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ORIGINAL ARTICLE

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

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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 ($P=141$ 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.

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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 in the liver to form 25(OH)D, which is the storage form and reflects intake during the previous month. The second hydroxylation occurs mainly in the kidneys but also in the placenta.[1] In normal pregnancy, the maternal serum concentration of $1,25(OH)_2D$ increases with gestation and this increase, which has been attributed to placental production, has an important role in the doubling of calcium absorption during pregnancy.[2]

In addition to it role in calcium homeostasis, vitamin D may be involved in placentation and several studies reported that in preeclampsia (PE), which is thought to be the consequence of impaired placentation, the maternal serum level of vitamin D is reduced.[3–7] It was 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.[8,9] There is also contradictory evidence that reduced vitamin D levels may predate the onset of PE. One study of 55 pregnancies that subsequently developed PE reported that the mean maternal serum concentration of vitamin D at 5–19 weeks was reduced,[5] and another reported no significant differences at 9–13 weeks in 39 pregnancies that developed PE from normal controls.[10] There is extensive evidence that in some cases of PE, particularly those with early-onset severe disease requiring delivery before 34 weeks (early PE) there is impaired placental perfusion and function, manifested in increased pulsatility index (PI) in the uterine arteries and reduced maternal serum concentration of pregnancy-associated plasma protein-A (PAPP-A).[11–13]

The aim of this study is to investigate further whether maternal serum levels of vitamin D are altered in pregnancies that subsequently develop early and late PE, and whether such changes are related to the racial origin of the women and alterations in placental perfusion and function, reflected in uterine artery PI and serum levels of PAPP-A.

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^{+0}–13^{+6} 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.[14,15] We also measure the mean arterial pressure (MAP) by automated devices,[16] uterine artery PI by transabdominal pulsed Doppler[12] and store serum and plasma at $-80\,^{\circ}C$ 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

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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.[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 Scientific Instruments, Columbia, MD, USA) equipped with a Phenomenex Luna C8 3 × 50 mm column (Phenomenex Inc., Torrence, CA, USA) and AB Sciex API-5000 ESI triple quadrupole (AB Sciex 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 6.6 and 7.3%, respectively, and the intra-assay coefficient of variations 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.[18] The systolic blood pressure should be ⩾140 mm Hg and/or the diastolic blood pressure should be ⩾90 mm Hg on at least two occasions, 4 h apart developing after 20 weeks of gestation in previously normotensive women and there should be proteinuria of ⩾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.[19]

Statistical analysis

Comparison between outcome groups was done by Kruskal-Wallis test with *post hoc* Dunn's procedure for continuous variables and χ^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 probability plots. The distributions of PAPP-A, uterine artery PI and MAP were made Gaussian after logarithmic transformation. In each case and control the measured vitamin D, PAPP-A, uterine artery PI and MAP were converted into the respective multiple of the unaffected median (MoM) after appropriate adjustment for maternal characteristics, including fetal crown-rump length, maternal age, body mass index (BMI), racial origin, parity, smoking, method of conception and season of blood testing, as previously described.[17,20,21] Kruskal-Wallis test with *post hoc* Dunn's procedure was used to compare median MoM values of vitamin D, PAPP-A, uterine artery PI and MAP with the outcome groups. Logistic regression analysis was used to examine the significance of contribution to prediction of early and late PE from square root vitamin D MoM, $\log_{10}$ PAPP-A MoM, $\log_{10}$ MAP MoM and $\log_{10}$ uterine artery PI MoM. The significance of difference in the incidence of vitamin D levels below the 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 (ρ)) 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.

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 ($P=141$ 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 $\log_{10}$ PAPP-A MoM ($P<0.0001$), $\log_{10}$ MAP MoM ($P<0.0001$) and $\log_{10}$ uterine artery PI MoM ($P<0.0001$), but not from square root vitamin D MoM ($P=0.234$). In the prediction of late-PE, there was a significant contribution from $\log_{10}$ PAPP-A MoM ($P=0.013$) and $\log_{10}$ MAP MoM ($P<0.0001$) but not from $\log_{10}$ 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%, respectively, $P=0.772$ and $P=0.067$, respectively).

In the control group, there was no significant association of vitamin D MoM with either PAPP-A MoM ($\rho=-0.059$, $P=0.061$), uterine artery PI MoM ($\rho=-0.021$, $P=0.502$), MAP MoM ($\rho=0.045$, $P=0.152$), gestation at delivery ($\rho=-0.052$, $P=0.103$) or birth weight percentile ($\rho=0.056$, $P=0.075$). Similarly, in the PE group, there was no significant association of vitamin D MoM with either PAPP-A MoM ($\rho=0.030$, $P=0.775$), uterine artery PI MoM ($\rho=0.095$, $P=0.371$), MAP MoM ($\rho=0.121$, $P=0.258$), gestation at delivery ($\rho=-0.069$, $P=0.516$) or birth weight percentile ($\rho=0.142$, $P=0.182$). However, there was a significant association of both uterine artery PI MoM and PAPP-A MoM with gestation at delivery ($\rho=-0.308$, $P=0.003$; $\rho=0.288$, $P=0.006$, respectively) and birth weight percentile ($\rho=-0.466$, $P<0.0001$; $\rho=0.287$, $P=0.006$, 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

