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

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50

51 **Abstract**

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

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

60 **Results:** 83 cases of preeclampsia were matched with 319 controls. Mean 25(OH)D
61 levels in the first trimester were 20.1 ± 9.3 ng/mL in cases and 22.3 ± 11.1 ng/mL in
62 controls ($p=0.09$). The risk for preeclampsia with 25(OH)D level ≥ 30 ng/mL in the first
63 trimester was decreased, but did not achieve statistical significance (OR, 0.57; 95%
64 CI, 0.30–1.01; $p=0.09$). High 25(OH)D during the 3rd trimester was associated with a
65 significantly decreased risk of preeclampsia (OR, 0.43; 95%CI, 0.23-0.80; $p=0.008$).
66 When women with 25(OH)D levels <30 ng/mL both in the first and 3rd trimesters
67 (“low-low”) were taken as references, OR for preeclampsia was 0.59 (95% CI, 0.31–
68 1.14; $p=0.12$) for “low-high” or “high-low” women and 0.34 (95% CI, 0.13–0.86; $p =$
69 0.02) for “high-high” women.

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

74

75

76 **Introduction**

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

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

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

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

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

113 The present case-control study in a French-Belgian cohort explored the relationship
114 between vitamin D insufficiency (defined by a 25(OH)D level <30 ng/mL) in the first
115 trimester and the occurrence of preeclampsia later in pregnancy. Secondary
116 objectives were to evaluate this potential relationship between preeclampsia and
117 25(OH)D during the third trimester of pregnancy.

118

119 **Materials and Methods**

120 **Study design**

121 We performed a nested case-control study within a prospective observational cohort
122 (FEPED cohort) including pregnant women in six centers: one in Belgium (latitude
123 50.83°N) and five in France. Four of the five French centers were located in Paris or
124 its suburbs (Béclère, Bicêtre, Cochin and Trousseau university hospitals, latitude
125 48.86°N) and the last one was located in Nantes (latitude 47.22°N).

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

130 Women were included in the cohort if they were at the first trimester (from 10 to <15
131 WA) of a singleton pregnancy. Exclusion criteria were the following: hypercalcemia
132 (> 2.65 mmol/L) or any other calcium-phosphorus imbalance, hypertension (>140/90
133 mmHg) from the first trimester, renal insufficiency (creatinine > 120 µmol/L), bone
134 disease, lithium therapy, bowel malabsorption or kidney stone disease.

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

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

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

146 **Selection of cases and controls**

147 We matched cases of preeclampsia to eligible controls randomly selected in a 1:4
148 ratio. Preeclampsia was defined as a blood pressure > 140 mmHg or a diastolic
149 blood pressure > 90 mmHg and proteinuria > 0.3 g/24 h. This definition is the one
150 currently used in France and it was also used internationally at the time of the trial set
151 up. Matching factors were parity (primiparous or not), season at conception, skin
152 color (< 5 or ≥ 5 according to Fitzpatrick scale), body mass index (< 25 or ≥ 25 kg/m²)
153 and maternal age (< 35 or ≥ 35 years). In addition, cases were matched to controls
154 with the closest age whenever possible. Skin color was preferred instead of the more
155 commonly used variable ethnicity, because its use seemed more reliable.

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

164 **Assessment of vitamin D status**

165 25(OH)D was measured in maternal blood samples obtained during the first (11 to
166 <15 WA) and third (28–40 WA) trimesters. The patients did not need to be fasting

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

174 **Statistical analysis**

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

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

187
188 All statistical analyses were undertaken using R 2.11.1 software. Statistical tests
189 were two-sided and p values less than 0.05 were considered statistically significant.
190 Baseline characteristics of the two groups were described as mean \pm standard
191 deviation for quantitative variables and frequencies (%) for qualitative variables.

192 Primary outcome was analyzed using conditional logistic regression. Among

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

199

200 **Results**

201 **Demographic and clinical characteristics of cases and controls**

202 Pregnant women were included from April 2012 to July 2014 with the last delivery in
203 February 2015. From this total cohort of 3093 women, we identified 84 cases of
204 preeclampsia. Mean term at the onset of preeclampsia was 36.2 ± 3.8 WA, 9 cases
205 occurring before or at 32 WA. A total of 296 patients were excluded and the
206 prevalence of preeclampsia in the remaining 2797 women was therefore 3.0%
207 (84/2797). Finally, 83 cases of preeclampsia were randomly matched among the
208 eligible controls. Only one case out of the 84 was excluded because of incomplete
209 data at inclusion and over follow-up. For six cases with the least common profiles, we
210 could not match the four controls required. Overall, the 83 cases were matched to
211 four controls (n=77), three controls (n=1), two controls (n=3) or one control (n=2),
212 leading to a total number of 319 controls. The selection of cases and controls is
213 summarized in **Figure 1**.

