Impact of maternal vitamin D status during pregnancy on neonatal vitamin D status

Mohamed A. El Koumi[1], Yasser F. Ali[1], Rehab N. Abd El Rahman[2]

Departments of [1]Pediatrics, and [2]Clinical Pathology, Zagazig University Maternity and Children's Hospital, Zagazig, Sharkia, Egypt. E-mail: mohamed_197228@hotmail.com

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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 are important in order to prevent neonatal vitamin D deficiency and related complications. The aim of the current study was 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 Zagazig University Maternity and Children's Hospital, Egypt during the year 2011. Maternal and cord blood samples were taken at the beginning of the third trimester 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±SD 33.44±18.33 nmol/L vs. 55.39±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) D3 was related to maternal third trimester levels (r=0.89, p=0.01). The newborn serum 25(OH)D3 concentrations are reliant on maternal vitamin D status, and the poor maternal vitamin D status may adversely affect neonatal vitamin D status and consequently Ca^{++} homeostasis.

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

Vitamin D, especially its most active metabolite 1,25 dihydroxyvitamin D3, plays an important role not only in calcium (Ca^{++}) homeostasis and bone remodeling, but also in the control of hormone secretion, immune dysfunction, and cell proliferation and differentiation[1]. During pregnancy, maternal serum concentrations of 25(OH)D3, the circulating form of vitamin D, correlates with dietary vitamin D intake[2]. Maternal serum concentrations of 1,25-dihydroxyvitamin D3, the hormonal circulating and active from 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 Ca^{++} absorption. The fetus is entirely dependent on the mother for adequate supply of 25(OH)D3, which is believed to cross the placenta[3]. Hypocalcemia and increased parathyroid hormone (PTH) secretion induce synthesis of 1,25-dihydroxyvitamin D3 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 D3 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 Ca^{++} concentration declines progressively throughout pregnancy and reaches a nadir of 2-2.2 mmol/L by the second month[4]. Because 50% of Ca^{++} is bound to serum albumin, hypoalbuminemia resulting from expansion of the extracellular volume accounts in part for this decrease; by contrast, serum

ionized Ca^{++} concentration undergoes minimal changes. As alluded to above, serum 25(OH) D3 concentration varies according to vitamin D intake and synthesis, season, and geographic location[6]. Low maternal vitamin D levels during pregnancy have been linked to various health outcomes in the offspring, including higher incidence of abortion, low birth weight, neonatal hypocalcemia, impaired development, and rickets[1,7].

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

Material and Methods

This case-control study was carried out at Zagazig University Maternity and Children's Hospital, Egypt. 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 non-full-term babies or failure to obtain a neonatal blood sample. Thus, a total of only 92 women and their newborn infants were actually included.

Inclusion criteria included healthy women between 20 and 40 years of age and singleton full-term pregnancies.

Exclusion criteria were those with a history of thyroid or parathyroid diseases, diabetes mellitus, or any kind of Ca^{++} or vitamin D supplements in the current pregnancy. Neonates with congenital anomalies or who were small for gestational age (SGA) were excluded as well, and retrospectively, their mothers.

All women participating in the study provided a 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, sociodemographic

Table I. Characteristics of Pregnant Women According to Circulating $25(OH)D_3$ Concentrations

Variable(s)	Maternal serum 25(OH)D3 (nmol/L)						Total		$\chi 2$ test	P value
	30 < (n=13)		30–50 (n=48)		> 50 (n=31)					
	n	%	n	%	n	%	n	%		
Socioeconomic 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 BMI										
Under weight (<24.9)	7	53.8	14	29.2	7	22.6	28	30.4		
Normal weight (25-29.9)	4	30.8	12	25.0	10	32.2	26	28.2	5.84	0.211
Overweight (≥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		
1	2	15.4	13	27.1	16	51.6	31	33.7	10.3	0.036*
≥ 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 II. Levels of Maternal 25(OH)D_3 and Other Biochemical Markers

Variable	Mothers at risk (n = 64)	Mothers without risk (n = 28)	p-value
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: Non-significant.

