Vitamin D Status in Premature Infants at Risk of Metabolic Bone Disease of Prematurity

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Abstract

Objectives: The purpose of this study is to report the vitamin D status in a group of premature infants with increased risk of MBD and to describe the relationship between their vitamin D status and intake.

Methods: Retrospective descriptive study in in a level IV neonatal intensive care unit.

Results: One hundred and fifty-two subjects were included in the study. Mean gestational age was 26.8 ± 3.3 weeks and mean age was 157 ± 93 days. Mean serum 25OHD concentration was 57.8 ± 2.0 mg/mL. The prevalence of 25OHD <20 ng/mL and 25OHD >100 ng/mL was 5.9% (n=9) and 8.6% (n=13), respectively. No association between 25OHD level and vitamin D intake with respect to total daily dose (p=0.43) or total daily dose based on body weight (p=0.812) was found. Younger gestational age and younger chronological age were associated with wider range of 25OHD levels.

Conclusions: Both suboptimal and elevated 25OHD concentrations are found with our current protocol for supplementation. Vitamin D status in premature infants does not correlate with their vitamin D intake, but younger gestational age and chronological age are both associated with more diverse range of 25OHD.

Keywords: Vitamin D; Prematurity; Infants; Metabolic bone disease of prematurity

Introduction

Metabolic bone disease (MBD) of prematurity is common among preterm, low-birth weight infants. This compromised bone is attributed to a combination of factors including lower calcium and phosphorus stores at birth, dependence on total parenteral nutrition (PN) with difficulty in achieving adequate enteral intake of calcium, phosphorus and vitamin D, relative immobility and adverse effects of medications (e.g., diuretics and Glucocorticoids) [1]. MBD is associated with increased risk of fractures, compromised pulmonary status, and growth failure. By promoting intestinal and renal calcium absorption, vitamin D plays an important role in bone mineralization. In addition, it also has potential extra-skeletal effects on immune functions [2] and respiratory outcome [3] in preterm infants. Since most preterm infants are hospitalized after birth and have minimal sunlight exposure, dermal vitamin D synthesis is expected to be negligible, and preterm infants are dependent upon an exogenous vitamin D source.

Thus, most professional bodies recommend routine vitamin D supplementation to preterm infants. However, current guidelines for vitamin D supplementation differ. For example, the American Academy of Pediatrics (AAP) recommends a vitamin D intake of 200 to 400 IU/day [4], while the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) suggests 800-1000 IU/day for infants [5]. Nevertheless, these recommendations were mainly based on data extrapolated from term infants. The optimal dose of vitamin D supplementation required to achieve sufficient vitamin D levels in preterm infants remains unclear, and the extent to which differences in weight impact these outcomes is unclear.

Therefore, this study aims to describe the vitamin D status and the relationship with vitamin D intake in a group of preterm infant of gestational age <37 weeks followed at a single center and at risk of MBD.

Materials and Methods

This study was conducted in the Division of Endocrinology at the Children's Hospital of Philadelphia (CHOP) with approval from the institutional review board.

Study design

In this retrospective descriptive study, subjects were identified from the list of patients who were assessed by the multidisciplinary bone team. Clinical data were retrieved by retrospective chart review.

Setting/Subjects

The neonatal intensive care unit in the Children's Hospital of Philadelphia is a level IV unit. All infants with gestation age younger than 37 weeks who were screened for MBD and were seen by the multidisciplinary bone team in the neonatal intensive care unit from 1st January 2011 to 31st December 2015 were included in our study. Subjects were excluded for significant renal insufficiency/renal failure or suspected vitamin D metabolism defect/vitamin D resistance. Subjects were also excluded if they were treated with calcitriol.

