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Effects of maternal serum 25-hydroxyvitamin D concentrations in the first trimester on subsequent pregnancy outcomes in an Australian population

Francisco J Schneuer, Christine L Roberts, Cyrille Guilbert, Judy M Simpson, Charles S Algert, Amina Z Khambalia, Vitomir Tasevski, Anthony W Ashton, Jonathan M Morris, Natasha Nassar

Clinical and Population Perinatal Health Research, Kolling Institute of Medical Research, University of Sydney, Sydney, NSW, Australia (FJS, NN, CG, CSA, AZK, AWA, JMM and CLR); Sydney School of Public Health, University of Sydney, Sydney, New South Wales, Australia (JMS); Fetal Maternal Medicine (PaLMs), Royal North Shore Hospital, St Leonards, NSW, Australia (VT).

Names for PubMed indexing: Schneuer, Roberts, Guilbert, Simpson, Algert, Khambalia, Tasevski, Ashton, Morris, Nassar

Address correspondence to: Francisco J Schneuer. Email: francisco.schneuer@sydney.edu.au
Address: Building 52, Royal North Shore Hospital, St Leonards NSW 2065, Australia
Telephone: +61 2 9926 6031 Fax: +61 2 9906 6742. Reprints will not be available from the author.

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Abbreviations used: APDC, Admitted Patient Data Collection; AUC, area under the receiver operating characteristic curve; GDM, gestational diabetes mellitus;
NPV, negative predictive value; NSW, New South Wales; PDC, Perinatal Data Collection; PPV, positive predictive value; SGA, small for gestational age; 25(OH)D, 25-hydroxyvitamin-D

Running title: Serum 25(OH)D concentrations in first trimester and pregnancy outcomes

ABSTRACT

Background: Low serum 25-hydroxyvitamin-D [25(OH)D] concentration during pregnancy have been associated with adverse pregnancy outcomes by a few studies, and not by others.

Objective: To assess serum 25(OH)D concentration at 10-14 weeks of pregnancy and its association with adverse pregnancy outcomes, and examine the predictive accuracy.

Design: This nested case-control study measured serum 25(OH)D in 5,109 women with singleton pregnancies attending first trimester screening in New South Wales, Australia. Multivariate logistic regression was conducted to examine the association between low 25(OH)D concentrations and adverse pregnancy outcomes (small for gestational age, preterm birth, preeclampsia, gestational diabetes, miscarriage and stillbirth). The predictive accuracy of models was assessed.

Results: Median (interquartile range) 25(OH)D concentrations for the total population was 56.4 nmol/L (43.3-69.8). Serum 25(OH)D concentrations showed significant variation by parity, smoking, weight, season of sampling, country of birth and socio-economic status. After adjusting for maternal and clinical risk factors, low 25(OH)D concentrations were not associated with most adverse pregnancy outcomes. The area under the Receiver Operating Characteristic curve (AUC) and likelihood ratio (LR) for a composite of severe adverse pregnancy outcomes of 25(OH)D <25nmol/L were 0.51 and 1.44; and for risk factors alone were 0.64 and 2.87, respectively. Adding 25(OH)D information to maternal and clinical risk factors did not improve the ability to predict severe adverse pregnancy outcomes (AUC=0.64; LR=2.32; P=0.39).

Conclusion: Low 25(OH)D serum concentrations in first trimester of pregnancy are not associated with adverse pregnancy outcomes and do not predict complications any better than routinely assessed clinical and maternal risk factor information.

INTRODUCTION

The prevalence and significance of vitamin D deficiency during pregnancy has attracted much recent public health interest. The main function of vitamin D is to maintain normal ranges of serum calcium and phosphorus by enhancing calcium absorption from the intestine and promoting the mobilization of calcium and other minerals from the skeleton (1). It also has immunomodulatory roles during pregnancy that enables successful implantation and stimulates antimicrobial activity (2). From early in pregnancy, there are high demands for calcium to support both maternal calcium homeostasis and fetal skeletal development and growth (2).

The definition and the terminology used to describe adequate concentrations of vitamin D are inconsistent. A recent Institute of Medicine report recommends serum 25-hydroxyvitamin D [25(OH)D] concentrations >50 nmol/L for adequate bone health (3), but, clinical guidelines from The Endocrine Society have defined vitamin D deficiency and insufficiency as serum concentration <50 nmol/L and 50–72.5 nmol/L, respectively (4). The National Institute for Health and Clinical Excellence (NICE) guidelines (5) defines 25(OH)D concentrations <25 nmol/L as insufficiency while other researchers (6) propose that 25(OH)D concentrations <37.5 nmol/L are associated with lower bone mass density and increased risk of fracture. These definitions refer to the general population, are not universally accepted and are not specific to pregnancy populations. In addition to the increased physiological demands in pregnancy, geographical and/or ethnic differences in 25(OH)D concentrations suggest the important need to establish locally determined population reference ranges and normative concentrations during pregnancy (7).