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

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

222 **Routine vitamin D supplementation**

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

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

230 25(OH)D levels measured in the first trimester after a mean 12.8 WA were
231 comparable in cases and matched controls (20.1 ± 9.3 vs. 22.3 ± 11.1 ng/mL;
232 $p=0.09$) (**Table 2**). Women with vitamin D sufficiency represented 16.9% of cases
233 and 26.0% of controls ($p = 0.17$). In the first trimester, the risk for preeclampsia was
234 decreased in these patients, but did not achieve statistical significance: the odds ratio
235 (OR) of preeclampsia was 0.57 (95% CI, 0.30–1.01; $p = 0.09$; conditional logistic
236 regression estimation) (**Table 3**). However, 25(OH)D levels in the first trimester in
237 women who developed preeclampsia ≤ 32 WA (12.0[5-25]) was lower than in the
238 group after 32 WA (19.5 [5-42]) ($p=0.028$). The association between maternal
239 25(OH)D in the first trimester and the risk of preeclampsia was analyzed after
240 adjustment for matching factors (Lowess plot). As shown in **Figure 2**, the probability
241 of preeclampsia decreased with the level of 25(OH)D. For each 10 ng/mL increase,

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

244 25(OH)D levels were measured in the third trimester after a mean 35.0 ± 2.4 WA in
245 cases and 35.1 ± 2.2 WA in controls (**Table 2**). The mean 25(OH)D level was
246 significantly higher in controls compared with cases (30.8 ± 11.0 vs. 27.7 ± 12.2
247 ng/mL; $p = 0.049$). Patients with vitamin D sufficiency (≥ 30 ng/mL) were significantly
248 less frequent in cases compared with controls: 34.9% versus 53.6% ($p = 0.009$). The
249 OR for preeclampsia associated with vitamin D sufficiency during the third trimester
250 was 0.43 (95% CI, 0.23–0.80; $p = 0.008$; conditional logistic regression estimation)
251 (**Table 3**).

252 Exploratory analyses were performed by combining measurements of 25(OH)D in the
253 first and third trimesters. When women with 25(OH)D <30 ng/mL in both the first and
254 third trimesters (“low-low”) were taken as references, the OR for preeclampsia was
255 0.59 (95% CI, 0.31–1.14, $p = 0.12$) for “low-high” or “high-low” women and 0.34 (95%
256 CI, 0.13–0.86; $p = 0.023$) for “high-high” women (**Table 3**).

257 **Discussion**

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

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

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

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

276 Our study was the only one performed in Europe among the case-control studies
277 assessing vitamin D status in early pregnancy on preeclampsia. The three other
278 case-control studies were performed in North American cohorts with various
279 prevalence rates of preeclampsia: 4.9% in the US cohort of Bodnar et al. (16), 1.2%
280 (only severe preeclampsia) in the US cohort of Baker et al. (18) and 1.8% in the
281 Canadian cohort of Achkar et al. (17). Another difference was a 30 ng/mL cut-off in
282 defining vitamin D insufficiency in our study; however, exploratory analyses using a
283 20 ng/mL cut-off did not change the conclusions (OR=1.27, 95% CI 0.78-2.08; p =
284 0.34). Many other differences between the 3 studies and ours are summarized in
285 Table 4. Patient selection varies between the 4 studies, as do gestational age at
286 sampling and adjustment factors. We used a conditional logistic regression for
287 analysis, which generates unbiased estimates when using matched data, a 30 ng/mL
288 25(OH)D cut-off and a short timeframe for sampling in order to be able to answer the
289 specific question of the association of 25(OH)D deficiency in the first trimester and
290 preeclampsia.

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

303 Mirzakahani et al. have recently explored the effect of early vitamin D
304 supplementation on preeclampsia in the Vitamin D Antenatal Asthma Reduction Trial
305 (VDAART) (23). They evaluated daily administration of vitamin D3 (4400 or
306 400 IU/day) starting early in pregnancy (10–18 WA). There was no difference
307 between treatment groups for the rates of preeclampsia. Nevertheless, regardless of
308 treatment groups, women with 25(OH)D \geq 30 ng/mL in both early and late pregnancy
309 developed preeclampsia less frequently (OR, 0.28; 95% CI, 0.10–0.96) (23). We
310 confirmed these results in an exploratory analysis where women with high vitamin D
311 status in both the first and third trimesters were significantly protected against
312 preeclampsia (OR, 0.34; 95% CI, 0.13–0.86).

313 Overall, vitamin D deficiency appears to be a risk factor of preeclampsia, but
314 available studies do not support vitamin supplementation as an effective treatment
315 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

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

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

357

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367 **Conflict of Interest Statement**

368

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371

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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, prepregnancy 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.

