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Paediatrics Section

# Association of Hypocalcaemia and Vitamin D Deficiency in Newborns Admitted in NICU: A Cross-sectional Study from a Tertiary Care Centre, Prayagraj, Northern India

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## ABSTRACT

**Introduction:** Hypocalcaemia is very common in sick newborns and is frequently associated with severe Vitamin D Deficiency (VDD). VDD can lead to both acute and long-term complications in newborns. Estimating vitamin D levels in hypocalcaemic newborns may therefore be cost-effective.

**Aim:** To determine the association between hypocalcaemia and VDD in newborns admitted to the Neonatal Intensive Care Unit (NICU) of a tertiary care centre.

**Materials and Methods:** The present cross-sectional study was conducted in the NICU of the Department of Paediatrics, MLN Medical College, Prayagraj, Uttar Pradesh, India, from 1st August 2021 to 31st July 2022. A total of 100 newborns (up to 28 days old) were enrolled and divided into two groups of 50 each. Group 1 included hypocalcaemic newborns (serum ionic calcium <1.2 mmol/L in term and <1.0 mmol/L in preterm infants), and Group 2 included newborns with normocalcaemia. Vitamin D levels were estimated in both groups. Maternal history regarding the intake of calcium (1000 mg/day) and vitamin D (500 IU/day) from 14 weeks of pregnancy until delivery was also recorded. The Chi-square test was used to assess the association of hypocalcaemia with vitamin D levels.

**Results:** The mean age of the newborns was 4.93±4.61 days, with a male-to-female ratio of 2.03:1. VDD was observed in 74% (74/100) of the newborns. Among hypocalcaemic newborns, 46 (92%) were vitamin D deficient. Hypocalcaemia was significantly associated with VDD (p<0.001). Sixty-five mothers had a history of either no or inadequate calcium and vitamin D intake. Among these, 32 mothers of hypocalcaemic newborns had inadequate or no intake, which was not significantly associated with neonatal hypocalcaemia (p=0.580). Breastfeeding was significantly associated with normocalcaemia (p=0.037).

**Conclusion:** There was a very high prevalence of VDD in the study population, with 74% of NICU-admitted newborns being vitamin D deficient. VDD was significantly associated with hypocalcaemia in the present cross-sectional study.

**Keywords:** Ionic calcium, Neonatal intensive care unit, Neonate, 25 hydroxy vitamin D

## INTRODUCTION

Neonatal hypocalcaemia occurs in approximately 76% of the general newborn population [1] and in 36% of newborns admitted to the NICU [2]. It is defined as ionic serum calcium <1.2 mmol/L (4.8 mg/dL) in term infants and <1.0 mmol/L (4 mg/dL) in preterm infants [3]. Neonatal hypocalcaemia is classified based on age of onset: early-onset hypocalcaemia occurs within 72 hours of birth, whereas late-onset hypocalcaemia occurs after 72 hours [3].

The primary causes of early-onset hypocalcaemia (<72 hours) include reduced sensitivity of Parathyroid Hormone (PTH) to low calcium levels and target organ hyporesponsiveness to PTH, especially in preterm infants. Other causes include prematurity, pre-eclampsia, Infants of Diabetic Mothers (IDM), perinatal stress or asphyxia, Intrauterine Growth Restriction (IUGR), maternal use of anticonvulsants (e.g., phenobarbitone, phenytoin), maternal hyperparathyroidism, and iatrogenic causes such as phototherapy [3].

Late-onset hypocalcaemia (>72 hours) is commonly caused by VDD, high phosphate intake (e.g., cow's milk), hypomagnesaemia, hyperparathyroidism, and genetic syndromes such as DiGeorge syndrome, CATCH 22 syndrome, Kenny-Caffey syndrome, and Kearns-Sayre syndrome, as well as hepatobiliary disease [3]. Among these, VDD and high phosphate intake are the most frequent causes of neonatal hypocalcaemia [3].

Vitamin D deficiency (VDD) is highly prevalent among pregnant women in India [4]. Neonatal vitamin D status is closely associated with maternal vitamin D levels [1]. VDD in newborns can cause neonatal hypocalcaemia and may also lead to hypocalcaemic seizures, tetany, and acute respiratory distress syndrome [5,6]. Additionally, VDD in newborns has been linked to long-term morbidities such as asthma, type 1 diabetes mellitus, atopic dermatitis, florid rickets, schizophrenia, autism, and abnormal neurocognitive outcomes [7-13].