Studies reporting the association between 25(OH)D concentrations in early pregnancy and adverse pregnancy outcomes are conflicting. Some report low 25(OH)D concentrations are associated with an increased risk of preeclampsia (8), small for gestational age (9-11), gestational diabetes mellitus (12, 13) and miscarriage (14), while others have not reported a significant association with pregnancy complications (15-23). However these studies have limitations including: small sample size, limited generalizability due to low response rates or no information on several pregnancy outcomes. No large study has assessed these associations. Furthermore, there is no information about the predictive accuracy of low 25(OH)D concentrations in early pregnancy in identifying pregnancies at-risk. First trimester screening provides an ideal opportunity for early identification of pregnancies at-risk. The aims of this study were three-fold: i) describe normative concentrations of serum 25(OH)D in the first trimester of pregnancy; ii) examine the association between maternal 25(OH)D and risk of adverse pregnancy outcomes; and iii) assess the diagnostic accuracy of low 25(OH)D in predicting adverse pregnancy outcomes.

SUBJECTS AND METHODS

Participants and study design

A nested case-control study was conducted based on a study population of 11,358 women attending first trimester Down syndrome screening between October 2006 and September 2007 in New South Wales (NSW), Australia. Existing data from banked serum and routinely collected electronic health databases were used. Serum samples were collected by the Pacific Laboratory Medicine Services, and then archived and stored at -80°C. During this period, this was the state's only public screening service and received samples from throughout NSW. Serum samples were stored according to time of collection in boxes containing 81 samples in 9x9 rows. Cases of each study outcome of interest (small for gestational age (SGA), preterm birth, preeclampsia,

gestational diabetes mellitus, miscarriage and stillbirth) were randomly selected from the maternal screening population using a computerized random-number function, with the specific box and row of the case identified. Controls were then sourced using the remaining eight samples in each row, regardless of their outcome status. These were selected by laboratory technicians from the relevant box containing the case and who analysed the full row (9 samples) unaware of the case's position within the row. This ensured random selection of samples, allowed laboratory technicians to remain blinded to pregnancy outcomes and increased the efficiency of processing. Half of cases from the study population were initially selected but in the end, 63% were identified because some samples in the control group were cases of other adverse pregnancy outcomes.

Biochemical analysis

Serum samples were thawed once and serum concentrations of 25(OH)D were measured by an automated immunoassay system (LIAISON, Diasorin S.p.A. Saluggia, Italy). Intra-assay and inter-assay coefficient of variation were <9.5% and reported analytic sensitivity of the immunoassay was 4.9 - 368 nmol/L. Commonly used cut-points to define 25(OH)D status were assigned: <15, <25, <37.5, <50 and <75 nmol/L (4-6).

Data sources

Maternal information for archived serum samples were derived from the laboratory database and corresponding pregnancy and birth outcomes were ascertained via record-linkage to the Perinatal Data Collection (PDC) and Admitted Patient Data Collection (APDC). The PDC is a statutory surveillance system of all births in NSW of at least 400 grams birthweight, or at least 20 weeks' gestation; it includes information on maternal demographic, pregnancy and delivery factors and infant outcomes. The APDC is a census of all in-patient hospital admissions from NSW public

and private hospitals, with records for both mothers and liveborn infants. Relevant diagnoses are recorded for each admission and coded according to the International Classification of Diseases version 10–Australian Modification (ICD10-AM). Validation studies of the PDC and the APDC show excellent level of agreement with the medical record and low rates of missing data (24, 25). Reporting in both datasets have high specificity (> 99%) indicating few false positive reports. Only factors and outcomes accurately reported in birth or hospital data were included in analyses (26). Record-linkage was conducted by the NSW Centre for Health Record Linkage with identifying information removed prior to the release of data for analysis. The study was approved by the NSW Population and Health Services Research Ethics Committee.

Study outcomes assessed included: small for gestational age (SGA) defined as birthweight <10th and <3rd centile of the distribution for gestational age and infant sex (27); preterm birth at <37 weeks' and very preterm birth <34 weeks' gestation; early-onset and all preeclampsia, gestational diabetes mellitus (GDM), miscarriage and stillbirth. During the study period, preeclampsia was defined as onset of hypertension (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) from 20 weeks' gestation accompanied by proteinuria (28), and early-onset preeclampsia classified for women requiring delivery at ≤34 weeks gestation. GDM was characterised as fasting glucose ≥5.5 mmol/l or 2-hour plasma glucose ≥8.0 mmol/l (29). Information on preeclampsia and gestational diabetes was obtained from both the APDC and PDC data, to maximize ascertainment. Preeclampsia (regardless of severity) and gestational diabetes were determined either the box was checked in the PDC record, or if any APDC record had a diagnosis in any of the 55 fields of gestational hypertension (ICD10-AM: O13 and O16), preeclampsia (O11 and O14) or eclampsia (O15) (for preeclampsia) and gestational diabetes (O24) (30, 31). Miscarriage was defined as spontaneous fetal loss between 10-20 weeks gestation and identified from APDC data (O00, O01, O02, O03, O05 or O06), while stillbirth

classified for spontaneous fetal death after 20 weeks gestation and was identified from PDC data. A composite of severe adverse pregnancy outcome variable was also developed comprising occurrence of either SGA<3rd centile, preterm birth <34 weeks, early-onset preeclampsia or stillbirth. Controls were defined as pregnancies unaffected by each outcome.

