferred monthly for centralized serum 25(OH)D measurement using DiaSorin RIA in the Necker University Hospital Department of Physiology (Paris, France), which has excellent results in the Vitamin D External Quality Assessment Scheme (DEQAS). An arbitrary value of 4 ng/mL corresponding to the limit of quantification that we determined in our laboratory was assigned to any undetectable concentration.

Statistical analysis

The study was designed to detect an odds ratio of 3 for preeclampsia in the 20% of women with the lowest vitamin D level, with 90% power and a two-sided alpha value of 5%. This low rate of exposure was chosen to insure sufficient power, because at this time no universally recognized threshold was available to define vitamin D insufficiency and there were few reliable published data on pregnant women. We chose to match 4 controls per case to maximize power in this case-control study. Defining a stratum as the combination of one case and its 4 associated controls, we assumed an intraclass correlation coefficient within strata = 0.2, thus producing a target sample size of 61 cases and 244 controls.

With an expected rate of pre-eclampsia at 1.5% in this population, a cohort of 4100 patients was needed. Enrolment of 4500 patients was thus finally planned to take into account potential loss to follow-up.

All statistical analyses were undertaken using R 2.11.1 software. Statistical tests were two-sided and p values less than 0.05 were considered statistically significant. Baseline characteristics of the two groups were described as mean ± standard deviation for quantitative variables and frequencies (%) for qualitative variables.

Primary outcome was analyzed using conditional logistic regression. Among

secondary outcomes studied, the association between preeclampsia and vitamin D insufficiency was also investigated at the third trimester, using two different thresholds for each timepoint and conditional logistic regression. Other secondary outcomes were compared between groups using the Chi-squared test (or Fisher's test when it was appropriate) for qualitative parameters and Student's t test for quantitative parameters.

Results

Demographic and clinical characteristics of cases and controls

Pregnant women were included from April 2012 to July 2014 with the last delivery in February 2015. From this total cohort of 3093 women, we identified 84 cases of preeclampsia. Mean term at the onset of preeclampsia was 36.2 ± 3.8 WA, 9 cases occurring before or at 32 WA. A total of 296 patients were excluded and the prevalence of preeclampsia in the remaining 2797 women was therefore 3.0% (84/2797). Finally, 83 cases of preeclampsia were randomly matched among the eligible controls. Only one case out of the 84 was excluded because of incomplete data at inclusion and over follow-up. For six cases with the least common profiles, we could not match the four controls required. Overall, the 83 cases were matched to four controls (n=77), three controls (n=1), two controls (n=3) or one control (n=2), leading to a total number of 319 controls. The selection of cases and controls is summarized in **Figure 1**.

The characteristics of the 83 preeclampsia cases and the 319 matched controls are summarized in **Table 1**. As expected, cases and controls were comparable for matching variables (age, body mass index before pregnancy, conception season, skin color and parity). Pregnancy terms at inclusion (12.8 ± 0.9 and 12.8 ± 0.8 WA)

were comparable. The rates of diabetes before pregnancy were comparable (< 5%). After inclusion, the rates of gestational diabetes were 14.5% in cases and 8.9% in controls (p = 0.13) (**Table 2**). Mean delivery term was significantly shorter in cases compared with controls (37.2 ± 3.0 vs 39.9 ± 1.2 WA; p < 0.001).

Routine vitamin D supplementation

Pregnant women received vitamin D supplementation as a routine procedure in agreement with national guidelines. There was no significant difference in the percentage of women who took vitamin D supplementation during pregnancy: 79.5% of cases and 85.7% of controls (p = 0.17). Women received vitamin D supplementation after a mean 28.1 ± 3.3 WA for cases and 27.9 ± 3.8 WA for controls (p = 0.64) (**Table 2**).

