Association between vitamin D deficiency at one month of age and bronchopulmonary dysplasia

Shin Yun Byun, MD, PhD, Mi Hye Bae, MD, Na Rae Lee, MD, Young Mi Han, MD, PhD, Kyung Hee Park, MD, PhD

Abstract
Vitamin D deficiency is common and increases the likelihood of neonatal morbidities in preterm infants. This study assessed vitamin D levels at 1 month of age after 4 weeks of vitamin D supplementation and determined the association between vitamin D levels and neonatal morbidities.

This retrospective study included preterm infants with birth weight <1500 g or gestational age <32 weeks born in our hospital between January 2018 and December 2019. They were administered 400 IU of oral vitamin D supplementation after birth according to our policy. The infants were then divided into sufficient (≥20 ng/mL) and deficient (<20 ng/mL) groups according to their serum vitamin D levels at 1 month of age.

The vitamin D deficient and sufficient groups included 49 and 41 patients, respectively. The mean gestational age and birth weight, GA = gestational age, were 29.1 ± 2.1 weeks and 1216.1 ± 308.1 g, respectively, and 30.0 ± 1.7 weeks and 1387.6 ± 350.8 g, respectively, in the sufficient group. No significant differences were observed between the 2 groups in demographic and clinical outcomes except for bronchopulmonary dysplasia (BPD), which occurred significantly more often in the vitamin D-deficient group (odds ratio 2.21; 95% confidence interval, 1.85–2.78; P = .02).

The results of our study suggest that vitamin D deficiency at 1 month of age is associated with BPD in preterm infants.

Abbreviations: 25-OHD = 25-hydroxyvitamin D, ALP = alkaline phosphatase, BPD = bronchopulmonary dysplasia, BW = birth weight, GA = gestational age, hemorrhage, NEC = necrotizing enterocolitis, RDS = respiratory distress syndrome, ROP = retinopathy of prematurity.

Keywords: bronchopulmonary dysplasia, preterm infants, vitamin D deficiency

1. Introduction
Vitamin D is a fat soluble vitamin that plays important role in calcium metabolism and bone health.[1] The reported roles of vitamin D were the following; musculoskeletal function, regulation of hormone secretion, immune system function, and regulation of cell proliferation and differentiation. And studies has been conducted to determine the association between vitamin D status and these health problems in infants and children including preterm infants as well.[2,3]

The role of vitamin D in nonskeletal diseases has been increasingly reported in recent decades and is especially associated with neonatal morbidities in preterm infants. Vitamin D deficiency is common in neonates and is present in 70% to 97.4% of preterm infants at birth.[4–6] Previous studies have reported the relationship between vitamin D deficiency and neonatal outcomes, including respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), sepsis, and necrotizing enterocolitis (NEC)[7–10], generally based on the evaluation of cord blood or serum in the first days of life.[10–12]

Our institution has prescribed 400 IU/day of vitamin D within the first days after birth according to guidelines published by Pudowski et al.[13] They recommended that preterm infants fed enterally should receive vitamin D supplementation of 400 to 800 IU/d within the first days of life and continue up to 40 weeks of GA. This study analyzed the vitamin D status at 1 month of age after 4 weeks of vitamin D supplementation after birth and assessed whether vitamin D deficiency at 1 month was associated with neonatal morbidities.

2. Patients and methods
2.1. Patients
This retrospective observational study included preterm infants born between January 2018 and December 2019 at Pusan National University Hospital. Infants were included if they were born at ≤32 weeks of GA or weighed ≤1500 g at birth. Infants were excluded if they had any of the following: major congenital cardiac anomaly, chromosomal anomaly, congenital infection, or severe medical problems requiring intensive care.
anomalies or chromosomal abnormality, fetal hydrops, metabolic disorders or central nervous system infection, or death before 4 weeks of life.

Since January 2018, our neonatal intensive care unit has prescribed 400 IU of vitamin D within the first days of life in all preterm infants with stable vital signs. Levels of vitamin D, parathyroid hormone, Ca, P, and alkaline phosphatase (ALP) were routinely checked at 1 month of age. Vitamin D levels were measured as 25-hydroxyvitamin D (25-OHD). In this study, vitamin D deficiency and sufficiency were defined as 25-OHD concentrations of <20ng/mL and ≥20ng/mL, respectively.

2.2. Methods

2.2.1. Data collection. Data including clinical characteristics and morbidities were reviewed retrospectively.

Perinatal data were collected from the medical charts of infants and included GA, birth weight (BW), intrauterine growth retardation (defined as a weight below the 10th percentile for the GA), sex, 1- and 5 min Apgar scores, and mode of delivery.

