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Maternal Serum Vitamin D at 11–13 Weeks in Pregnancies Delivering Small for Gestational Age Neonates

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Key Words
Vitamin D in pregnancy · Small for gestational age · First trimester screening · Pyramid of prenatal care

Abstract
Objectives: To determine if maternal serum levels of 25(OH)D at 11–13 weeks' gestation are altered in pregnancies that subsequently deliver small for gestational age (SGA) neonates and whether the levels are related to placental function reflected in serum concentration of pregnancy-associated plasma protein-A (PAPP-A). ***Methods:*** Serum 25(OH)D and PAPP-A were measured at 11–13 weeks in 150 singleton pregnancies that delivered SGA neonates and 1,000 appropriate for gestational age (AGA) controls. The median 25(OH)D and PAPP-A multiple of the unaffected median (MoM) in the outcome groups were compared. ***Results:*** In the SGA, the median serum 25(OH)D and PAPP-A were significantly decreased (0.78 vs. 1.00 MoM, $p < 0.0001$ and 0.78 vs. 1.00 MoM, $p < 0.0001$, respectively). The incidence of 25(OH)D levels below the 10th percentile was significantly higher in the SGA than the AGA group in Caucasian women ($p = 0.002$) but not in those of African racial origin ($p = 0.183$). There was no significant association between 25(OH)D MoM and PAPPA MoM in either the SGA or the AGA groups. ***Conclusion:*** Serum 25(OH)D levels at 11–13 weeks are decreased in pregnancies of Caucasian women that deliver SGA neonates but not in those of African racial origin. The decrease in 25(OH)D levels is unrelated to placental function.

Introduction

Vitamin D deficiency is a common and increasing problem in many parts of the world and concerns have been raised that there may be an epidemic of such deficiency in pregnancy [1, 2]. There is evidence that the condition is associated with adverse pregnancy outcome. Some studies reported that maternal vitamin D deficiency is associated with the delivery of small for gestational age (SGA) neonates [3, 4] and that the incidence of SGA is reduced by maternal vitamin D supplementation [5]. The association between vitamin D deficiency and impaired fetal growth may be mediated by the effect of vitamin D in enhancing endometrial decidualisation [6] and stimulating placental synthesis of estrogen [7, 8] which in turn upregulates uteroplacental angiogenic factor expression resulting in increase of uterine vascularisation and uteroplacental blood flow [7]. An early marker of placental function is maternal serum pregnancy-associated plasma protein-A (PAPP-A), and in pregnancies

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delivering SGA neonates serum PAPP-A at 11–13 weeks' gestation is decreased [9].

The aim of this study is to investigate whether in the first-trimester of pregnancy maternal serum levels of vitamin D are altered in pregnancies that subsequently deliver SGA neonates and if such changes are related to alterations in placental function reflected in serum levels of PAPP-A.

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 was held at 11^{+0}–13^{+6} weeks of gestation, we recorded maternal characteristics and medical history and performed combined screening for aneuploidies by measurement of the fetal crown-rump length and nuchal translucency thickness and maternal serum PAPP-A and free β-hCG [10, 11]. We stored 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 the King's College Hospital ethics committee.

In this study, we measured maternal serum vitamin D in 150 cases that subsequently delivered SGA neonates with birth weight below the 5th percentile for gestational age [12] and 1,000 controls that delivered phenotypically normal neonates at term with appropriate for gestational age (AGA) weight. We selected 75 cases of SGA from women of African racial origin and 75 from Caucasian women at random from our database of stored samples. The samples for the controls were also selected at random from our database of cases with no medical complications, such as hypertensive disorders or diabetes mellitus, resulting in the birth of neonates with birth weight between the 5th and 95th percentiles for gestational age [12, 13].

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 by a LC-MSMS method using a Shimadzu Prominence HPLC system equipped with a Phenomenex Luna C8 3 × 50 mm column and AB Sciex API-5000 ESI triple quadruple. The analysis was performed using the PerkinElmer MSMS Vitamin D (3075-0010) kit. Individual runs were calibrated using NIST SRM 2972 standards. The average inter-assay coefficients of variation for vitamin D2 and D3 were 6.6 and 7.3%, respectively, and the intra-assay coefficients of variation 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.