Levels of 25(OH)D during pregnancy and association with preeclampsia

25(OH)D levels measured in the first trimester after a mean 12.8 WA were comparable in cases and matched controls (20.1 ± 9.3 vs. 22.3 ± 11.1 ng/mL; p=0.09) (**Table 2**). Women with vitamin D sufficiency represented 16.9% of cases and 26.0% of controls (p = 0.17). In the first trimester, the risk for preeclampsia was decreased in these patients, but did not achieve statistical significance: the odds ratio (OR) of preeclampsia was 0.57 (95% CI, 0.30–1.01; p = 0.09; conditional logistic regression estimation) (**Table 3**). However, 25(OH)D levels in the first trimester in women who developed preeclampsia ≤ 32 WA (12.0[5-25]) was lower than in the group after 32 WA (19.5 [5-42]) (p=0.028). The association between maternal 25(OH)D in the first trimester and the risk of preeclampsia was analyzed after adjustment for matching factors (Lowess plot). As shown in **Figure 2**, the probability of preeclampsia decreased with the level of 25(OH)D. For each 10 ng/mL increase,

the risk of preeclampsia decreased by 19%, without however achieving statistical significance.

25(OH)D levels were measured in the third trimester after a mean 35.0 ± 2.4 WA in cases and 35.1 ± 2.2 WA in controls (**Table 2**). The mean 25(OH)D level was significantly higher in controls compared with cases (30.8 ± 11.0 vs. 27.7 ± 12.2 ng/mL; p = 0.049). Patients with vitamin D sufficiency (≥ 30 ng/mL) were significantly less frequent in cases compared with controls: 34.9% versus 53.6% (p = 0.009). The OR for preeclampsia associated with vitamin D sufficiency during the third trimester was 0.43 (95% CI, 0.23–0.80; p = 0.008; conditional logistic regression estimation) (**Table 3**).

Exploratory analyses were performed by combining measurements of 25(OH)D in the first and third trimesters. When women with 25(OH)D <30 ng/mL in both the first and third trimesters ("low-low") were taken as references, the OR for preeclampsia was 0.59 (95% CI, 0.31–1.14, p = 0.12) for "low-high" or "high-low" women and 0.34 (95% CI, 0.13–0.86; p = 0.023) for "high-high" women (**Table 3**).

Discussion

We investigated the relationship between vitamin D insufficiency and preeclampsia in a cohort of pregnant women where the prevalence of preeclampsia was 3.0%. After matching cases and controls, the risk for preeclampsia in women with serum vitamin D level ≥30 ng/mL in the first trimester was decreased, but did not achieve statistical significance.

In other nested case-control studies that assessed vitamin D status in early pregnancy (summarized in **Table 4**), a significantly lower 25(OH)D concentration was reported in cases compared with controls. The relative difference in mean

concentrations was 14.2% (18.2 vs. 21.2 ng/mL) in Bodnar et al. (16), 23.5% (30.0 vs. 39.2 ng/mL; median) in Baker et al. (18) and 9.6% (18.9 vs. 20.9 ng/mL) in Achkar et al. (17). In our study, the relative difference between controls and cases was 9.9% (20.1 vs. 22.3 ng/mL), but statistical significance was not achieved. In Achkar et al., which is the most recent study and probably the one out of the three with the most rigorous methodology, very few patients had 25(0H) D >30 ng/mL and therefore they were not able to draw any conclusions for this cut-off.

The decrease of 25(OH)D was translated into a significantly increased risk of preeclampsia in these previous studies (**Table 4**). In the present study, a level <30 ng/mL increased the risk by 1.75 without reaching statistical significance.

Our study was the only one performed in Europe among the case-control studies assessing vitamin D status in early pregnancy on preeclampsia. The three other case-control studies were performed in North American cohorts with various prevalence rates of preeclampsia: 4.9% in the US cohort of Bodnar et al. (16), 1.2% (only severe preeclampsia) in the US cohort of Baker et al. (18) and 1.8% in the Canadian cohort of Achkar et al. (17). Another difference was a 30 ng/mL cut-off in defining vitamin D insufficiency in our study; however, exploratory analyses using a 20 ng/mL cut-off did not change the conclusions (OR=1.27, 95% CI 0.78-2.08; p = 0.34). Many other differences between the 3 studies and ours are summarized in Table 4. Patient selection varies between the 4 studies, as do gestational age at sampling and adjustment factors. We used a conditional logistic regression for analysis, which generates unbiased estimates when using matched data, a 30 ng/mL 25(OH)D cut-off and a short timeframe for sampling in order to be able to answer the specific question of the association of 25(OH)D deficiency in the first trimester and preeclampsia.