The key maternal and clinical risk factors used in this analysis included maternal age and weight (kilograms) calculated at the time of first trimester screening, parity (nulliparae/multiparae), smoking during pregnancy, any previously diagnosed hypertension (chronic or pregnancy) or diabetes (pre gestational or gestational), season at sampling, country of birth and socio-economic disadvantage. Season of sample collection (Southern Hemisphere) was defined as spring (September-November), summer (December-February), autumn (March-May) and winter (June-August). Socio-economic disadvantage was determined using the Socio-Economic Indexes for Areas (SEIFA) relative disadvantage scores developed by the Australian Bureau of Statistics (32) and categorised into quintiles. Maternal weight was missing in 831 (16.3%) of the records. Multiple imputation was used to account for the missing maternal weight, a technique that predicts missing values using existing values from other variables (33). Other missing data were infrequent and were excluded from the analyses: maternal age missing in 1 record (0.002%), smoking in 42 (0.8%), country of birth in 6 (0.1%) and socio-economic disadvantage information missing in 13 (0.25%) records.

Statistical analysis

As each case and control had a known probability of selection calculated as the prevalence of each pregnancy outcome, concentrations of 25(OH)D were weighted to account for sampling probabilities to determine normative concentrations for our pregnancy sample. Weight calculation involved multiplying each case and control by the inverse proportion of the

probability of selection. The 25(OH)D distribution was then calculated applying individual weights. Similarly, to ensure comparability with the NSW state maternity population, we weighted all observations for the population rates of age, parity, smoking and socio-economic disadvantage using SEIFA quintiles (34).

Normative concentrations in the overall population and by maternal characteristics were examined at various percentiles (5^{th}, 10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th}, 95^{th}) and differences assessed using Kruskall-Wallis test. Spearman coefficient was used to determine correlations between 25(OH)D and maternal weight. Comparison of maternal characteristics and concentrations of 25(OH)D between women with and without each study outcome was performed using contingency table, student's t-test or Wilcoxon-rank sum test for categorical, normal or non-normally distributed data, respectively. Conditional univariate and multivariate logistic regression analysis was performed to asses crude and adjusted association between low 25(OH)D and adverse pregnancy outcomes, taking into account important maternal and clinical risk factors in the latter analysis.

In assessing predictive accuracy we defined low 25(OH)D concentrations at <25 and <37.5 nmol/L. The performance in predicting severe pregnancy outcomes in the total population and among a group of women at high-risk (weight >85kg or born in countries most likely to have dark skin or covered) was determined by examining the area under the Receiver Operating Characteristics (ROC) curves (AUC), derived from logistic regression models. AUC results were also examined to determine whether models performed better than chance (0.5). Models for 25(OH)D alone, those including maternal and clinical risk factors only and with 25(OH)D and risk factors combined were compared. Comparison of predictive accuracy of the models was performed by evaluating the maximum likelihood estimates and applying likelihood ratio (X^2)

test. Finally, estimates of predictive accuracy were calculated including sensitivity, specificity, positive (PPV), negative predictive values (NPV) and positive likelihood ratio with exact binominal confidence intervals. A P-value of <0.05 was considered statistically significant and analyses performed using SAS software 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

A total of 5,762 serum samples were tested for 25(OH)D, with linked health information relevant to the pregnancy available for 5,397 (93.7%) women. We excluded 288 women whose blood sample was taken before 10 or after 14 weeks gestation; 25(OH)D concentrations were outside limits of assay detection; or who had a medical abortion, twin pregnancy or infant with major congenital anomaly. A total of 5,109 women were included in the analysis. The demographic characteristics and percentile distribution of serum 25(OH)D concentrations are presented in **Table 1**. Median (interquartile range, IQR) serum concentrations of 25(OH)D for the total population were 56.4 (43.4 – 69.9) nmol/L; 294 (5.8%) women had serum <25 nmol/L, 835 (16.3%) <37.5 nmol/L and 999 (19.6%) >75 nmol/L. Women having a first baby, who smoked during pregnancy, women born in Middle Eastern, Asian, African and Caribbean countries had lower 25(OH)D concentrations, as well as women living in socially disadvantaged areas (P<0.05). There was a negative correlation with maternal weight (r = −0.36, P=0.01) (Table 1). There was also significant variation by season of sampling (P<0.001). 25(OH)D concentrations were highest in Autumn (median 63.5; IQR: 52.0 – 78.4) and lowest in Winter (median 51.5, IQR: 37.5 – 64.4) (P<0.001) (**Figure 1**).

Table 2 presents maternal characteristics and 25(OH)D serum concentrations by adverse pregnancy outcome. Compared with unaffected pregnancies (median 56.9, IQR 43.9 - 70.8 nmol/L), median concentrations of 25(OH)D in the first trimester were lower for women

diagnosed with GDM (median 52.1, IQR 41.5 - 63.6 nmol/L; P<0.001). The association between 25(OH)D and adverse pregnancy outcomes are presented in **Table 3**. After adjusting for risk factors, women with 25(OH)D <25 nmol/L had increased risk of having an SGA <10th centile infant (Adjusted odds ratio (aOR) 1.58 95% CI: 1.06, 2.35). In univariate analysis, women with 25(OH)D concentrations <37.5 nmol/L had increased risk of GDM; however this association was attenuated after adjusting for maternal factors (aOR 1.08 95% CI: 0.74, 1.56). Women with low 25(OH)D concentrations had a tendency towards an increased risk of spontaneous preterm birth (P=0.09) or a severe pregnancy outcome (P=0.07), but a reduced risk of preeclampsia (P=0.07) (Table 3). There was also a slight tendency for high 25(OH)D concentrations >75 nmol/L to be protective against early-onset preeclampsia (P=0.09), however, numbers were small and results imprecise. There was no association between women with low 25(OH)D concentrations and any other of the adverse pregnancy outcomes (Table 3), and regardless of the various cut-points applied (data not presented). In particular, there was no relationship between 25(OH)D <15nmol/L and the composite measure of any adverse pregnancy outcome (aOR 1.20; 95% CI: 0.69, 2.07) (data not included in tables). When the association between 25(OH)D concentrations and severe adverse pregnancy outcomes were analysed in a sub-group of high risk women (N=758), the adjusted odds for <25 and <37.5nmol/L was 2.34 (95% CI: 1.01, 5.50) and 2.42 (95% CI: 1.20, 4.92), respectively. In absolute terms, up to 11 in 100 women with 25(OH)D <37.5nmol/L compared with 6 in 100 without low 25(OH)D had a severe adverse pregnancy outcome.