Statistical Analysis

Comparison between outcome groups was done by Mann-Whitney U-test for continuous variables and χ^2 test or Fisher's exact test for categorical variables. Bonferroni correction was used for multiple comparisons. Data are presented as median and interquartile range (IQR).

The distribution of serum 25(OH)D and PAPP-A was made Gaussian by square root and logarithmic transformation, respectively, and normality was assessed using histograms and probability plots. The measured concentration of 25(OH)D and PAPP-A in each case in the SGA and AGA groups was converted into a multiple of the AGA median (MoM) after appropriate adjustment for maternal characteristics, including maternal age, body mass index, racial origin, smoking, method of conception and season of blood testing, as previously described [13, 14]. Mann-Whitney U-test was used to compare median MoM values of 25(OH)D and PAPP-A between the outcome groups. The significance of difference in the incidence of 25(OH)D levels below the 10^{th} percentile in the SGA group, compared to the AGA group was estimated. Non-parametric correlation analysis was used to determine the significance of association between maternal serum 25(OH)D and PAPP-A.

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, Ill, USA) was used for data analyses.

Results

The maternal characteristics of each of the outcome groups are compared in table 1. In the SGA group, compared to the AGA group, more women delivered SGA neonates in their previous pregnancy, there were more cigarette smokers and more women required assisted conception techniques and delivered neonates with a lower birth weight percentile in the current pregnancy.

In the SGA group, compared to the AGA group, maternal serum 25(OH)D and PAPP-A were decreased (0.78 vs. 1.00 MoM, $p < 0.0001$ and 0.78 vs. 1.00 MoM, $p < 0.0001$, respectively). The maternal serum 25(OH)D in the SGA group compared to the AGA group was decreased in Caucasian women (0.69 vs. 1.03 MoM, $p < 0.0001$) but not in those of African racial origin (0.80 vs. 1.00 MoM, $p = 0.038$) (table 2).

The incidence of 25(OH)D levels below the 10th percentile was significantly higher in the SGA (28 of 150) than in the AGA group (104 of 1,000) ($p = 0.005$), but a stratified analysis according to racial origin showed that there was a significantly higher incidence of levels below the 10th percentile in Caucasian women (16 of 75 vs. 53 of 580, $p = 0.002$) but not in those of African racial origin (12 of 75 vs. 32 of 293, $p = 0.183$). Similarly, there was a significantly higher incidence of 25(OH)D insufficiency (serum level below 30 ng/ml) in the SGA than the AGA group ($p = 0.013$) in Caucasian women (61 of 75 vs. 385 of 580) but not in those of African racial origin (74 of 75 vs. 321 of 325, $p = 0.943$).

Table 1. Maternal and pregnancy characteristics in the outcome groups

Maternal characteristics	Non-small for gestation (n = 1,000)	Small for gestation (n = 150)
Maternal age in years, median (IQR)	31.5 (27.2–35.4)	30.2 (24.6–36.0)
Maternal body mass index, median (IQR)	24.2 (21.9–27.5)	23.9 (20.6–29.3)
Crown-rump length in mm, median (IQR)	63.3 (58.0–68.5)	62.3 (57.1–66.5)
Season of sampling		
Summer, n (%)	246 (24.6)	25 (16.7)*
Other seasons, n (%)	754 (75.4)	125 (83.3)
Racial origin		
Caucasian, n (%)	580 (58.0)	75 (50.0)
African, n (%)	325 (32.5)	75 (50.0)**
Asian, n (%)	95 (9.5)	–
Parity		
Nulliparous, n (%)	481 (48.1)	83 (55.3)
Parous – no previous SGA, n (%)	475 (47.5)	45 (30.0)**
Parous – SGA, n (%)	44 (5.4)	22 (14.7)**
Cigarette smoker, n (%)	73 (7.3)	34 (22.7)*
Conception		
Spontaneous, n (%)	980 (98.0)	141 (94.0)
Assisted, n (%)	20 (2.0)	9 (6.0)*
Birth weight percentile, median (IQR)	50.2 (28.0–69.3)	1.0 (0.2–2.0)*

Comparisons between outcome groups (χ^2 test and Fisher's exact test for categorical variables and Mann Whitney U-test for continuous variables).

* Significance level was $p < 0.05$. ** In the subgroup analysis according to racial origin and parity, Bonferroni correction was used and the adjusted significance level for comparisons was $p < 0.025$.