Table III. Levels of Cord Blood 25(OH)D_3 and Other Biochemical Markers

Variable	Neonates of mothers at risk (n=64)	Neonates of mothers without risk (n=28)	*P* value
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	278±18.7	221.1±10.8	<0.01
Range	234-296	200-237	

data, dietary habits, lifestyle, and Ca^{++} or vitamin D supplements. Maternal pre-pregnancy body mass index (BMI) was based on measured height at recruitment and pre-pregnancy self-reported weight.

Of the 92 women included in the study, 64 had risk factors for vitamin D deficiency (less exposure to sunshine, more indoor work, less dairy product intake, 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

Two ml of venous blood were obtained from each mother at the beginning of the third trimester as well as 3 ml of cord blood of each neonate into EDTA-containing tubes and

centrifuged at 3000 rpm for 10 minutes; 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 immunoassay (EIA) developed by Immunodiagnostic, 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 phosphorus (PO4) and Ca^{++} levels were estimated according to Jakubowski et al.[11] Serum alkaline phosphatase (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 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 characteristic (ROC) curve as mean±2SD of control. Data were tabulated and statistically analyzed with the Statistical Package for the Social Sciences (SPSS) version 14 software. P-values less than 0.05 were considered significant[13].

Results

The study included 92 pregnant women with ages ranging from 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 third trimester are shown in Table I; a total of 13 (14.2%) pregnant women had 25(OH) D3 concentration 30< nmol/L, 46 (50%) had vitamin D insufficiency 30–50 nmol/L, and 33 (35.8%) had a level >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 II). Despite lower vitamin D concentration in women with risk factors, serum Ca^{++} and PO4 were found to be non-significantly different in both mothers' groups (p>0.05). 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 II).

Among the newborn infants of mothers with risk factors for vitamin D deficiency, vitamin D concentrations were found to be significantly lower than in 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 Ca^{++} levels were found to be significantly lower in neonates of mothers with risk factors for vitamin D deficiency than in neonates of mothers without risk factors (8.04±0.47 vs. 9.07±0.62 mg/dl, p<0.05). Meanwhile, serum PO4 and ALP were significantly higher in neonates of mothers with risk factors for vitamin D deficiency (Table III).

A positive linear relationship was found between circulating concentrations of maternal 25(OH)D3 in pregnancy and both serum Ca^{++} (r=0.81, p=0.01) and serum PO4 levels (r=0.88, p=0.01). On the other hand, there was a negative correlation with serum ALP (r= - 0.98, p=0.01), as shown in Table IV. Cord blood serum levels of 25(OH)D3 correlated negatively with both serum ALP (r= -0.74, p=0.01) and serum PO4 levels (r= -0.82, p<0.05), while there was a significantly positive correlation with serum Ca^{++} levels (r=0.72, p=0.01) (Table V). Maternal serum 25(OH)D3 strongly correlated with cord blood 25(OH) D3 (r=0.89, p=0.01) and serum PO4 levels (r=0.83, p=0.01). However, there was a significantly negative correlation with ALP (r= -0.78, p=0.01) (Table VI).

Discussion

Vitamin D deficiency is a public health issue worldwide[14]. In most countries, the routine monitoring of serum 25(OH)D3 levels during pregnancy does not occur. 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 socioeconomic state, history of inadequate sun exposure and two or more previous births