Maternal data were collected from the medical charts of mothers and included premature rupture of membranes (defined as >18 hours after membrane rupture), preeclampsia, diabetes mellitus, and other systemic diseases. We collected maternal 25-OHD concentrations at mid trimester and defined vitamin D deficiency as 25-OHD concentrations of <20ng/mL as well. The reviewed neonatal clinical outcomes included RDS; patent ductus arteriosus (defined as the need for ibuprofen medication or surgical ligation), NEC (defined as Bell criteria stage > III), BPD (defined as moderate and severe by Jobe and Bancalari), intraventricular hemorrhage (defined as grade 3 and above by Papile criteria), and retinopathy of prematurity (ROP; defined as stage 2 and above).

2.2.2. Ethics approval. This study was approved by the Institutional Review Board of Pusan National University Hospital (2006-013-091). The requirement for informed consent was waived by the Institutional Review Board of Pusan National University Hospital. All methods were carried out in accordance with relevant guidelines and regulations.

2.3. Statistical analysis

Data were stored in a dedicated Microsoft Access database and verified for accuracy. Statistical analyses of the raw scores were performed using IBM SPSS Statistics for Windows, version 22.0. The analyses in the present study focused on comparisons between vitamin D deficient and sufficient groups. Statistical comparisons of the continuous and categorical variables were performed using t and chi-square tests, as appropriate. In addition to univariate nonparametric statistical tests, the results of the t and chi-square tests were confirmed using Mann–Whitney and Fisher exact tests. Pearson correlation analysis was used to evaluate the relationships between vitamin D levels and other laboratory factors, including ALP concentration. Logistic regression analysis was used to assess the relationship between vitamin D levels and neonatal diseases. Statistical significance was set at P < .05.

3. Results

During the study period, 107 preterm infants born at ≤32 weeks of GA or ≤1500 g BW were delivered at our hospital. Of these, 17 were excluded for chromosomal abnormalities (n=2), death before 4 weeks after birth (n=11), or lack of parental consent (n=4).

Of the 90 included infants, 49 and 41 were categorized as vitamin D deficient and sufficient, respectively, based on their vitamin D levels at 1 month of age. The demographic and clinical characteristics of both groups are shown in Table 1. The mean GA and BW were 29.1 ± 2.1 weeks and 1216.1 ± 308.1 g, respectively, in the vitamin D deficient group and 30.0 ± 1.7 weeks and 1387.6 ± 350.8 g, respectively, in the sufficient group (Table 1). Both GA and BW were slightly higher in the sufficient group than in the deficient group, but only BW differed significantly. No significant differences were observed between the 2 groups in demographic and clinical characteristics, including sex, delivery mode, singleton or twin, intruterine growth restriction, and Apgar scores. No significant differences were observed between the 2 groups in maternal characteristics, including vitamin D levels at mid-trimester (Table 2).

Laboratory outcomes are presented in Table 3. The mean vitamin D levels were 11.7 ± 4.5 and 31.3 ± 9.7 in the 2 groups, respectively. Although parathyroid hormone, Ca, and P did not differ significantly between the groups, ALP levels were significantly higher in the deficient group (P=.001). Pearson correlation analysis showed a negative correlation between vitamin D levels and ALP at 1 month of age (P=.002, r=.32).

Table 4 shows the comparison of neonatal morbidities between the groups. The prevalence of RDS, patent ductus arteriosus, NEC, intraventricular hemorrhage, and sepsis did not differ significantly between the groups. The incidences of BPD and ROP were significantly higher in the deficient group. However, BPD and ROP may be influenced by GA and BW. Using variables significant on univariate analyses (BPD and ROP), logistic

| Table 1 | Comparison of perinatal characteristics between vitamin D deficient group and sufficient group. |
|---------|----------------------------------------------------------------------------------|
|          | Deficient group (N=49) | Sufficient group (N=41) | P-value  |
| Gestational age, mean (range), wk | 29.1±2.1 | 30.0±1.7 | .058 |
| Birth weight, mean (range), g | 1216.1±308.1 | 1387.6±350.8 | .028 |
| Male gender, n (%) | 16 (57%) | 36 (60%) | .526 |
| IUGR, n (%) | 8 (25%) | 8 (13%) | .896 |
| Cesarean section, n (%) | 24 (85%) | 44 (73%) | .36 |
| Twin, n (%) | 8 (45%) | 24 (40%) | .336 |
| Apgar score at 1 min, mean | 5.25 | 5.06 | .555 |
| Apgar score at 5 min, mean | 6.87 | 7.0 | .366 |

| Table 2 | Comparison of maternal characteristics and vitamin D levels between deficient group and sufficient group. |
|---------|----------------------------------------------------------------------------------|
|          | Deficient group (N=49) | Control group (N=41) | P-value  |
| Age (yr) | 33.2±3.2 | 33.9±4.4 | .358 |
| Preeclampsia | 17 (35%) | 11 (27%) | .432 |
| PROM | 21 (43%) | 14 (35%) | .397 |
| GDM | 7 (15%) | 7 (18%) | .713 |
| < 20 ng/mL of 25-OHD at mid trimester, n/available (%) | 30/38 (78%) | 24/30 (80%) | .546 |