Table 4 presents the predictive accuracy results for severe adverse pregnancy outcomes. The AUC of the univariate model was not different from chance and the predictive accuracy of prior risk factors was poor. Adding 25(OH)D information to maternal and clinical risk factors did not improve the ability to predict adverse pregnancy outcomes (X^2=0.75, P=0.39). When restricting

the analysis to women at high risk of low 25(OH)D, the predictive accuracy of 25(OH)D alone was inferior compared with information from maternal and clinical history risk factors (X^2=0.75, P=0.39) (Table 4).

DISCUSSION

This is the largest study to examine maternal vitamin D concentrations among women in first trimester, and to assess the association with adverse pregnancy outcomes. Our results indicate that there is variation in serum 25(OH)D concentrations in first trimester by a range of maternal characteristics and season. In general, low 25(OH)D concentrations were not associated with most adverse pregnancy outcomes, although women at high risk, such as those overweight or from Middle-Eastern and Asian countries, were more likely to develop a severe outcome. In spite of this, on further analysis, our findings revealed that low 25(OH)D concentrations did not predict complications any better than routinely collected maternal and clinical risk factor information, even when limiting analyses to these high risk women. Overall, our findings suggest that routine screening for 25(OH)D concentrations in early pregnancy would not help predicting those pregnancies at-risk of adverse pregnancy outcomes. These also support recent Australian Government antenatal care clinical practice guidelines which only recommend 25(OH)D testing of pregnant women at high risk of vitamin D deficiency (35).

Our results provide important information and a moderating message for the current enthusiasm for screening and vitamin D supplementing in pregnancy (36). Support for vitamin D testing and supplementation is based on certain studies suggesting low 25(OH)D concentrations are associated with potential harms in pregnancy (8-13). However, a recent randomised trial of vitamin D supplementation in pregnancy has suggested that there is no impact or decrease on adverse pregnancy outcomes, including preeclampsia, GDM and fetal growth (37). Despite not

having information regarding vitamin D supplementation amongst women, screening and supplementation were uncommon during the study period. Since then, vitamin D testing amongst Australian women aged 15-44 years has increased over 13-fold and with significant cost to the public healthcare system of $31.8 million in 2011 (38). Moreover, some studies have found elevated maternal concentrations >75 nmol/L were associated with increased risk of SGA infants (9), while ours did not. Subsequent development of atopic eczema in the offspring has also been identified (39). Although, our study did not assess childhood health, the potential long-term effect of vitamin D supplementation on the mother and offspring is unknown.

Our study found no significant association between low 25(OH)D concentrations and most adverse pregnancy outcomes and is consistent with the majority of previous studies (15-23) assessing 25(OH)D in early pregnancy. Although we found women with 25(OH)D <25nmol/L to be more likely to have an infant diagnosed SGA< 10^{th} centile, this may be a chance finding due to multiple testing, given the result was not replicated in infants SGA< 3^{rd} centile and results were imprecise. Other studies have evaluated 25(OH)D later in pregnancy when it may be too late to intervene and testing may provide no additional benefit. Only few studies have reported increased risks for women tested in early pregnancy; two found an association between 25(OH)D <30 and <37.5 nmol/L and SGA infants amongst white women (9, 11), one reported a five-fold increased risk of preeclampsia in white women with 25(OH)D <37.5 nmol/L at <22 weeks of pregnancy (8) and one study showed a two-fold risk of GDM in women with 25(OH)D <73.5 nmol/L at 15-18 weeks (12). Unique to our study, we assessed the predictive accuracy of low 25(OH)D concentrations for severe adverse pregnancy outcomes and found these were poor, also revealing that 25(OH)D adds very little information in addition to maternal risk factors.

Variation in findings between studies may also be explained by differences in cut-points used, population characteristics and methods used to measure 25(OH)D concentrations. Cut-points to define vitamin D sufficiency/insufficiency have been traditionally determined based on the threshold above where 25(OH)D serum concentrations have no correlation with serum parathyroid hormone (PTH) concentrations (PTH plateau) (40). A recent systematic review of 70 studies found 25(OH)D level thresholds varied between 25-150 nmol/L (41), suggesting large individual variation. Therefore, use of particular cut-points to define vitamin D status may be unreliable given this heterogeneity. Furthermore, a recent study found no overall correlation between concentrations of PTH with 25(OH)D in pregnant women tested in first trimester. The authors concluded that 25(OH)D threshold estimates could not be precisely defined and are, therefore, less useful for determining optimum vitamin D status during pregnancy (42). In addition, recent studies have demonstrated that genetic differences in vitamin D receptor (*VDR*) has a strong influence on individual vitamin D metabolism which may impact on susceptibility to disease (43) and low birth size in infants from 25(OH)D deficient mothers (44). Thus, future studies stratified by genetic variation are warranted.

