Maternal serum vitamin D levels at 11–13 weeks of gestation in preeclampsia

CKH Yu¹, R Ertl¹, E Skyfta¹, R Akolekar¹ and KH Nicolaides¹,²

This study is aimed to determine whether the maternal serum levels of vitamin D in the first trimester of pregnancy are altered in cases that develop preeclampsia (PE) and whether the levels are related to biochemical and biophysical markers of impaired placental perfusion and function. Maternal total serum vitamin D, pregnancy-associated plasma protein-A (PAPP-A), uterine artery pulsatility index (PI) and mean arterial pressure (MAP) were measured at 11–13 week gestation in 90 cases that developed PE, including 30 that required delivery before 34 weeks (early PE) and 1000 unaffected controls. The median values of vitamin D, PAPP-A, uterine artery PI and MAP expressed as a multiple of the unaffected median (MoM), in the patients developing early PE and late PE were compared with the controls. There was no significant difference in the median serum vitamin D MoM or raw values within the outcome groups (P = 0.141 and P = 0.231, respectively) whereas the median PAPP-A MoM, uterine PI MoM and MAP MoM were significantly different (P = 0.031, P = 0.001 and P < 0.0001, respectively). Serum PAPP-A was decreased in both early PE and late PE (0.54 and 0.88 versus 1.03 MoM, P < 0.0001 and P = 0.010, respectively), MAP was increased in both early PE and late PE (1.09 and 1.06 versus 0.99 MoM, P < 0.0001 and P < 0.0001, respectively) and uterine artery PI was increased in early PE but not in late PE (1.32 and 1.12 versus 1.01 MoM, P < 0.0001 and P = 0.083, respectively). In pregnancies that subsequently develop PE maternal serum total vitamin D levels at 11–13 weeks are not altered.

Keywords: vitamin D; first trimester screening; preeclampsia; pregnancy-associated plasma protein-A; mean arterial pressure; uterine artery doppler

INTRODUCTION

The active form of vitamin D, 1,25(OH)₂D, is the result of two stages of hydroxylation of pro-hormone calciferol, which is synthesized in the skin from 7-dehydrocholesterol after exposure to the ultraviolet B radiation of sunlight. The first hydroxylation occurs mainly in the kidneys but also in the placenta.¹ In normal pregnancy, the maternal serum concentration of vitamin D, PAPP-A, uterine artery PI and MAP expressed as a multiple of the unaffected median (MoM), in the patients developing early PE and late PE were compared with the controls. There was no significant difference in the median serum vitamin D MoM or raw values within the outcome groups (P = 0.141 and P = 0.231, respectively) whereas the median PAPP-A MoM, uterine PI MoM and MAP MoM were significantly different (P = 0.031, P = 0.001 and P < 0.0001, respectively). Serum PAPP-A was decreased in both early PE and late PE (0.54 and 0.88 versus 1.03 MoM, P < 0.0001 and P = 0.010, respectively), MAP was increased in both early PE and late PE (1.09 and 1.06 versus 0.99 MoM, P < 0.0001 and P < 0.0001, respectively) and uterine artery PI was increased in early PE but not in late PE (1.32 and 1.12 versus 1.01 MoM, P < 0.0001 and P = 0.083, respectively). In pregnancies that subsequently develop PE maternal serum total vitamin D levels at 11–13 weeks are not altered.

Keywords: vitamin D; first trimester screening; preeclampsia; pregnancy-associated plasma protein-A; mean arterial pressure; uterine artery doppler

MATERIALS AND METHODS

Study population

This was a case-control study drawn from a large prospective observational study for early prediction of pregnancy complications in women attending for their routine first hospital visit in pregnancy at King’s College Hospital, London, UK. In this visit, which is held at 11–13 weeks of gestation, we record maternal characteristics and medical history and perform combined screening for aneuploidies by measurement of the fetal crown-rump length and nuchal translucency thickness, and maternal serum PAPP-A and free ß-human chorionic gonadotropin.¹³ We also measure the mean arterial pressure (MAP) by automated devices,¹⁶ uterine artery PI by transabdominal pulsed Doppler¹⁴ and store serum and plasma at –80 °C for subsequent biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study, which was approved by King’s College Hospital ethics committee.

