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ORIGINAL ARTICLE

Vitamin D deficiency in pregnant women and newborns in Lagos, Nigeria

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ABSTRACT
We aimed to determine the prevalence of Vitamin D deficiency among pregnant women and their newborns in order to make recommendations for Vitamin D supplementation, if necessary. It was a cross-sectional study carried out over a period of 12 months. Information such as use of Vitamin D supplements, number of daytime hours spent outdoors and dressing style was obtained from 166 pregnant women in Lagos, Nigeria; maternal and cord blood 25(OH)D concentration was determined using ELISA. The prevalence of Vitamin D deficiency (<20 ng/mL) in the mothers and newborns was 4.8% and 29.5%, respectively, while insufficiency (21–29 ng/mL) was 28.3% and 46.1%, respectively. Vitamin D supplement use during pregnancy, daytime outdoor exposure and mothers' dressing style were significantly associated with maternal serum 25(OH)D concentration ($p < .05$). Our study showed that despite a sunny environment like ours, inadequate serum 25(OH)D concentration is still considerable among pregnant women and their newborns and suggests a need for Vitamin D supplementation in pregnant women.

KEYWORDS
Vitamin D deficiency; prevalence; pregnant women; newborns; cord blood; maternal blood

IMPACT STATEMENT

- **What is already known on this subject?** Vitamin D is an essential vitamin that plays a major role in maintaining pregnancy and ensuring adequate skeletal formation in the foetus. Studies have shown that there is high Vitamin D deficiency in pregnant women in the temperate regions of the world and thus Vitamin D supplements are being offered to these pregnant women. Studies have also shown that the foetal/neonatal serum Vitamin D level is a reflection of the maternal level.
- **What the results of this study add?** The results of this study adds that there may be some factors preventing adequate delivery of Vitamin D from the maternal circulation to the foetal circulation, because despite a low prevalence of Vitamin D deficiency in the mothers, their neonates had a high deficiency rate.
- **What the implications are of these findings for clinical practice and/or further research?** The implications of these findings are; further research is warranted in order to find what could be causing a reduced delivery of Vitamin D from the mothers to their foetuses, so as to prevent it if possible. Second, these findings suggest that our pregnant women should still receive a form of Vitamin D supplements, so as to raise their serum Vitamin D to a level which would guarantee optimal foetal concentration.

Introduction

Vitamin D [25(OH)D] is an essential fat-soluble vitamin and a key modulator of calcium metabolism in children and adults. During pregnancy calcium demand increases, making Vitamin D is very important for maternal health, foetal skeletal growth and optimal maternal and foetal outcomes (Mulligan et al. 2010). Vitamin D deficiency has been found to be very common and high in pregnancy and breastfed infants in the Middle East and Asia ranging from 46% to 90.3% and 81% to 90.3%, respectively (Agarwal and Arya 2011; Karim et al. 2011; Halicioglu et al. 2012). Vitamin D deficiency has also been noted to have increased incidences among persons of African-American race. This deficiency is likely the result of an increased melanin content that prevents adequate exposure to ultraviolet B radiation for conversion of 7-dehydrocholesterol within the skin to Vitamin D (Nesby-O'Dell et al. 2002; Hollis 2005).

There is no absolute agreement as to what a normal range for 25(OH)D should be. In recent years, as a result of numerous studies, most authors agree that Vitamin D deficiency should be defined by a 25(OH)D concentration ≤20 ng/mL (<50 nmol/L) in children and adults (Holick and Chen 2008; Holick 2009; Henry et al. 2010). A serum 25(OH)D concentration of 21–29 ng/mL designates Vitamin D insufficiency. Moreover, the preferred concentration for 25(OH)D is recommended by many authors to be >30 ng/mL (Holick 2009; Henry et al. 2010).

Vitamin D is responsible for regulating calcium and bone metabolism by enhancing intestinal calcium absorption and mobilising calcium from the skeleton (Mulligan et al. 2010). Data suggest that Vitamin D aids implantation and maintains normal pregnancy, supports foetal growth through delivery of calcium and limits production of proinflammatory cytokines (Shin 2010).

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Many studies have shown that 1,25(OH)$_2$D levels in plasma increase by twofold early in pregnancy compared with pre-pregnancy values, reaches a maximum in the third trimester and returns to normal or below normal during lactation (Wilson et al. 1990; Ritchie et al. 1998; Kovacs 2008). It has also been shown that 25(OH)D readily crosses the human placenta and that the Vitamin D pool of the foetus is entirely dependent on that of the mother (Salle et al. 2000). So, cord blood 25(OH)D levels are strongly correlated with maternal Vitamin D status (Greer 2008).

