Chapter

Trends in Plasma Vitamin D Levels among Exclusively Breastfed Preterm Infants

Victor Ayodeji Ayeni, Tinuade Adetutu Ogunlesi, Iyabode Olabisi Florence Dedeke and Olatunbosun Oladipupo Olawale

Abstract

Exclusively breastfed preterm babies are dependent almost entirely on the available vitamin D content of breast milk for their metabolic and physiologic needs. The breast milk content of vitamin D correlates with the serum levels of vitamin D in the mother. This study investigates the trend in plasma vitamin D levels of preterm neonates who are exclusively breastfed between birth and 10 weeks of life. The proportion of mothers with low plasma vitamin D levels at birth was 41.7%, while it was 50% in the preterm babies. The plasma vitamin D levels in infants showed a strong positive correlation with maternal plasma levels at birth. By the 10th week postnatal age, the proportion of mothers with low plasma vitamin D had reduced slightly to 39.6%, but the proportion of babies with low plasma vitamin D had increased significantly to 81.3% while being fed exclusively on breast milk. It is, therefore, recommended that pregnant women should have vitamin D supplementation and that babies delivered preterm should have vitamin D supplementation.

Keywords: preterm infant, vitamin D, exclusive breastfeeding, Nigeria, pregnant women

1. Introduction

Sources of vitamin D in early infancy include transplacental transfer, breast milk, and cutaneous production through sunlight exposure. The maternal vitamin D status significantly influences the amount of vitamin D transported across the placenta during foetal development, consequently affecting the vitamin D reserves at birth [1, 2]. The vitamin D content in human milk typically ranges between 12 and 60 U/L and is generally insufficient to meet the physiologic needs of the growing infant [1]. After delivery, infants who are breastfed, therefore, depend primarily on cutaneous synthesis, which tends to be low due to limited sun exposure, to maintain adequate

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vitamin D levels. The half-life of 1,25-dihydroxyvitamin D is only 4 hours, so it does not reflect the vitamin D stores in the body [3]. On the other hand, the half-life of 25-hydroxyvitamin D is estimated to be approximately 2 to 3 weeks [4]. This suggests that 25-hydroxyvitamin D is both more reflective of stores in the body and also more likely to be related to an infant's postnatal nutrition when assayed after 9 weeks postnatal age rather than what the baby acquired *in utero.*

Current evidence does not affirm that the metabolism of vitamin D in preterm infants significantly differs from that in term infants, particularly with respect to the intestinal absorption of vitamin D, as well as its 25- and 1α-hydroxylation [5]. The vitamin D3 levels in human breast milk are low, and only minimal amounts of 25-hydroxyvitamin D are transferred from maternal circulation to breast milk [6]. Additionally, the vitamin D content of breast milk is correlated with the serum levels of vitamin D in the mother [7]. Furthermore, several studies have shown that the level of vitamin D in the newborn depends on the level of vitamin D in the mother while pregnant, with possible deleterious effects on the pregnancy outcome and growth of the newborn [8–11].

Preterm delivery constitutes about 12% of all births in Nigeria [12], and with the wide adherence to the tenets of the Baby Friendly Initiative (BFI), which prescribes exclusive breastfeeding, irrespective of the length of gestation of the infant, unless non-exclusive breastfeeding is medically indicated [13, 14], it is essential to examine the perinatal maternal vitamin D status, and the pattern of vitamin D among Nigerian preterm infants, who are exclusively breastfed. According to the recommendations provided by the World Health Organisation (WHO) for the optimal feeding of low-birth-weight infants, the evidence for feeding babies exclusively on breastmilk is strong for low- and middle-income countries. However, the evidence supporting supplementation with substances other than breast milk (including vitamin D) is weak [15]. In contrast, some studies in Caucasian and Asian countries [16–18] suggest that the supplementation with vitamin D is beneficial in preterm and low-birth-weight babies. The study, therefore, aims to determine the trend in mean plasma levels of vitamin D from birth to 10 weeks postnatal age in a Nigerian population of exclusively breastfed preterm infants while comparing these with plasma levels in their corresponding mothers at birth and at 10 weeks postnatal age.

2. Methods

2.1 Study design

This was a hospital-based panel study that took place in three hospitals within Ogun State, southwest Nigeria. The study sites were Olabisi Onabanjo University Teaching Hospital, Sagamu, Federal Medical Centre, Abeokuta, and Sacred Heart Hospital, Abeokuta, all in Ogun State. All three hospitals were designated centres for specialised obstetric and paediatric care.