Standard of Care of Vitamin D Supplementation in Preterm Infants in CHOP

In the Children's Hospital of Philadelphia, infants at risk of MBD (most commonly includes infants with low birth weight ≤ 1000 gram or gestational age ≤ 28 weeks; infants with other risk factors e.g., prolonged total parenteral nutrition, cholestasis, surgical necrotizing

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enterocolitis/malabsorption, steroids or loop diuretics exposure ≥ 2 weeks and those with features of osteopenia on radiographs) are assessed by the multidisciplinary bone team, which consists of a pediatric endocrinologist specializing in bone health and dietitians with expertise in neonates. The dietitians estimate the total vitamin D, calcium and phosphorus intake from both enteral and parenteral sources for each patient.

The standard of care of MBD of prematurity includes maximizing mineral intake with early feeds, fortification, and direct mineral supplementation. For infants on PN, vitamin D is given intravenously, based on body weight (less than 1kg: 120 IU daily; 1 to 3 kg: 260 IU daily; greater than 3 kg: 400 IU daily). Once full feeding is established, 400 IU of enteral cholecalciferol is given to all infants (same dose for all weights).

Assays and analyses

Serum 25-hydroxyvitamin D (25OHD) concentrations were measured using high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) based on the procedure of Maunsell et al. [6] with modifications. Instrumental analysis was performed on an Applied Biosystems API 4000 LC-MS/MS instrument equipped with a Perkin-Elmer Series 200 autosampler (PerkinElmer, Waltham, Massachusetts, USA) and two Perkin-Elmer Micro LC pumps controlled from a computer system using Analyst software (Applied Biosystems, Darmstadt, Germany).

Serum calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), magnesium (Mg), albumin, creatinine (Cr) and urine electrolytes were measured with a Microslide chemistry system using Vitros 5600 and Vitros 5,1 (Ortho Clinical Diagnostics, Raritan, NJ). Serum intact parathyroid hormone (PTH) values were measured using the immunoassay system IMMUNOLITE 2000 (Siemens Healthcare Diagnostics, Deerfield, IL).

Outcome measures and definition

The primary endpoint was the average 25OHD level and percentages of infants with 25OHD <20 ng/mL and with 25OHD>100ng/mL. The secondary endpoints included the relationship between vitamin D status and vitamin D intake as well as risk factors for suboptimal 25OHD concentrations, defined as 25OHD <20 ng/mL, and elevated 25OHD concentrations, defined as 25OHD>100 ng/mL, for the purposes of this study.

Data Analyses

Data were summarized using descriptive statistics using means and standard deviations or medians and minimum/maximum depending upon the normality of the continuous variables, and using frequency distributions and proportions for categorical variables. Serum 25OHD concentrations were measured more than once in some infants; these data are presented as first 25OHD and follow-up 25OHD.

For the secondary outcomes, the relationships between 25OHD and vitamin D intake were examined using regression models (both linear and logistic). In order to further examine relationships between 25OHD and vitamin D supplementation and to account for the correlation between multiple 25OHD measures within the same subject and for differences in baseline 25OHD, mixed effect models were developed. Analyses were performed using Stata 13 (StataCorp, College Station, TX).

Results

Subjects' characteristics and mean serum 25OHD concentrations

One hundred fifty-two subjects were included in the study. Some subjects had more than one 25OHD level measured and the total number of 25OHD measurements was 209 (41 had two measurements, 13 had three measurements and 3 had four measurements). Mean gestational age was 26.8 ± 3.3 weeks and mean age at time of assessment was 157 ± 93 days. Demographic data and biochemical profiles were summarized (Table 1). Mean serum 25OHD concentration was 57.8 ± 2.0ng/mL; concentrations were slightly higher in infants born <28 weeks (59.5 ± 2.2ng/mL) versus ≥ 28 weeks (52.7 ± 4.1 ng/mL) (p=0.01). Serum 25OHD concentration was also higher in infants with corrected gestational age (CGA) <40 weeks (64.2 ± 4.6 ng/mL) than ≥ 40 weeks (55.7 ± 2.1 ng/mL) (p=0.13).