were significantly more likely to have lower 25(OH)D3 concentrations compared to those with higher socioeconomic state, adequate sun exposure, and 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 maternal plasma concentrations of 25(OH)D3 did not differ according to maternal pre-pregnancy BMI, maternal 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 result is consistent with Dijkstra et al.[18] Regarding maternal serum ALP, the current study revealed significantly higher levels in mothers with risk factors for vitamin D deficiency compared with mothers not at risk, which can be attributed to the lower serum 25(OH)D3 levels6. Newborns of mothers at risk for vitamin D deficiency had lower cord blood 25(OH)D3 compared with 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 ALP concentrations were found to be significantly higher compared with newborns of mothers without risk, indicating increased bone turnover. Our results were comparable with Zeghoud et al.[20], who reported that neonatal 25(OH)D3 concentrations <30 nmol/L (12 ng/ml) were associated with elevated PTH and serum ALP, and they proposed that concentration 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 <25 nmol/L (10 ng/ml), whereas only 2% of those who had serum 25(OH)D3 concentrations >25 nmol/L had elevated ALP. Also, an Indian study done by Marya et al.[22] 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

Table IV. Correlation Between Maternal Serum 25 (OH)D_3 and Other Biochemical Parameters

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

Table V. Correlation Between Neonatal Serum 25(OH) D_3 and Other Biochemical Parameters

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

Table VI. Correlation Between Maternal Serum 25(OH) D_3 and Neonatal Biochemical Markers

Parameter	r	P value
25(OH)D_3	0.89	0.01
Calcium	0.54	> 0.05
Alkaline phosphatase	-0.78	0.01
Phosphorus	0.83	0.01

supplemented with vitamin D (600,000 IU twice in the 7th and 8th months of gestation) had elevated ALP.

The present study revealed a significant positive correlation between cord blood 25(OH)D3 and serum Ca^{++} 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, could be a factor affecting placental Ca^{++} transfer and fetal bone mineralization[23]. Also, many studies report that maternal vitamin D concentration plays a crucial role in neonatal and maternal Ca^{++} homeostasis, and that infants of mothers with low vitamin D intake during pregnancy had low serum Ca^{++} 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].

The study has some limitations. First, only a single 25(OH)D3 measurement per subject was available, which could not reflect the maternal long-term status during the entire pregnancy. Second, dealing with misclassification of estimated long-term vitamin D exposure by season of blood sampling was accounted for by estimating 25(OH)D3 concentrations independent of seasons.

In conclusion, vitamin D deficiency was common among high-risk women and their newborn infants. Further researches are required to determine the levels of vitamin D 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 or at the beginning of the third trimester at the latest, especially for those with risk factors, and treatment of women who are found to be vitamin D-deficient in order to avoid neonatal morbidities.

REFERENCES

1. Brown EM, Chattopadhyay N, Yano S. Calcium-sensing receptors in bone cells. J Musculoskelet Neuronal Interact 2004; 4: 412-413.
2. Javaid MK, Crozier SR, Harvey NC, et al. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. Lancet 2006; 367: 36–43.
3. Novakovic B, Galati JC, Chen A, Morley R, Craig JM, Saffery R. Maternal vitamin D predominates over genetic factors in determining neonatal circulating vitamin D concentrations. Am J Clin Nutr 2012; 96: 188-195. Epub 2012 May 30.
4. Czech-Kowalska J, Pludowski P, Dobrzanska A. Impact of vitamin D supplementation on markers of bone mineral metabolism in term infants. Bone 2012; 51: 781-786.
5. Namgung R, Tsang RC. Factors affecting newborn bone mineral content: in utero effects on newborn bone mineralization. Proc Nutr Soc 2000; 59: 55-63.
6. Grover SR, Morley R. Vitamin D deficiency in veiled or dark-skinned pregnant women. Med J Aust 2001; 175: 251-252.
7. Tavakoli M, Jeddi-Tehrani M, Salek-Moghaddam A. Effects of 1,25(OH)2 vitamin D3 on cytokine production by endometrial cells of women with recurrent spontaneous abortion. J Fertil Steril 2011; 96: 751-757.
8. Wallace AM, Gibson S, de la Hunty A, Lamberg-Allardt C, Ashwell M. Measurement of 25-hydroxyvitamin D in the clinical laboratory: current procedures, performance characteristics and limitations. Steroids 2010; 75: 477-488.
9. Grootjans-Geerts I. Vitamin D: important from before the cradle and to the grave (in Dutch). Ned Tijdschr Geneeskd 2006; 150: 470–472.
10. Roth HJ, Schmidt-Gayk H, Weber H, Niederau C. Accuracy and clinical implications of seven 25-hydroxyvitamin D methods compared with liquid chromatography-tandem mass spectrometry as a reference. Ann Clin Biochem 2008; 45(Pt 2): 153-159.
11. Jakubowski Z, Kabata J, Kalinowski L, Szczepanska-Konkel M, Angielski S. Laboratory tests in every day practice. MAK Med Gdansk 1996.
12. Berth M, Delanghe J. Protein precipitation as possible important pitfall in the clinical chemistry analysis of blood samples containing monoclonal immunoglobulins: 2 case reports and a review of literature. ACTA Clin Belg 2004; 59: 263.
13. Noursis MJ. Statistical Package for the Social Sciences (SPSS), base 10.0 for Windows Users Guide. Chicago, IL: SPSS; 1997.
14. Cranney A, Horsley T, O'Donnell S, et al. Effectiveness and safety of vitamin D in relation to bone health. Evid Rep Technol Assess 2007; 158: 1-23.