Recent studies have shown a staggering increase in the prevalence of Vitamin D deficiency (Bowyer et al. 2009; Davis et al. 2010) and very high prevalence rates of Vitamin D deficiency have been recorded in pregnant women and their neonates in India; a country where Vitamin D deficiency is unexpected because of the abundant overhead sun for most or all of the day (Sachan et al. 2005; Agarwal and Arya 2011). The US National Academy of Sciences recommends 400 IU as the dietary reference intake for Vitamin D during pregnancy (Sachan et al. 2005); and some countries now give Vitamin D supplements to their antenatal women to boost the serum level of Vitamin D (Davis et al. 2010; Halicioglu et al. 2012).

In our institution, Vitamin D supplements are not part of the routine antenatal drugs prescribed for our pregnant patients and there is no documented local or national study showing the prevalence of serum Vitamin D deficiency in pregnancy. Therefore, this study was designed to determine the prevalence of Vitamin D deficiency in the pregnant women presenting for delivery at Lagos University Teaching Hospital (LUTH), to examine the relationship between their serum Vitamin D levels and that of neonatal cord blood; and to investigate the demographic and lifestyle factors associated with maternal serum Vitamin D concentrations. This study will serve as a basis for further studies on Vitamin D in pregnancy and in the neonates. Also, advocacy for routine prescription of Vitamin D supplements may become necessary based on the findings of this study.

Materials and methods

The study was carried out at the Labour ward complex of LUTH. It was a prospective cross-sectional study, carried out over a period of 12 months on pregnant women in the peripartum period and their neonates.

A total of 166 pregnant women were recruited using the consecutive sampling method. They were pregnant women who presented in labour and for elective caesarean section at term. Term was defined as pregnancy lasting between 37 weeks and 42 weeks (Carey 2008). Pregnant women with preterm pregnancies, gestational diabetes mellitus, gestational hypertension and multiple pregnancy were excluded from the study.

The procedure was explained to all the subjects and a written consent was obtained from them. With well-designed pro forma, socio-demographic information was obtained, as well as information on the use of Vitamin D supplements during pregnancy (i.e. the use of multivitamins containing at least 400 IU of Vitamin D), the number of daytime hours per day spent outdoor, dressing style; whether covered or uncovered. Covered dressing was defined as covering of the entire body except the hands and feet, while uncovered dressing was usual dressing with exposure of face, neck, hand and feet.

Four millilitres of venous blood were collected from the women before delivery or caesarean section as the case was; and the same volume of venous cord blood was collected immediately after clamping and severing the umbilical cord. After centrifugation, the sera were separated from all blood samples and stored at $-20\,^{\circ}$C at the Central Research Laboratory, LUTH, until analysis. Numbers were assigned to the samples, and maternal and neonatal samples were matched.

The laboratory analysis was carried out at the Nigerian Institute for Medical Research (NIMR) laboratory. Serum Vitamin D level was measured as the total concentration of 25(OH)D2 and 25(OH)D3 in serum, using a solid phase, competitive enzyme-linked immunosorbent assay (ELISA) method, according to the manufacturer's guidelines (CALBIOTECH, El Cajon, CA, Catalogue no. VD2208). Serum 25(OH)D level of $\leq$20 ng/mL ($\leq$50 nmol/L) was classified as deficiency, 21–29 ng/mL (52.5–72.5 nmol/L) as insufficiency and $\geq$30 ng/mL ($\geq$75 nmol/L) as sufficiency (Agarwal and Arya 2011).

Data analysis was done using SPSS version 15 statistical package (SPSS Inc., Chicago, IL). Linear regression model was used to measure as regression coefficient (r), the relationship of serum 25(OH)D concentrations of mothers with those of their newborns. The mean maternal and neonatal 25(OH)D concentrations were compared using the Student t-test. Multiple linear regression model was used to identify variables that were independently associated with maternal 25(OH)D status. Data were also presented as a histogram. For all analyses done, statistical outcomes with p values less than .05 were considered to be significant.

Ethical approval was obtained from the hospital's Health Research and Ethics Committee, prior to the commencement of the study and written consent was obtained from each participant before involvement in the study.