Table 1. Maternal and pregnancy characteristics in the outcome groups

Maternal characteristics	*Unaffected controls* (n = *1,000)*	*Early preeclampsia* (n = *30)*	*Late preeclampsia* (n = *60)*
Maternal age in years, median (IQR)	31.5 (27.2-35.4)	31.6 (25.5-36.5)	32.4 (27.1-37.3)
Maternal BMI in kg m^{-2}, median (IQR)	24.2 (21.9-27.5)	28.3 (23.8-33.3)*	26.2 (23.2-31.5)*
Crown-rump length in mm, median (IQR)	63.3 (58.0-68.5)	62.0 (56.0-67.9)	61.4 (58.0-68.9)
Gestation at sampling (weeks), median (IQR)	12.4 (12.1-12.9)	12.4 (12.0-12.8)	12.4 (12.1-12.9)
Season of sampling			
Summer, *n* (%)	246 (24.6)	6 (20.0)	11 (18.3)
Other seasons, *n* (%)	754 (75.4)	24 (80.0)	49 (81.7)
Racial origin			
Caucasian, *n* (%)	580 (58.0)	11 (36.7)	30 (50.0)
African, *n* (%)	325 (32.5)	14 (46.7)	23 (38.3)
Asian, *n* (%)	95 (9.5)	5 (16.7)	7 (11.7)
Parity			
Nulliparous, *n* (%)	481 (48.1)	17 (56.7)	36 (60.0)
Parous - no previous preeclampsia, *n* (%)	495 (49.5)	10 (33.3)	18 (30.0)*
Parous - previous preeclampsia, *n* (%)	24 (2.4)	3 (10.0)	6 (10.0)*
Family history of preeclampsia, *n* (%)	33 (3.3)	4 (13.3)*	5 (8.3)
Cigarette smoker, *n* (%)	73 (7.3)	1 (3.3)	4 (6.7)
Conception			
Spontaneous, *n* (%)	980 (98.0)	28 (93.3)	55 (91.7)
Assisted, *n* (%)	20 (2.0)	2 (6.7)	5 (8.3)*
History of chronic hypertension, *n* (%)	7 (0.7)	3 (10.0)*	4 (6.7)*
Birth weight <5th percentile, *n* (%)	0	13 (43.3)*	14 (23.3)*

Abbreviations: BMI, body mass index; IQR, interquartile range. Comparisons between outcome groups (χ^2-test and Fisher's exact test for categorical variables and Kruskal-Wallis test with *post hoc* Dunn's procedure for continuous variables). *Significance value $P<0.025$.

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

Variable	*Unaffected controls* (n = *1,000)*	*Early preeclampsia* (n = *30)*	*Late preeclampsia* (n = *60)*
Vitamin D			
ng ml^{-1}	18.75 (11.12-28.03)	12.90 (9.09-20.20)	15.71 (8.87-25.25)
MoM	0.99 (0.71-1.33)	0.89 (0.70-1.14)	0.86 (0.59-1.39)
PAPP-A			
mU ml^{-1}	3.04 (1.95-4.78)	1.39 (0.83-3.54)*	2.38 (1.62-3.62)*
MoM	1.03 (0.73-1.44)	0.54 (0.37-1.17)*	0.88 (0.59-1.20)*
Uterine artery PI			
Unit	1.65 (1.35-2.00)	2.10 (1.69-2.67)*	1.78 (1.41-2.16)
MoM	1.01 (0.82-1.23)	1.32 (1.03-1.71)*	1.12 (0.83-1.35)
MAP			
mm of Hg	83.3 (78.5-88.5)	96.3 (89.1-101.6)*	91.9 (84.5-98.8)*
MoM	0.99 (0.94-1.05)	1.09 (1.03-1.19)*	1.06 (0.99-1.15)*

Abbreviations: 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. Significance value $P<0.025$.