15. Camadoo L, Tibbott R, Isaza F. Maternal vitamin D deficiency associated with neonatal hypocalcaemic convulsions. Nutr J 2007; 6: 23.

16. Merewood A, Mehta SD, Grossman X, et al. Widespread vitamin D deficiency in urban Massachusetts newborns and their mothers. Pediatrics 2010; 125: 640-647.

17. Shand A, Nassar N, Von Dadelszen P, Innis S, Green T. Maternal vitamin D status in pregnancy and adverse pregnancy outcomes in a group at high risk for pre-eclampsia. BJOG 2010; 117: 1593–1598.

18. Dijkstra SH, van Beek A, Janssen JW, de Vleeschouwer LH, Huysman WA, van den Akker EL. High prevalence of vitamin D deficiency in newborn infants of high-risk mothers. Arch Dis Child 2007; 92: 1049.

19. Namgung R, Tsang RC, Lee C, Han DG, Ho ML, Sierra RI. Low total body bone mineral content and high bone resorption in Korean winter-born versus summer-born newborn infants. J Pediatr 1998; 132(3 Pt 1): 421-425.

20. Zeghoud F, Vervel C, Guillozo H, Walrant-Debray O, Boutignon H, Garabedian M. Subclinical vitamin D deficiency in neonates: definition and response to vitamin D supplements. Am J Clin Nutr 1997; 65: 771–778.

21. Brooke OG, Brown IR, Cleeve HJ, Sood A. Observations on the vitamin D state of pregnant Asian women in London. Br J Obstet Gynaecol 1981; 88: 18–26.

22. Marya RK, Rathore S, Dua V, Sangwan K. Effect of vitamin D supplementation during pregnancy on foetal growth. Ind J Med Res 1988; 88: 488–492.

23. Bassir M, Laborie S, Lapillonne A, Claris O, Chappuis MC, Salle BL. Vitamin D deficiency in Iranian mothers and their neonates: a pilot study. Acta Paediatr 2001; 90: 577-579.

24. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr 2005; 35: 317-322.

25. Sachan A, Gupta R, Das V, Agarwal A, Awasth PK, Bhatia V. High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India. Am J Clin Nutr 2005; 81: 1060-1064.

26. Weiler H, Fitzpatrick-Wong S, Veitch R, et al. Vitamin D deficiency and whole-body and femur bone mass relative to weight in healthy newborns. CMAJ 2005; 172: 757-761.

27. Mallet E, Gugi B, Brunelle P, Henocq A, Basuyau JP, Lemeur H. Vitamin D supplementation in pregnancy: a controlled trial of two methods. Obstet Gynecol 1986; 68: 300-304.