Maternal intake of calcium is recommended from 14 weeks of pregnancy until six months postpartum, but the intake and nutritional status of mothers in India are often inadequate, which may contribute to neonatal hypocalcaemia and its associated complications [14]. Studies have shown a strong correlation between maternal and foetal vitamin D levels [14-16].

Since hypocalcaemia is a common neonatal disorder often associated with VDD, and given the potential for both early and late complications, it is important to study this association. However, previous studies on neonatal hypocalcaemia and VDD [1,16] lacked control groups. To establish a stronger association, we included a control group of newborns with normal calcium levels. Considering the high prevalence of VDD in India and the absence of Indian studies on this specific topic, the aim of the present study was to investigate the association between hypocalcaemia and VDD in sick newborns.

## MATERIALS AND METHODS

The present cross-sectional study was conducted in the NICU of the Department of Paediatrics, MLN Medical College, Prayagraj, Uttar Pradesh, India, from 1st August 2021 to 31st July 2022. The study was approved by the Institutional Ethical Committee (IEC no. ECR/922/Inst/UP/2017/RR-22, issued under rule 122DD of the Drugs and Cosmetics Rule 1945).

**Inclusion criteria:** Newborns aged 0-28 days were enrolled. Newborns with hypocalcaemia were designated as cases, and those with normal calcium levels were designated as controls.

**Exclusion criteria:** Newborns with metabolic syndromes (DiGeorge syndrome, CATCH 22 syndrome, Kenny-Caffey syndrome, Kearns-Sayre syndrome), congenital heart diseases, or congenital malformations such as cleft lip or cleft palate were excluded.

**Sample size calculation:** Due to time constraints and the COVID-19 pandemic, all available newborns with hypocalcaemia were enrolled. An equal number of newborns with normal calcium levels were selected as controls using purposive sampling.

### Study Procedure

After obtaining informed consent, data were collected using a predefined proforma. Detailed maternal history regarding calcium and vitamin D intake during pregnancy was recorded. Adequate intake was defined as 1000 mg/day of calcium and 500 IU/day of vitamin D from 14 weeks of gestation until delivery [17].

Blood samples for serum calcium, phosphorus, alkaline phosphatase, and vitamin D levels were collected on the day of admission. Newborns were divided into two groups:

**Group 1:** Hypocalcaemic babies (serum ionic calcium <1.2 mmol/L in term infants; <1.0 mmol/L in preterm infants)

**Group 2:** Normocalcaemic babies (serum ionic calcium 1-1.5 mmol/L in preterm infants; 1.2-1.5 mmol/L in term infants) [4].

The 25 hydroxy vitamin D (25(OH)D) levels were measured using a fully automated immunoassay system (MINI-VIDAS) with an Enzyme-Linked Fluorescence Assay (ELFA). Vitamin D status was classified as deficient (<20 ng/mL), insufficient (20-29 ng/mL), or sufficient (30-100 ng/mL) [18].

Serum ionic calcium was estimated using the direct calorimetric and complexometric method. Serum phosphorus was classified as hypophosphatemia (<4.3 mg/dL), normal (4.3-5.4 mg/dL), or hyperphosphatemia (>5.4 mg/dL). Serum Alkaline Phosphatase (ALP) was considered normal if between 83-469 U/L [4].

## STATISTICAL ANALYSIS

Data were entered into an Excel sheet and analysed using the same software. Means were calculated for continuous variables, and percentages were calculated for categorical variables. The $\chi^2$ (chi-square) test was used to determine the association of hypocalcaemia with vitamin D deficiency and maternal calcium and vitamin D intake. A p-value of <0.05 was considered statistically significant.

## RESULTS

A total of 100 Newborns (NB) were enrolled, with 50 in each group. The mean age of the newborns was 4.93 ± 4.61 days, with 67 (67%) males and 33 (33%) females, resulting in a male-to-female ratio of 2.03:1. Most neonates (87, 87%) were admitted to the NICU within the first seven days of life. Among the hypocalcaemic newborns, 20 had early-onset hypocalcaemia, and 30 had late-onset hypocalcaemia.

Regarding feeding patterns, 84 (84%) neonates were breastfed, 10 (10%) were formula-fed, and 6 (6%) received cow's milk. There was a statistically significant association between cow's milk intake and hypocalcaemia (p=0.037). No significant associations were observed between neonatal illness, gestational age, Birth Weight (BW), place of delivery, or maternal illnesses and hypocalcaemia.

