

Impact of maternal vitamin D status during pregnancy on the prevalence of neonatal vitamin D deficiency

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Abstract

Maternal vitamin D deficiency is not uncommon. The lack of vitamin D during pregnancy may result in poor fetal growth and altered neonatal development that may persist into later life. Recognition of risk factors and early detection of vitamin D deficiency during pregnancy is important in order to prevent neonatal vitamin D deficiency and related complications. The aim of the current study is to assess the effect of maternal vitamin D status on the neonatal vitamin D stores. A total of 92 pregnant women at the end of the 3rd trimester and their newborns were recruited from Al Khafji Joint Operation Hospital, Saudi Arabia, during the year 2011. Maternal and cord blood samples were taken for determination of serum levels of circulating 25-hydroxyvitamin D₃ [25(OH)D₃] concentration, serum calcium (Ca⁺⁺), phosphorus (PO₄) and alkaline phosphatase (ALP). Compared with pregnant women with adequate vitamin D levels, women deficient in vitamin D had infants with vitamin D deficiency ($X \pm SD$ 33.44 \pm 18.33 nmol/L vs 55.39 \pm 17.37 nmol/L, $P=0.01$). Maternal and neonatal serum 25(OH)D₃ levels showed a positive correlation with serum Ca⁺⁺ and negative correlation with serum PO₄ and ALP. Neonatal 25(OH)D was related to maternal 3rd trimester levels ($r=0.89$, $P=0.01$). The newborn serum 25(OH)D₃ concentrations rely on maternal vitamin D status. Poor maternal vitamin D status may adversely affect neonatal vitamin D status and, consequently, calcium homeostasis.

Introduction

Vitamin D, especially its most active metabolite 1,25 dihydroxyvitamin D₃, plays an important role not only in calcium homeostasis and bone remodeling, but also in the control of hormone secretion, immune dysfunction, cell-proliferation and differentiation.¹ During preg-

nancy, maternal serum concentrations of 25(OH) D₃, the circulating form of vitamin D, correlate with dietary vitamin D intake.² Maternal serum concentrations of 1,25-dihydroxyvitamin D₃, the hormonal circulating and active form of vitamin D, are elevated during pregnancy.² During the intrauterine development, 1,25-dihydroxyvitamin D₃ is synthesized mainly by the decidual cells of the placenta and allows for increased calcium absorption. The fetus is entirely dependent on the mother for an adequate supply of 25(OH)D₃, which is believed to cross the placenta.³ Hypocalcemia and increased parathyroid hormone secretion induce synthesis of 1,25-dihydroxyvitamin D after birth in both full term and preterm neonates. Nevertheless, serum concentrations of 25(OH)D₃, a rate limiting factor in the synthesis of 1,25-dihydroxyvitamin D concentration, are higher than those observed in older infants.⁴ In countries where dairy products are not routinely supplemented with vitamin D, maternal vitamin D supplementation during pregnancy is necessary.⁵ Maternal total serum calcium concentration declines progressively throughout pregnancy and reaches a nadir of 2-2.2 mmol/L by the 2nd month.⁴ Because 50% of calcium is bound to serum albumin, hypoalbuminemia resulting from expansion of the extracellular volume partly account for this decrease. In contrast, serum ionized calcium concentration undergoes minimal changes. As mentioned above, serum 25(OH)D₃ concentration varies according to vitamin D intake and synthesis, season, and geographical location.⁶ Low maternal vitamin D levels during pregnancy have been linked to various health outcomes in the offspring, including a higher incidence of abortion, low birth weight, neonatal hypocalcemia, impaired development and rickets.^{1,7}

The aim of the current study is to assess the effect of maternal vitamin D status on neonatal vitamin D stores.

Materials and Methods

This case-control study was carried out at Al Khafji Joint Operation Hospital, Saudi Arabia. Initially, the study enrolled 118 pregnant women and their newborns, during the year 2011. Twenty-six women were excluded from the study either due to delivery outside the hospital, delivery of a non-full term baby, or failure to obtain a neonatal blood sample. Therefore, a total of only 92 women and their newborn infants were actually included in the study.

Inclusion criteria

The study included healthy women between 20 and 40 years of age and singleton full term pregnancies.

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Key words: maternal vitamin D, neonatal 25-hydroxyvitamin D₃, calcium homeostasis.

Contributions: MAEK is responsible for study concept and design, designed the data collection, carried out the initial analyses, drafted the initial manuscript, reviewed and revised the manuscript, and approved the final manuscript to be submitted; YA contributed to study design, carried out the initial analyses, drafted the initial manuscript, reviewed and revised the manuscript, and approved the final manuscript to be submitted; RAER performed the laboratory workup, revised the manuscript, and approved the final manuscript to be submitted.

