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Maternal and neonatal vitamin-D status in twin versus singleton pregnancies

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Abstract

Aim: There is a paucity of information on vitamin D status of women with twin pregnancy and their newborns. This case–control study compared maternal and neonatal vitamin-D status in twin versus singleton pregnancies.

Methods: Subjects included 50 women with twin pregnancy delivering at >28 weeks and 50 gestational-age-matched women with singleton pregnancy delivering during the same period. Maternal and neonatal serum 25-hydroxy vitamin D [25(OH)D] was compared between the two groups using the independent Student's *t*-test on log values. Serum albumin-adjusted calcium, inorganic phosphate, and intact parathormone levels were also compared.

Results: Maternal vitamin-D deficiency (VDD; serum 25(OH)D < 30 nmol/L) was present in 90% of twin and 88% of singleton pregnancies. The prevalence of neonatal VDD was 89% in twin and 74% in singleton pregnancies ($P = 0.03$). Maternal serum 25(OH)D was lower in the twin group as compared to the singleton group (14.3 ± 10.47 vs 18.5 ± 12.36 nmol/L; $P = 0.02$). Mean serum calcium, intact parathormone, and inorganic phosphate were comparable between the women in the two groups. Maternal and neonatal 25(OH)D showed positive correlation in the two groups ($P < 0.001$). Mean cord blood 25(OH)D was significantly lower in the twins than in singleton newborns (14.8 ± 12.63 vs 22.6 ± 16.68 nmol/L; $P = 0.002$). The difference persisted even after adjustment for birthweights and maternal serum 25(OH)D. Mean serum calcium was significantly lower in the twins.

Conclusion: Twin newborns and their mothers have higher VDD as compared to singleton newborns and their mothers in the VDD population.

Key words: newborn, pregnancy, twin pregnancy, vitamin D deficiency.

Introduction

The probability of twins in spontaneous pregnancy is 1:89 resulting in approximately 13–32 twins/1000 live births across the world.[1] With increasing use of assisted reproductive techniques, the prevalence of twin births is increasing.[2] Twin pregnancies put an extra stress on mothers and their newborns due to increased metabolic demand for essential micronutrients.[3,4]

Vitamin-D is an important micronutrient in pregnancy and its deficiency has been associated with pre-eclampsia, low birthweight and neonatal respiratory tract infections.[5,6] Vitamin D deficiency (VDD) in pregnancy is a global health problem. The US National Health and Nutrition Examination Survey reported vitamin-D insufficiency in 69% of pregnant women.[7] Similarly, VDD is common in South Asian and Caucasian women of childbearing age in the UK.[8] A large proportion of Indian women suffer from VDD during pregnancy due to poor sunshine exposure and skin pigmentation.[9–11] There is a lack of clarity on maternal vitamin-D status in twin pregnancy. It is possible that increased demand in twin pregnancy would put women at a higher risk of VDD as compared to singleton

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pregnancy. There is a paucity of systematic information on the vitamin D status of women with twin pregnancy and their newborns. The present study from India reports on maternal and neonatal vitamin-D status in twin pregnancy and compares it with that of gestational-age-matched singleton pregnancies.

Methods

The study subjects were 50 north Indian women with twin pregnancy delivering after 28 weeks of gestation during 2011–2012 in the Department of Obstetrics & Gynecology at Maulana Azad Medical College, New Delhi (28.4°N; 77.12°E). Gestational age at delivery was calculated on the basis of their last menstrual period or ultrasound scan findings. Controls included 50 gestational-age-matched (±1 week) women delivering a singleton baby who were recruited at a ratio of 1:1 with study subjects during the same period (±1 month). Women with stillbirths, fetal congenital malformations, chronic liver, renal disease, and those on anticonvulsants or anti-tubercular treatment were excluded. Socioeconomic status of cases and controls was assessed with the Kuppuswamy scale, which is a commonly used socioeconomic assessment scale for the urban Indian population.[12]

Daily calcium intake was recorded by a trained dietician using a food frequency questionnaire.[13,14] Dietary calcium and calcium tablets consumed as antenatal supplementation were taken into account while calculating daily calcium intake. All of the subjects were asked on the day after delivery about the average duration of sunshine exposure during the month prior to delivery and the body surface area (BSA) exposed to sun. BSA exposed to sun was assessed by the rule of nine and sun index was calculated by multiplying hours of sun exposure per week with fraction of BSA exposed to sunlight.[15,16] Body mass indexes (BMI) of the women with twin and singleton pregnancies were calculated using maternal weight and height recorded within 24 h of delivery. Information regarding hypertension and postpartum hemorrhage was obtained from the case records. Gestational hypertension was defined as a blood pressure ≥ 140/90 mmHg after 20 weeks of pregnancy in women with previously normal blood pressure.