GDM = gestational diabetes mellitus; PPROM = premature rupture of membranes.
regression analysis adjusted for BW showed that vitamin D deficiency at 1 month of age was a risk factor for BPD (odds ratio 2.21; 95% confidence interval, 1.85–2.78; P = .02) (Table 5).

4. Discussion

Neonatal vitamin D storage at birth is dependent on maternal vitamin D levels in newborn infants including preterm infants. In pregnant women, low vitamin D levels are common, and cord blood levels tend to be even lower.[14,15] In recent studies, vitamin D concentrations of Korean women were the lowest among studied international groups.[16,17] In a Korean study by Choi et al., three-fourths of Korean pregnant women had a risk of vitamin D deficiency and one-fourth had severe vitamin D deficiency. The incidence of vitamin D deficiency was significantly higher during the first and second trimesters than during the third trimester in pregnant women.[19]

Thus, vitamin D deficiency has been reported in preterm infants. One study observed vitamin D deficiency at birth in 79.8% of preterm infants in Korea.[12] In addition, Lee et al.[18] reported vitamin D deficiency in 80% of preterm infants in their study in Busan, Korea, where our hospital is also located. Studies from other countries have reported vitamin D deficiency incidence rates of up to 97%.[6] In fact, our study showed that maternal vitamin D deficiency was 78% and 80% in both groups, respectively.

We checked vitamin D levels at 1 month of age after 4 weeks of supplementation according to the guidelines published by Pudlowska et al.[13] We tried to analyze the correlation between vitamin D levels at 1 month and neonatal morbidities because the majorit of recent studies analyzed cord blood or samples collected within the first days of life.[10,11,12]

The immune functions of vitamin D have been extensively studied in recent years. Adequate vitamin D concentration was found to stimulate antimicrobial peptide expression, especially cathelicidin, in human monocytes, neutrophils, and other cell lines.[21,22] An association between infections and vitamin D deficiency has been reported in neonates. Dhnada et al.[10] reported that neonates with vitamin D deficiency were at a greater risk of late-onset sepsis than those with sufficient vitamin D levels. However, these findings are controversial, as Say et al.[11] reported no significant relationship between cord blood vitamin D levels and the risk of neonatal sepsis in premature infants. In this study, the rates of sepsis did not differ significantly between the study groups.

Vitamin D has receptors on retina and effects on angiogenesis.[23] In several studies, it has been shown that vitamin D inhibits endothelial cell proliferation in vitro and angiogenesis in vivo.[24] Several studies have assessed the relationship between vitamin D levels and ROP. Emrah et al.[25] recently reported that lower 25(OH)D levels in the first days of life may be related to ROP. In the present study, ROP was increased in the vitamin D-deficient group at 1 month of age. However, logistic regression analysis did not show a relationship between vitamin D deficiency at 1 month and ROP.

Vitamin D deficiency during pregnancy is associated with reduced placental development and weight, leading to a presumed risk of preterm birth, which may lead to RDS and BPD. Apart from prematurity, some reports have suggested that vitamin D deficiency may be a risk factor for neonatal respiratory morbidities such as RDS and BPD.[13] Kim et al.[12] reported, using logistic analysis, that a low serum vitamin D level at birth was a risk factor for RDS and BPD, with odds ratios of 4.32 and 4.11, respectively. Although a low vitamin D status was associated with respiratory morbidity, the exact mechanism is unknown. Some studies have suggested a role for vitamin D in lung maturation in RDS. The correlation between lung maturation and vitamin D is explained by phospholipid production and secretion on the surface of alveolar type II cells.[7] Therefore, BPD is increased in patients with vitamin D deficiency because RDS is a well-known risk factor for BPD. In addition, animal studies support the hypothesis that vitamin D status might affect the risk of BPD and the vitamin D receptor have important roles in perinatal lung development.[26,27]