Prevalence of suboptimal and elevated 25OHD concentrations

The prevalence of having suboptimal 25OHD concentration was 5.9% (n=9) while the prevalence of elevated 25OHD concentration was 8.6% (n=13). Suboptimal 25OHD was more common among infants born ≥28 weeks, while elevated 25OHD was more common in infants born <28 weeks (Table 2).

Among those with suboptimal 25OHD, six (66.7%) had x-ray changes consistent with MBD and one (11.1%) had history of long bone fracture. In the 11 episodes with serum 25OHD concentrations <20 ng/mL, 4 (36.4%) were associated with elevated ALP of >800 U/L (none with cholestasis) and 3 (27.3%) were also associated with elevated PTH >100 pg/mL.

Of the 15 episodes with serum 25OHD concentrations >100 ng/mL, one (6.7%) was associated with hypercalciuria (urine Ca/Cr ratio >1, not on furosemide). None had documented nephrocalcinosis, hypercalcemia (>11 mg/dL) or suppressed PTH (<10 pg/mL).

Variable	Value
Male sex	82 (53.9%)
Gestational age (weeks)	26.8 ± 3.3
Birth weight (gram)	885 ± 54
Age at assessment (days)	157 ± 93
Chronological age at assessment (weeks)	49 ± 13
Ethnic	47/105
(Black/Non-black)	(31%/69%)
Laboratory findings	
25OHD (mg/mL)	
In all subjects (n: 209)	57.8 ± 2.0
In all subjects (episode 1 only, n: 152)	55.8 ± 2.3
Chronological age <40 weeks (n: 49)	64.2 ± 4.6
Chronological age ≥40 weeks (n: 160)	55.7 ± 2.1
Gestation <28 weeks (n: 154)	59.5 ± 2.2
Gestation ≥28 weeks (n: 55)	52.7 ± 4.1
25OHD < 20 mg/mL	9 subjects (5.9%); 11 episodes (5.3%)
25OHD > 100 mg/mL	13 subjects (8.6%); 15 episodes (7.2%)
Alkaline phosphatase (U/L)	406.0 ± 19.8
Calcium (mg/dL)	9.7 ± 0.05
Phosphorus (mg/dL)	6.7 ± 0.8
PTH (mg/mL)	97.5 ± 7.5

Table 1: Demographics and clinical characteristics of preterm infants (n: 152).

Gestation	25OHD<20 ng/mL	25OHD>100 ng/mL	Vit. D intake (IU/day)	Vit. D intake (IU/kg/day)
All	8/152 (5.3%)	13/152 (8.6%)	567.4 ± 22.8	180.3 ± 9.1
<28 weeks	6/108 (5.6%)	11/108 (10.2%)	591.2 ± 28.1	193.2 ± 11.3
≥ 28 weeks	4/44 (9.1%)	2/44 (4.5%)	500.8 ± 35.0	144.1 ± 12.5

Table 2: Distribution of 25OHD concentrations.

25OHD levels (mg/mL)	Total Vit D/day (IU/day)	Total Vit D/kg/day (IU/kg/day)
All levels (n: 209)	567.4 ± 22.8 (Episode 1 only: 540.9 ± 24.9)	180.2 ± 9.0 (Episode 1 only: 188.9 ±10.9)
<20 (n: 12)	834.7 ± 108.1 (Episode 1 only: 667.3 ± 112.0)	277.5 ± 39.4 (Episode 1 only: 229.5 ± 49.7)
20-100 (n: 182)	541.2 ± 317.3	170.62 ± 128.6
>100 (n: 15)	671.1 ± 89.0 (Episode 1 only: 677.9 ±110.4)	219.8 ± 31.7 (Episode 1 only: 235.3 ± 38.2)

Table 3: Vitamin D intake and serum 25OHD concentrations.

Vitamin D intake and 25(OH)D levels

The mean vitamin D intake was 567.4 ± 22.8 IU per day (180.3 ± 9.1 IU/kg/day). No association between 25(OH)D and vitamin D intake, by total daily dose (p=0.43) or total daily dose based on body weight (p=0.812), was found (Figure 1). The average vitamin D intakes for low, normal and high 25(OH)D levels were summarized in Table 3.