2.2 Study population, sampling, and eligibility criteria

The study subjects included singleton preterm neonates born before an estimated gestational age (EGA) of 37 completed weeks and aged between 0 and 24 hours in any of the study sites, as well as their paired mothers. The exclusion criteria included milk feeding prior to presentation at the study sites, maternal diabetes, maternal prolonged use of anticonvulsant drugs, tocolytic agents and magnesium sulphate in pregnancy and perinatally, presence of gross malformations of the digestive tract and central nervous system and any other severe illness, which precluded oral feeding in the infant. The studied infants were the number of infants who were eligible for follow-up for vitamin D levels at 10 weeks, from a larger study. The rest of the recruited baby in the larger study had died (19%), were erroneously fed artificial milk (6.6%), were lost to follow-up (19%) or were given medications that could alter vitamin D metabolism (15.7%), by the time of follow-up at 10 weeks postnatal age. This is shown in **Figure 1**. The mothers and their babies were consecutively recruited at the Postnatal Ward in the Maternity Unit of each of the hospitals (for in-born babies), as well as the Neonatal Unit (for out-born babies).

2.3 Data collection process

Data were collected between April 2018 and February 2020. A research proforma in the form of a structured questionnaire was used for the study. The documented data included maternal age, maternal parity, last confinement, mode of feeding following birth, history of medications used during pregnancy, and perinatally, age and sex of the baby. The body weight, body length, and occipitofrontal circumference (OFC) of the babies were taken at each contact. The body weight was measured to the nearest 10 g, while the recumbent body length and occipitofrontal circumference were measured to the nearest 0.1 cm [19]. The EGA by Ballard Scoring was determined for each neonate, using the New Ballard Scoring system [20].

Contacts were made with the subjects within the first 24 hours of life and at the 10th week of postnatal age. All recruited infants received 0.3 ml/day of multivitamin drops, which contained 200 IU of ergocalciferol, in accordance with the unit protocols at the study sites and as ethically recommended. The parents were counselled on exclusive breastfeeding, using the method prescribed by the managing physicians (either direct suckling only or direct suckling with supplemental expressed breast milk).

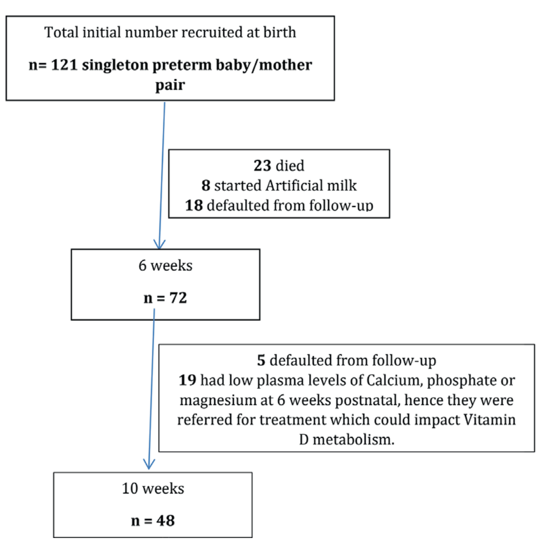

Figure 1.
Flow chart of selected babies.

2.4 Blood sample collection, storage, and analysis

At both contacts, 2 millilitres of venous blood samples were taken from each mother and infant for the laboratory assay of plasma vitamin D. The blood sample was promptly taken to the laboratory and was centrifuged at 2000–3000 g for 15 minutes, to separate the plasma from the cells, within 1 hour of sample collection [21]. The plasma was then decanted into a clean serum bottle and transported/stored at a temperature of −20°C. The assay was performed using a solid phase 25-OH vitamin D Total ELISA (Enzyme-Linked Immunosorbent Assay) Kit manufactured by Demeditec Diagnostics®, GmbH, Germany. The reference range for normal plasma levels of 25-OH vitamin D was 30–100 ng/ml.

2.5 Data management

The data were analysed, using the Statistical Package for the Social Sciences (SPSS) 26.0 software (IBM Corp). Analyses were done using descriptive and inferential statistics. Mean values of continuous variables were compared using the *Student's t-test* or Analysis of variance *(ANOVA).* Proportions of continuous variables were compared using the *Chi-Square* test. Correlation analysis (*Pearson's r or Spearman's rho*) was used to describe relationships between continuous variables. Statistical significance was determined using *p*-values less than 0.05.

2.6 Ethical considerations

Ethical approval was obtained from the Health Research Ethics Committees of Olabisi Onabanjo University Teaching Hospital, Sagamu (OOUTH/HREC/119/2017); Federal Medical Centre, Abeokuta (FMCA/47/HREC/10/2017); and Sacred Heart Hospital, Abeokuta (SHH/EC/EA/003/08/17). The mothers/—caregivers of all eligible participants were given detailed information about the study. Written informed consent was obtained from the mothers/caregivers present at the point of enrolment into the study.