Although GA and the incidence of RDS did not differ significantly between our study groups, the occurrence of BPD was higher in the low vitamin D level group at 1 month of age. Therefore, we concluded that BPD per se is influenced by vitamin D deficiency at 1 month of age, regardless of RDS. However, Jung et al.[28] reported that vitamin D levels in both cord blood and the

### Table 3

|                  | Deficient group (N = 49) | Sufficient group (N = 41) | P-value |
|------------------|-------------------------|--------------------------|---------|
| Serum 25 (OH)D (ng/mL) | 11.7 ± 4.5              | 31.3 ± 9.7               | .000    |
| PTH              | 50.3 ± 36.9             | 63.2 ± 81.9              | .324    |
| Calcium          | 9.4 ± 0.7               | 9.6 ± 0.7                | .166    |
| Phosphorus       | 5.3 ± 1.5               | 5.9 ± 1.0                | .41     |
| ALP              | 619.6 ± 295.0           | 417.7 ± 230.7            | .001    |

ALP = alkaline phosphatase, PTH = parathyroid hormone.

### Table 4

|                  | Deficient group (N = 49) | Sufficient group (N = 41) | P-value |
|------------------|-------------------------|--------------------------|---------|
| RDS, n (%)       | 44 (89%)                | 32 (78%)                 | .126    |
| PDA, n (%)       | 20 (40%)                | 9 (22%)                  | .133    |
| NEC, n (%)       | 5 (10%)                 | 3 (7%)                   | .094    |
| Sepsis, n (%)    | 8 (16%)                 | 5 (12%)                  | .204    |
| IVH, n (%)       | 10 (20%)                | 4 (9%)                   | .165    |
| BPD, n (%)       | 35 (73%)                | 17 (41%)                 | .004    |
| ROP, n (%)       | 15 (30%)                | 8 (19%)                  | .036    |
| Duration of TPN, d | 31.5 ± 12.4             | 28.5 ± 11.7              | .236    |
| Duration of hospital stay, d | 64.5 ± 24.6           | 58.6 ± 19.4              | .098    |

BPD = bronchopulmonary dysplasia, IVH = intraventricular hemorrhage, NEC = necrotizing enterocolitis, PDA = patent ductus arteriosus, RDS = respiratory distress syndrome, ROP = retinopathy of prematurity.

### Table 5

|                  | Unadjusted | Adjusted |
|------------------|------------|----------|
|                  | Exp(B)     | 95% CI | P-value | Exp(B)     | 95% CI | P-value |
| BPD              | 2.54       | 1.89–2.95 | .01 | 2.21       | 1.86–2.78 | .02 |
| ROP              | 1.32       | 0.89–1.45 | .11 | 1.01       | 0.98–1.22 | .41 |

BPD = bronchopulmonary dysplasia, ROP = retinopathy of prematurity.

* Adjusted for birth weight.
36 week’s corrected age were not associated with BPD development.

Our study has several limitations, including the small number of patients and the limitations inherent to retrospective review studies. First, we did not check vitamin D levels at birth. Vitamin D levels of 1 month of age might be affected by those level of at birth. However, we think that vitamin D deficiency might be present at birth and similar in both groups because maternal vitamin D deficiency in the mid-trimester was not different between the 2 groups. Fetal and newborn concentrations of vitamin D correlate with maternal serum levels and depend on transplacental transfer. This occurs mainly in the third trimester. In this study, the majority of preterm infants were born before 29 to 30 weeks’ GA. Second is that we were not able to explain why vitamin D deficiency occurred after 4 weeks of supplementation, although there was no difference in the duration of TPN and incidence of NEC affecting the intestinal absorption of vitamin D. In addition, we didn’t check the nutritional aspects of breast milk or formula, which may also impact on the vitamin D levels though. Despite these limitations, our results suggest that vitamin D deficiency at 1 month of age is associated with a higher incidence of BPD. Therefore, detection of vitamin D deficiency and proper supplementation of vitamin D before 1 month of age is important to prevent BPD in preterm infants. Further well-designed prospective studies with larger case numbers are needed to understand the role of vitamin D deficiency in BPD, and guidelines for vitamin D supplementation for prevention of BPD are required. In addition, we planned that our data combined with others for a meta-analysis may explain the mechanism between vitamin D deficiency and neonatal morbidities including BPD.

Author contributions
Conceptualization: Kyung Hee Park.
Data curation: Mi Hae Bae, Narae Lee, Young Mi Han.
Formal analysis: Shin Yun Byun.
Supervision: Shin Yun Byun, Kyung Hee Park.
Writing – review & editing: Mi Hae Bae, Narae Lee, Young Mi Han, Kyung Hee Park.

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