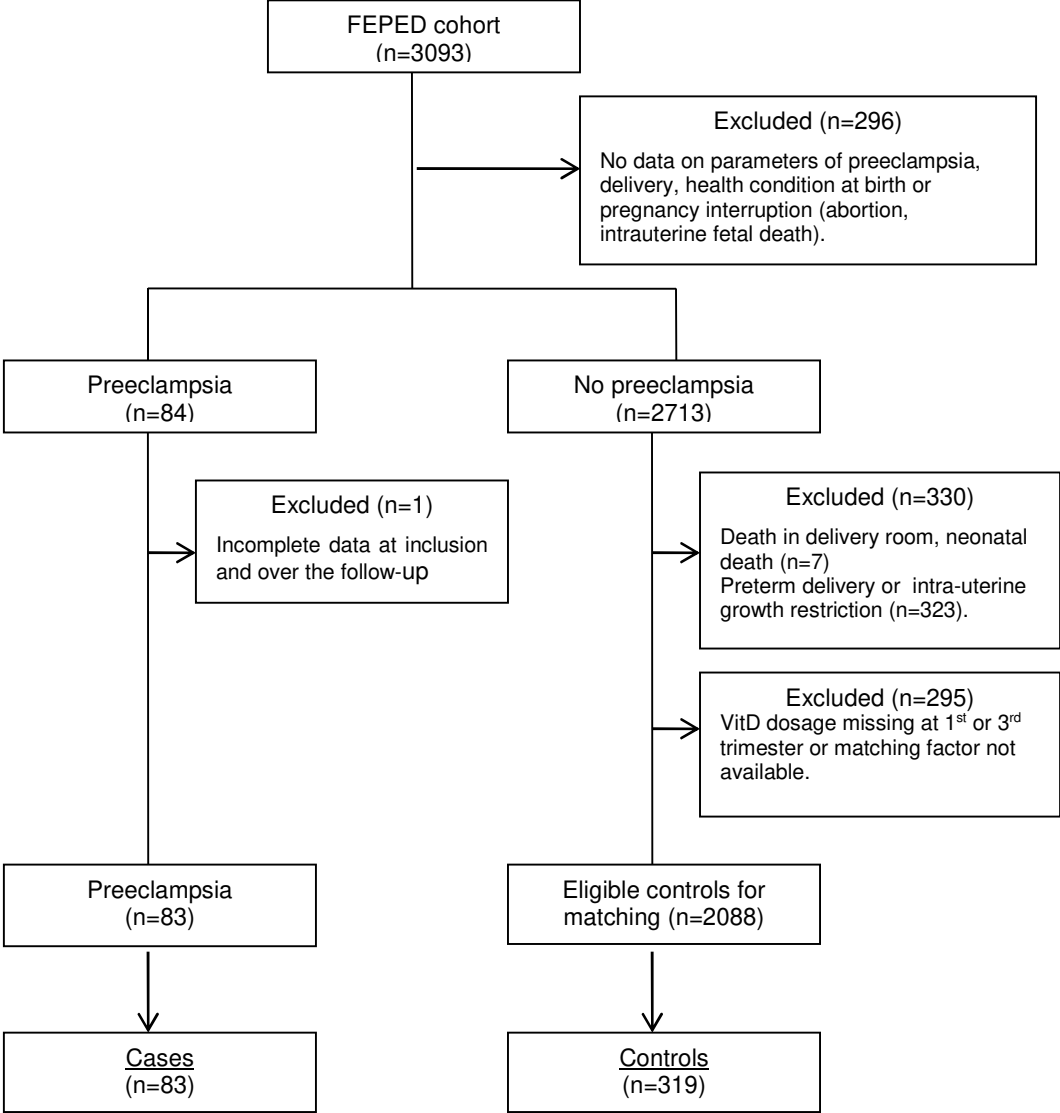


Figure 2. Association between maternal serum 25(OH)D at first trimester of gestation and predicted probability of preeclampsia (Lowess plot). Probability of preeclampsia was calculated with a logistic model adjusted for matching factors (parity, maternal age, prepregnancy BMI, season of conception, skin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