3. Results

A total of 48 mother-preterm infant pairs recruited were followed up for 10 weeks. The babies' birth weights ranged from 0.68 to 3.31 kg, with a mean of 1.997 ± 0.571 kg. The mean length at birth was 43.0 ± 4.1 cm, with a range of 32.9–51.0 cm. The occipitofrontal circumference at birth ranged from 22.5 to 35.0 cm, with a mean of 30.3 ± 3.0 cm. The EGA ranged from 26 to 36 weeks, with a mean of 33.3 ± 2.3 weeks and a median of 34 weeks. There were slightly more males, 25/48 (52.1%). The ages of the mothers ranged from 21 to 45 years, with a mean of 31.2 ± 5.7 years. Parity at the time of recruitment ranged from one to six (1 to 6). Mothers whose last confinement fell within 2–10 years had the highest frequency of 27 (56%), and primiparous mothers were 14 (29.2%).

3.1 Plasma vitamin D levels at birth

The mean plasma vitamin D level in the mothers was 33.26 ± 11.79 ng/mL (range = 2.69–78.20 ng/mL), and hypovitaminosis D was recorded in 20/48 (41.7%)

of the mothers (**Figure 1**). The mean plasma vitamin D level at birth in the babies was 30.95 ± 11.11 ng/mL, with a range of 8.70–59.96 ng/mL. Twenty-four (50%) babies had low levels of vitamin D (**Figure 2**). There was a moderate positive correlation between maternal plasma vitamin D levels at birth and their corresponding neonatal substrate levels at birth (r = 0.612; $p < 0.001$).

Most of the infants of mothers who had normal plasma vitamin D levels at birth also had normal plasma vitamin D, while most of the infants of mothers who had low plasma vitamin D levels at birth also had low plasma vitamin D ($\chi^2 = 16.800$; $p < 0.001$).

3.2 Plasma vitamin D levels at 10 weeks

At 10 weeks, the mean plasma vitamin D in the mothers was 32.72 ± 8.27 ng/mL, with a range of 17.80–58.73 ng/mL. Plasma vitamin D levels were low in 19/48 (39.6%) of the mothers (**Figure 3**). The mean plasma vitamin D in the infants at 10 weeks age was 23.56 ± 8.64 ng/mL with a range of 3.40–47.13 ng/mL. Plasma vitamin D levels were low in 39/48 (81.3%) babies (**Figure 3**). There was no significant relationship between the categories of plasma vitamin D in the infant at 10 weeks when compared with the category of corresponding maternal plasma vitamin D at 10 weeks ($\chi^2 = 0.109$; p = 0.741).

3.3 Comparison of demographic characteristics of the mothers and babies in relation to vitamin D levels

Table 1 shows the distribution of maternal vitamin D levels in mothers at birth in relation to their educational level and ages, while **Table 2** shows the distribution at 10 weeks. The difference was not statistically significant in the categories. **Table 3** shows the babies' plasma vitamin D levels in relation to the babies' sex at birth and at 10 weeks. The sex had no significant association with the category of vitamin D levels both at birth and at 10 weeks postnatal ages.

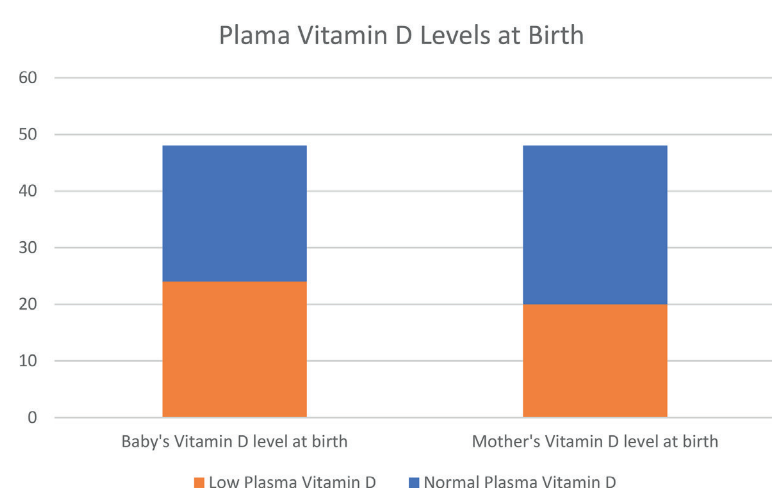

Figure 2.
Plasma vitamin D levels in the babies and mothers at birth.