In France, vitamin D supplementation is recommended with one vial of 100.000 IU of cholecalciferol administered at 28 WA. In our study, the percentages of women who received this vitamin D supplementation did not differ between cases (79.5%) and controls (85.7%). The third-trimester maternal serum 25(OH)D concentrations indicated an improvement of the rates of patients with 25(OH)D ≥ 30 ng/mL in both controls and cases. Interestingly, despite vitamin D supplementation in both groups and a marginal difference in first-trimester 25(OH)D concentration, only 34.9% of cases achieved 25(OH)D ≥ 30 ng/mL at 35 WA vs. 53.6% in controls (p=0.009). These results might suggest that the metabolism of vitamin D is different in women who have preeclampsia. Nevertheless, in the absence of a more precise understanding of the role of vitamin D and its metabolites in the pathogenesis of preeclampsia, it is difficult to speculate further.

Mirzakahani et al. have recently explored the effect of early vitamin D supplementation on preeclampsia in the Vitamin D Antenatal Asthma Reduction Trial (VDAART) (23). They evaluated daily administration of vitamin D3 (4400 or 400 IU/day) starting early in pregnancy (10–18 WA). There was no difference between treatment groups for the rates of preeclampsia. Nevertheless, regardless of treatment groups, women with 25(OH)D ≥ 30 ng/mL in both early and late pregnancy developed preeclampsia less frequently (OR, 0.28; 95% CI, 0.10–0.96) (23). We confirmed these results in an exploratory analysis where women with high vitamin D status in both the first and third trimesters were significantly protected against preeclampsia (OR, 0.34; 95% CI, 0.13–0.86).

Overall, vitamin D deficiency appears to be a risk factor of preeclampsia, but available studies do not support vitamin supplementation as an effective treatment for the prevention of this risk. In order to understand better the possible role of

316 vitamin D in the pathogenesis of eclampsia, Mirzakhani et al. studied which genes
317 were activated in the peripheral blood of pregnant women after vitamin D
318 supplementation (23). They reported a series of vitamin D-associated genes that
319 were closely connected with genes involved in preeclampsia. These findings are
320 therefore arguments for further studies aimed at defining the timing and dosage of
321 vitamin D supplementation for greater efficacy in preeclampsia prevention.

322 One possible explanation for the failure of trials on vitamin D supplementation in
323 preeclampsia is that the current cut-offs defining vitamin D deficiency/insufficiency,
324 which are based on osteoporosis prevention, are inappropriate for the prevention of
325 preeclampsia. Another possibility is that the timing of vitamin D supplementation is
326 not adequate. Indeed, preeclampsia is the late consequence of abnormal
327 placentation, which occurs at the very beginning of pregnancy, and sufficient vitamin
328 D concentrations must be present at that time (24). Therefore, one could suggest that
329 the effect of vitamin D supplementation before pregnancy on the risk of preeclampsia
330 should be assessed in clinical trials, preferably in at-risk women.

331 There are some limitations in this case-control study, which was nested in a large
332 cohort. Each case was paired with controls according to known risk factors of
333 preeclampsia. Although all precautions were taken in matching cases with controls,
334 we cannot exclude that some unknown confounding factors could have introduced a
335 bias, even after matching for known risk factors. Although this is not a real limitation
336 in our opinion, it must be noted that we used the DiaSorin RIA to measure 25(OH)D
337 in the present study in accordance with the protocol that was submitted to the *Clinical*
338 *Trial* website in 2011. At that time, this assay was the most appropriate immunoassay
339 to test the association between serum 25OHD concentration and pregnancy
340 outcomes because it was little influenced by the well-known increase in vitamin D

binding protein during pregnancy thanks to a pre-treatment of the serum samples with acetonitrile. However, the DiaSorin RIA is no longer available, and there is still a significant inter-method variability in the measurement of 25(OH)D with the current automated immunoassays. We thus believe that, according to the recommendations of the Vitamin D standardization Program (VDSP) [25], future studies should use a VDSP-traceable 25(OH)D assay performed by a certified laboratory. This would allow pooling research data from different studies and a better definition of a 25(OH)D level below which the risk of a given disease is significantly increased.