Risk factors for suboptimal and elevated 25OHD concentrations

Lower serum 25OHD value is associated with older gestation (coefficient= -1.64, p=0.001) and presence of cholestasis (coefficient=-14.8, p=0.004) by linear regression model, after corrected for ethnicity, mode of nutrition (TPN vs. partial PN vs. full enteral feeding) and vitamin D intake (IU/kg/day) (Figure 2).

Younger gestational age and chronological age were associated with wider range of 25OHD levels (Figure 3).

Discussion

In our cohort, both suboptimal and elevated 25OHD concentrations are found with our current protocol of vitamin D supplementation. Interestingly, suboptimal 25OHD concentration was more common among infants born at older gestations (≥ 28 weeks) while hypervitaminosis D was more common among infants born at younger gestation (<28 weeks). This difference could partly be explained by higher daily weight-based dosing in the younger gestation group, implying the potential problem with the recommendation of 'one vitamin D dose fits all' and that the problem of elevated 25OHD does occur with routine supplementation. No relationship could be found between suboptimal or elevated 25OHD and vitamin D intake, but this could be due to the low prevalence in this relatively small cohort. Published data on the response to vitamin D supplementation in preterm infants are limited. Roberta et al. reported a beneficial effect with vitamin D supplementation 400 IU daily in a group of 148 preterm infants in Ireland, with the percentage of hypovitaminosis D (defined as serum 25OHD <20 ng/mL) decreasing from 78% at a median age of 18 days to 13% at a median age of 104 days [7]. A smaller study conducted by Natarajan et al. also reported daily vitamin D supplementation at both 400 IU and 800 IU reduced the prevalence of VDD at 40 weeks' CGA and at 3 months' CGA in preterm infants in India, with the 800 IU arm achieving better effect (from 37% to 14%

