Early Human Development 112 (2017) 54–59

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Multi-dose vitamin d supplementation in stable very preterm infants: Prospective randomized trial response to three different vitamin D supplementation doses

Ozlem Bozkurt*, Nurdan Uras, Fatma Nur Sari, Funda Yavanoglu Atay, Suzan Sahin, Ayse Dogan Alkan, Fuat Emre Canpolat, Serife Suna Oguz

Department of Neonatology, Zekai Tahir Burak Maternity Teaching Hospital, Ankara, Turkey

ARTICLE INFO

Keywords:
Vitamin D supplementation
Preterm
High dose
Vitamin D deficiency

ABSTRACT

Background: Preterm newborns are born with lower vitamin D stores. Although vitamin D supplementation is recommended there is no consensus regarding the adequate dose of supplementation for preterm infants.
Aims: To assess the effect of three different doses of vitamin D supplementation (400, 800 and 1000 IU/d) in preterm infants ≤ 32 weeks gestation on the prevalence of vitamin D deficiency and 25(OH) D levels at 36 weeks postmenstrual age (PMA).
Study design: Prospective randomized trial.
Subjects: 121 preterm infants with gestational age of 24–32 weeks were randomly allocated to receive 400, 800 or 1000 IU/d vitamin D.
Outcome measures: Serum concentration of 25(OH) D and the prevalence of vitamin D deficiency at 36 weeks PMA. Vitamin D deficiency was defined as serum 25(OH) D concentrations < 20 ng/ml.
Results: Of the 121 infants 72% had deficient vitamin D levels before supplementation. The average 25(OH) vitamin D concentrations at 36 weeks PMA were significantly higher in 800 IU (40 ± 21.4 ng/ml) and 1000 IU group (43 ± 18.9 ng/ml) when compared to 400 IU group (29.4 ± 13 ng/ml). The prevalence of vitamin D deficiency (2.5 vs 22.5; RR: 0.09; CI:0.01–0.74) and insufficiency (30 vs 57.5; RR:0.32; CI:0.13–0.80) was significantly lower in 1000 IU group when compared to 400 IU group at 36 weeks PMA.
Conclusion: 1000 IU/d of vitamin D supplementation in preterm infants ≤ 32 weeks gestation age effectively decreases the prevalence of vitamin D deficiency and leads to higher concentrations of 25(OH) vitamin D at 36 weeks PMA
Trial registration: Clinical Trials.gov: NCT02941185.

1. Introduction

Vitamin D is a fat-soluble vitamin that is either taken by dietary sources or synthesized upon exposure to sunlight [1]. Although major function is on bone metabolism, in recent years other effects of Vitamin D attracted attention. There is evidence that it is involved in regulation of immune system [2,3], lung development [4–6] and differentiation of nervous system [7]. Previous studies demonstrated that low maternal vitamin D has been associated with increased risk of low birth weight, asthma [8] diabetes mellitus [9] and multiple sclerosis [10] in the offspring. Low neonatal vitamin D levels were reported to be associated with increased risk of respiratory distress syndrome (RDS) [11] bronchopulmonary dysplasia (BPD) [12] and sepsis [12,13].

Vitamin D deficiency is very common among infants especially the preterm infants, children, pregnant and lactating women and elderly people [1,14,15]. Serum 25(OH) D concentrations < 20 ng/ml are defined as vitamin D deficiency by most of the authorities. But specific range for neonates is not defined [1,16]. Most of the vitamin D is transferred to the fetus during third trimester and correspondingly preterm newborns especially those with < 32 weeks gestational age born with lower vitamin D stores [17]. Knowing that most of the mothers have insufficient vitamin D levels, preterm newborns born with lower stores, the breast milk is inadequate regarding vitamin D, and the required volume of preterm formulas to achieve sufficient intake of vitamin D is too high, vitamin D supplementation is recommended to infants. But there is no consensus regarding the adequate dose of vitamin D supplementation for preterm infants. The American Academy of Pediatrics recommends supplementation of 200–400 IU/d vitamin D

* Corresponding author at: Zekai Tahir Burak Maternity Teaching Hospital, Department of Neonatology, 06230 Ankara, Turkey.
E-mail address: dr_kalyoncu@hotmail.com (O. Bozkurt).

http://dx.doi.org/10.1016/j.earlhumdev.2017.07.016
Received 8 March 2017; Received in revised form 13 July 2017; Accepted 24 July 2017

for preterm infants [18] And the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends 800–1000 IU/d vitamin D supplementation for preterm infants [19].