Biochemical estimations

Maternal blood samples were drawn without venostasis in fasting state on the morning after delivery. Cord blood was collected from the placental end of the umbilical cord (n = 100 for twin births and n = 50 for singleton births). Blood samples were transported on ice to the biochemistry laboratory. Serum was separated by cold centrifugation and stored in multiple aliquots at −20°C for estimation of biochemical parameters. Serum 25-hydroxy vitamin D [25(OH)D)] and intact parathyroid hormone (iPTH) were analyzed by electrochemiluminescence immunoassay (2010 Cobas E411, Roche). The minimal detectable limits for 25(OH)D and iPTH assays were 3.0 ng/mL and 1.20 pg/mL, respectively. Serum total calcium, albumin and inorganic phosphate were measured using an automated analyzer (SYNCHRON CX 5 PRO; Beckman Coulter). In order to correct for pregnancy-related hypoalbuminemia, serum total calcium values were adjusted for albumin. Serum 25(OH)D < 30 nmol/L (12 ng/mL) was considered as deficient, 30.0–49.9 nmol/L as insufficient and ≥50 nmol/L (20 ng/mL) as sufficient.[17]

The Institutional Ethics Committee approved the study protocol. None of the pregnant women refused to participate in the study and written informed consent was obtained from all of the subjects.

Statistical analysis

Results are shown as mean ± SD and frequencies in percentages. Serum 25(OH)D values for the twin and singleton groups were compared using the independent Student's t-test on log values. Other biochemical variables were also compared using the independent Student's t-test. Differences in the mean serum 25(OH)D between the two groups of newborns were further assessed after adjustment for their birthweights and the maternal serum 25(OH)D levels using linear regression. Difference in sun exposure between the two groups of mothers was assessed using the Mann–Whitney U-test. Fisher's exact test was used to study the difference in the prevalence of maternal and neonatal VDD between the twin and singleton groups. Pearson's correlation coefficients of various characters with maternal serum 25 (OH)D and cord 25(OH)D levels along with their statistical significance were assessed separately for twin and singleton pregnancies. Statistical analysis was performed with SPSS (version 18.0). A two-tailed P-value < 0.05 was considered significant.

Results

The clinical and biochemical characteristics of the women with twin and singleton pregnancies and their newborns are given in Table 1. Ninety-two percent of

Table 1 Maternal and neonatal variables in twin and singleton pregnancies

Study variables	Mothers			Newborns		
	Twins(n = 50)	*Singleton(n = 50)*	*P*	*Twins(n = 100)*	*Singleton(n = 50)*	*P*
Age (years)	27.4 ± 4.18	25.3 ± 3.47	0.01	—	—	—
Period of gestation (weeks)	35.8 ± 1.89	36.1 ± 1.87	0.46	—	—	—
Weight (kg)	61.8 ± 11.99	57.8 ± 10.73	0.08	2.0 ± 0.47	2.6 ± 0.43	<0.001
Height/crown–heel length (cm)	159.2 ± 5.01	155.9 ± 7.58	0.01	45.2 ± 3.31	47.4 ± 3.2	<0.001
Post-delivery BMI (kg/m^2)	24.3 ± 4.45	23.6 ± 3.37	0.40	—	—	—
Head circumference (cm)	—	—	—	31.5 ± 1.86	33.4 ± 1.65	<0.001
Fraction of body surface area exposed	0.04 ± 0.05	0.04 ± 0.06	0.54			
Median (IQR)	0.02 (0.02–0.02)	0.02 (0.02–0.02)				
Sun exposure (h/week)	1.57 ± 0.83	1.62 ± 0.89	0.83			
Median (IQR)	1.17 (1.17–2.33)	1.17 (1.17–2.33)				
Sun indexMedian (IQR)	0.08 ± 0.17	0.11 ± 0.21	0.81			
	0.02 (0.02–0.05)	0.02 (0.02–0.05)				
Calcium intake(mg/day)	1023 ± 275	910 ± 267	0.04	—	—	—
Serum calcium (mg/dL)	8.7 ± 0.52	9.1 ± 0.82	0.004	9.6 ± 0.63	9.9 ± 0.87	0.03
Serum albumin (gm/dL)	2.4 ± 0.52	2.8 ± 0.74	0.001	2.8 ± 0.48	3.3 ± 0.59	<0.001
Adjusted serum calcium (mg/dL)	9.9 ± 0.66	10.4 ± 0.91	0.49	10.1 ± 0.83	10.4 ± 0.94	0.04
Serum phosphate (mg/dL)	3.9 ± 0.76	3.8 ± 0.82	0.69	4.2 ± 0.65	4.0 ± 0.74	0.30
Serum 25(OH)D (nmol/L)	14.3 ± 10.47	18.5 ± 12.36	0.02	14.8 ± 12.63	22.6 ± 16.68	0.002
						0.03†
						0.04‡
Serum iPTH (pg/mL)	11.46 ± 7.05	14.43 ± 8.46	0.06	20.60 ± 15.18	16.20 ± 13.54	0.085