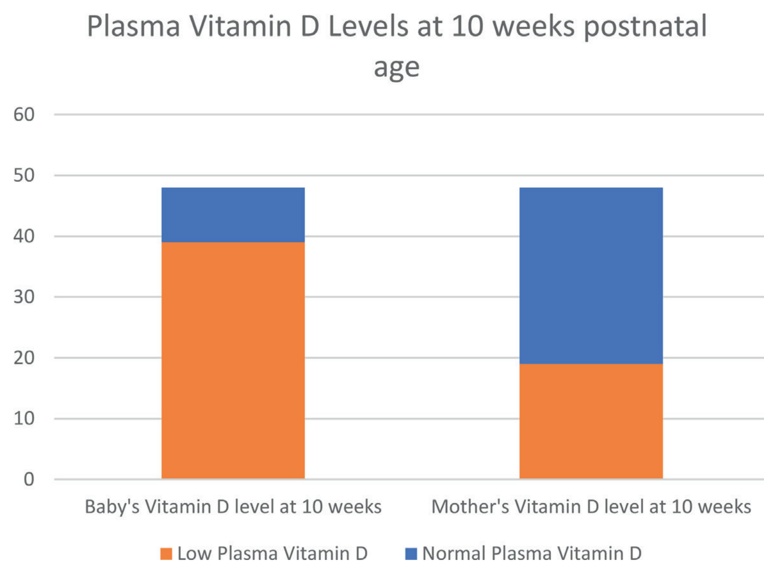

Figure 3.
Plasma vitamin D levels in the babies and mothers at 10 weeks postnatal age.

Characteristic	Low maternal vitamin D (%)	Normal maternal vitamin D (%)	Chi-Square	P-value
Educational Level			1.317	0.251
Primary & Secondary	6 (31.6)	13 (68.4)		
Post-Secondary	14 (48.3)	15 (51.7)		
Maternal Age			0.148	0.701
20–35 years	14 (40.0)	21 (60.0)		
> 35 years	6 (46.2)	7 (53.8)		

Table 1.
Plasma vitamin D categories in the mothers at birth in relation to maternal sociodemographic characteristics.

Characteristic	Low maternal vitamin D (%)	Normal maternal vitamin D (%)	Chi-Square	P-value
Educational Level			0.084	0.772
Primary & Secondary	8 (42.1)	11 (57.9)		
Post-Secondary	11 (37.9)	18 (62.1)		
Maternal Age			0.322	0.571
20–35 years	13 (37.1)	22 (62.9)		
> 35 years	6 (46.2)	7 (53.8)		

Table 2.
Plasma vitamin D categories in the mothers at 10 weeks in relation to maternal sociodemographic characteristics.

Characteristic	Low vitamin D (%)	Normal vitamin D (%)	Chi-Square	P-value
At birth				
Female	10 (43.5)	13 (56.5)	0.751	0.386
Male	14 (56.0)	11 (44.0)		
At 10 weeks				
Female	18 (78.3)	5 (21.7)	0.259	0.611
Male	21 (84.0)	4 (16.0)		

Table 3.
Plasma vitamin D categories in the babies at birth and at 10 weeks in relation to the sex of the babies.

Variables	Pearson coefficient (r)	p-value
Maternal vitamin D at birth and Maternal vitamin D at 10 weeks	0.245	0.094
Baby's vitamin D at birth and Baby's vitamin D at 10 weeks	0.279	0.054
Maternal vitamin D at 10 weeks and Baby's vitamin D at 10 weeks	−0.070	0.636

Table 4.
Correlations between plasma vitamin D levels at birth and 10 weeks among infants and their mothers.

3.4 Comparison of mean plasma vitamin D levels at birth and at 10 weeks

Table 4 shows weakly positive and insignificant correlations between maternal plasma vitamin D levels at birth and at 10 weeks, as well as between the babies' plasma vitamin D levels at birth and at 10 weeks. Similarly, the correlation between plasma vitamin D levels in infants and mothers at 10 weeks was negative, weak, and insignificant. There was no significant relationship between the categories of plasma vitamin D in the infant at 10 weeks when compared with the category of corresponding maternal plasma vitamin D at birth (χ^2 = 0.031; p = 0.854). Similarly, the relationship between categories of plasma vitamin D in the babies at birth and at 10 weeks was not significant (χ^2 = 0.137; p = 0.712).

4. Discussion

Fifty percent of the babies in the present study had low plasma vitamin D at birth. The finding on neonatal plasma vitamin D in the present study is also similar to that reported earlier by Terek et al. in Turkey [22], in which about half of the babies had low vitamin D levels. However, a study in India [23] found a vitamin D deficiency prevalence of 92% in preterm neonates, using the cutoff of 30 ng/dL used in this study. The Indian study did not include maternal vitamin D levels, which have been shown to have a direct correlation with the baby's vitamin D levels [11, 24]. Hence it would be difficult to explain the marked variation without also considering the maternal values.