Results

Table 1 shows the general characteristics of the mothers in the study. The mean age of the women in the study was 31.4 ± 0.3 (18–42) years. The majority of the women (50.6%) had Body Mass Index (BMI) of $\geq$30 kg/m^2. About 24% of the women used Vitamin D supplements during pregnancy and 9% used a covered dressing style. The vast majority of the women (78.3%) spent one hour and above outdoor per day, during pregnancy.

Table 2 shows that Vitamin D deficiency was seen in 4.8% of the mothers and in 29.5% of the neonates. The mean maternal serum concentration of 25(OH)D was 35.0 ± 0.8 ng/mL, while that of the umbilical cord was 25.4 ± 0.6 ng/mL. The mean umbilical cord serum 25(OH)D concentration was found to be 72.6% of the maternal and this was significant ($p < .0001$). The mean maternal serum level of 25(OH)D was positively correlated with cord serum level ($r = 0.57$, $p < .05$) (Figure 1). A comparison of the Vitamin D status, based on serum concentrations of 25(OH)D in the mothers and neonates, is shown in Figure 2.

Table 1. General characteristics of the mothers in the study.

Age (years)	*n* (%)
<25	13 (7.8)
≥25	153 (92.2
Mean age ± SEM	31.4 ± 0.3
Parity, *n* (%)	
0	47 (28.3)
1	55 (33.1)
≥2	64 (38.6)
BMI, kg/m^2, *n* (%)	
<25	23 (13.9)
25–29	59 (35.5)
≥30	84 (50.6)
Dressing style, *n* (%)	
Covered	15 (9)
Uncovered	151 (91)
Use of Vitamin D supplement, *n* (%)	
Yes	40 (24.1)
No	126 (75.9)
Occupation, *n* (%)	
Employed	92 (55.4)
Unemployed	74 (44.6)
Education, *n* (%)	
Secondary	22 (13.3)
Tertiary	144 (86.7)
Daytime outdoor hours, *n* (%)	
<1	36 (21.7)
≥1	130 (78.3)
Mode of delivery, *n* (%)	
Caesarean section	90 (54.2)
Vaginal	76 (45.8)

SEM: standard error of mean.

Table 2. Serum concentrations of 25(OH) Vitamin D of the mothers and their neonates.

Serum Vitamin D status	Mother ($N = 166$), *n* (%)	Neonate ($N = 166$), *n* (%)	*p*
Vitamin D deficiency (<20 ng/mL)	8 (4.8)	49 (29.5)	
Insufficiency (21–29 ng/mL)	47 (28.3)	60 (36.1)	
Normal (>30 ng/mL)	101 (66.9)	57 (34.4)	
Overall mean ± SEM 25(OH) Vitamin D	35 ± 0.8	25.4 ± 0.6	<.0001

Data are expressed as frequency (%) and mean + SD. Disparity between mean values was analysed using Student's *t*-test. $p < .05$ was considered to be significant.

Table 3 shows that the mean maternal and neonatal 25(OH)D concentrations were significantly ($p < .001$) higher in uncovered dressing group (36.3 ng/mL and 26.4 ng/mL vs. 23.1 ng/mL and 15.6 ng/mL), Vitamin D supplement group (44.2 ng/mL and 31.8 ng/mL vs. 32.4 ng/mL and 23.4 ng/mL) and those who reported 1 h and above daytime outdoor exposure (36.8 ng/mL and 27.1 ng/mL vs. 28.5 ng/mL and 19.7 ng/mL) compared to the covered dressing group, those not taking Vitamin D supplement and those who reported <1 h daytime outdoor exposure. Other variables compared did not show significance ($p > .05$) as determinant of mean maternal and neonatal 25(OH)D concentrations.

The demographic and lifestyle variables relating to serum 25(OH)D concentration of the mothers were investigated using multiple linear regression analysis (Table 4). Three factors: Vitamin D supplement use during pregnancy, daytime outdoor exposure and mothers' dressing style were found to be significantly associated with serum 25(OH)D concentration ($p < .05$), even after adjusting for one another.

Discussion

The low prevalence of Vitamin D deficiency in our study is in contrast with the high prevalence rates (46–80%) got in other tropical countries (Goswami et al. 2000; Bassir et al. 2001; Agarwal and Arya 2011; Karim et al. 2011). In those studies, many of the women wore veils, which might have played an important role in the high deficiency rates. In this study, however, just a handful of our women used veils and a very large percentage of them had good sun exposure. Our women are used to being outdoors, even while pregnant, in order to cope with the hustle and bustle of the city life, like ours.