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[17] 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.[22] 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.[17] 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,[20] 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)D2 or total 25(OH)D are 15−57%

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 in that of Powe *et al.*[10] 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^{-1} 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.[8,9] 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 be a consequence of impaired placentation.
- 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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REFERENCES

1 Weisman Y, Harell A, Edelstein S, David M, Spirer Z, Golander A. 1 alpha, 25-Dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3 *in vitro* synthesis by human decidua and placenta. *Nature* 1979; **281**: 317-319.
2 Reddy GS, Norman AW, Willis DM, Goltzman D, Guyda H, Solomon S *et al.* Regulation of vitamin D metabolism in normal human pregnancy. *J Clin Endocrinol Metab* 1983; **56**: 363-370.
3 August P, Marcaccio B, Gertner JM, Druzin ML, Resnick LM, Laragh JH. Abnormal 1,25-dihydroxyvitamin D metabolism in preeclampsia. *Am J Obstet Gynecol* 1992; **166**: 1295-1299.
4 Frølich A, Rudnicki M, Storm T, Rasmussen N, Hegedüs L. Impaired 1,25-dihydroxyvitamin D production in pregnancy-induced hypertension. *Eur J Obstet Gynecol Reprod Biol* 1992; **47**: 25-29.
5 Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. *J Clin Endocrinol Metab* 2007; **92**: 3517-3522.
6 Halhali A, Trovar AR, Torres N, Bourges H, Garabedian M, Larrea F. Preeclampsia Is Associated with Low Circulating Levels of Insulin-Like Growth Factor I and 1,25-Dihydroxyvitamin D in Maternal and Umbilical Cord Compartments. *J Clin Endocrinol Metab* 2000; **85**: 1828-1833.
7 Robinson CJ, Alanis MC, Wagner CL, Hollis BW, Johnson DD. Plasma 25-hydroxyvitamin D levels in early-onset severe preeclampsia. *Am J Obstet Gynecol* 2010; **203**: 366.e1-6.
8 Harris SS, Harris SS, Dawson-Hughes B. Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women. *Am J Clin Nutr* 1998; **67**: 1232-1236.
9 Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM. High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. *J Nutr* 2007; **137**: 447-452.
10 Powe CE, Seely EW, Rana S, Bhan I, Ecker J, Karumanchi SA *et al.* First trimester vitamin D, vitamin D binding protein and subsequent preeclampsia. *Hypertension* 2010; **56**: 758-763.
11 Yu CKH, Smith GCS, Papageorghiou AT, Cacho AM, Nicolaides KH. An integrated model for the prediction of preeclampsia using maternal factors and uterine artery Doppler velocimetry in unselected low risk women. *Am J Obstet Gynecol* 2005; **193**: 429-436.
12 Plasencia W, Maiz N, Bonino S, Kaihura C, Nicolaides KH. Uterine artery Doppler at 11+0 to 13+6 weeks in the prediction of preeclampsia. *Ultrasound Obstet Gynecol* 2007; **30**: 742-749.
13 Poon LC, Maiz N, Valencia C, Plasencia W, Nicolaides KH. First-trimester maternal serum pregnancy-associated plasma protein-A and pre-eclampsia. *Ultrasound Obstet Gynecol* 2009; **33**: 23-33.
14 Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. *Lancet* 1998; **352**: 343-346.
15 Kagan KO, Wright D, Baker A, Sahota D, Nicolaides KH. Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta-human chorionic gonadotropin, and pregnancy associated plasma protein-A. *Ultrasound Obstet Gynecol* 2008; **31**: 618-624.
16 Poon LC, Kametas NA, Valencia C, Chelemen T, Nicolaides KH. Hypertensive disorders in pregnancy: screening by systolic diastolic and mean arterial pressure at 11-13 weeks. *Hypertens Pregnancy* 2011; **30**: 93-107.
17 Yu CKH, Ertl R, Samaha R, Akolekar A, Nicolaides KH. Normal range of vitamin D in maternal serum at 11-13 weeks' gestation. *Fetal Diagn Ther* 2011; **30**(2): 94-99.
18 Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy* 2001; **20**: IX-XIV.
19 Poon LC, Karagiannis G, Staboulidou I, Shafiei A, Nicolaides KH. Reference range of birth weight with gestation and first-trimester prediction of small for gestation neonates. *Prenat Diagn* 2011; **31**: 58-65.
20 Akolekar R, Syngelaki A, Sarquis R, Wright D, Nicolides KH. Prediction of preeclampsia from biophysical and biochemical markers at 11-13 weeks. *Prenat Diagn* 2011; **31**: 66-74.
21 Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. *Ultrasound Obstet Gynecol* 2008; **31**: 493-502.
22 Nicolaides KH. A model for a new pyramid of prenatal care based on the 11 to 13 weeks' assessment. *Prenat Diagn* 2011; **31**: 3-6.
23 Hewison M. Vitamin D and the immune system. *J Endocrinol* 1992; **132**: 173-175.
24 Evans KN, Bulmer JN, Kilby MD, Hewison M. Vitamin D and placentaldecidual function. *J Soc Gynecol Investig* 2004; **11**: 263-271.
25 Cardus A, Parisi E, Gallego C, Aldea M, Fernandez E, Valdivielso JM. 1,25-Dihydroxyvitamin D3 stimulates vascular smooth muscle cell proliferation through a VEGF-mediated pathway. *Kidney Int* 2006; **69**: 1377-1384.
26 Hypponen E. VitaminD for the prevention of preeclampsia? A hypothesis. *Nutr Rev* 2005; **63**: 225-232.