†After adjustment for birthweight. ‡After adjustment for birthweight and maternal serum 25(OH)D levels. BMI, body mass index; IQR, interquartile range; iPTH, intact parathormone.

women in the twin group and 88% in the singleton group had received antenatal care at our institution. There is no mass vitamin-D food fortification strategy in India and vitamin-D is not a routine supplementation during pregnancy. In the present study, no vitamin-D supplementation in any form was prescribed during the antenatal period. The calcium carbonate tablets prescribed during the antenatal period were generic and contained 500 mg of elemental calcium without any vitamin D. Seventy-eight percent of women in the twin group and 81% in the singleton group belonged to the lower-middle socioeconomic status.

The women with twin pregnancy were on an average 2 years older than those with singleton pregnancy (27.4 ± 4.18 vs 25.3 ± 3.47 years, $P = 0.01$). The mean gestational age and BMI were comparable between the two groups (35.8 ± 1.89 vs 36.1 ± 1.87 weeks, $P = 0.46$ and 24.3 ± 4.45 vs 23.6 ± 3.37 kg/m^2, $P = 0.40$). One woman (from the twin group) required blood transfusion for post-partum hemorrhage. Only two of the women in the twin pregnancy group and none in the singleton group had gestational hypertension.

Daily calcium intake was higher in the twin pregnancy than in the singleton pregnancy group (1023 ± 275 vs 910 ± 267 mg; $P = 0.04$). Seventy percent of all the study subjects were non-vegetarian. However, intake of meat products and fish was occasional (1–2 days/week and 1–2 days/month, respectively). The sun index was comparable between women with twin and singleton pregnancies.

The mean birthweight, crown–heel length and head circumference of the newborns in the twin group ($n = 100$) were significantly less than those recorded for the singleton newborns ($n = 50$) (Table 1).

Maternal vitamin D and calcium status in twin and singleton pregnancies

Ninety percent of the women in the twin group and 88% in the singleton pregnancy group had VDD ($P = 0.99$, Figure 1). The mean serum 25(OH)D was significantly less in women with twin pregnancy than in those with singleton pregnancy (14.3 ± 10.47 vs 18.5 ± 12.36 nmol/L; $P = 0.02$, Table 1, Figure 2). Mean albumin-adjusted serum total calcium, iPTH and inorganic phosphate were comparable in the two groups. Maternal serum 25(OH)D showed a positive correlation with albumin-adjusted serum calcium and inverse correlation with serum iPTH. However, this attained significance only in the singleton group (Table 2).