IQR = Interquartile range.

Table 2. MoM (interquartile range) for maternal serum vitamin D and PAPP-A in the outcome groups

Variable	Appropriate for gestation (n = 1,000)	Small for gestation (n = 150)	p value
Vitamin D			
ng/ml	18.75 (11.12–28.03)	12.16 (8.09–20.54)	
MoM (All)	1.00 (0.72–1.35)	0.78 (0.58–1.14)	<0.0001
Caucasians	1.02 (0.75–1.32)	0.69 (0.52–1.12)	<0.0001*
African	1.00 (0.69–1.41)	0.80 (0.65–1.24)	0.038*
Insufficiency (<30 ng/ml), n (%)	796 (79.6)	135 (90.0)	0.004
Deficiency (<20 ng/ml), n (%)	548 (54.8)	108 (72.0)	<0.0001
PAPP-A			
mU/ml	3.04 (1.95–4.78)	2.16 (1.45–3.78)	
MoM	1.00 (0.71–1.40)	0.78 (0.46–1.07)*	<0.0001

Comparisons between outcome groups by Mann Whitney U-test.

* In the subgroup analysis according to racial origin, Bonferroni correction was used and the adjusted significance level for comparisons was $p < 0.025$.

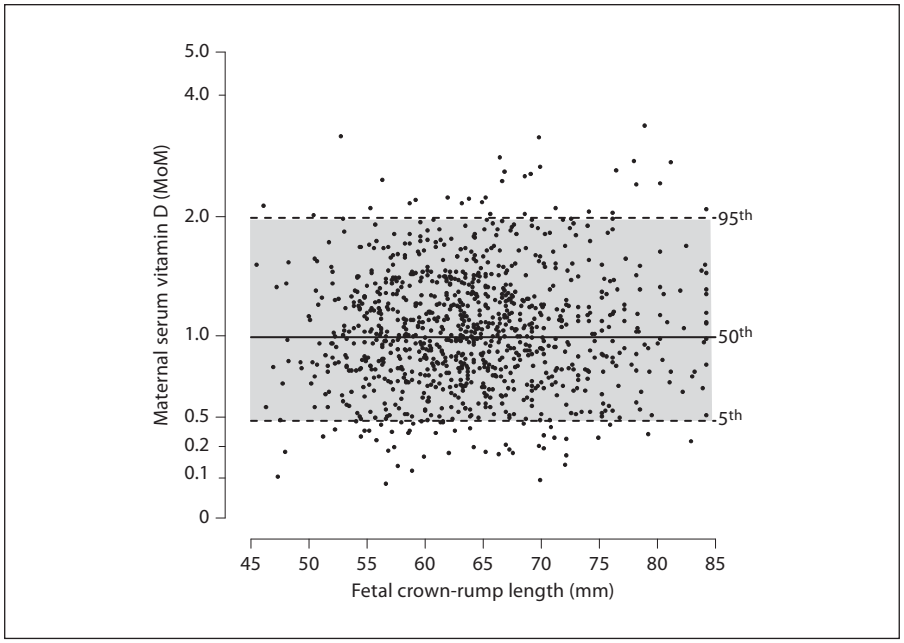

Fig. 1. Reference range of vitamin D multiple of the median (MoM) plotted against fetal crown-rump length demonstrating the 5th, 50th and 95th percentiles of the normal range.