Conflict of interests: the authors declare no potential conflicts of interests.

Received for publication: 5 December 2012.

Accepted for publication: 9 January 2013.

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Licensee PAGEPress, Italy
Pediatric Reports 2013; 5:e6
doi:10.4081/pr.2013.e6

Exclusion criteria

Those women with a history of thyroid or parathyroid diseases, diabetes mellitus, or any kind of calcium or vitamin D supplements in the current pregnancy were excluded. Neonates with congenital anomalies or who were small for gestational age (SGA) were also excluded, as were, retrospectively, their mothers. All women participating in the study provided their written informed consent in accordance with the Declaration of Helsinki. During their regular antenatal visits, the participating women were requested to complete a questionnaire that included obstetric history, socio-demographic data, dietary habits, life style and calcium or vitamin D supplements. Maternal pre-pregnancy body mass index (BMI) was calculated according to height measured at recruitment and self-reported pre-pregnancy weight. Of the 92 women included in the study, 64 had risk factors of vitamin D deficiency (less exposure to sunshine, more work indoors, less intake of dairy products, dark skin, veiled clothing). The remaining women were considered as a comparison group.

Newborn clinical examination and anthropometric measurements including weight,

length, and head circumference were performed at birth.

Laboratory measurements

Measurement of serum 25(OH)D₃: 2 mL of venous blood were collected from each mother at the end of the 3rd trimester as well as 3 mL of cord blood from each neonate in EDTA-containing tubes and centrifuged at 3000 rpm for 10 min. Serum was separated and stored as 1 mL aliquots at -20°C until analysis. Serum level of 25(OH)D₃ was measured after extraction using the immunodiagnostic enzyme immuno-assay (EIA) developed by Immuno-diagnostic, Bensheim and Biomedica, Wien, Australia.⁸ Cut-off levels of vitamin D were distinguished as follows: <30 nmol/L deficient, 30-50 nmol/L insufficient, and 50 nmol/L adequate.^{9,10} Serum PO₄ and Ca⁺⁺ levels were estimated according to Kalliomaki JL *et al.*¹¹ Serum ALP was assayed according to Berth and Delanghe.¹²

Statistical analysis

Data were presented as mean ± standard deviation (X±SD) or percentage (%). The means of two groups were compared using Student's t-test. Linear correlation and regression were used to test the correlation between the measured parameters. Cut-off values were calculated from the receiver operating characteristics (ROC) curve as mean ± 2 SD of control. Data were tabulated and statistically analyzed with the statistical package for Social

Sciences (SPSS) version 14 software. P<0.05 was considered significant.¹³

Results

The study included 92 pregnant women aged 20-40 years (X±SD:33±6.2 years). The characteristics of pregnant women according to clinically defined cut-off points of circulating 25(OH)D₃ concentrations during the 3rd trimester are shown in Table 1. A total of 13 (14.2%) pregnant women had 25(OH)D₃ concentration less than 30 nmol/L, 46 (50%) had vitamin D insufficiency 30-50 nmol/L, and 33 (35.8%) had a level over 50 nmol/L. Decreasing trends across the categories of 25(OH)D₃ were found for lower social class, those living in rural areas with a history of inadequate sun exposure, and multiparous women.

Vitamin D status in women with risk factors for deficiency was found to be significantly lower compared with women without risk factors (41.5±18.8 vs 58.2±11.25 nmol/L, P=0.01) (Table 2). Despite lower vitamin D concentration in women with risk factors, there was no significant difference in serum calcium and phosphorus between the mothers' groups (P>0.01). On the other hand, the mean serum ALP was found to be significantly higher in mothers with risk factors for vitamin D deficiency than in those without risk factors

(P<0.05) (Table 2).

Among the newborn infants of mothers with risk factors of vitamin D deficiency, vitamin D concentrations were found to be significantly lower than newborns of mothers without risk (33.44±18.33 vs 55.39±17.37 nmol/L; P<0.01). As regards the neonatal serum biochemical markers, serum calcium levels were found to be significantly lower in neonates of mothers with risk factors of vitamin D deficiency than those in neonates of mothers without risk factors (8.04±0.47 vs 9.07±0.62 mg/DL; P<0.05). Meanwhile, serum phosphorus and alkaline phosphatase were significantly higher in neonates of mothers with risk factors of vitamin D deficiency (Table 3).