Additional methodological strengths of the study were the early gestational age and short timeframe (11-14 WA) at sampling, the multicenter collection of samples and the prospective measurement of serum 25(OH)D before any symptom of preeclampsia. No significant association between preeclampsia and vitamin D deficiency in the first trimester was found. Nevertheless, women with vitamin D sufficiency in both the first and third trimesters had a significantly lower risk of preeclampsia. Studies aimed at evaluating vitamin D supplementation from early pregnancy and even before pregnancy in at-risk women are needed.

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Conflict of Interest Statement

JC. Souberbielle reports lecture fees and/or travel/hotel expenses from DiaSorin, Roche Diagnostics, Abbott, Amgen, Shire, MSD, Lilly, and Rottapharm/Meda.

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453 **Figure Legends**

454 **Table 1.** Clinical and demographic characteristics of the women with preeclampsia
455 (cases) and matched women without preeclampsia (controls).

456 **Table 2.** 25(OH) D concentration in maternal serum, intakes of vitamin D and other
457 data recorded during pregnancy.

458 **Table 3.** Odds ratios for preeclampsia according to vitamin D status in the first and/or
459 third trimesters.

460 **Table 4.** Nested case-control studies aimed to assess the relationship between
461 vitamin D status in early pregnancy and preeclampsia.

462 **Figure 1.** Flow chart for the nested case-control study.

463 **Figure 2.** Association between maternal serum 25(OH)D at first trimester of gestation
464 and predicted probability of preeclampsia (Lowess plot). Probability of preeclampsia
465 was calculated with a logistic model adjusted for matching factors (parity, maternal
466 age, prepregancy BMI, season of conception, skin color). Upper and lower dashed
467 lines are the respective upper and lower limits of the 95% confidence interval.

Figure 1. Flow chart for the nested case-control study.