We designed this prospective randomized trial to assess the 25(OH) D levels in response to three different doses of vitamin D supplementation (400, 800 or 1000 IU) at 36 weeks PMA in preterm infants born at ≤ 32 weeks gestational age and to determine the optimal dose of vitamin D supplementation to prevent vitamin D deficiency in preterm infants.

2. Methods

This multiple-dose randomized prospective study was conducted at Zekai Tahir Burak Maternity Teaching Hospital between January 2014 and March 2016. Preterm infants with gestational age 24 to 32 weeks admitted to our neonatal intensive care unit and achieved at least 75% of total nutrition by enteral feedings in postnatal 2 weeks were included in the study. Infants with perinatal asphyxia, major congenital or chromosomal anomalies, twin-twin transfusion syndrome, requirement of dopamine ≥ 15 μg/kg/min or more than one inotrope, those with no expectation of survival in first 2 weeks and those that total parenteral nutrition was not ceased by the first 2 weeks were excluded.

Informed consent was obtained from parents before enrollment.

After parental consent, infants were randomly allocated to either of the 3 groups designating oral Vitamin D_3 dose of: 1) 400 IU/day; 2) 800 IU/day; 3) 1000 IU/day by sealed opaque envelopes. Randomization cards were generated using computer generated random number list and concealed in opaque, sequentially numbered, sealed envelopes. The envelopes were opened and each infant was randomized just after achieving 75% of total nutrition as enteral feeding.

After randomization blood sample was drawn for the measurement of serum 25(OH) D, parathyroid hormone (PTH), calcium, phosphorus and alkaline phosphatase (ALP) levels. The intervention was started after blood sampling and continued until 36 weeks PMA. The study drug (Devit-3 Oral Drop, 50,000 IU/15 ml, Deva Company, Turkey) was administered once daily through orogastric tube or orally either directly or mixed with enteral feedings. For those discharged before 36 weeks PMA, the study drug was given by the mother as prescribed daily. If the mother was not in compliance with drug intake instructions, patient was excluded from the study. In our unit, human milk fortifier (Eoprotin, Nutriceal Foods, S.A.) was used in all infants receiving breast milk as a standard procedure providing similar Vitamin D contents in fortified human milk and preterm formula at 160 ml/kg/day enteral feeding. Vitamin D intake was assumed to be 283–320 IU/d from fortified human milk and 288–300 IU/d from preterm formula at 160 ml/kg/d enteral feeding volume for infant weighing 1.5 kg.

For estimation of 25(OH) D and PTH levels 1 ml whole blood was collected just before intervention and at 36 weeks PMA. The samples were centrifuged and collected serum was divided in to 2 aliquots and stored at − 20 °C for subsequent analysis. Serum 25(OH) D levels were determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS, Waters Quattro Premier XE, US). Serum PTH levels were analyzed by using electrochemiluminometric assay (Roche Cobas e601, Roche Diagnostics, US). Serum calcium, phosphorus and ALP levels were analyzed on the same day of sampling by Beckman Coulter assay (Beckman Coulter, Inc.). To assess adverse effects of vitamin D excess urine calcium to creatinine ratio was analyzed at 36 weeks PMA.

Vitamin D deficiency was defined as 25(OH) D < 20 ng/ml. Recommended target level of 25(OH) D is >30 ng/ml. The values of 25(OH) D < 30 ng/ml were defined as insufficiency according to the Endocrine Society's Clinical Guidelines [20,21]. Vitamin D excess was defined as concentrations > 100 ng/ml [22].

