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Research Article

Monitored Supplementation of Vitamin D in Preterm Infants. A Randomised Controlled Trial

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Abstract

Neonatal vitamin D stores at birth are dependent on maternal levels and neonates receive 50–70% of their mother's 25-hydroxyvitamin D [25(OH)D]. The incidence of vitamin D deficiency could be up to 55% in pre term infants weighing <1000 g. The aim of this study was to assess the effectiveness of monitored supplementation of vitamin D in a population of preterm infants.

Methods: Preterm infants born at 27–36 weeks of gestation were recruited within the first 7 days of life. Depending on the type of feeding, and after reaching partial enteral feeding or at 7 days of life, vitamin D supplementation of 400 IU and an additional 150–300 IU/kg was included in human milk fortifiers (if fed exclusively with breast milk) or 190 IU/kg in milk formulas. Subjects were randomised to either monitored supplementation group with an option of dose modification based on 25(OH)D levels as per protocol) or standard therapy up to 40 weeks of Post-Conceptional Age (PCA).

The primary outcome measure is the number of neonates with deficiency or excess levels of 25(OH)D at 40 ±2 weeks of PCA. Additionally, 25(OH)D levels were measured at birth, 4 weeks, 8 weeks of age, and 40 +/- 2 weeks of PCA. Secondary objectives were to note incidence of osteopenia, nephrocalcinosis and nephrolithiasis. Serum parameters of calcium phosphorus and alkaline phosphatase were measured.

Results: The 25 OHD levels in both groups were significantly higher at 40 weeks PCA as compared to the baseline in both groups but levels were statistically more significant in monitored group (group A) ($p < 0.001$). 16 of the 30 infants in group B were noted to have vitamin D deficiency at 40 weeks PCA as compared to 2 in group A which was statistically significant ($p < 0.001$). A statistically significant difference was also recorded in calcium, phosphorous and ALP values between 2 groups at 40 weeks PCA ($P < 0.05$). No infant in the monitored supplementation group depicted metabolic bone disease of prematurity.

Conclusion: The results of our study suggest that monitored vitamin D supplementation in preterm infants significantly improves 25 OHD levels and other metabolic bone parameters and decreases the risk of development of metabolic bone disease in premature infants.

Introduction

The pivotal role of vitamin D in skeletal health is well known. The discovery of receptors for vitamin D in most tissues and cells has provided new insights on its role in non-skeletal health. These actions include among others regulation of cellular proliferation, apoptosis and angiogenesis [1]. Studies have shown that in both term and preterm infants, neonatal vitamin D storage at birth is dependent on maternal 25-hydroxyvitamin D [25(OH)D] because the foetus secures all its vitamin D from the mother. Neonates usually receive 50–70% of their mother's 25-hydroxyvitamin D [25(OH)D] [2–4].

Deficiency of vitamin D in preterm neonates can lead to metabolic bone disease. The incidence of this morbidity can reach up to 55% in infants weighing <1000 g [5]. VDD is also associated with increased risk of respiratory tract infections, Bronchopulmonary Dysplasia (BPD), seizures and growth disturbance in preterm neonates [6–10].

The American Academy of Paediatrics guidelines recommend supplementation of 200–400 IU/day in enterally fed preterm infants [11], whereas according to European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines, supplementation of vitamin D for preterm infants should reach 800–1000 IU/day [12]. Guidelines published for Central Europe recommend preterm infants fed enterally should receive vitamin D supplementation of 400–800 IU/day within the first days of life and continue up to 40 weeks of GA. This should be followed by 400 IU/day [13].

In a randomised, double-blind trial done in North India, investigators enrolled 96 infants (28–32 weeks of GA) into two groups: vitamin D 400 IU/day vs vitamin D 800 IU/day. The primary outcome was prevalence of VDD at 40 weeks of post-conceptional

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age._Secondary outcomes included VDD, bone mineral content and density at 3 months of Corrected Age (CA). The prevalence of VDD (defined as<20 ng/ml) in the 800 IU/day group was significantly lower than in the 400 IU/day group at 40 weeks of PCA (38.1% vs 66.7%, Relative Risk [RR] 0.57, 95% CI 0.37–0.88) and at 3 months of CA (12.5% vs 35%, RR 0.36, 95% CI 0.14–0.90). One infant in the 800 IU/day group had vitamin D hypervitaminosis (>100 ng/ml). This trial did not include infants born ELBW, who are at most risk of VDD, as well as supplementation-induced hypervitaminosis [14].