vs. 40% to 30%) [8] Similarly, a recent study by Hanson et al reported vitamin D supplementation at 400 IU and 800 IU are both effective in raising 25(OH)D concentrations, with mean serum 25(OH)D concentrations improved from 16.4ng/mL and 18.4 ng/mL to 54.1 ng/mL and 65.7 ng/mL, respectively) in a small group of preterm neonates in America [9]. In contrast, Hitnova et al. reported no significant effect of vitamin D supplementation at 1334 IU daily from day 20 onwards upon hypovitaminosis D in their cohort of preterm infants (≤32 weeks, n: 41) in Bulgaria. The percentage of VDD was 32% and 30% at birth and at 8 weeks of age respectively despite supplementation [10]. Tergesti et al. also reported no improvement in vitamin D deficiency with supplementation at 400 IU daily. In fact, the percentage of VDD was 12.6% at birth and it increased to 52.2% at 6 weeks [11]. In a recently published randomized doubled-blind controlled trial comparing the effect of vitamin D supplementation at 400 vs. 1000 IU daily in a group of preterm neonate at 24 to 27 weeks gestation, mean 25OHD concentration increased from 21.3 ng/mL to 47.5 ng/mL at 40 weeks CGA in the 1000 IU daily group, but mean 25OHD concentration in 400 IU daily group decreased from 31.3ng/mL to 17.5 ng/mL. Five (9.8%) subjects in the 1000 IU daily group had elevated 25OHD concentration of >70 ng/mL but the exact number of subjects with hypervitaminosis D was not known as 70 ng/mL was the upper limit of