Despite the low deficiency rate of Vitamin D in this study, almost one third (28.3%) of the women had insufficient serum Vitamin D levels. Similar results were obtained in three other studies (Hamilton et al. 2010; Karim et al. 2011; Parisaei et al. 2011). The percentage of insufficiency of Vitamin D in this study calls to question whether the sun exposure alone is enough to maintain adequate serum Vitamin D level. Perhaps other sources of Vitamin D, like supplements and diets rich in Vitamin D, are needed to maintain sufficient serum Vitamin D levels.

A disturbingly high serum Vitamin D deficiency (29.5%) was seen in neonatal umbilical cord blood compared to the mothers, and the total mean concentration of Vitamin D in cord blood was 72.6% of the maternal. These significant, lower neonatal levels were also in studies in India (Goswami et al. 2000) and Iran (Bassir et al. 2001). It has been shown that 25(OH)D readily crosses the placenta; and that the Vitamin D pool of the foetus is entirely dependent on that of the mother (Salle et al. 2000); therefore, it is important that our women have sufficient serum Vitamin D in order to deliver enough to their growing foetuses, since their foetuses are not getting 100% according to this study. Curiously, it is not known why the mothers are not delivering 100% of their Vitamin D concentration to their foetuses. It may be due to *malaria parasitaemia* of the placenta. Griffin et al. (2012) found out that *malarial parasitaemia* reduces uteroplacental blood flow, thereby reducing nutrients transfer to the foetus. The prevalence of malaria in pregnancy and placental malarial parasitaemia in Nigeria has been found to range from 59.9% to 95.4% (Ogbodo et al. 2009; Agan et al. 2010) and 65.2% to 90.8% (Nwali et al. 2014; Bassey et al. 2015), respectively.

Similar to other investigators (Bassir et al. 2001; Nicolaidou et al. 2006; Kazemi et al. 2009), a strong positive correlation was found between maternal and cord blood 25(OH)D in this study, thus making the neonatal Vitamin D levels predictable by maternal levels. This is important because deficiency and insufficiency in the mothers would mean the newborns would also have inadequate Vitamin D stores to draw on in early life, with its attendant complications like infantile rickets, asthma and neurological diseases (Camargo et al. 2011; Grayson and Hewison 2011).

Covered dressing style, use of Vitamin D supplements and daytime outdoor exposure were the statistically significant