In this study, we measured maternal serum vitamin D in 90 singleton pregnancies that developed PE, including 30 that required delivery before 34 weeks (early PE) and 60 with late PE, and 1000 unaffected controls who
did not develop any hypertensive disorder of pregnancy and delivered a
phenotypically normal neonate at term with weight appropriate for
gestational age. Cases were selected at random from our database of
stored samples and the data were compared with those of previously
published unaffected controls.\textsuperscript{17} All samples were collected between 2006 and
2010, none of the women were receiving vitamin D supplements and
none had any known liver or renal disease.

Sample analysis
None of the samples in this study were previously thawed and refrozen.
Duplicate samples of 100 µL were used to analyse vitamin D2 and D3 using a
liquid chromatography tandem mass spectrometry (LC-MS/MS) method
using a Shimadzu Prominence HPLC system (Shimadzu ScientificInstru-
mements, Columbia, MD, USA) equipped with a Phenomenex Luna C8
3 \times 50 mm column (Phenomenex Inc., Torrence, CA, USA) and AB Sciec API-
5000 ESi triple quadrupole (AB Sciec Pte. Ltd., Foster City, CA, USA).
The analysis was performed using the PerkinElmer MSMS Vitamin D (3075-0010)
kit, (Perkin Elmer Life and Analytical Sciences, Turku, Finland). Individual
runs were calibrated using National Institute of Standards and Technology
(NIST) Standard Reference Material (SRM) 2972 standards. The average
inter-assay coefficient of variation for vitamin D2 and D3 were 6.3 and 6.5%,
respectively. The total vitamin D concentration in maternal serum was
calculated by adding together measured vitamin D2 and D3 concentrations.

Outcome measures
Data on pregnancy outcome were obtained from the maternity
computerized records or the general medical practitioners of the women
and were recorded in our database. The obstetric records of all women
with preexisting or pregnancy-associated hypertension were examined to
determine whether the condition was chronic hypertension, PE or
gestational hypertension. The definition of PE was that of the International
Society for the Study of Hypertension in Pregnancy.\textsuperscript{18} The systolic blood
pressure should be \( \geq 140 \) mmHg and/or the diastolic blood pressure
should be \( \geq 90 \) mmHg on at least two occasions, 4 h apart developing
after 20 weeks of gestation in previously normotensive women and there
should be proteinuria of \( \geq 300 \) mg in 24 h or two readings of at least + +
on dipstick analysis of midstream or catheter urine specimens if no 24 h
collection is available. In PE superimposed on chronic hypertension
significant proteinuria (as defined above) should develop after 20 weeks of
gestation in women with known chronic hypertension (history of
hypertension before conception or the presence of hypertension at the
booking visit before 20 weeks of gestation in the absence of trophoblastic
disease). The birth weight percentile corrected for gestation at delivery
was calculated using a reference range derived from our population.\textsuperscript{19}

Statistical analysis
Comparison between outcome groups was done by Kruskal-Wallis test
with post hoc Dunn’s procedure for continuous variables and \( \chi^2 \)-test or
Fisher’s exact test for categorical variables. The distribution of serum vitamin D was made Gaussian by square root
transformation, and normality was assessed using histograms and
Fisher's exact test for categorical variables.

RESULTS
The maternal characteristics of each of the outcome groups are
compared in Table 1. In the early PE group compared with controls, the median maternal BMI was higher, more women had
chronic hypertension, mothers who had developed PE and
delivered neonates with a lower birth weight percentile. n late
PE compared with controls, the median maternal BMI was higher,
more women had PE in their previous pregnancy, had chronic
hypertension, required assisted conception techniques and
delivered neonates with a lower birth weight percentile.