A positive linear relationship was found between circulating concentrations of maternal 25(OH)D₃ in pregnancy and both serum calcium (r=0.81, P=0.01) and serum phosphorus (r=0.88, P=0.01) levels. On other hand, it correlates negatively with serum alkaline phosphatase (r=-0.98, P=0.01) as shown in Table 4. Cord blood serum levels of 25(OH)D₃ correlated negatively with both serum alkaline phosphatase (r=-0.74, P=0.01) and serum phosphorus levels (r=-0.82, P<0.05), while it significantly correlated positively with serum calcium levels (r=0.72, P=0.01) (Table 5). Maternal serum 25(OH)D₃ strongly correlated with cord blood 25(OH)D₃ (r=0.89, P=0.01) and serum phosphorus (r=0.83, P=0.01) levels. Meanwhile, it significantly correlated neg-

Table 1. Characteristics of pregnant women according to circulating 25(OH)D₃ concentrations.

Variable(s)	Maternal serum 25(OH)D ₃ (nmol/L)						n	Total %	χ ² test	P
	<30 (n=13)		30-50 (n=48)		>50 (n=31)					
	n	%	n	%	n	%				
Socio-economic level										
Low	10	76.9	34	70.8	8	25.8	52	56.6	18.1	0.000**
Middle	3	23.1	14	29.2	23	74.2	40	43.4		
Residence										
Urban	5	38.5	15	31.3	22	71.0	42	45.7	12.3	0.002**
Rural	8	61.5	33	68.7	9	29.0	50	54.3		
Sun exposure										
Positive	2	15.4	14	29.2	24	77.4	40	43.4	22.7	0.000*
Negative	11	84.6	34	70.8	7	22.6	52	56.6		
Maternal pre-pregnancy										
Body mass index										
Under weight (<24.9)	7	53.8	14	29.2	7	22.6	28	30.4	5.84	0.211
Normal weight (25-29.9)	4	30.8	12	25.0	10	32.2	26	28.2		
Over weight (≥30)	2	15.4	22	45.8	14	45.2	38	41.3		
Maternal age (years)										
20-30	7	53.8	33	68.7	17	54.8	57	62.0	1.97	0.374
30-40	6	46.2	15	31.3	14	45.2	35	38.0		
Parity										
0	4	30.8	6	12.5	2	6.5	12	13.0	10.3	0.036*
1	2	15.4	13	27.1	16	51.6	31	33.7		
≥2	7	53.8	29	60.4	13	41.9	49	53.3		

*Statistically significant at P<0.05; **Highly statistically significant at P<0.01.

Table 2. Levels of maternal 25(OH)D₃ and other biochemical markers.

Variable	Mothers at risk (n=64)	Mothers without risk (n=28)	P
Serum 25 (OH) D, nmol/L			
X±SD	41.5±18.8	58.2±11.25	0.01
Range	21.3-57.2	39.5-69.2	
Serum calcium, mg/dL			
X±SD	8.4±0.57	9.7±0.66	>0.05
Range	7.5-10.2	8.3-10.9	
Serum phosphorus, mg/dL			
X±SD	4.5±0.61	3.9±0.56	>0.05
Range	2.7-5.4	3-5	
Serum alkaline phosphatase, U/L			
X±SD	255±10.3	207.9±11.47	<0.05
Range	231-271.5	184.4-223.2	

P<0.05, significant. P>0.05, not significant.

Table 3. Levels of cord blood 25(OH)D₃ and other biochemical markers.

Variable	Neonates of mothers at risk (n=64)	Neonates of mothers without risk (n=28)	P
Serum 25 (OH) D ₃ , nmol/L			
X± SD	33.44±18.33	55.39±17.37	<0.01
Range	19.3-67.1	39-78.5	
Serum calcium, mg/dL			
X± SD	8.04±0.47	9.07±0.62	< 0.05
Range	7.2-9.3	8-10.2	
Serum phosphorus, mg/dL			
X± SD	5.1±0.48	4.4±0.62	< 0.05
Range	3.8-5.9	3.3-5.5	
Serum alkaline phosphatase, U/L			
X± SD	78±18.7	221.1±10.8	< 0.01
Range	234-296	200-237	

atively with alkaline phosphatase ($r=-0.78$, $P=0.01$) (Table 6).