Significant population and geographic variation based on latitude and ethnic background have also been shown in a recent review of serum concentrations of 25(OH)D amongst women in first trimester (7). Regional differences in 25(OH)D concentrations are confirmed when comparing our results (latitude 34°S, median 56.4 nmol/L) with a recent study examining 25(OH)D concentrations in pregnant women before 20 weeks living in tropical Australia (latitude 16.9°S), with mean 25(OH)D of 114 nmol/L and no women with concentrations <50nmol/L (45). Therefore, our findings are relevant to sun-rich latitudes with low prevalence of vitamin D deficiency and may not be applicable to other settings. Additionally, maternal risk factors for low 25(OH)D concentrations in pregnancy such as obesity (due to storage of vitamin D in fat) and

race/ethnicity are also risk factors for preeclampsia and gestational diabetes. Therefore, adjusting for such factors may not completely control for the dual effect that these can have on adverse pregnancy outcomes (46). Finally, studies have found a lack of standardisation of 25(OH)D measurement methods and significant variability in results between assay methods and laboratories (47, 48).

Strengths of the study were the assessment of a large sample of pregnant women attending first trimester screening over four seasons. Record-linkage of laboratory to birth and hospital data ensured follow-up and ascertainment of pregnancy outcomes for 93% of samples with minimal missing information. Missing health and pregnancy information was mostly attributable to women residing in bordering towns and giving birth in hospitals out of state, but these women had similar characteristics compared with those included in the study. Given limited resources, we were unable to include all women undergoing first trimester screening in our study and conducted a nested case-control, instead. To overcome this potential limitation and ensure representativeness of our sample, cases were randomly selected according to population prevalence rates and corresponding controls randomly identified based on unknown outcome status and matched by timing of collection. Furthermore, we adjusted and weighted our results back to population rates of maternal characteristics to ensure comparability with the NSW maternity population. A possible weakness of the study is that outcomes were not verified with individual medical records, but we only used data in this study that have been previously validated (26). However, other potential confounders such as ethnicity, BMI, sun exposure, education and lifestyle behaviour were not measured or available in the datasets and could not be taken into account.

In conclusion, our findings confirm that there is variation in 25(OH)D serum concentrations in first trimester by maternal characteristics and season of testing. Low 25(OH)D concentrations were not associated with an increased risk of most adverse pregnancy outcomes and have poor predictive accuracy. Results reveal that widespread screening for vitamin D deficiency in the first trimester of pregnancy would not efficiently identify women at-risk of adverse pregnancy outcomes. Our findings suggest that current screening and testing for vitamin D in early pregnancy without taking into account individual variability leads to over-diagnosis and unnecessary and potentially harmful treatment of women, and causes excessive burden and costs to healthcare providers.

The authors' responsibilities were as follows: FS, NN, AK, CA, VT, AA, JM and CR conceived and designed the study. NN, VT, JM and CR acquired the data. FS and CG performed the laboratory analysis. FS, NN, and JS conducted the statistical analysis. FS and NN drafted the manuscript which was approved by all authors. All authors critically reviewed the manuscript for important intellectual content. FS takes responsibility for the integrity of the data and the accuracy of the data analyses.

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TABLE 1 First trimester 25(OH)D serum levels by maternal characteristics