There was no significant difference in the median serum vitamin
D MoM or raw values within the outcome groups (\( P = 0.141 \) and
\( P = 0.231 \), respectively) whereas the median PAPP-A MoM, uterine PI MoM,
and MAP MoM were significantly different (\( P = 0.031 \),
\( P = 0.001 \) and \( P < 0.0001 \), respectively) (Table 2). Serum PAPP-A was
decreased in both early PE and late PE (0.54 and 0.88 versus
1.03 MoM, \( P < 0.0001 \) and \( P = 0.009 \), respectively), MAP was
increased in both early PE and late PE (1.09 and 1.06 versus 0.99
MoM, \( P < 0.0001 \) and \( P < 0.0001 \), respectively) and uterine artery PI
was increased in early PE but not in late PE (1.32 and 1.12 versus
1.01 MoM, \( P < 0.0001 \) and \( P = 0.083 \), respectively). Logistic regression analysis demonstrated that in the prediction of early PE there was a
significant contribution from log10 PAPP-A MoM (\( P = 0.001 \), log10
MAP MoM (\( P < 0.0001 \) and log10 uterine artery PI MoM (\( P < 0.0001 \),
but not from square root vitamin D MoM (\( F = 0.234 \)). In the prediction of late-PE, there was a significant contribution from
log10 PAPP-A MoM (\( P = 0.013 \) and log10 MAP MoM (\( P < 0.0001 \))
but not from log10 uterine artery PI MoM (\( P = 0.338 \) or square root vitamin
D MoM (\( P = 0.056 \)). The incidence of vitamin D levels below 10th
percentile was not significantly different in early PE and late PE
compared with unaffected controls (13.3, 18.3 and 10.0%, respec-
tively, \( P = 0.772 \) and \( P = 0.067 \), respectively).

In the control group, there was no significant association of
vitamin D MoM with either PAPP-A MoM (\( P = 0.059 \), \( P = 0.061 \)), uterine
artery PI MoM (\( P = 0.021 \), \( P = 0.502 \)), MAP MoM (\( P = 0.045 \), \( P = 0.152 \)),
gestation at delivery (\( P = 0.103 \) or birth weight percentile (\( P = 0.056 \),
\( P = 0.075 \)). Similarly, in the PE group, there was no significant association of
vitamin D MoM with either PAPP-A MoM (\( P = 0.030 \), \( P = 0.775 \)),
uterine artery PI MoM (\( P = 0.095 \), \( P = 0.371 \)), MAP MoM (\( P = 0.121 \),
\( P = 0.258 \), gestation at delivery (\( P = 0.069 \), \( P = 0.516 \)) or birth
weight percentile (\( P = 0.142 \), \( P = 0.182 \)). However, there was a
significant association of both uterine artery PI MoM and PAPP-A
MoM with gestation at delivery (\( P = 0.308 \), \( P = 0.003 \), \( P = 0.288 \),
\( P = 0.006 \), respectively) and birth weight percentile (\( P = 0.466 \),
\( P < 0.0001 \); \( P = 0.287 \), \( P = 0.006 \), respectively).

In the study population, there were 362 (33.2%) women of
African racial origin, including 14 with early PE, 23 with late PE and
325 unaffected controls. In women of African racial origin, there was
no significant difference in the median vitamin D MoM in early
and late PE compared with unaffected controls (\( P = 0.703 \) and
\( P = 0.999 \), respectively).

DISCUSSION
The findings of this study confirm that at 11–13 week gestation in
pregnancies that subsequently develop PE, especially early PE, there is evidence of impaired placentation manifested in increased

10th percentile in the early PE and late PE groups, compared with the
unaffected population was estimated. Non-parametric correlation analysis
(Spearman’s correlation coefficient (\( \rho \)) was used to estimate the
significance of association between maternal serum vitamin D and
PAPP-A, uterine artery PI, MAP, gestation at delivery and birth weight
percentile in the outcome groups.

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was
used for data analyses.
uterine artery PI and decreased maternal serum PAPP-A. In contrast, there is no significant difference in maternal serum total 25(OH)D concentration between pregnancies that develop PE and normotensive controls.

The strengths of our study are firstly, distinction between early and late PE, secondly, measurement of vitamin D by LC-MSMS, which is currently the most accurate technique for such estimation, thirdly, adjustment of measured concentrations of serum vitamin D for the maternal factors that affect this measurement and fourthly, examination of a large number of cases within a narrow window at 11–13 weeks, which is emerging as the gestation of choice for risk assessment for a wide range of pregnancy complications. The main weakness resides in the design of the study that was case-control rather than prospective.

