RESEARCH PAPER

Vitamin D Levels in Neonates With and Without Seizures: A Single Center Cross-Sectional Study

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Received: December 28, 2020; Initial review: February 13, 2021; Accepted: May 13, 2021.

Objective: To study the serum vitamin D levels in neonatal seizures and vitamin D status of the mothers whose babies had vitamin D deficiency. **Methods**: For this cross-sectional study, vitamin D levels were studied in term and late preterm neonates admitted to NICU with seizures at our tertiary care center. Controls were term and late preterm healthy neonates admitted in the postnatal ward with the mothers in the same center. **Results**: 30 cases and 30 controls were enrolled. The mean (SD) serum vitamin D was 19.33 (7.76) ng/mL among cases and 16.83 (6.74) ng/mL among controls (P=0.18). We tested maternal vitamin D levels in babies with seizures and low vitamin D levels. The mean (SD) serum vitamin D level among these mothers (n=11) was 13.25 (6.17) ng/mL. ***Conclusions***: There was no statistically significant association between serum vitamin D levels and seizures among neonates in our study.

Keywords: *Hypocalcemia, Neonates, Seizures, Vitamin D*.

Trial registration: CTR1/2018/12/023028

Published online: May 20, 2021; ***PII***:S097475591600330

The incidence of neonatal seizures is 2-4 per 1000 live births. Seizures may be due to electrolyte abnormalities, underlying brain injury, or initial presentation of an underlying inborn error of metabolism [1,2]. Early recognition and management of biochemical disturbances in neonatal seizures are important to prevent further brain damage. Neonates with hypocalcemia may present with seizures secondary to increased excitability of the cell membrane, thus resulting in exaggerated startles, jitteriness, myoclonic jerks, and seizures [2]. Hypocalcemia due to vitamin D deficiency constitutes a major cause of neonatal seizures in developing countries [3]. Maternal vitamin D deficiency results in a poor trans-placental transfer of vitamin D during pregnancy and reduced stores in the newborns [4,5]. Neonates born to vitamin-deficient mothers are at a significantly higher risk to develop hypocalcemic seizures [6]. In addition to hypocalcemic seizures, some neonates with idiopathic seizures also have low vitamin D levels [5].

During early infancy, vitamin D stores depend on intrauterine accretion and breastmilk, in addition to sunlight exposure in the mother. Breastfed neonates born to and nursed by vitamin D deficient mothers have low serum vitamin D levels [7]. We compared vitamin D levels in neonates with seizures with those without seizures, and also studied vitamin D levels among mothers whose babies had seizures and low vitamin D levels.

METHODS

This was a cross-sectional study done from November, 2018 to August, 2020 in a tertiary care center in southern India, after institutional ethics committee clearance.

Term and late preterm (35-40 weeks) neonates admitted to the neonatal intensive care unit (NICU) of our institute with seizures were enrolled as cases. Controls were healthy term and late preterm neonates admitted in the postnatal ward along with their mothers. Exclusion criteria were: neonates with congenital anomalies, meningitis, hypoglycemia, birth asphyxia, or inborn errors of metabolism; neonates with mothers having hepatic, renal, or bone disorders, mothers on enzyme-inducing drugs and COVID-positive neonates; and neonates with vitamin D supplementation or neonates who were administered antiepileptic drugs before admission.

Blood was drawn for 25-hydroxy vitamin D levels (25(OH)D) from enrolled neonates admitted with seizures. The mothers' vitamin D levels were also evaluated in those neonates with seizures who had vitamin D deficiency. Controls were evaluated for vitamin D levels during day 3-7 investigations like serum bilirubin levels.

Informed consent was obtained from both parents. A detailed antenatal, intranatal and postnatal history was taken in a pre-designed proforma. Whether mothers had received antenatal calcium and vitamin D supple-

mentation and compliance history was also taken. Baseline anthropometry was carried out for all neonates at admission. All babies with seizures who satisfied the inclusion criteria were examined at admission with a detailed examination of the central nervous system. Clinical details of the witnessed seizure episode were noted and details at the time of first seizure and the type of seizure were noted. The neonatal seizures were classified as per Volpe classification into subtle, multifocal tonic, focal clonic, focal tonic, and myoclonic.

Blood samples of all neonates with seizures included in the study were sent for vitamin D levels, calcium, and magnesium along with other investigations like sepsis screen (to rule out septicemia), ammonia, lactate, pyruvate, ABG, TMS/GCMS (to rule out IEM), blood glucose, ionized calcium and total calcium, and serum albumin. Blood samples were taken immediately after seizures and before administration of any specific treatment. Second-line investigations were done in cases, as and when indicated. These included electro-encephalography (EEG), cerebrospinal fluid analysis, and neurosonogram/magnetic resonance imaging. Serum vitamin D estimation was done by electro-chemiluminescence immunoassay.

Serum vitamin D concentrations >20 ng/mL was considered as sufficient, between 12-20 ng/mL as insufficient and <12 ng/mL as deficient [7]. Neonatal hypocalcemia was defined as a total serum calcium concentration of <7 mg/dL or an ionized calcium concentration of <4 mg/dL (1 mmol/L) [8].