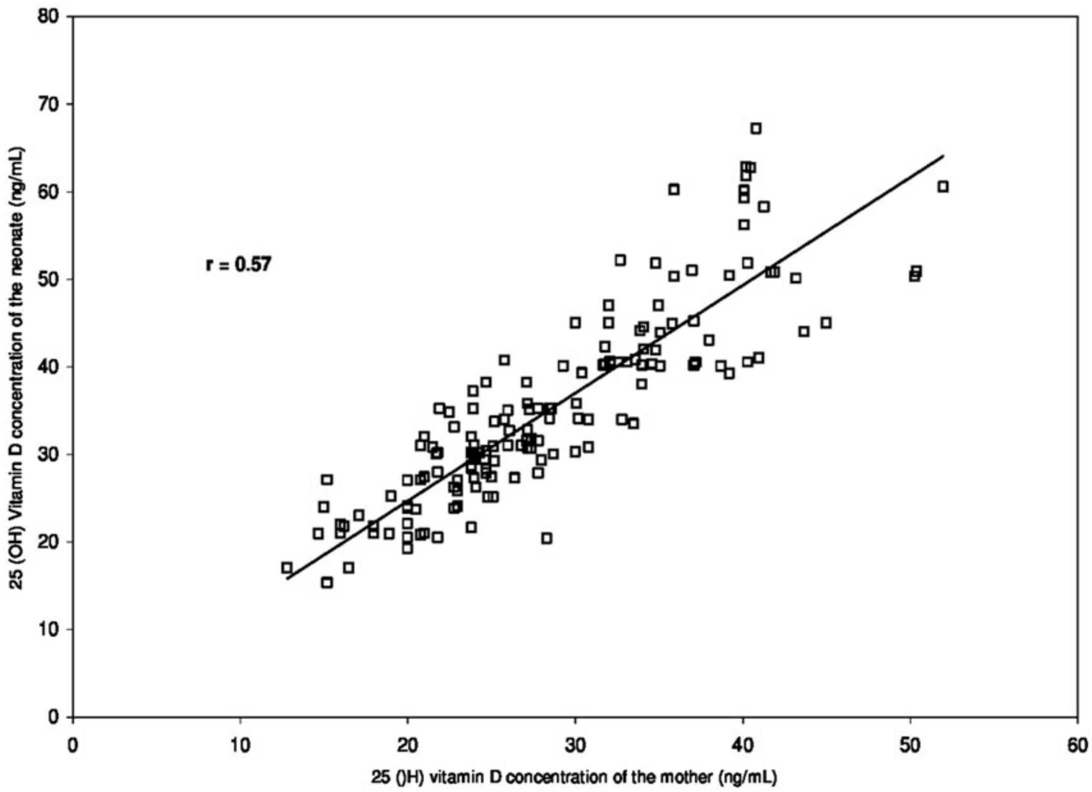

Figure 1. The relationship between serum 25(OH) Vitamin D concentrations of the mothers and their neonates (solid line shows linear regression).

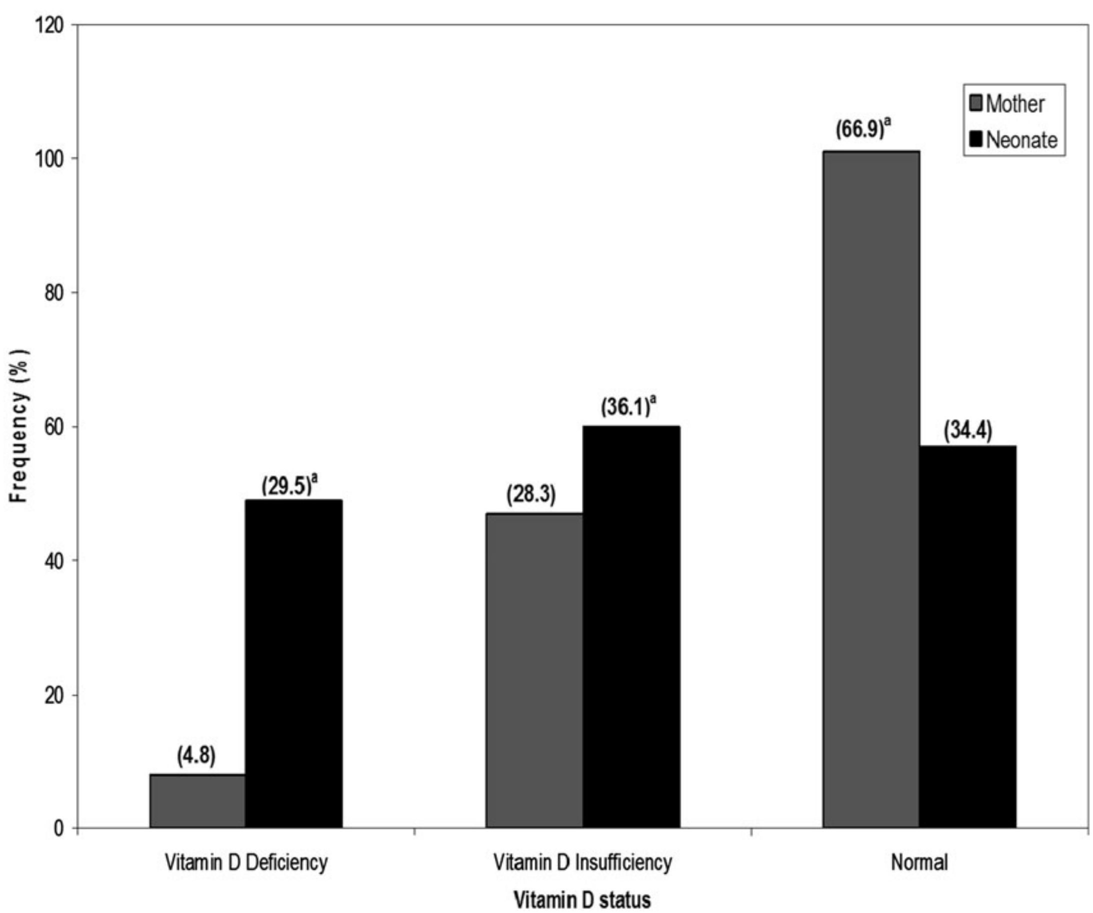

Figure 2. The comparison of Vitamin D status based on serum concentrations of 25(OH) Vitamin D in the mothers and their neonates (the figures in parenthesis on each bar represent the percentage frequency). Disparity in frequency between mother and neonates by Vitamin D status was evaluated by chi square(×2) test. [a]$p < .05$.(mother vs. neonate).