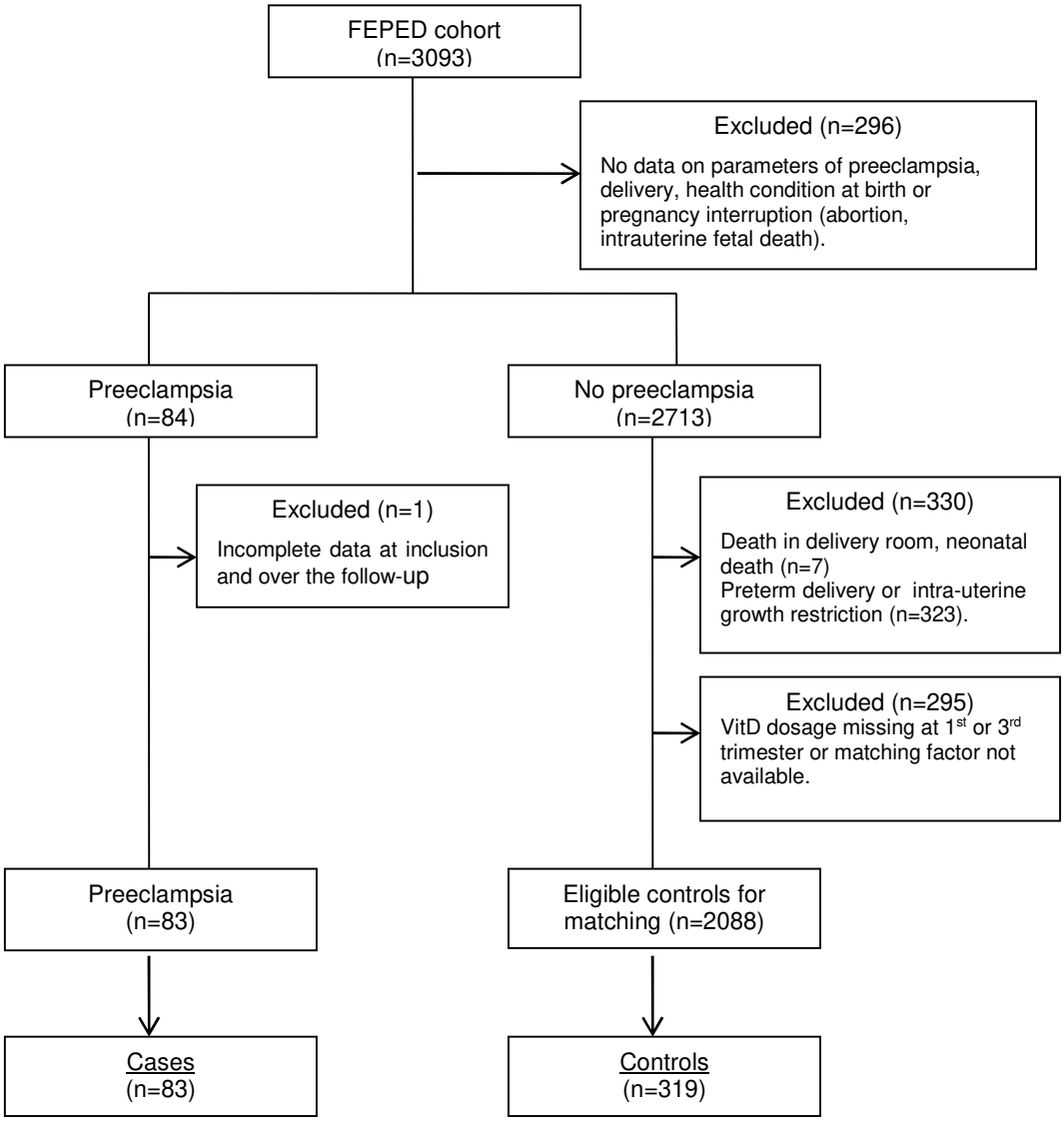

Figure 2. Association between maternal serum 25(OH)D at first trimester of gestation and predicted probability of preeclampsia (Lowess plot). Probability of preeclampsia was calculated with a logistic model adjusted for matching factors (parity, maternal age, prepregancy BMI, season of conception, skin color). Upper and lower dashed lines are the respective upper and lower limits of the 95% confidence interval.