their assay [12]. Thus, available data in the literature are conflicting and inconclusive. Moreover, none of these studies addressed the impact of body weight in vitamin D supplementation, and only one study assessed the potential problem of hypervitaminosis D with routine supplementation. On the other hand, the exact physiological implication of measured 25OHD level in preterm infants remains controversial. Vitamin D is essential for enteral absorption of calcium. However, the exact timing and proportion of vitamin D-dependent calcium enteral absorption in preterm infants is unclear [4]. Ideally, optimal 25OHD levels should be defined based on enteral calcium absorption, PTH concentrations and degree of bone mineralization. However, these data in preterm infants are currently unavailable and the commonly used 'optimal' 25OHD level is extrapolated from adult and pediatric populations only. In our cohort, among the 11 episodes with 25OHD <20 ng/mL, only 4 episodes were associated with elevated ALP and only 3 of these 4 episodes were associated with elevated PTH. Similarly, in the 15 episodes of 25OHD >100 ng/mL, none fulfilled the criteria for vitamin D intoxication based on the definition in the latest Global Consensus Recommendation on Prevention and Management of Nutritional Rickets, which defined
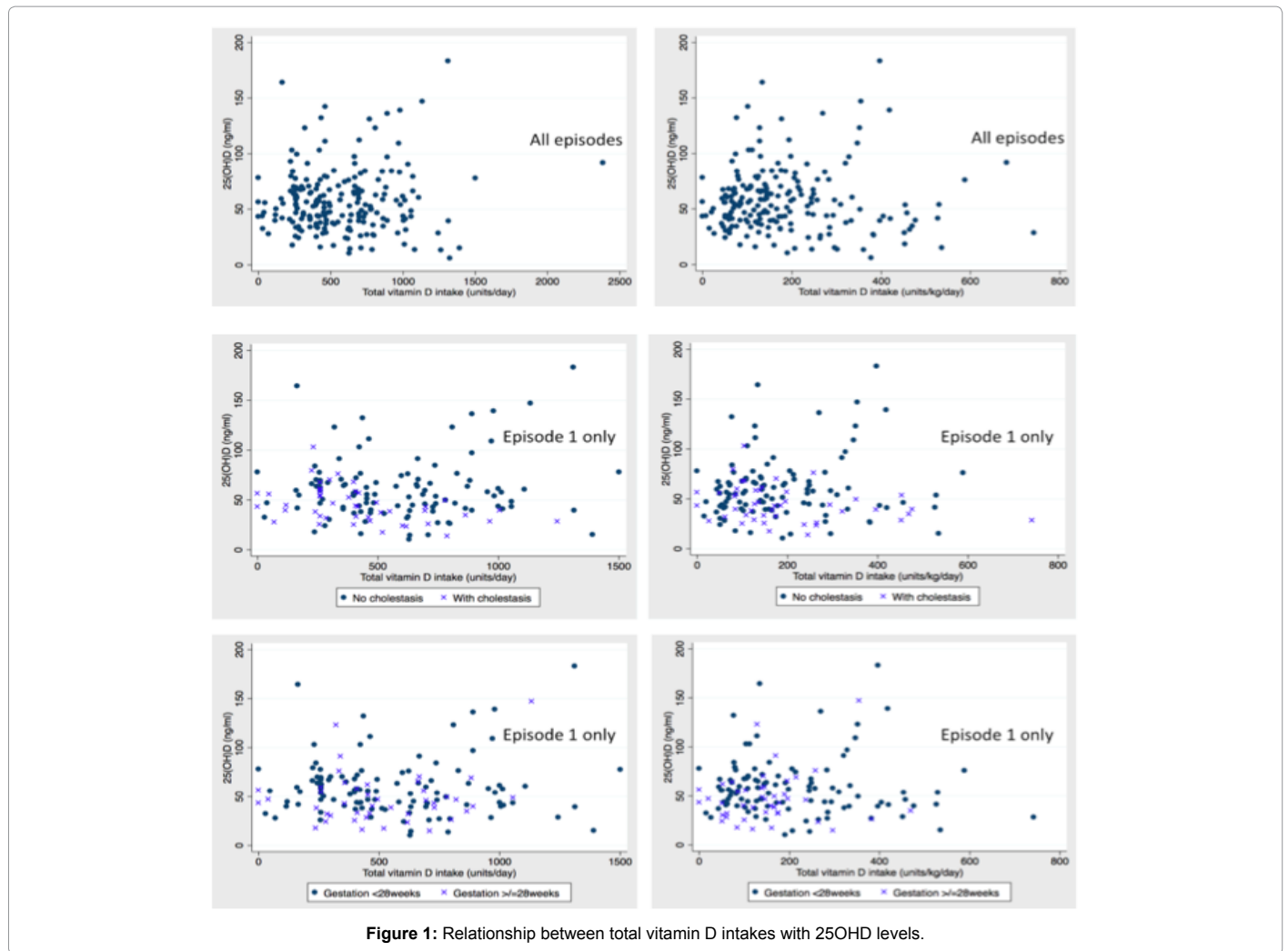
Figure 1: Relationship between total vitamin D intakes with 25OHD levels.
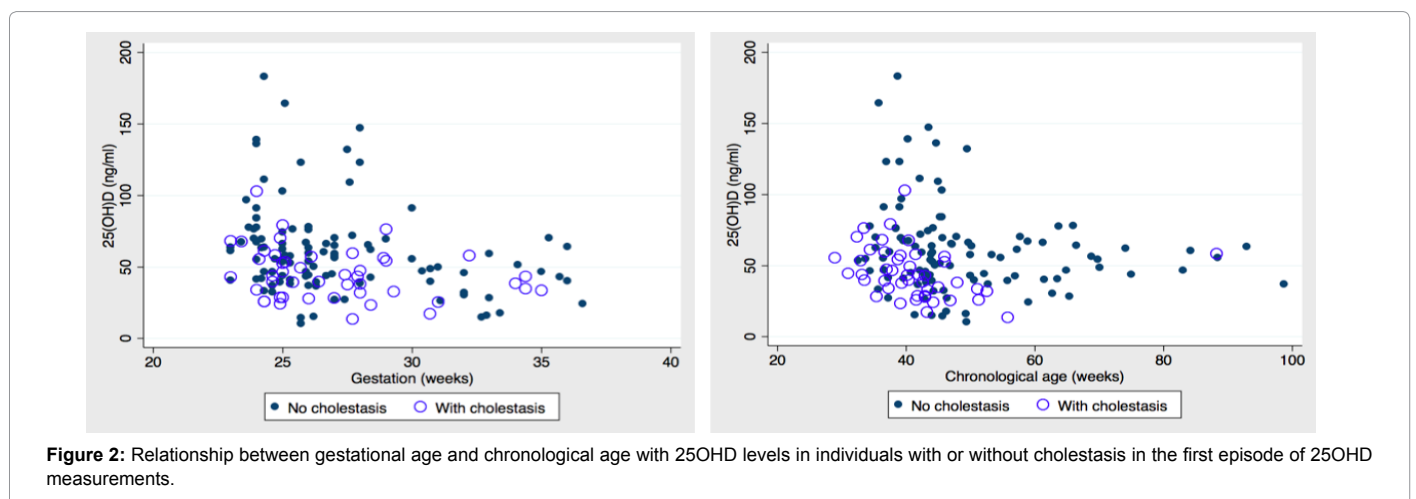