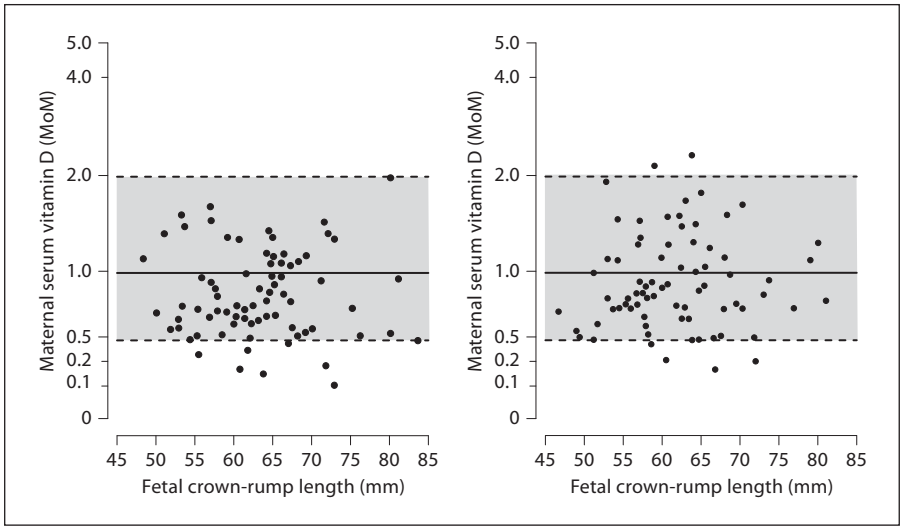

Fig. 2. Maternal serum vitamin D levels (ng/ml) in pregnancies delivering small for gestational age neonates in Caucasian women (left) and women of African racial origin (right) plotted on the reference range for fetal crown-rump length (shaded area with lines demonstrating the 5th, 50th and 95th percentiles).

There was no significant association between 25(OH)D MoM and PAPP-A MoM in either the AGA group (Spearman's correlation coefficient [ρ] = –0.059, p = 0.061) or in the SGA group (ρ = 0.136, p = 0.096).

Discussion

The findings of this study demonstrate that at 11–13 weeks' gestation in pregnancies of Caucasian women that subsequently deliver SGA neonates, but not in those of African racial origin, serum 25(OH)D is reduced. The data confirm that in such pregnancies there is evidence of impaired placentation manifested in decreased maternal serum PAPP-A [9]. However, there was no significant association between 25(OH)D and PAPPA suggesting that the underlying mechanism for the vitamin D deficiency in SGA pregnancies may be unrelated to impaired placentation.

In normal pregnancy, the measured maternal serum 25(OH)D is affected by maternal characteristics [13]. The levels are lower in cigarette smokers and in women of Af-

rican racial origin compared to Caucasians, they increase with maternal age and decrease with body mass index 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. This is particularly important for those variables that are known to be related to the risk for delivery of SGA neonates, including maternal racial origin, age, weight, height and smoking status [12]. Additional strengths of our study are firstly, examination of a large number of pregnancies resulting in delivery of SGA neonates 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 [15], secondly, distinction between Caucasian women and those of African racial origin, and thirdly, measurement of vitamin D by LC-MSMS which is currently the most accurate technique for such estimation [16, 17]. The main weakness resides in the design of the study which was case-control rather than prospective.

There is no absolute consensus as to what a normal range for circulating 25(OH)D should be, mainly because of the difficulty in defining the methodology for determining such a range [18]. Nevertheless, many experts suggest that the patient should be considered to be deficient or insufficient in vitamin D if the serum level of 25(OH)D is less than 20 and 20–29 ng/ml, respectively [18]. On the basis of such definitions about 90% of our SGA group, but also 80% of the AGA group, would be classified as being deficient or insufficient in vitamin D. In our study, we used the pragmatic approach of examining the distribution of serum 25(OH)D values in a diverse inner city population of singleton pregnancies with normal outcome [13]. On the basis of this normal range, serum 25(OH)D was below the 5th percentile in about 9% of the SGA group in Caucasian women and in 5% of those in the African racial group.

Our finding of the maternal race-related effect on the association between SGA and reduction in serum 25(OH)D is compatible with the results of a previous study which reported that maternal serum vitamin D levels before 22 weeks' gestation were lower in pregnancies of Caucasian women delivering SGA than AGA neonates but not in women of African origin [3]. The main source of vitamin D is synthesis in the skin after exposure to the ultraviolet B radiation of sunlight and this is reduced in women of African racial origin because the higher content of melanin in their skin absorbs ultraviolet B radiation, thereby limiting its availability to the deeper layers involved in the synthesis of calciferol [19, 20]. However, women of African racial origin may be less sensitive than Caucasians to the negative impact of vitamin D deficiency possibly because of skeletal and renal adaptations to such deficiency [21, 22].

Maternal total vitamin D levels at 11–13 weeks were found to be significantly lower in Caucasian women, who subsequently deliver SGA neonates but not in women of African racial origin. The extent to which vitamin D supplementation in Caucasian women with low serum 25(OH)D in early pregnancy can reduce the incidence of SGA remains to be determined.

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