Maternal characteristics		25(OH)D percentiles (nmol/L)							
	n (%)	5th	10th	25th	50th	75th	90th	95th	P-value[1]
All woman	5109	23.4	31.2	43.4	56.4	69.9	84.8	92.8	
Maternal age (y)									0.91
<25	313 (6.1)	25.5	35.9	45.5	56.5	68.2	83.3	89.5	
25 - 29	1035 (20.3)	21.2	28.4	42.2	56.9	71.3	85.6	93.4	
30 - 34	1957 (38.3)	24.0	31.0	43.1	55.9	69.8	85.1	95.5	
35 - 39	1538 (30.1)	24.4	31.4	43.1	56.4	70.3	84.6	92.3	
40+	265 (5.2)	20.0	27.1	38.9	55.5	69.4	85.1	104.0	
Number of previous pregnancies									<0.001
0	2448 (47.9)	23.1	31.5	42.0	54.5	67.1	80.5	88.7	
1	1722 (33.7)	23.8	31.5	44.6	57.5	71.4	85.8	93.0	
2+	929 (18.2)	21.4	30.2	42.5	57.1	71.8	90.5	96.1	
Smoking during pregnancy									0.01
Yes	326 (6.4)	29.0	36.2	45.2	58.4	71.3	88.7	93.4	
No	4739 (93.6)	22.6	30.4	42.6	56.0	69.6	84.2	92.7	
Maternal weight (kg)									<0.001
<55	798 (18.6)	23.2	29.5	43.1	56.2	71.0	86.8	92.5	
55 - 64	1434 (33.5)	23.7	31.5	43.7	57.4	71.5	86.6	96.9	
65 - 74	1076 (25.1)	25.1	31.5	43.7	57.5	70.4	85.0	93.3	
75 - 84	494 (11.5)	21.3	32.3	43.2	55.8	67.6	84.2	90.1	
85+	478 (11.2)	24.0	32.2	41.4	51.2	61.9	76.5	85.8	
Gestational week at sampling									0.21
10	467 (9.1)	26.0	33.5	42.4	57.5	72.5	85.9	95.1	
11	1609 (31.5)	21.9	30.9	43.2	56.5	68.8	82.3	91.3	
12	2092 (41)	24.6	31.4	44.2	56.4	69.8	85.8	93.2	
13	806 (15.8)	20.6	29.5	42.0	56.4	70.1	85.3	95.7	
14	135 (2.6)	26.9	32.5	40.3	53.7	73.3	83.3	90.7	
Country of birth									<0.001
Australia & New Zealand	3343 (65.5)	28.2	35.7	46.1	58.4	72.5	86.8	94.9	
Pacific Islands	39 (0.8)	17.9	21.2	36.4	51.8	61.6	74.5	74.5	
Europe, North America & South Africa	552 (10.8)	28.9	33.2	48.9	60.3	72.6	88.2	96.9	
Middle East	114 (2.2)	17.0	17.9	30.8	44.5	59.4	67.9	89.2	
South east Asia	341 (6.7)	19.5	24.6	37.3	49.2	62.5	77.1	84.0	
China, Hong Kong & Taiwan	269 (5.3)	23.5	29.7	39.0	52.7	64.5	80.3	86.2	
Japan & Koreas	144 (2.8)	20.6	24.0	33.5	45.9	59.5	68.2	85.3	
India, Pakistan, Sri-Lanka, Nepal & Bangladesh	195 (3.8)	11.3	13.6	20.0	34.3	46.0	56.3	60.0	
Central & South America	70 (1.4)	26.9	30.4	40.2	51.9	61.1	83.3	87.7	
Africa & Caribbean	36 (0.7)	21.3	22.0	23.4	44.4	51.8	69.5	87.4	
Socio-economic disadvantage quintiles									<0.001
1 (most disadvantage)	387 (7.6)	20.8	25.4	39.5	52.7	65.4	82.0	88.4	
2	626 (12.3)	27.1	33.6	44.9	57.9	70.2	85.9	94.1	
3	811 (16)	24.1	31.5	42.1	54.5	67.5	83.1	90.4	
4	737 (14.5)	23.2	30.1	43.2	56.3	70.8	86.1	93.0	
5 (least disadvantage)	2524 (49.6)	24.5	33.7	45.7	58.7	73.3	88.8	98.1	

[1] P-values for Kruskall-Wallis test; 25(OH)D: 25-hydroxyvitamin D

TABLE 2 Maternal characteristics and 25(OH)D serum levels by adverse pregnancy outcomes

Maternal characteristics	Unaffected N=3714 n (%)	SGA<10th centile N=580 n (%)	SGA<3rd centile N=149 n (%)	Preterm birth <37 weeks N=388 n (%)	Preterm birth <34 weeks N=115 n (%)	All preeclampsia N=223 n (%)	Early-onset preeclampsia N=29 n (%)	Gestational diabetes N=376 n (%)	Miscarriage N=39 n (%)	Stillbirth N=33 n (%)
Age (y)	33.1 ± 4.7[1]	32.8 ± 5.0	33.6 ± 5.2	32.6 ± 4.8	32.7 ± 5.2	32.8 ± 5.1	31.2 ± 6.0	34.5 ± 4.6 *	34.9 ± 5.5 *	32.7 ± 5.8
Smoking	200 (5.4)	61 (10.7) *	19 (12.9) *	36 (10) *	12 (13.5) *	12 (5.4)	1 (3.6)	23 (6.2)	2 (5)	5 (12.5) *
Nulliparous	1634 (44.2)	368 (64.2) *	94 (64) *	191 (53.1) *	48 (53.9)	150 (67.6) *	21 (75) *	188 (50)	-	18 (45)
Maternal weight (kg)	66.4 ± 13.8	61.0 ± 13.4 *	59.3 ± 12.5 *	67.3 ± 15.9	66.8 ± 14.9	72.6 ± 16.8 *	68.9 ± 18.1	69.8 ± 17.8 *	69.0 ± 13.6	75.6 ± 17.9 *
Season of sampling										
Summer	884 (23.8)	142 (24.5)	30 (20.1)	100 (25.8)	29 (25.2)	67 (30.0)	11 (37.9)	97 (25.9)	8 (20.5)	4 (12.1)
Autumn	859 (23.1)	133 (22.9)	43 (28.9)	91 (23.5)	30 (26.1)	53 (23.8)	3 (10.3)	83 (22.2)	10 (25.6)	10 (30.3)
Winter	1008 (27.1)	158 (27.2)	35 (23.5)	99 (25.5)	30 (26.1)	51 (22.9)	5 (17.2)	97 (25.9)	8 (20.5)	12 (36.4)
Spring	963 (25.9)	147 (25.3)	41 (27.5)	98 (25.7)	26 (22.6)	52 (23.3)	10 (34.5)	97 (25.9)	13 (33.3)	7 (21.2)
Median 25(OH)D [nmol/L (IQR)]	56.9 (43.9 - 70.8)	55.3 (41.1 - 68.1)	54.5 (40.1 - 69.1)	56.5 (43.0 - 69.6)	55.9 (40.2 - 69.8)	54.6 (45.5 - 69.6)	53.5 (42.4 - 61.7)	52.1 (41.5 - 63.6) *	52.7 (43.0 - 64.8)	59.1 (33.2 - 71.4)
<25 nmol/L	198 (5.3)	52 (9.0)	13 (8.7)	25 (6.4)	9 (7.8)	8 (3.6)	2 (6.9)	24 (6.4)	3 (7.7)	4 (12.1)
<37.5 nmol/L	588 (15.8)	114 (19.6)	33 (22.2)	67 (17.3)	23 (20.0)	23 (10.3)	6 (20.7)	77 (20.5)	6 (15.4)	7 (21.2)
37.5 - 49.9 nmol/L	702 (18.9)	110 (19.0)	20 (13.2)	78 (20.1)	25 (21.7)	58 (26.1)	9 (31.0)	95 (25.3)	10 (25.6)	8 (24.2)
50 - 75 nmol/L	1663 (44.8)	256 (44.1)	70 (47.0)	171 (44.1)	49 (42.6)	105 (47.1)	13 (44.8)	155 (41.2)	14 (35.9)	12 (36.4)
>75 nmol/L	761 (20.5)	100 (17.2)	26 (17.5)	72 (18.6)	18 (15.7)	37 (16.6)	1 (3.5)	49 (13.0)	9 (23.1)	6 (18.2)