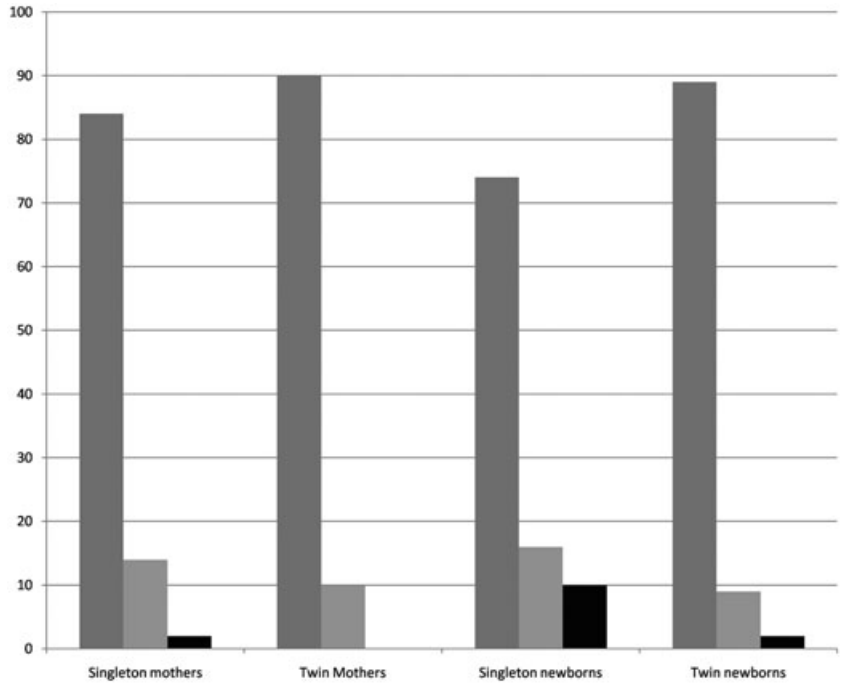

Figure 1 Percentage prevalence of maternal and neonatal vitamin-D deficiency in twin and singleton pregnancies. (■) Vitamin D deficient. (■) Vitamin D insufficient. (■) Vitamin D sufficient.

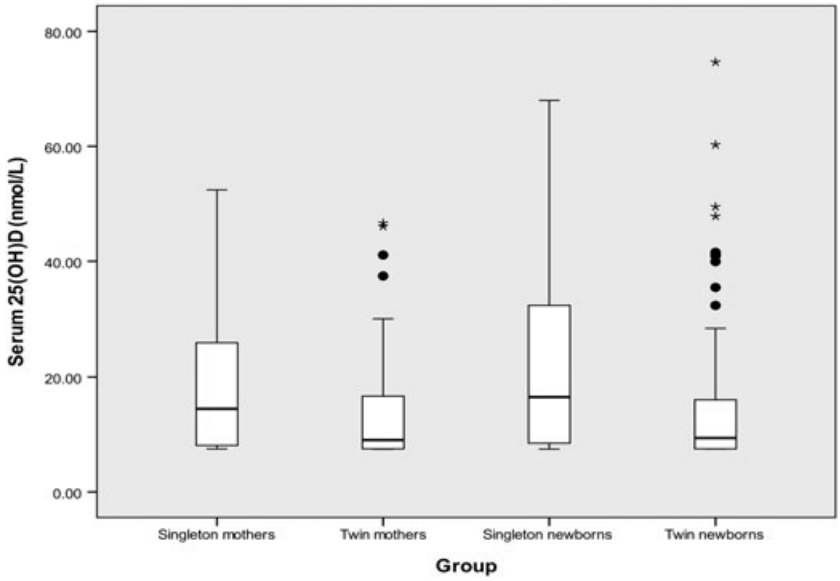

Figure 2 Maternal and neonatal serum 25(OH)D levels in twin and singleton pregnancies.

Table 2 Correlations of maternal and cord blood 25(OH)D with other variables

Study variable	Correlations with	Twin pregnancy		Singleton pregnancy	
		r	*P*	*r*	*P*
Maternal serum 25(OH)D	Cord serum 25(OH)D	0.64 (1st twin) 0.65 (2nd twin)	<0.001 <0.001	0.75	<0.001
	Serum adjusted calcium	0.25	0.84	0.39	0.005
	Serum iPTH	−0.22	0.12	−0.39	0.005
Cord serum 25(OH)D	Cord serum adjusted calcium	0.15	0.14	0.24	0.10
	Cord serum iPTH	−0.27	0.01	−0.20	0.16

iPTH, intact parathormone.

Neonatal vitamin D and calcium status in twin and singleton pregnancies

VDD was observed in 89% of the twin newborns and in 74% of the singleton newborns ($P = 0.03$, Figure 2). The mean cord blood 25(OH)D of twins was significantly less than that of singleton newborns (14.8 ± 12.63 vs 22.6 ± 16.68 nmol/L; $P = 0.002$; Table 1, Figure 2). The difference in the mean 25(OH)D levels of the two groups of newborns remained significant even after adjustment for the differences in their birthweights and for maternal serum 25(OH)D levels. The mean albumin-adjusted serum total calcium was significantly lower in the twins as compared to the singleton newborns. The mean serum iPTH and inorganic phosphate levels were comparable in the two groups. The neonatal serum 25(OH)D showed inverse correlation with serum iPTH. However, this attained significance only in the twin group (Table 2).