Sixty-four (64%) mothers reported no calcium or vitamin D supplementation, 35 (35%) had adequate intake, and 1 (1%) had inadequate intake. Maternal supplementation of calcium and vitamin D during pregnancy was not significantly associated with neonatal hypocalcaemia (p=0.580). Forty-three (86%) newborns were successfully discharged in both groups, while 3 (6%) in the hypocalcaemic group and 2 (4%) in the normocalcaemic group died. There was no significant difference in neonatal outcomes between the two groups (p=0.856) [Table/Fig-1].

| Baseline characteristics | | Group 1 | Group 2 | p-value |
|---|---|---|---|---|
| Mean age (days) | | 4.45±3.37 | 5.4±4.61 | 0.311 |
| Sex ratio | Male | 34 (68%) | 33 (66%) | 0.832 |
| | Female | 16 (32%) | 17 (34%) | |
| Mode of delivery | Lower section caesarean section | 18 (36%) | 12 (24%) | 0.190 |
| | Normal vaginal delivery | 32 (64%) | 38 (76%) | |
| Place of delivery | Institutional | 46 (92%) | 45 (90%) | 0.845 |
| | Home (referred for some neonatal illness) | 4 (8%) | 5 (10%) | |
| Maternal illness | GDM | 3 (6%) | 6 (12%) | 0.120 |
| | Pre-eclampsia | 1 (2%) | 5 (10%) | |
| | Eclampsia | 0 (0%) | 0 (0%) | |
| | No | 46 (92%) | 39 (78%) | |
| Gestational age | Full-term | 42 (84%) | 45 (87%) | 0.372 |
| | Pre-term | 8 (16%) | 5 (13%) | |
| BW | Small for Gestational Age (SGA) | 9 (18%) | 10 (20%) | 0.576 |
| | Appropriate for Gestational Age (AGA) | 41 (82%) | 39 (78%) | |
| | Large for Gestational Age (LGA) | 0 | 1 (2%) | |
| Associated illness | Seizures | 23 (46%) | 25 (50%) | 0.559 |
| | Breathing difficulty | 25 (50%) | 21 (42%) | |
| | Neonatal jaundice | 1 (2%) | 4 (8%) | |
| | Neonatal sepsis | 26 (52%) | 21 (42%) | |
| | HIE | 22 (44%) | 25 (50%) | |
| Maternal calcium and vitamin D intake | Inadequate intake | 31 (62%) | 33 (66%) | 0.580 |
| | Adequate intake | 18 (36%) | 17 (34%) | |
| | No intake | 1 (2%) | 0(0%) | |
| Feeding | Breast milk | 40 (80%) | 44 (88%) | 0.037 |
| | Formula milk | 4 (8%) | 6 (12%) | |
| | History of Cow's milk intake before admission in NICU | 6 (12%) (mean age is 4 days, so they were fed with cow milk during their stay at home) | 0 | |
| Neonatal outcome | Discharge | 43 (86%) | 43 (86%) | 0.856 |
| | LAMA | 4 (8%) | 5 (10%) | |
| | Expired | 3 (6%) | 2 (2%) | |

**[Table/Fig-1]:** Baseline characteristics of newborn in two groups.
p-value <0.05 was taken as significant; Chi-square test was used for comparison; *GDM: Gestational diabetes mellitus; *LAMA: Leave against medical advice

Vitamin D deficiency (VDD) was observed in 74% of neonates. In Group 1, 46 (92%) neonates had VDD, and 4 (8%) had normal or insufficient vitamin D levels. In Group 2, 28 (56%) neonates had VDD, and 22 (44%) had normal or insufficient vitamin D levels [Table/Fig-2]. The mean vitamin D level was 17.79±12.42 ng/mL. Group 1 had a mean vitamin D level of 12.37±5.87 ng/mL, whereas Group 2 had a mean of 23.28±14.37 ng/mL. There was a statistically significant relationship between hypocalcaemia and vitamin D levels (p<0.001), as well as with serum ALP, but not with serum phosphorus levels [Table/Fig-2,3].