Researchers in a U.K. trial randomised 100 infants (from 23 to 28 weeks of GA) to three groups: placebo (routine vitamin D supplementation in parenteral and enteral nutrition), additional 200 IU/day or 800 IU. Infants in the 800 IU/day group presented with higher 25(OH)D concentration (p<0.05). The incidence of death, BPD, necrotizing Enterocolitis (NEC) or intracranial haemorrhage did not differ between the study groups. The authors concluded that ESPGHAN recommendations led to increased vitamin D (>50 ng/ml) in many infants, whereas routine intake of an additional 200 IU/day allowed more infants to reach recommended levels [15].

The dosage, safety and effectiveness of vitamin D supplementation in preterm infants remain controversial and clear criteria for adequate 25(OH)D levels in preterm infants have not been established. In view of inconsistent and insufficient data, several authors have suggested that vitamin D supplementation could be monitored in the preterm population [10, 11].

We hypothesise that monitored therapy in preterm infants is more effective and safer than standard therapy in infants given vitamin D supplementation.

Aim and Objectives

Aim

To assess the effectiveness of monitored supplementation of vitamin D3 in a population of preterm infants.

- **Primary objectives**:25 OHD level at 40 weeks' gestation

Secondary objectives:

- Incidence metabolic bone diseases of prematurity.
- Serum parameters of calcium and phosphorus and Alkaline Phosphatase (ALP) at 40 weeks PCA

Material and Methods

Study Design

Case control study

Study Location

SRCC Children's Hospital Mumbai

Study Duration

1 year

Study Population

Inclusion criteria:

- Preterm babies born between 27-36 weeks of gestation

Exclusion criteria:

- Major congenital anomaly
- Cholestasis
- Severe illness deemed incompatible with survival

Sample Size: Approximately 60 (30 in each group)

The sample size was calculated on the basis of the main outcome, defined as the number of neonates with deficient or excess levels of 25(OH)D at 40 ± 2 weeks of PCA. To meet acceptable recruitment rates and to obtain statistically significant results, we chose to detect a decrease of 40% in the number of patients with VDD with a power of 80% and α value of 0.05; hence, 26 infants were included in each study group. In order to account for 20% loss to follow-up, we recruited a total of 60 infants for the study.

Source of Funding for the Study: Institutional.

Methodology

Preterm infants were randomly assigned to a monitored group or a standard group. A blood sample was obtained within the first week of life to assess 25(OH)D levels at birth. At day 7 of life or when reaching 100 ml/day of enteral feeding, all infants received 400 IU of vitamin D with an additional 160 IU/kg of vitamin D included in parenteral nutrition. Infants received supplementation up to 40 weeks of PCA. After full enteral feeding was reached, depending on the type of feeding, vitamin D supplementation of 400 IU and 150–300 IU/kg included in human milk fortifiers (if fed exclusively with breast milk) or 190 IU/kg in milk formulas was initiated. In the monitored supplementation group, vitamin D supplementation dose was appropriately modified on the basis of 25(OH)D levels. An automated quantitative test available at the study site, VIDAS® (bioMérieux, Marcy l'Etoile, France) was used to measure 25(OH)D levels.

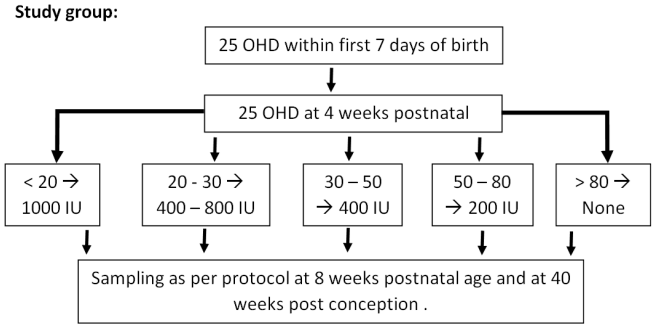
We chose to use the undermentioned stratification of 25 OHD levels to denote deficiency, suboptimal concentration, optimal concentrations, increased level, potentially toxic level and toxic levels of vitamin D for the purpose of the study [17].