Figure 2: Relationship between gestational age and chronological age with 25OHD levels in individuals with or without cholestasis in the first episode of 25OHD measurements.

vitamin D toxicity as serum 25OHD >100ng/mL with hypercalcemia, hypercalciuria and suppressed PTH [13]. However, these outcomes were not systematically measured, and transient occurrences cannot be excluded. Nonetheless, the extent to which the commonly defined 'optimal' range of serum 25OHD concentration applies in preterm infants is unknown. Our study has several limitations. First, maternal vitamin D status and cord blood 25OHD levels were not available for analysis. However, despite the fact that infant cord blood 25OHD level is strongly associated with maternal vitamin D status [14], the mean age of our cohort was 157 days, and therefore, their 25OHD level should be less affected by maternal vitamin D status or their own vitamin D status at birth, but more reflective of their intake and
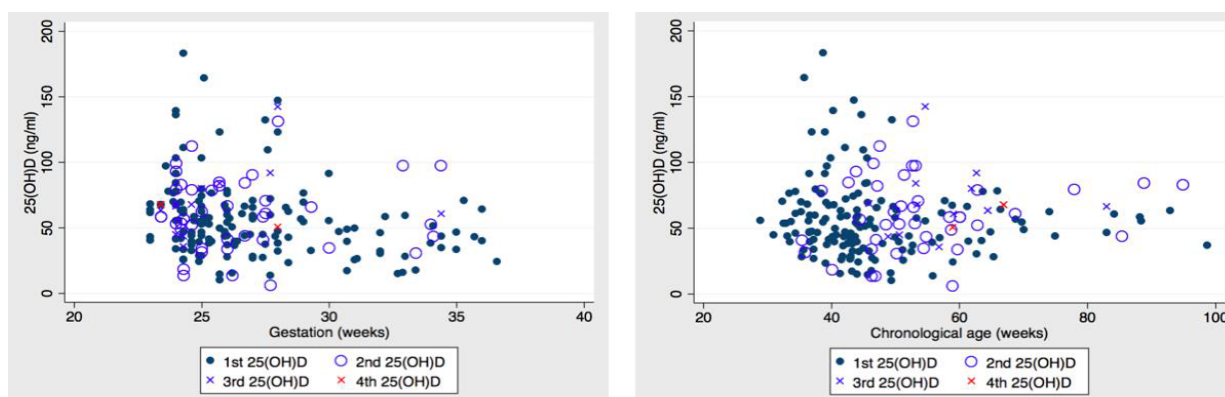
Figure 3: Relationship between gestational age and chronological age with 25OHD levels in various episodes of measurements.

metabolism. Second, the effect of vitamin D binding protein (VDBP) and polymorphisms could not be assessed. Genetic polymorphisms in VDBP may contribute to variations in serum 25OHD concentrations [15,16]. Due to retrospective nature of this study, this aspect could not be addressed. In addition, the effect of VDBP in this preterm population is still unclear [17,18].

An epimeric form of 25OHD, 3-epi-25-hydroxyvitamin D3, has recently been reported to contribute 36 to 55% of total 25OHD in infants with highest level in preterm neonates, partly related to liver immaturity. Its clinical significance is unknown but it could be erroneously measured as 25OHD even with liquid chromatography-tandem mass spectrometry [19,20]. Nevertheless, in a recent study on the time-course analysis of 3-epi-25-hydoxyvitamin D3 in infants, 3-epi-25-hydroxyvitamin D3 level normalized to <10% in infants older than 3 months of age. Since 81% of our infants (170 out of 209 observations) were older than 3 months at the time of assessment, the effect of 3-epi-25-hydroxyvitamin D3 would be less significant in our group.

Finally, given the retrospective nature of the study, iPTH, urine calcium, skeletal radiographs, and renal ultrasounds were not available for all subjects who contributed data. The extent to which differences in vitamin D status contributed to differences in these other markers of bone mineral metabolism cannot be assessed. Additionally, the measured 25OHD is a function of not only the supplementation received under our institution's current protocol but the duration of this supplementation as well as the supplementation (dose and duration) received prior to transfer; these factors were not addressed in this retrospective study.

Conclusion

Both suboptimal and elevated 25OHD concentrations are observed with our current protocol for vitamin D supplementation. Vitamin D status in premature infants did not correlate with their intake but younger gestations and chronological age are both associated with more diverse range of 25OHD levels. Interestingly, hypovitaminosis D was more common among the older gestation group while hypervitaminosis D was more common among the younger group, implying the potential problem of 'one dose fits all' approach in vitamin D supplementation. Further studies assessing the optimal weight-based dosing in vitamin D supplementation are needed.

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