2.1. Outcome variables

The primary outcome was the serum concentration of 25(OH) D and the prevalence of vitamin D deficiency at 36 weeks PMA. The secondary outcomes were serum levels of calcium, phosphorus and ALP at PMA 36 weeks and frequency of BPD, clinical or proven sepsis, retinopathy of prematurity (ROP), days on mechanical ventilation, nasal CPAP and oxygen requirement, duration of hospitalization, discharge weight and head circumference.

2.2. Sample size

By ANOVA design, α-error set 0.05 and β-error set at 0.2 and a 30% change in serum 25(OH) D concentration at 36 weeks PMA, the number needed to verify our hypothesis was 37 infants for each arm. For sample size collection mean ± SD of serum 25(OH) D concentration was used as 16 ± 6.5 based on previous data of our unit.

2.3. Statistical analysis

Categorical data were expressed as count and percentage and continuous data were expressed as means, standard deviation, medians, minimum and maximum. Student's *t*-test was used for continuous data that were normally distributed, whereas Kruskal Wallis test was used for skewed data. χ^2 test was used to analyze the categorical data, along with Fischer exact test when applicable. We also calculated the relative risk (RR) with 95% confidence intervals (CI) for categorical variables. All statistics were done using the SPSS for windows software version 17.0 (SPSS Inc., Chicago, IL, USA). A P value < 0.05 was considered significant.

The study was approved by the local ethics committee of the hospital.

3. Results

Between January 2014 and March 2016, 138 infants with gestational age of 24–32 completed weeks were randomized to one of the 3 vitamin D supplementation dose. After intervention 17 infants were excluded for the declared reasons in consort diagram, eventually 121 infants completed the study and a total of 40 infants in the 400 IU, 41 infants in the 800 IU, 40 infants in the 1000 IU groups were analyzed (Fig. 1). The baseline maternal and infant characteristics were similar between 3 groups except that the frequency of multiple birth was higher in 400 IU group (Table 1).

The frequency of vitamin D deficiency (25(OH) D < 20 ng/ml) was 75%, 73% and 68% in the 400, 800 and 1000 IU groups respectively ($p = 0.738$). In total, 72% had deficient vitamin D levels before supplementation. Mean serum 25(OH) D concentrations were 16.8 ± 6.6, 15.2 ± 5.9 and 17.2 ± 7.2 respectively at baseline ($p = 0.53$). Also there were no difference in baseline levels of serum calcium, phosphorus, ALP and PTH between 3 groups (Table 1). The rates of vitamin D supplementation during pregnancy and exclusive breastfeeding were similar between the groups ($p = 0.395$; $p = 0.088$). The median starting day of intervention was on day 9 in all 3 groups.

The average 25(OH) vitamin D levels at 36 weeks PMA were significantly higher in 800 IU (40 ± 21.4) and 1000 IU groups (43 ± 18.9) compared to 400 IU group (29.4 ± 13) ($p = 0.014$; $p \leq 0.001$ respectively). There were no significant difference in average 25(OH) vitamin D levels between 800 and 1000 IU groups at 36 weeks PMA ($p = 0.24$) (Table 2). The rate of vitamin D deficiency was significantly lower in 1000 IU group when compared to 400 IU group (2.5 vs 22.5; RR = 0.09; 95% CI = 0.01–0.74; $p = 0.007$). The rate of vitamin D deficiency was also lower in 800 IU group compared to 400 IU group (9.8 vs 22.5) but the difference was not statistically significant. The rate of infants having vitamin D insufficiency (< 30 ng/ml) was also significantly lower in 1000 IU group compared to 400 IU group (30 vs 57.5; RR = 0.32; 95%CI = 0.13–0.80; $p = 0.013$). There was a tendency for lower rate of vitamin D insufficiency in 800 IU group compared to 400 IU group that did not reach statistical significance