Statistical analysis: Statistical analysis was done by using the statistical package for social sciences (SPSS) version 20. Mann-Whitney *U* test was used to compare median vitamin D levels among cases and controls. Comparison of mean values was done by paired and unpaired Student *t*-test and chi-square test. *P* value <0.05 was considered significant.

RESULTS

A total of 91 babies with seizures were admitted to the NICU during the study period, of which 61 were excluded. Thirty neonates were enrolled as controls. Baseline characteristics of cases and controls were not significantly different (**Table I**).

Based on the semiology, the most common seizures were multifocal clonic type (*n*=9), followed by focal clonic (*n*=7). Mixed type and subtle seizures were seen in six neonates each, and tonic and myoclonic types in one each. Based on the etiology, idiopathic seizures were the most common (*n*=21) followed by hypocalcemic seizures (*n*=6).

The serum vitamin D levels were higher in cases than the controls (*P*=0.18); although, both groups had levels in the insufficient range (15-20 ng/mL) (**Table I**). There were 16 neonates with seizures with low vitamin D levels (<20 ng/mL). Out of which, five mothers' samples could not be done as they were not willing and/or were not admitted to the same Institute as the babies were outborn. The mean (SD) serum vitamin D levels of remaining mothers (*n*=11) was 13.25 (6.17) ng/mL and the mean serum vitamin D levels of their babies (*n*=11) was 13.04 (4.55) ng/mL. There was no significant association (*P*=0.84) between maternal and neonatal vitamin D levels.

There was no significant association (*P*=0.18) between onset of seizures (within and beyond 72 hours) and vitamin D levels. Levels of vitamin D were low among neonates with hypocalcemic seizures but it was not statistically significant [16.17 (8.79) vs 20.91 (6.88); P=0.11]. Among the cases, EEG was done in 19 babies. Out of the 19 EEGs, only three were abnormal.

DISCUSSION

We found that majority of mother-neonate pairs in this study had low vitamin D levels. Vitamin D levels were low in both cases and controls, with no significant association of low vitamin D level in neonates and occurrence of seizures.

Vitamin D levels were low in controls probably because low birthweight and late preterm babies were more among controls than cases, and mean birth weight was less among controls than cases. Mean vitamin D levels among the mothers whose babies had low vitamin D levels were also low. Possibly mothers in this part of the country have low vitamin D levels as previously also reported [10], which did not improve even after antenatal vitamin D

Table I Baseline Characteristics and Serum Vitamin D Levels in Neonates With Seizures and Controls

Characteristics	*Cases (n=30)*	*Controls (n=30)*
Birthweight, g[a]	3083 (567)	2896 (488)
Low birthweight	5 (17)	7 (23)
Gestational age, wk[a]	38.13 (1.3)	37.5 (1.1)
Male gender	19 (63)	15 (50)
Late preterm	4 (13)	6 (20)
Maternal age, y[a]	29.9 (5.1)	28.9 (4.3)
Cesarean section	18 (60)	21 (70)
Vitamin D levels, ng/mL[b]	19.17 (14.3-21.9)	15.38 (12.3-19.9)

All values in no. (%) except [a]mean (SD) or [b]median (IQR). P>0.05 for all comparisons. All mothers received vitamin D and calcium supplementation during pregnancy.

WHAT THIS STUDY ADDS?

- There was no association of neonatal seizures and vitamin D deficiency.

supplementation. Aparna, et al. [11] also reported that vitamin D deficiency was highly prevalent among pregnant women, lactating mothers, neonates, and/or exclusively breastfed infants. Increasing the dose of antenatal vitamin D supplementation may be considered, if similar findings are seen in larger community-based studies.

Previously, one-third of infants have been reported to have vitamin D levels <10 ng/mL [10]. Fetal and newborn concentrations of 25 (OH) D depend on and correlate with maternal serum levels. Thus, newborns of vitamin D-insufficient mothers are at a greater risk of developing vitamin D deficiency [12]. Although there was no significant difference in vitamin D levels among the two groups in this study, it suggests that vitamin D levels are low among the normal neonate population in India and the mothers. In a similar study conducted by Singh, et al. [13], it was found that 85.7% of the neonates were vitamin D-deficient. Other studies [11,14] also show that majority of the neonates have vitamin D deficiency even in tropical climates.

The study population was small and selective because we did not include extreme, early, and moderate preterm babies as seizures are less common in these babies and we would not get matched controls. This study needs to be done in a larger sample to find the status of vitamin D levels among mothers and babies in the Indian population.

We conclude that hypovitaminosis D in mothers is also associated with hypovitaminosis D in neonates. There is a need to assess the vitamin D status of pregnant and lactating women and to consider routine vitamin D supplementation or to increase the dose of vitamin D supplementation among pregnant and lactating women in this region. Routine vitamin D supplementation among healthy newborns also need to strengthened.

Ethics clearance: Institutional ethics committee of KMC; No. 779/2018 dated November 13, 2018.
Contributors: JP, SJA, AB: conceptualization, methodology; JCR: data collection, analysis; JP: original draft preparation. AB, SJA, AV: correction of draft and final preparation. All authors approved the final manuscript.
Funding: None; *Competing interest*: None stated.

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