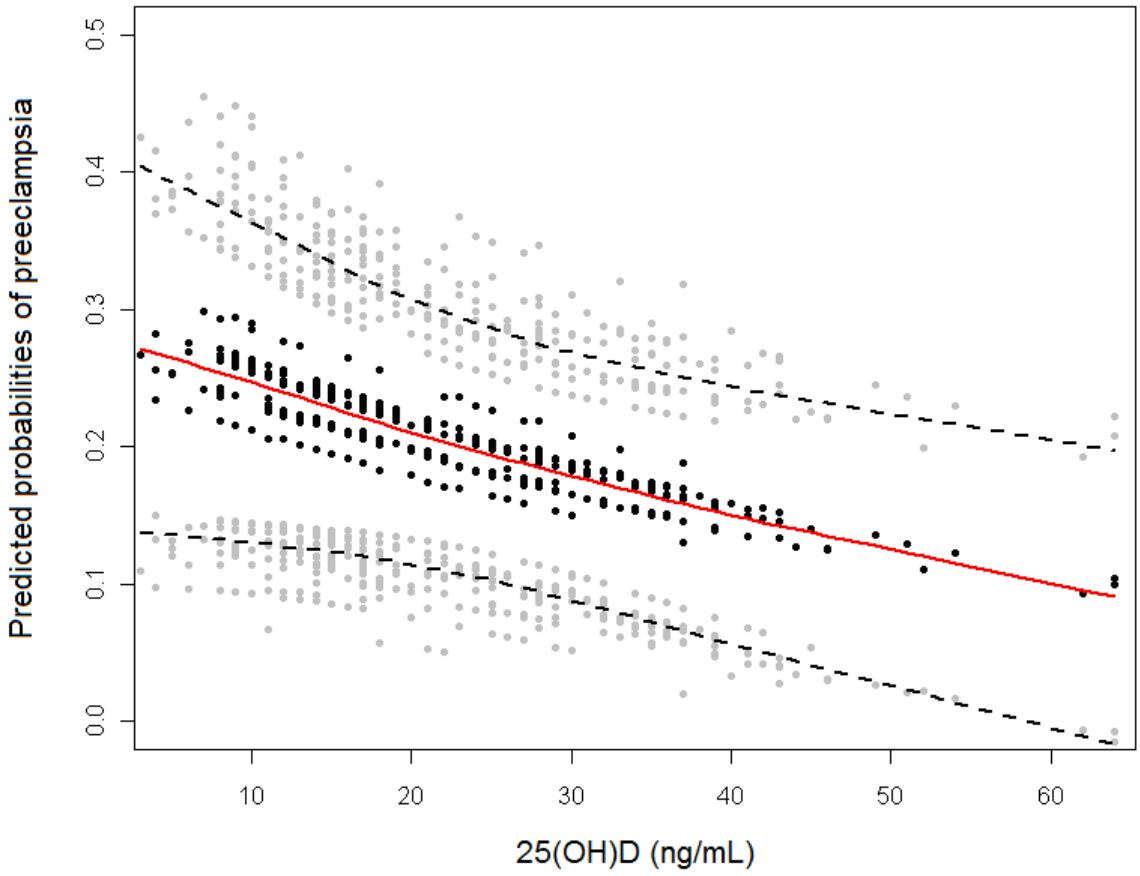

Table 1. Clinical and demographic characteristics of the women with preeclampsia (cases) and matched women without preeclampsia (controls).

Characteristics	**Cases** (N = 83)	**Controls**[a] (N = 319)
Pregnancy term at inclusion (WA), mean ± SD	12.8 ± 0.9	12.8 ± 0.8
Maternal age (years)		
Mean ± SD	32.2 ± 5.9	31.7 ± 5.0
< 35, n (%)	56 (67.5)	224 (70.2)
BMI before pregnancy (kg/m^2)		
Mean ± SD	25.6 ± 5.1	24.4 ± 4.5
< 25, n (%)	45 (54.2)	175 (54.9)
Conception during spring/summer, n (%)	50 (60.2)	187 (58.6)
Skin color 1-4 (Fitzpatrick scale), n (%)	48 (57.8)	192 (60.2)
Geographic origin, n (%)		
France	36 (43.4)	141 (44.3)
North Europa	0	8 (2.5)
South Europa	3 (3.6)	14 (4.4)
Maghreb	12 (14.5)	55 (17.3)
Sub-Saharan Africa	16 (19.3)	63 (19.8)
Overseas France	6 (7.2)	16 (5.0)
Asia	4 (4.8)	10 (3.1)
Other	6 (7.2)	11 (3.5)
Parity (previous deliveries), n (%)		
0	57 (68.7)	215 (67.4)
1	14 (16.9)	52 (16.3)
>1	12 (14.5)	52 (16.3)
Diabetes before pregnancy, n (%)	4 (4.8)	12 (3.8)
Intake of vitamin D within one month before inclusion, n (%)	0	8 (2.7)

BMI, body mass index; WA, weeks of amenorrhea.

[a] Age, BMI before pregnancy, conception season, skin color and parity were matching factors.

Table 2. 25(OH) D concentration in maternal serum, intakes of vitamin D and other data recorded during pregnancy.

	Cases (N = 83)	**Controls** (N = 319)	**P-value**
Gestational diabetes	12 (14.5)	28 (8.9)	0.13
Delivery term (WA), mean ± SD	37.2 ± 3.0	39.9 ± 1.2	< 0.001
Intake of vitamin D after inclusion	62 (79.5)	270 (85.7)	0.17
Time (WA) at first vitamin D intake, mean ± SD	28.1 ± 3.3	27.9 ± 3.8	0.64
Number of doses of vitamin D[a]			
1	56 (91.8)	255 (95.5)	0.14
≥ 2	5 (8.2)	12 (4.5)	
Measurement of 25(OH)D in 1st trimester			
Time of dosage (WA), mean ± SD	12.8 ± 0.9	12.8 ± 0.8	0.70
Serum 25(OH)D level (ng/mL), mean ± SD	20.1 ± 9.3	22.3 ± 11.1	0.091
Classes, n (%)			
< 10	8 (9.6)	35 (11.0)	0.17
10–30	61 (73.5)	201 (63.0)	
≥ 30	14 (16.9)	83 (26.0)	
Measurement of 25(OH)D in 3rd trimester			
Time of blood sampling (WA), mean ± SD	35.1 ± 2.2	35.0 ± 2.4	0.91
Serum 25(OH)D level (ng/mL), mean ± SD	27.7 ± 12.2	30.8 ± 11.0	0.049
Classes, n (%)			
< 10	0	7 (2.2)	0.009
10–30	41 (65.1)	141 (44.2)	
≥30	22 (34.9)	171 (53.6)	
Increase of serum 25(OH)D level from 1st to 3rd trimester (ng/mL), mean ± SD	6.3 ± 12.6	8.5 ± 11.9	0.20

[a]Vitamin D3 100.000 IU per os.
WA, weeks of amenorrhea.