| Parameters | | Deficiency (N=74) | Insufficiency (N=8) | Sufficiency (N=18) | Total (N=100) | p-value |
|---|---|---|---|---|---|---|
| | | N (%) | N (%) | N (%) | N (%) | |
| Ionic calcium | Hypocalcaemia | 46 (92.0) | 3 (6%) | 1 (2%) | 50 (50) | <0.0001 |
| | Normocalcaemia | 28(56.0) | 5 (10%) | 17 (34%) | 50 (50) | |
| Serum phosphorus | Hypophosphatemia | 9 (100) | 0 | 0 | 9 (9) | 0.169 |
| | Normal | 61 (71) | 6 (4.7) | 18 (21.2) | 85 (85) | |
| | Hyperphosphatemia | 4 (66.6) | 2 (33.3) | 0 | 6 (6) | |
| ALP | Normal | 1 (14.2) | 4 (57.1) | 2 (28.5) | 7 (7) | <0.0001 |
| | High | 73 (75.2) | 4 (43) | 16 (17.2) | 93 (93) | |

**[Table/Fig-2]:** Biochemical markers of VDD.
p-value <0.05 was taken as significant

| Parameters | Total | Group 1 | Group 2 | p-value |
|---|---|---|---|---|
| Serum ionic Calcium (mmol/L) Mean (Standard deviation) | 1.2894 (0.572) | 0.82 (0.18) | 1.71 (0.48) | 0.00001 |
| Serum Phosphorus (mg/dL) Mean (Standard deviation) | 6.613 (1.642) | 6.34 (1.61) | 6.86 (1.65) | 0.057 |
| Serum Alkaline phosphatase (U/L) Mean (Standard deviation) | 563.2 (62.73) | 551.62 (68.41) | 573.48 (55.9) | 0.041 |
| Serum Vitamin D (ng/dL) Mean (Standard deviation) | 17.79 (12.42) | 12.37 (5.87) | 23.28 (14.37) | 0.00001 |

**[Table/Fig-3]:** Mean values of calcium, phosphorus, alkaline phosphatase and vitamin D level.

## DISCUSSION

Preterm infants are more prone to hypocalcaemia because approximately 80% of calcium is actively transferred from the mother to the foetus during the third trimester. After delivery, the newborn is deprived of maternal calcium supply. During this period, Parathyroid Hormone (PTH) acts to normalise calcium levels. Vitamin D plays a minimal role during the first two weeks of life as it primarily metabolizes calcium afterward [19]. Mild jitteriness may occur at serum calcium levels of 6-8 mg/dL, but severe symptoms such as seizures, tetany, apnoea, or cardiomyopathy may present at lower levels [19].

The present study did not find any significant association between neonatal hypocalcaemia and age, sex, mode or place of delivery, maternal illness, gestational age, birth weight, or onset (early vs. late). In contrast, Bošnjak I et al., (2017) reported significant associations of hypocalcaemia with male gender, low gestational age (<36 weeks), birth weight of 2501-3500 grams, vaginal delivery, and maternal diabetes [3]. The differences may be due to their larger sample size, while the current study included similar patient types across both groups.

In the current study, 32 (64%) hypocalcaemic newborns had perinatal asphyxia, 4 (8%) had spontaneous preterm labour, and 1 (2%) had chorioamnionitis. Among all neonates, 48% experienced seizures, 46% had breathing difficulties, and 5% had neonatal jaundice. There was no significant association between these perinatal illnesses and hypocalcaemia. Elsary AY et al., (2018) also reported no statistically significant relationship between perinatal illnesses and serum calcium levels [1]. Lenarčič Ž et al., (2018) indicated that neonatal sepsis is a risk factor for hypocalcaemia [19], while Bošnjak I et al., (2017) found associations with sepsis, jaundice, respiratory distress syndrome, and seizures [3].

Regarding feeding patterns, 84% of neonates were breastfed, 10% formula-fed, and 6% received cow's milk. A statistically significant relationship was observed between cow's milk intake and hypocalcaemia, with more normocalcaemic neonates being breastfed (P=0.037). In contrast, Elsary AY et al., (2018) found no significant association between feeding type and hypocalcaemia [1]; the discrepancy may be due to the inclusion of a control group in the current study. Similarly, Cho WI et al., (2015) in Korea reported that hypocalcaemia was more common in formula-fed neonates, which aligns with the findings of this study [20].

Maternal nutritional status has a strong impact on foetal development; maternal supplementation of vitamin D and calcium during pregnancy influences neonatal vitamin D and calcium levels. Calcium supplementation in pregnancy reduces the risk of preterm birth, which is a leading cause of neonatal mortality [1]. In the current study, most mothers (64%) had no history of calcium or vitamin D3 supplementation. There was no significant association between maternal supplementation of vitamin D and calcium and neonatal hypocalcaemia.