In normal pregnancy, the measured maternal serum total 25(OH)D is affected by maternal characteristics. The levels are lower in cigarette smokers and in women of African and Asian racial origin compared with Caucasians, they increase with maternal age and decrease with BMI, and they are higher if blood sampling is in the summer than other months. Consequently, in comparing levels between normal and pathological pregnancies it is important to make the appropriate adjustments for these variables. As the risk for PE is higher in women of African and Asian racial origin than in Caucasians and increases with BMI, failure to adjust for these variables could result in the erroneous conclusion that serum levels of 25(OH)D in PE are reduced even if in reality they are not.

In pregnancies with PE the reported mean maternal serum or plasma levels of either 25(OH)D or total 25(OH)D are 15–57%. There was no significant difference in maternal serum total 25(OH)D concentration between pregnancies that develop PE and normotensive controls.

### Table 1. Maternal and pregnancy characteristics in the outcome groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unaffected controls (n = 1,000)</th>
<th>Early preeclampsia (n = 30)</th>
<th>Late preeclampsia (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age in years, median (IQR)</td>
<td>31.5 (27.2–35.4)</td>
<td>31.6 (25.5–36.5)</td>
<td>32.4 (27.1–37.3)</td>
</tr>
<tr>
<td>Maternal BMI in kg m⁻², median (IQR)</td>
<td>24.2 (21.9–27.5)</td>
<td>28.3 (23.8–33.3)*</td>
<td>26.2 (23.2–31.5)*</td>
</tr>
<tr>
<td>Crown-rump length in mm, median (IQR)</td>
<td>63.3 (58.0–68.5)</td>
<td>62.0 (56.0–67.9)</td>
<td>61.4 (58.0–68.9)</td>
</tr>
<tr>
<td>Gestation at sampling (weeks), median (IQR)</td>
<td>12.4 (12.1–12.9)</td>
<td>12.4 (12.0–12.8)</td>
<td>12.4 (12.1–12.9)</td>
</tr>
<tr>
<td>Season of sampling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer, n (%)</td>
<td>246 (24.6)</td>
<td>6 (20.0)</td>
<td>11 (18.3)</td>
</tr>
<tr>
<td>Other seasons, n (%)</td>
<td>754 (75.4)</td>
<td>24 (80.0)</td>
<td>49 (81.7)</td>
</tr>
<tr>
<td>Racial origin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian, n (%)</td>
<td>580 (58.0)</td>
<td>11 (36.7)</td>
<td>30 (50.0)</td>
</tr>
<tr>
<td>African, n (%)</td>
<td>325 (32.5)</td>
<td>14 (46.7)</td>
<td>23 (38.3)</td>
</tr>
<tr>
<td>Asian, n (%)</td>
<td>95 (9.5)</td>
<td>5 (16.7)</td>
<td>7 (11.7)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous, n (%)</td>
<td>481 (48.1)</td>
<td>17 (56.7)</td>
<td>36 (60.0)</td>
</tr>
<tr>
<td>Parous - no previous preeclampsia, n (%)</td>
<td>495 (49.5)</td>
<td>10 (33.3)</td>
<td>18 (30.0)*</td>
</tr>
<tr>
<td>Parous - previous preeclampsia, n (%)</td>
<td>24 (2.4)</td>
<td>3 (10.0)</td>
<td>6 (10.0)*</td>
</tr>
<tr>
<td>Family history of preeclampsia, n (%)</td>
<td>33 (3.3)</td>
<td>4 (13.3)*</td>
<td>5 (8.3)</td>
</tr>
<tr>
<td>Cigarette smoker, n (%)</td>
<td>73 (7.3)</td>
<td>1 (3.3)</td>
<td>4 (6.7)</td>
</tr>
</tbody>
</table>