Table 3. Mean maternal and neonatal serum 25(OH)D concentration by demographic and life style variables.

Parameter	Maternal 25(OH)D Mean ± SEM	p	Neonatal 25(OH)D Mean ± SEM	p
Vitamin D supplement use during pregnancy				
Yes ($n = 40$)	44.2 ± 1.7	<.0001	31.8 ± 1.1	<.0001
No ($n = 126$)	32.1 ± 0.8		23.4 ± 0.7	
Parity				
0 ($n = 47$)	35.6 ± 1.6	>.05	26.2 ± 1.2	>.05
1 ($n = 55$)	35.1 ± 1.5		25.4 ± 1.1	
≥2 ($n = 64$)	34.6 ± 1.6		24.9 ± 1.0	
BMI, kg/m^2				
<25 ($n = 23$)	33. 8 ± 1.5	>.05	24.3 ± 1.1	>.05
25–29 ($n = 59$)	35.9 ± 2.6		25 ± 1.9	
≥30 ($n = 84$)	35.6 ± 1.2		26.1 ± 0.9	
Delivery mode				
Caesarean section ($n = 90$)	35.1 ± 1.1	>.05	25.8 ± 0.8	>.05
Vaginal ($n = 76$)	34.9 ± 1.3		24.8 ± 1.0	
Dressing				
Covered ($n = 15$)	23.1 ± 1.3	<.0001	15.6 ± 1.2	<.0001
Uncovered ($n = 151$)	36.3 ± 2.4		26.4 ± 1.7	
Occupation				
Employed ($n = 92$)	37.4 ± 1.1	>.05	26.6 ± 1.4	>.05
Unemployed ($n = 74$)	33.5 ± 2.4		24.4 ± 0.9	
Age				
<25 years ($n = 13$)	33.3 ± 2.8	>.05	24.4 ± 2.3	>.05
≥25 years ($n = 153$)	35.2 ± 0.9		25.5 ± 0.7	
Daytime outdoor				
<1 h ($n = 36$)	28.5 ± 1.3	<.0001	19.7 ± 0.7	<.0001
≥1 h ($n = 130$)	36.8 ± 2.1		27.1 ± 1.5	
Education				
Secondary ($n = 22$)	34.2 ± 2.2	>.05	24.5 ± 1.8	>.05
Tertiary ($n = 144$)	35.2 ± 0.9		25.6 ± 0.7	

Table 4. Multiple linear regression analysis of factors associated with maternal serum 25(OH)D concentrations ($n = 116$).

Variable	b^a (SE)	p Value^a
Vitamin D supplement use during pregnancy	3.04 (0.73)	.00001
Daytime outdoor exposure	2.74 (0.635)	.0002
Mother's dressing style	3.15 (0.672)	<.0001
Parity	0.247 (0.827)	>.05
Age	0.371 (0.783)	>.05
BMI	0.297 (0.728)	>.05
Mode of delivery	0.395 (0.639)	>.05

b: regression coefficient; SE: standard error.
[a]All variables are adjusted for one another.

variables associated with serum 25(OH)D concentrations. Our study is in accordance with previous studies, showing maternal Vitamin D deficiency to be high in mothers with covered dressing style because of their ethnic or religious principles (Sachan et al. 2005; Van der Meer et al. 2006).

This study found a statistically significant association between the use of Vitamin D supplement and serum 25(OH)D concentration; and a similar association was also found in other studies (Bikle et al. 1999; Nesby-O'Dell et al. 2002). Aly et al. (2013) and Merewood et al. (2010) reported significant association between sun exposure and maternal serum Vitamin D concentrations. These findings are in consonance with the one in our study.

In conclusion, this study showed a low prevalence of Vitamin D deficiency among our antenatal women, but a considerable high rate of deficiency in their newborns. These findings therefore suggest Vitamin D supplementation for our pregnant women especially those at risk of Vitamin D deficiency, like women who use veils and women who are always indoors (for one reason or the other except going for antenatal visits) in order to raise their serum levels of Vitamin D, thereby increasing neonatal levels.

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Disclosure statement

The authors report no conflicts of interest.

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