Table 3. Odds ratios for preeclampsia according to vitamin D status in the first and/or third trimesters.

Vitamin D status [a]	Cases (n)	Controls (n)	Odd ratio of preeclampsia	95% confidence interval	P-value
First trimester	*83*	*319*			
Low	*69*	*236*	1.00 (Ref)		
High	*14*	*83*	0.57	0.30–1.01	0.092
Third trimester	*63* [b]	*239* [b]			
Low	*41*	*111*	1.00 (Ref)		
High	*22*	*128*	0.43	0.23–0.80	0.008
First-Third trimesters combined	*63* [b]	*239* [b]			
Low-Low	*36*	*98*	1.00 (Ref)		
Low-High or High-Low	*20*	*89*	0.59	0.31–1.14	0.12
High-High	*7*	*52*	0.34	0.13–0.86	0.023

OR, odds ratio.

[a] Low: 25(OH)D < 30 ng/ml; High: 25(OH)D ≥ 30 ng/mL

[b] Dosage of third trimester were unavailable for 20 cases (the corresponding 80 controls were removed from this analysis).

Table 4. Nested case-control studies aimed to assess the relationship between vitamin D status in early pregnancy and preeclampsia.

Authors	Country	Period of inclusion	Details and specificities of the design	Number of Cases/ Controls	Pre eclampsia rate in cohort	Limit GA at 25(OH)D dosagefor inclusion	Observed GA at 25(OH)D dosage	25(OH)D (ng/mL) [b] Cases vs. Controls	Risk of preeclampsia	
									Cut-offs 25(OH)D (ng/mL) [b]	OR [c] (95% CI)
Bodnar et al (16)	United States (Pittsburg)	1997 - 2001	Nulliparous women involved only No matching used	55/219 (49/216 for analysis)	4.9%	<22 WA	10.4 WA (median)	18.2 vs. 21.2 (p<0.01)	<15 vs. >15	5.0 (1.7–14.1)
Baker et al (18)	United States (North Carolina)	2004 - 2008	Cases = severe preeclampsia only Matching (1:4) by race/ethnicity	51/204 (43/198 for analysis)	1.2%[a]	15-20 WA	17 WA (median)	30.0 vs. 39.2 (p=0.01)	<20 vs. >30	5.41 (2.02–14.52)
Achkar et al. (17)	Canada	2002-2010	Frequency matching on study site, GA at recruitment, season and year of blood collection	169/1975	1.8%	<20 WA	13.6 WA (mean)	18.9 vs. 20. 9 (p=0.0002)	<12 vs. >20	2.23 (1.29–3.83)
Present study	France/ Belgium	2012 - 2014	Matching (1:4) on parity, maternal age, prepregancy BMI, season of conception, skin color	83/319	3.0%	11-14 WA	12.8 WA (mean)	20.1 vs. 22.3 (p=0.09)	<30 vs. >30	1.75 (0.91–3.33)

GA: Gestational Age

[a] Severe preeclampsia rate

[b] Original results and cut-offs in nmol/L have been converted in ng/mL for inter-study comparisons (30 ng/mL = 75 nmol/L)

[c] Bodnar et al: OR adjusted for race/ethnicity, season, sample gestational age, prepregnancy BMI, and education.

Baker et al: OR adjusted for season of blood draw, maternal age, multiparity, body mass index, and gestational age at serum collection

Achkar et al: OR adjusted for prepregnancy body mass index, parity, maternal age, smoking, season of blood collection, year of blood collection, gestational age at blood collection, and study site

Present study:crude OR using conditional logistic regression (matching factors used were parity, maternal age, prepregancy BMI, season of conception, skin color)