### Table 2. Median multiple of the median (MoM) (IQR) for maternal serum vitamin D, PAPP-A, uterine artery PI and MAP in the outcome groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unaffected controls (n = 1,000)</th>
<th>Early preeclampsia (n = 30)</th>
<th>Late preeclampsia (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D ng ml⁻¹</td>
<td>18.75 (11.12–28.03)</td>
<td>12.90 (9.09–20.20)</td>
<td>15.71 (8.87–25.25)</td>
</tr>
<tr>
<td>MoM</td>
<td>0.99 (0.71–1.33)</td>
<td>0.89 (0.70–1.14)</td>
<td>0.86 (0.59–1.39)</td>
</tr>
<tr>
<td>PAPP-A mU ml⁻¹</td>
<td>3.04 (1.95–4.78)</td>
<td>1.39 (0.83–3.54)*</td>
<td>2.38 (1.62–3.62)*</td>
</tr>
<tr>
<td>MoM</td>
<td>1.03 (0.73–1.44)</td>
<td>0.54 (0.37–1.17)*</td>
<td>0.88 (0.59–1.20)*</td>
</tr>
<tr>
<td>Uterine artery PI Unit</td>
<td>1.65 (1.35–2.00)</td>
<td>2.10 (1.69–2.67)*</td>
<td>1.78 (1.41–2.16)</td>
</tr>
<tr>
<td>MoM</td>
<td>1.01 (0.82–1.23)</td>
<td>1.32 (1.03–1.71)*</td>
<td>1.12 (0.83–1.35)</td>
</tr>
<tr>
<td>MAP mm of Hg</td>
<td>83.3 (78.5–88.5)</td>
<td>96.3 (89.1–101.6)*</td>
<td>91.9 (84.5–98.8)*</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; IQR, interquartile range; MAP, mean arterial pressure; PAPP-A, pregnancy-associated plasma protein-A; PI, pulsatility index. Comparisons between outcome groups by Kruskal-Wallis test with post hoc Dunn’s procedure for continuous variables. *Significance value P < 0.025.
lower than in normal pregnancies.3-7 Such reduced levels could have been the consequence of the disease possibly reflecting impaired hepatic function and hydroxylation of 7-dehydrocholesterol to 25(OH)D. However, the findings of reduced levels preceding the clinical onset of the disease raised the possibility that vitamin D deficiency may be implicated in the pathogenesis of PE through direct or indirect effects on immune regulation, placental implantation, angiogenesis, inflammation and hypertension.23-26

Our findings, as well as those of another first trimester study, do not provide support for the hypothesis that vitamin D deficiency is involved in the pathogenesis of PE. In our study as well as that of Powe et al., the vitamin D levels were inversely related to the BMI, which was estimated from maternal weight and height measured at the time of the first trimester visit. In contrast, Bodnar et al.5,9 relied on self-reported pre-pregnancy weight, which may lead to inadequate adjustment for this factor. As maternal BMI is a known risk factor for PE, inadequate adjustment of serum 25(OH)D for BMI may lead to erroneous conclusions that the levels are altered. Bodnar et al.5,9 reported that levels of 25(OH)D < 37.5 nmol/L were associated with increased risk for PE but in our study as well that by Powe et al.10 this finding was not confirmed, with no significant difference in the incidence of 25(OH)D below the 10th percentile between the PE group and controls. Additionally, there were no significant associations between serum 25(OH)D and either uterine artery PI or serum PAPP-A. It was previously suggested that racial differences in the incidence of PE, which is more common in Black than in White women, may be mediated by deficiency in vitamin D, which is more common in Black women.6,9 However, although we confirmed that serum vitamin D is lower in women of African racial origin than in Caucasians, within the African group there was no significant difference in the median vitamin D levels between those who developed PE and the unaffected controls.

In conclusion, the maternal serum levels of vitamin D in the first trimester of pregnancy are not altered in cases that subsequently develop PE and the levels are not significantly associated with biochemical and biophysical markers of impaired placental perfusion and function.

What is known about topic
- Vitamin D has an important role in calcium homeostasis in pregnancy.
- Preeclampsia is thought to be a consequence of impaired placentaion.
- Vitamin D is reported to be decreased in established preeclampsia, but there is contradictory evidence regarding its levels prior to onset of preeclampsia.

What this study adds
- First-trimester serum vitamin D levels are not altered in pregnancies that subsequently develop early or late preeclampsia.
- There is no significant association of maternal serum vitamin D levels with biochemical or biophysical markers of impaired placental perfusion or function.
- Measurement of vitamin D levels at 11-13 weeks is unlikely to be useful in screening for preeclampsia.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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