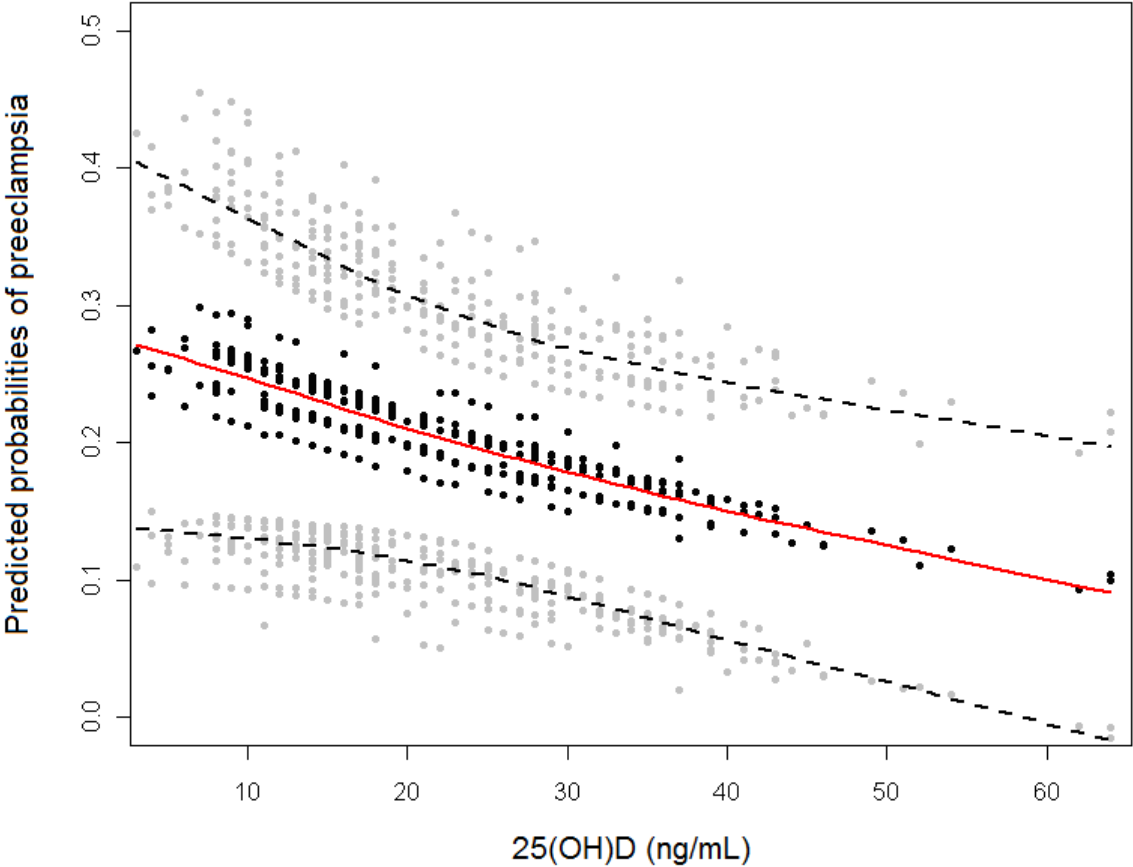


Table 1. Clinical and demographic characteristics of the women with preeclampsia (cases) and matched women without preeclampsia (controls).

Characteristics	Cases (N = 83)	Controls ^a (N = 319)
Pregnancy term at inclusion (WA), mean \pm SD	12.8 \pm 0.9	12.8 \pm 0.8
Maternal age (years)		
Mean \pm SD	32.2 \pm 5.9	31.7 \pm 5.0
< 35, n (%)	56 (67.5)	224 (70.2)
BMI before pregnancy (kg/m ²)		
Mean \pm SD	25.6 \pm 5.1	24.4 \pm 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 \pm SD	37.2 \pm 3.0	39.9 \pm 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 \pm SD	28.1 \pm 3.3	27.9 \pm 3.8	0.64
Number of doses of vitamin D ^a			
1	56 (91.8)	255 (95.5)	0.14
\geq 2	5 (8.2)	12 (4.5)	
Measurement of 25(OH)D in 1 st trimester			
Time of dosage (WA), mean \pm SD	12.8 \pm 0.9	12.8 \pm 0.8	0.70
Serum 25(OH)D level (ng/mL), mean \pm SD	20.1 \pm 9.3	22.3 \pm 11.1	0.091
Classes, n (%)			
< 10	8 (9.6)	35 (11.0)	0.17
10–30	61 (73.5)	201 (63.0)	
\geq 30	14 (16.9)	83 (26.0)	
Measurement of 25(OH)D in 3 rd trimester			
Time of blood sampling (WA), mean \pm SD	35.1 \pm 2.2	35.0 \pm 2.4	0.91
Serum 25(OH)D level (ng/mL), mean \pm SD	27.7 \pm 12.2	30.8 \pm 11.0	0.049
Classes, n (%)			
< 10	0	7 (2.2)	0.009
10–30	41 (65.1)	141 (44.2)	
\geq 30	22 (34.9)	171 (53.6)	
Increase of serum 25(OH)D level from 1 st to 3 rd trimester (ng/mL), mean \pm SD	6.3 \pm 12.6	8.5 \pm 11.9	0.20

^aVitamin 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)	Odds 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 dosage for 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 Matching (1:4) on parity, maternal age, prepregnancy BMI, season of conception, skin color	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, prepregnancy 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, prepregnancy BMI, season of conception, skin color)