The mean neonatal plasma vitamin D at birth was significantly lower than the mean maternal plasma levels of vitamin D in the present study. The relative disparity in the levels of low vitamin D in the neonates, accords with the findings in an

Australian study by Panda et al. [25], This may be simply related to the normal physiology in foetal life requiring a relatively low parathormone and a low 1,25-dihydroxyvitamin D for optimal bone mineralisation *in utero* [5, 26]. On the other hand, the mean neonatal plasma vitamin D levels had a direct positive correlation with the maternal levels, as previously established in previous studies [11, 24].

The low neonatal plasma vitamin D levels found in this study are a direct reflection of the maternal vitamin D levels, and this accords with previous reports [1, 2, 8, 25, 27]. Vitamin D deficiency is known to be common in pregnant women and breastfed infants, despite the use of vitamin supplementation in pregnant women [28], with negative implications for both pregnancy outcomes and infant growth and development [29, 30].

The present study showed that there was no correlation between plasma vitamin D at 10 weeks and plasma vitamin D at birth. This is to be expected because the half-life of plasma 25-hydroxyvitamin D is only 3 weeks [4], so the plasma vitamin D levels at 10 weeks could be adjudged to be dependent on the available dietary sources, which is breast milk in this cohort of babies. It should be noted that the babies in this study were given a supplement of daily 200 IU of vitamin D, according to the standard protocols in the hospitals at the time of the study, but this did not significantly improve the vitamin D levels. Conversely, the proportion of babies with low plasma vitamin D increased to four-fifths at 10 weeks postnatal age, from only half at birth, despite supplementation with a daily dose of 200 IU of vitamin D! This suggests that not only is there the need for supplementation in the Nigerian population studied, but also that the optimal doses should be more than 200 IU. This aligns with the doses of 400 IU or higher for vitamin D supplementation in preterm neonates highlighted in previous studies on neonates in Asia, Europe, North America, and Australia/New Zealand [16–18]. Different studies, specifically in preterm neonates, have reported varying recommendations. Some studies carried out in Republic of Ireland and Turkey [31, 32] suggest that supplementing with 400 IU of vitamin D daily achieved sufficient levels of 25 hydroxyvitamin D in infants. On the other hand, some trials conducted in India [16, 33] reported that doses of 1000 IU daily of oral vitamin D are more effective to achieve optimal levels.

Furthermore, it is important to pay attention to the reducing levels of vitamin D in preterm neonates if they are given only breastmilk without oral vitamin D supplementation at optimal quantities. Supplementation with vitamin D in deficient infants has been known to reduce the risk of respiratory infections and autoimmune diseases [34], protect against nutritional rickets [17], and improve the growth of preterm neonates [18]. These effects could not be investigated in this cohort of preterm neonates because of the small sample size and early follow-up period. Nonetheless, in the face of WHO recommendations for low- and middle-income countries about the need for supplementation or not for exclusively breastfed preterm infants [15], the findings from this study are a wakeup call for more high-powered studies to investigate the long-term consequences of non-supplementation of breastmilk in preterm babies. The study also highlights the need to conduct trials to determine the optimal dosing regimen of enteral supplemental vitamin D to maintain adequate plasma levels of vitamin D in babies who are delivered preterm in the population studied. The limitations of the present study include its small sample size and short follow-up duration.

5. Conclusion and recommendations

Fifty percent of the singleton preterm babies had low plasma levels of vitamin D at birth. There were strongly positive correlations between maternal and neonatal

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plasma vitamin D levels at birth. The proportion of babies with low vitamin D levels increased to 81.3% by 10 weeks while on exclusive breastfeeding and supplementation with 200 IU of vitamin D daily. However, the proportion of mothers with low vitamin D reduced slightly, meaning that the worsening vitamin D levels in the babies were due to inadequate quantities from the breastmilk source. Hence, routine supplementation of vitamin D and preterm infants who are exclusively breastfed is recommended at doses greater than 200 IU per day. Further studies are required to determine optimal quantities of vitamin D to give as a supplement for pregnant women and preterm neonates in the population studied.

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Conflict of interest

The authors declare no conflict of interest.

List of abbreviations

BFI	baby friendly initiative
EGA	estimated gestational age
ELISA	enzyme-linked immunosorbent assay
OFC	occipitofrontal circumference
SPSS	statistical package for the social sciences
WHO	world health organisation

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