Correlation between maternal and neonatal vitamin D levels

Serum 25(OH)D values in the cord blood of both the first and second newborns in the twin group showed a significant correlation with maternal 25(OH)D ($r = 0.64$ and 0.65, respectively; $P < 0.001$, Table 2). Similar correlation was observed between the neonatal and maternal 25 (OH)D values in the singleton group ($r = 0.75$, $P < 0.001$). The difference between maternal and corresponding neonatal 25(OH)D ranged from 0–42 nmol/L in the twin group and from 0–43 nmol/L in the singleton group.

Correlation of study variables between twin siblings

The mean serum 25(OH)D of the two siblings showed a significant correlation with each other ($r = 0.88$, $P <$

0.001). Similarly their birthweights ($r = 0.67$), crown–heel lengths ($r = 0.79$), head circumferences ($r = 0.67$), serum albumin levels ($r = 0.56$), albumin-adjusted total calcium levels ($r = 0.44$) and iPTH ($r = 0.67$) showed significant correlation with each other ($P < 0.001$ for all). Within-pair differences in cord blood 25(OH)D ranged from 0 to 33 nmol/L in twin newborns.

Discussion

The present study from India shows a high prevalence of maternal VDD in pregnancy; especially in twin pregnancy. On average, maternal serum 25(OH)D was lower by 4.0 nmol/L in twin than in singleton pregnancy. The present study also documented a direct correlation between maternal and neonatal 25(OH)D levels in twin pregnancy akin to singleton pregnancy. The impact of lower maternal serum 25(OH)D levels in twin pregnancy was evident in their newborns, whose average serum 25(OH)D was lower by 8.0 nmol/L than that of the singleton newborns.

In the present study, lower 25(OH)D in twin pregnancy seems to be functionally relevant for mothers and their newborns. Low maternal vitamin D would have adverse affect on intestinal calcium absorption, which in turn would stimulate the secretion of PTH from the parathyroid glands. In the present study, there was an inverse correlation between maternal serum 25(OH)D and iPTH; however, this attained significance only in the singleton group. Although the reason for this observation is not clear, this could possibly be due to higher placental parathyroid hormone-related protein (PTHrP) production in twin pregnancy. PTHrP has a PTH-like action and thus can affect the relation between serum 25(OH)D and iPTH. [18]

The functional impact of higher severity of VDD in twin newborns was indicated by a significant inverse correlation between their serum 25(OH)D and serum iPTH and lower mean albumin-adjusted serum calcium, unlike the singleton newborns. A strong correlation was observed between the maternal and cord blood 25(OH)D levels of both newborns in twin pregnancies. This indicates that the physiological relation between the maternal and neonatal 25(OH)D is maintained in twin pregnancy akin to that in singleton pregnancies.[19] Furthermore, the serum 25(OH)D values of the newborn siblings in twin pregnancy also correlated with each other. Recently, Novakovic *et al.* reported a similar observation on cord blood 25(OH)D correlation between newborns in twin pregnancy.[20] Although in the present study no newborns in the twin and singleton groups showed overt neonatal hypocalcemic seizures, severe VDD is a known predisposing factor for this complication.[6] Cord blood 25(OH)D levels showed no correlation with birthweight, crown–heel length and head circumference of the newborns in either of the two study groups. Smaller size of newborns in the twin group seems to be the effect of twin pregnancy per se, because none of these anthropometric parameters correlated with cord blood 25(OH)D levels.

There is limited information on vitamin D status of mothers and their newborns in twin pregnancy. There are only four previous studies on this subject and the results are variable.[21–24] Two of these studies showed lack of significant differences in maternal 25(OH)D levels in twin versus singleton pregnancies, one showed higher, and one showed lower levels in twin pregnancies (Table 3). The maternal serum 25(OH)D was lower by 5–25 nmol/L in twin pregnancies in three of these studies.[21,22,24] The study reporting higher maternal 25 (OH)D levels in twin pregnancy had attributed it to greater vitamin D consumption.[23] The variability in the extent of lower maternal 25(OH)D in twin pregnancy could be related to the severity of VDD. In fact, women in the present study had the lowest mean serum 25(OH)D as compared to the previous studies. Only one of these studies compared cord blood 25(OH)D of twin and singleton newborns and reported it to be lower by 6 nmol/L in twins; which is similar to that observed in the present study.[21]

The causal factors for lower 25(OH)D in twin pregnancy are not clear. A greater restriction of physical activity of the women with twin pregnancy in the last trimester might limit their sun exposure thereby leading to lower maternal 25(OH)D. Though the mean values of sun index in this study were lower than the values reported for normal subjects in Delhi, these were comparable between the singleton and twin groups.[27] Other possible mechanisms might include 15% higher maternal physiological hemodilution in twin pregnancy.[25,26] The lower serum albumin in women with twin pregnancy in the present study could be a reflection of the increased hemodilution in them. Sharing of limited maternal 25(OH)D stores between two fetuses in twin pregnancy might also lead to low cord blood 25(OH)D levels in them.