Calcium transfer from mother to foetus is an active process that occurs primarily during the third trimester. This could explain why maternal calcium supplementation had no apparent effect on neonatal serum calcium levels. Furthermore, the high prevalence of VDD among pregnant women in India [2] may have contributed to the limited effect of routine supplementation with the recommended daily allowance of vitamin D.

In contrast, Elsary AY et al., (2018) reported a significant association between neonatal hypocalcaemia and maternal calcium and vitamin D supplementation [Table/Fig-4] [1,16,20]. Benali AI et al., found only a weak correlation between maternal serum calcium and neonatal calcium levels, though their study did not specifically assess the effect of maternal calcium and vitamin D supplementation on neonatal hypocalcaemia [15].

The current study observed a high prevalence of VDD among newborns. There was a statistically significant association between hypocalcaemia and vitamin D levels (p<0.001). Hypocalcaemia in VDD occurs in two stages: initially, before PTH has acted to normalise calcium levels, and later, when PTH becomes refractory due to prolonged VDD. This suggests that infants with VDD and

| Study parameters | Current study | Kozgar SAM et al., (2020) [16] | Cho WI et al., (2015) [20] | Elsary AY et al., (2018) [1] |
|---|---|---|---|---|
| Place of study | India | Sydney, Australia | Seoul, Korea | Egypt |
| Sample size | 100 | 600 | 53 | 100 |
| Mean age | 4.93±4.61 days | 0-1 day- 81%<br>1-2 days -8%<br>2-7 days 10%<br>(Mean age not described) | Not given (newborns less than 28 Days) | 9.5±8.1 days |
| Male | 67 (67%) | 284 (47.5%) | 35 (66%) | 56 (56%) |
| Female | 33 (33%) | 315 (52.5%) | 18 (34 %) | 44 (44%) |
| Percentage of newborns with Vitamin D Deficiency (VDD) | 74% | 21.3% | 85.7% | 38% |
| Percentage of newborn with hypocalcaemia | Equal number of cases (hypocalcaemia) and controls (normal calcium levels) enrolled | 1.2 % (samples taken on Day one of age) | 39 % hypocalcaemic tetany), 5 % had hypocalcaemia | 76% |
| association of vitamin D level and hypocalcaemia | Positive (P=<0.001) | Positive (P=<0.0001) | No association (p=0.794) | No association (p=0.23) |
| Association of maternal calcium and vitamin D supplementation | No association (p=0.580) | - | - | Positive (p-value=0.002) |

**[Table/Fig-4]:** Comparison of various studies with current study (cited) [1,16,20].

hypocalcaemia may have had decreased vitamin D and calcium accretion from their mothers during the intrauterine period due to poor maternal nutritional status.

Studies by Kozgar S et al., and Cho WI et al., also found a positive correlation between vitamin D and calcium levels, consistent with the current study [Table/Fig-4] [16,20]. However, Elsary AY et al., (2018) reported no significant association between vitamin D levels and hypocalcaemia (P=0.23) [Table/Fig-4]. The difference may be due to the higher prevalence of VDD in the current study (74%) compared to only 14% in Elsary AY's study [1]. No significant association was found between late-onset hypocalcaemia and VDD, possibly because of the high prevalence of both conditions in sick neonates.

In the present study, no significant differences were observed between neonatal outcomes and serum calcium or vitamin D levels, consistent with findings by Mosayebi Z et al., (2021) in Iran [21]. A meta-analysis in the paediatric population reported a high rate of VDD among critically ill children [22]. The current study did not assess the severity of illness among vitamin D-deficient neonates.

### Limitation(s)

The present study was limited by the inability to assess the full spectrum of calcium metabolism in neonates. Maternal serum calcium and vitamin D levels could not be measured due to financial constraints.

## CONCLUSION(S)

Neonatal hypocalcaemia is significantly associated with VDD in sick newborns. Top feeding (formula or cow's milk) is an important risk factor for neonatal hypocalcaemia. VDD is highly prevalent in sick neonates. Hypocalcaemia was not associated with sex, age, mode or place of delivery, maternal illness, gestational age, birth weight, or any neonatal illness. It also did not affect neonatal outcomes at discharge. No association was found between maternal antenatal calcium and vitamin D intake and neonatal hypocalcaemia. Further studies are needed to explore the relationship between maternal supplementation and neonatal hypocalcaemia. Screening for vitamin D levels in newborns with hypocalcaemia is recommended to prevent future complications.

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