[1] Mean ± SD (all such values)

* P<0.05 for assessment of outcomes compared with pregnancies unaffected by each outcome using chi-square test, *t*-test or Kruskall-Wallis test as appropriate; SGA: Small for gestational age ; 25(OH)D: 25-hydroxyvitamin D

TABLE 3 Association between first trimester 25(OH)D serum levels and adverse pregnancy outcomes

Adverse pregnancy outcome	Univariate OR					Adjusted OR[1]				
	<25 nmol/L	<37.5 nmol/L	37.5 - 49.9 nmol/L	50 - 75 nmol/L	>75 nmol/L	<25 nmol/L	<37.5 nmol/L	37.5 - 49.9 nmol/L	50 - 75 nmol/L	>75 nmol/L
SGA<10th centile (n=580)	1.77 (1.22, 2.56)[2]	1.28 (0.99, 1.66)	1.02 (0.79, 1.31)	1.0 (Ref)	0.87 (0.66, 1.13)	1.58 (1.06, 2.35)	1.10 (0.83, 1.46)	0.94 (0.72, 1.23)	1.0 (Ref)	0.96 (0.73, 1.27)
SGA<3rd centile (n=149)	1.84 (0.91, 3.73)	1.32 (0.82, 2.11)	0.73 (0.43, 1.26)	1.0 (Ref)	0.81 (0.49, 1.36)	1.80 (0.82, 3.95)	1.12 (0.66, 1.90)	0.70 (0.39, 1.23)	1.0 (Ref)	0.90 (0.52, 1.55)
All preterm birth <37 weeks (n=388)	1.21 (0.75, 1.96)	1.08 (0.78, 1.48)	1.04 (0.77, 1.41)	1.0 (Ref)	0.90 (0.66, 1.23)	1.23 (0.75, 2.00)	1.06 (0.77, 1.47)	1.01 (0.74, 1.37)	1.0 (Ref)	0.92 (0.68, 1.27)
Spontaneous preterm birth <37 weeks (n=217)	1.44 (0.75, 2.75)	1.32 (0.87, 2.01)	1.04 (0.69, 1.56)	1.0 (Ref)	0.92 (0.61, 1.38)	1.47 (0.77, 2.82)	1.32 (0.86, 2.01)	1.01 (0.67, 1.53)	1.0 (Ref)	0.96 (0.63, 1.46)
All preterm birth <34 weeks (n=115)	1.51 (0.68, 3.36)	1.39 (0.80, 2.42)	1.09 (0.65, 1.85)	1.0 (Ref)	0.76 (0.42, 1.36)	1.75 (0.77, 3.96)	1.41 (0.80, 2.48)	1.10 (0.64, 1.87)	1.0 (Ref)	0.74 (0.41, 1.34)
Spontaneous preterm birth <34 weeks (n=56)	2.66 (0.93, 7.61)	1.80 (0.81, 4.01)	1.14 (0.52, 2.49)	1.0 (Ref)	1.15 (0.53, 2.50)	2.71 (0.92, 7.97)	1.61 (0.71, 3.63)	1.11 (0.50, 2.49)	1.0 (Ref)	1.25 (0.57, 2.75)
All preeclampsia (n=223)	0.53 (0.24, 1.17)	0.66 (0.40, 1.07)	1.45 (0.98, 2.08)	1.0 (Ref)	0.83 (0.54, 1.26)	0.46 (0.19, 1.10)	0.63 (0.37, 1.06)	1.30 (0.87, 1.94)	1.0 (Ref)	0.90 (0.57, 1.41)
Early-onset preeclampsia (n=29)	1.67 (0.28, 9.98)	1.27 (0.43, 3.69)	1.24 (0.49, 3.14)	1.0 (Ref)	0.18 (0.02, 1.42)	1.40 (0.20, 9.89)	1.01 (0.31, 3.29)	1.50 (0.50, 4.47)	1.0 (Ref)	0.11 (0.01, 1.18)
Gestational diabetes (n=376)	1.40 (0.85, 2.32)	1.48 (1.08, 2.04)	1.46 (1.09, 1.95)	1.0 (Ref)	0.61 (0.43, 0.87)	0.97 (0.56, 1.69)	1.08 (0.74, 1.56)	1.16 (0.83, 1.62)	1.0 (Ref)	0.88 (0.59, 1.30)
Miscarriage (n=39)	1.13 (0.27, 4.84)	0.81 (0.29, 2.23)	1.27 (0.52, 3.08)	1.0 (Ref)	1.36 (0.55, 3.35)	1.41 (0.31, 6.50)	0.90 (0.32, 2.54)	1.39 (0.56, 3.46)	1.0 (Ref)	1.09 (0.42, 2.80)
Stillbirth (n=33)	2.21 (0.62, 7.92)	1.23 (0.44, 3.46)	1.35 (0.51, 3.5)	1.0 (Ref)	1.20 (0.41, 3.51)	2.66 (0.70, 10.11)	1.10 (0.38, 3.16)	1.44 (0.54, 3.87)	1.0 (Ref)	1.27 (0.42, 3.87)
Severe outcomes (n=272)	1.81 (1.05, 3.10)	1.38 (0.96, 1.98)	1.02 (0.71, 1.47)	1.0 (Ref)	0.82 (0.56, 1.20)	1.73 (0.97, 3.08)	1.25 (0.85, 1.85)	0.99 (0.68, 1.45)	1.0 (Ref)	0.86 (0.58, 1.27)