- Deficiency: 0–20 ng/ml
- Suboptimal concentration: >20–30 ng/ml
- Optimal concentration: > 30–50 ng/ml
- Increased level: > 50–70 ng/ml
- Potentially toxic level: >70-100ng/ml
- Toxic level: > 100 ng/ml

We decided to define MBD as serum levels of ALP>500 IU and P<1.8 mmol/L. All babies underwent a single point of focus USG at 40 weeks PCA to determine development of osteopenia prematurity. The exact incidence of osteopenia remains unknown, in part owing to the lack of consensus on its definition. Assessment of Average Bone Mass (ABM) was done using quantitative ultrasound (Sunlight PREMIER 7000; Beam Med, Petah Tikva, Israel). This safe, non-invasive, radiation-free and easy-to-use method has been suggested as a screening tool for detecting osteopenia in premature infants [21-23]. With placement of a small ultrasound probe (CRB probe RoHS 900–

1000 kHz) along the mid-tibia, this device measures Speed of Sound (SOS) in meters per second in the axial transmission mode. High intra-individual variation does not allow definition of normal values. However, in a recently published study, preterm infants (24–28 weeks of GA) examined at 40 weeks of PCA showed significantly lower SOS than term infants [22]. Two previously trained neonatologists blinded to group allocation assessed the ABM in each enrolled patient at 40 weeks of PCA. The measurements were made on the tibia. The mid-tibial shaft length was determined by measuring the distance from the knee to the heel. The probe was placed over the medial aspect of the mid-shaft tibia to obtain an SOS measurement. Three measurements were performed. The mean value of these measurements was used for the data analysis [16].

Study group:



Control group: 400 IU, additional 150-300 IU/ kg in HMF if breastfed, 190 IU/ kg in formula fed babies.

Results

There was no significant difference in mean gestational age between group A (31.89 weeks) and group B (30.99 weeks) and in 25 OHD levels at birth. There was statistically significant difference in the mean birth weight between the two groups (p value=0.003). (p value = 0.79).

Baseline Characteristics

Baseline Characteristics			Group A (N=30)	Group B (N=30)	P value
Gestational Age		Mean ± SD	31.89 ± 2.05	30.99 ± 2.47	0.127[a]
Gestational Weight			1977.77 ± 501	1545.77 ± 558.1	**0.003[a]**
Gender	Males	Number (Percentage)	17 (56.7)	20 (66.7)	0.426[b]
	Females		13 (43.3)	10 (33.3)	
25 OHD levels At birth		Median (IQR)	13.85 (9.07 - 23.05)	13.85 (10.15 - 20.87)	0.79[c]

The 25 OHD levels in both groups were significantly higher at 40 weeks PCA as compared to the baseline in both groups but levels were statistically more significant in monitored group (group A) (p <0.001). 16 of the 30 infants in group B were noted to have vitamin D deficiency at 40 weeks PCA as compared to 2 in group A which was statistically significant (p <0.001). However, osteopenia of prematurity was noted in only 10 % infants in group B which was statistically insignificant (p value = 0.237)

Comparison of Vitamin D Levels at 40 weeks

		Group A (N=30)	Group B (N=30)	P value
Vitamin D Levels	Mean ± SD	49.14 ± 12.73	28.12 ± 8.8	**<0.001[a]**

Other Investigations

Investigations		Group A (N=30)	Group B (N=30)	P value
Calcium	Mean ± SD	9.85 ± 0.69	8.94 ± 0.82	**<0.001[a]**
Phosphorus		6.17 ± 0.57	5.79 ± 0.36	**0.003[a]**
ALP		347.73 ± 87.6	546.87 ± 177.11	**<0.001[a]**