Discussion

Vitamin D deficiency is a public health issue worldwide.¹⁴ In most countries, there is no routine monitoring of serum 25(OH)D₃ levels during pregnancy. A 2009 review has recommended that women with one or more risk factors for low serum 25(OH)D₃ should be monitored at the beginning of gestation and in mid-pregnancy.¹⁵ In the current study of women at high risk for vitamin D deficiency, women with low socio-economic state, women with history of inadequate sun exposure, and women with two or more previous births were significantly more likely to have lower 25(OH)D₃ concentrations compared with those of higher socio-economic state, adequate sun exposure, and women with one previous birth respectively ($P<0.05$). Similar results were also reported by Merewood *et al.*¹⁶ and Shand *et al.*¹⁷ Also, women from rural areas had lower vitamin D than women from urban areas, while there

were no significant associations between 25(OH)D₃ concentration and BMI or maternal age. Merewood *et al.*¹⁶ reported that there was no difference in maternal plasma concentrations of 25(OH)D₃ according to maternal pre-pregnancy BMI, social class or education level. In the current study, there was a significant difference in the prevalence of 25(OH)D₃ deficiency between mothers at high risk of vitamin D deficiency compared with a group presumed not to be at risk. This is in contrast to that of Dijkstra *et al.*¹⁸ The current study revealed significantly higher levels of maternal serum alkaline phosphatase in mothers with risk factors of vitamin D deficiency compared with mothers not at risk. This can be attributed to the lower serum 25(OH)D₃ levels.⁶ Newborns of mothers at risk of vitamin D deficiency had lower cord blood 25(OH)D₃ than a group of mothers not at risk. Similarly, Namgung *et al.*¹⁹ revealed that newborns born to mothers in winter with inadequate sun exposure had low 25(OH)D₃ compared with those in summer newborns. Among the newborns of mothers with risk of vitamin D deficiency, serum alkaline phosphatase concentrations were found to be significantly higher than newborns of moth-

Table 4. Correlation between maternal serum 25(OH)D₃ and other biochemical parameters.

Parameter	r	P
Calcium	0.81	0.01
Alkaline phosphatase	-0.98	0.01
Phosphorus	0.88	0.01

Table 5. Correlation between neonatal serum 25(OH)D₃ and other biochemical parameters.

Parameter	r	P
Calcium	0.72	0.01
Alkaline phosphatase	-0.735	0.01
Phosphorus	-0.821	<0.05

Table 6. Correlation between maternal serum 25(OH)D₃ and neonatal biochemical markers.

Parameter	r	P
25 (OH)D ₃	0.89	0.01
Calcium	0.54	> 0.05
Alkaline phosphatase	-0.78	0.01
Phosphorus	0.83	0.01

ers without risk, indicating increased bone turnover. Our results were comparable with those of Zeghoud *et al.*²⁰ who reported that neonatal 25(OH)D₃ concentrations below 30 nmol/L (12 ng/mL) were associated with elevated PTH and serum alkaline phosphatase, and they proposed this level as the cut-off for diagnosing hypovitaminosis D in the newborn.

In this study, there was a weak inverse correlation between maternal 25(OH)D₃ concentrations and serum ALP which was in agreement with Brooke *et al.*²¹ who reported elevation of ALP in 20% of Asian subjects from the United Kingdom with serum 25(OH)D₃ concentrations below 25 nmol/L (10 ng/mL), whereas only 2% of those who had serum 25(OH)D₃ concentrations over 25 nmol/L had elevated ALP. Also, an Indian study carried out by Marya *et al.*²¹ reported elevated ALP in 13% and hypocalcemia in 44% of their pregnant subjects who were not receiving vitamin D supplementation, whereas none of the subjects supplemented with vitamin D (600,000 IU twice in the 7th and 8th months of gestation) had elevated ALP.²² The present study identified a significant positive correlation between cord blood 25(OH)D₃ and serum calcium concentrations. Given a limited 25(OH)D₃ substrate availability, the fetal kidneys can try to overcome this deficiency by increasing the rate of production of active form of vitamin D and, theoretically, this could be a factor affecting placental calcium transfer and fetal bone mineralization.²³ Also, many studies report that maternal vitamin D concentration plays a crucial role in neonatal and maternal calcium homeostasis, and that infants of mothers with low vitamin D intake during pregnancy had low serum calcium concentrations in cord blood or during the first week of life.²⁴ Our results added to the evidence that, serum 25(OH)D₃ in pregnant mothers correlated with cord blood vitamin D, as reported by other several studies.²⁵⁻²⁷ This study has some limitations. First, only a single 25(OH)D₃ measurement per subject was available and this can not reflect the maternal long-term status during the entire pregnancy. Second, estimating 25(OH)D₃ concentrations did not take into consideration the season.

Conclusions

Vitamin D deficiency was common among high-risk women and their newborn infants. Further research is required to determine the levels of vitamin D₃ at different gestations and the dose of vitamin D to ensure adequate 25(OH)D₃ levels in pregnancy. Given the strong correlation of maternal 25(OH)D₃ with cord blood and neonatal 25(OH)D₃, and the

known risks for neonates associated with low maternal 25(OH)D₃ we suggest routine testing of 25(OH)D₃ early in the antenatal period, especially for those with risk factors, and treatment of women found to be vitamin D deficient to avoid neonatal morbidities.

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