[1] Adjusted for maternal age, parity, smoking during pregnancy, maternal weight, previously diagnosed hypertension, previously diagnosed diabetes, season at sampling, country of birth or socio-economic disadvantage;

[2] Odds ratio: 95% CI in parentheses (all such values) using logistic regression

SGA: Small for gestational age; COB: Country of birth

TABLE 4 Predictive accuracy results of 25(OH)D in first trimester on severe pregnancy outcomes[1]

Variable	AUC	P-value[2]	Sensitivity	Specificity	PPV	NPV	LR (+)
Total sample (n=5,109)							
25(OH)D <25 nmol/L	0.51	0.14	8.4 (5.3, 12.4)[3]	94.4 (93.7, 95.0)	7.6 (4.8, 11.3)	94.9 (94.2, 95.5)	1.44
25(OH)D <37.5 nmol/L	0.52	0.09	20.5 (15.8, 25.9)	83.8 (82.7, 84.9)	6.6 (5.0, 8.5)	95.0 (94.3, 95.7)	1.29
Maternal and clinical risk factors only[4]	0.64	<0.0001	14.4 (10.4, 19.1)	95.0 (94.3, 95.6)	13.9 (10.1, 18.5)	95.2 (94.5, 95.8)	2.87
25(OH)D <25 nmol/L + maternal and clinical risk factors	0.64	<0.0001	14.0 (10.1, 18.7)	94.0 (93.3, 94.6)	11.6 (8.3, 15.5)	95.1 (94.5, 95.7)	2.32
25(OH)D <37.5 nmol/L + maternal and clinical risk factors	0.64	<0.0001	12.9 (9.2, 17.5)	94.3 (93.6, 95.0)	11.3 (8.0, 15.4)	95.1 (94.4, 95.7)	2.27
High risk women (n=758)[5]							
25(OH)D <25 nmol/L	0.52	0.37	19.6 (10.2, 32.4)	85.3 (82.5, 87.8)	9.6 (4.9, 16.6)	93.0 (90.7, 94.8)	1.34
25(OH)D <37.5nmol/L	0.57	0.06	42.9 (29.7, 56.8)	70.2 (66.6, 73.6)	10.3 (6.7, 14.9)	93.9 (91.5, 95.8)	1.44

[1]Severe pregnancy outcome defined as a composite outcome of either SGA<3rd centile, preterm birth <34 weeks, early-onset preeclampsia or stillbirth

[2]P- value: test of AUC against chance (0.5) by likelihood ratio (X^2) test;

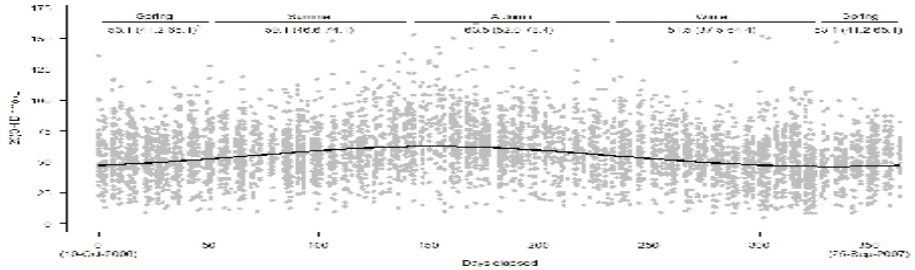
[3] Percentage; 95% confidence intervals in parentheses (all such values)

[4]Maternal and clinical risk factors: Maternal age, parity, smoking during pregnancy, maternal weight, previously diagnosed hypertension, previously diagnosed diabetes, season at sampling, country of birth or socio-economic disadvantage

[5]Sub-analysis among high risk women (weight >85 kg or born in India, Pakistan, Bangladesh, Sri-Lanka, Middle East or African countries) with severe pregnancy outcomes compared to those high-risk women without severe pregnancy outcomes

AUC: Area under the ROC curve; PPV: Positive predictive value; NPV: Negative predictive value; LR (+): Positive likelihood ratio; 25(OH)D: 25-hydroxyvitamin D

FIGURE 1 Seasonal variation of maternal **s**erum 25(OH)D concentrations at 10 to 14 weeks gestation (n=5,109)



[1] Median: IQR in parentheses (all such values); P<0.001 for overall difference in median values using Kruskall-Wallis test;

Black line represents polynomial regression trend; 25(OH)D: 25-hydroxyvitamin D.