Vitamin-D-binding protein is increased in pregnancy due to the effect of estrogen, which could lead to higher serum 25(OH)D levels.[28,29] As women with twin pregnancy are likely to have higher estrogen and thereby vitamin-D-binding protein, it is unlikely that lower maternal serum 25(OH)D levels observed in the twin group would be related to variations in this protein in pregnancy.[30] The role of hemodilution, volume of distribution of circulating 25(OH)D and vitamin-D-binding protein could be the subject of future studies in twin pregnancy.

Table 3 Studies comparing maternal and neonatal 25(OH)D levels in twin and singleton pregnancies

	Maternal 25(OH)D levels (ng/mL)†			Cord blood 25(OH)D levels (ng/mL)†		
References	**Twin**	**Singleton**	**Comparison**	**Twin**	**Singleton**	**Comparison**
Hillman & Haddad (1974)[21]	**38–42 weeks** 14.0 ± 6.6 (black, *n* = 6) — **31–37 weeks** 9.8 ± 3.7 (black, *n* = 5) 16.2 ± 9.0 (white, *n* = 5)	**38–42 weeks** 22.1 ± 9.7 (black, *n* = 20) 31 ± 11.5 (white, *n* = 14) **31–37 weeks** 17.8 ± 10.3 (black, *n* = 14) —	Not given	**38–42 weeks** 12.7 ± 6.5 (black, *n* = 12) — **31–37 weeks** 9.3 ± 5.7 (blacks, *n* = 10) 19.8 ± 7.8 (white, *n* = 10)	**38–42 weeks** 15.3 ± 6.8 (black, *n* = 20) 23.6 ± 8.3 (white, *n* = 14) **31–37 weeks** 14.0 ± 9.0 (black, *n* = 14) Not given	Not given
Reddy *et al.* (1983)[22]	**3rd trimester** 14 ± 0.9 (*n* = 19) Racially heterogeneous	**3rd trimester** 16 ± 1.6 (*n* = 22) Racially heterogeneous	Not given	—	—	—
Okah *et al.* (1996)[23]	**30.6 ± 0.9 weeks** 61 ± 5 (1 black, 16 white)	**32.4 ± 0.9 weeks** 39 ± 2 (6 black, 24 white)	Higher in twins $P < 0.001$	—	—	—
Nakayama *et al.* (2011)[24]	**30 & 36 weeks** 15.0 ± 6.6 (Japanese, *n* = 51)	**30 & 36 weeks** 25.3 ± 8.9 (Japanese, *n* = 109)	Lower in twins $P < 0.0001$	—	—	—
Present study (2015)	**35.8 ± 1.89 weeks** 5.7 ± 4.19 (Asian Indians, *n* = 50)	**36.1 ± 1.87 weeks** 7.4 ± 4.95 (Asian Indians, *n* = 50)	Lower in twins $P = 0.02$	5.9 ± 5.06 (*n* = 100)	9.1 ± 6.68 (*n* = 50)	Lower in twins $P = 0.002$

†1 ng/mL = 2.496 nmol/L.

To conclude, maternal serum 25(OH)D level was lower in twin pregnancy than in singleton pregnancy in a population not receiving vitamin D supplementation. The correlation between maternal and neonatal serum 25(OH)D is maintained in twin pregnancy. Twin neonates have a higher prevalence and greater severity of VDD than singleton newborns. The recommended dietary allowance for vitamin D in pregnancy is 600 IU/day.[17] There are no separate guidelines for vitamin D intake in women with twin pregnancy. In view of the higher maternal VDD in twin pregnancy, it seems that the daily requirement for vitamin D might be higher in twin pregnancies as compared to singleton pregnancies. Further studies should be designed to determine the optimal daily vitamin D intake in twin pregnancies.

Disclosure

The authors report no conflict of interest.

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