A statistically significant difference was also recorded in calcium, phosphorous and ALP values between 2 groups at 40 weeks PCA (P <0.05)

Metabolic bone disease of Prematurity

Metabolic Diseases of Prematurity		Group A (N=30)	Group B (N=30)	P value
Vitamin D Deficiency	Number (Percentage)	2 (6.7)	16 (53.3)	**<0.001[b]**
Suspected OOP		0 (0)	3 (10)	0.237[b]

Statistical Analysis

The Statistical analysis was performed by SPSS 23.0 version. Continuous variables were described as mean and variation of each observation from the mean value (Standard deviation) represented as mean ± SD (analysed using unpaired Student T test) or median (Interquartile range) if they failed to follow normal distribution (analysed using Mann-Whitney U test). Categorical variables were described by taking percentage and analysed using Chi square test of Fischer's Exact T test. Paired data was analysed using Paired Student T test if they followed normal distribution or Wilcoxon Signed Rank test if they fail to follow normal distribution. Variables with p value <0.05 was considered as statistically significant.

Discussion

The need for vitamin D supplementation in both term and preterm infants is widely acknowledged [11–13]. As 80% of placental transfer of calcium & phosphorous occurs between 24 and 40 weeks of gestation, preterm infants are particularly prone to adverse effects of vitamin D insufficiency. The results of our study may contribute to optimising vitamin D supplementation.

In both term and preterm infants, neonatal vitamin D stores at birth are dependent on maternal 25-hydroxyvitamin D [25(OH)D] as the foetus secures all its vitamin D from the mother. Neonatal 25(OH) D levels are approximately 50–70% of maternal vitamin D levels.[18-20]

Preterm infants often have low vitamin D stores due to decreased transplacental transfer from deficient mothers and tend to have a higher requirement of the same [24]. Most of the transplacental transfer of vitamin D occurs during the 3rd trimester, thus increasing the risk of vitamin D deficiency and bone mineral disease in preterm infants.

In our study we noted that at 40 weeks PCA, infants in the monitored dosing group had a significantly better 25OHD levels as compared to infants on standard dosing. Additionally better calcium, phosphorous and alkaline phosphatase levels were noted among infants in the monitored group. We also noted a 10% prevalence of osteopenia of prematurity in the standard therapy group during our study period as compared to about 16-40% in previous studies.

Natrajan CK et al [14] compared the effect of 800 vs 400 IU of daily oral vitamin D3 on the prevalence of Vitamin D Deficiency (VDD) at 40 weeks' Postmenstrual Age (PMA) in preterm infants of 28 to 34 weeks' gestation. Their study concluded that daily supplementation

with 800 IU of vitamin D reduces the prevalence of VDD at 40 weeks' PMA and at 3 months' CA in preterm infants without showing any improvement in bone mineralization. However, they did notice an increased risk of higher levels of 25 OHD with daily supplementation of 800IU among preterm infants. In our study, with monitored dosing there was no risk of vitamin D toxicity noted as the dosing was titrated based on serum vitamin D3 levels at regular intervals.

In a double-blind controlled trial by Tergestina et al,[25] vitamin D supplementation of 1000 vs. 400 IU in 99 preterm infants was analyzed (27–34 weeks of GA). At 40 weeks of PMA, the 1000 IU group had a significantly higher average of 25(OH)D levels (47 ng/mL vs. 17 ng/mL, $p < 0.001$) as compared to the 400 IU group.

To the best of our knowledge, there are very few randomised studies evaluating monitored supplementation using measurement of 25(OH) D concentrations. The results of our study suggest that monitored vitamin D supplementation in preterm infants significantly improves 25 OHD levels and other metabolic bone parameters and decrease the risk of development of metabolic bone disease in premature infants.

Conclusion

In preterm infants of 27-36 weeks' gestation with deficient 25 OHD levels at baseline, we found significant difference in 25 OHD levels at 40 weeks between groups receiving monitored supplementation of vitamin D3 versus the group receiving standard supplementation, suggesting the benefit of monitored supplementation of vitamin D in preterm infants.

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