

Vitamin D Deficiency Prevalence in Late Neonatal Hypocalcemia: A Multicenter Study

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What is already known on this topic?

Late neonatal hypocalcemia (LNH) occurs after the first 72 hours and the most common causes include excessive phosphate intake, hypomagnesemia, hypoparathyroidism, and vitamin D deficiency.

What this study adds?

Maternal vitamin D deficiency was found to be the most common cause of LNH in our study. Due to the immaturity of regulating factors of parathyroid hormone (PTH) and calcium, serum intact PTH levels may not reach expected levels and serum phosphorus levels may remain high in vitamin D deficient neonates, posing a diagnostic dilemma by mimicking primary hypoparathyroidism.



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Conflict of interest: None declared

Received: 14.09.2020

Accepted: 15.04.2021

Abstract

Objective: Late neonatal hypocalcemia (LNH) is a common metabolic problem associated with hypoparathyroidism, high phosphate intake and vitamin D deficiency, often presenting with seizures. In this cross-sectional study, we aimed to evaluate the role of vitamin D deficiency in LNH in Turkey and to describe the characteristics of affected newborns.

Methods: Conducted with a cross-sectional design and with the participation of 61 neonatal centers from December 2015 to December 2016, the study included term neonates with LNH (n = 96) and their mothers (n = 93). Data were registered on the FAVOR Web Registry System. Serum samples of newborns and mothers were analyzed for calcium, phosphate, magnesium, albumin, alkaline phosphatase, intact parathyroid hormone (iPTH) and 25 hydroxyvitamin D [25(OH)D] levels.

Results: The median (range) onset time of hypocalcemia was 5.0 (4.0-8.0) days of age, with a male preponderance (60.4%). The median (range) serum 25(OH)D levels of the neonates and their mothers were 6.3 (4.1-9.05) and 5.2 (4.7-8.8) ng/mL, respectively. The prevalence of vitamin D deficiency (< 12 ng/mL) was high in both the neonates (86.5%) and mothers (93%). Serum 25(OH)D levels of the infants and mothers showed a strong correlation (p < 0.001). While the majority (93.7%) of the neonates had normal/high phosphorus levels, iPTH levels were low or inappropriately normal in 54.2% of the patients.

Conclusion: Vitamin D deficiency prevalence was found to be high in LNH. Efforts to provide vitamin D supplementation during pregnancy should be encouraged. Evaluation of vitamin D status should be included in the workup of LNH.

Keywords: Vitamin D deficiency, hypocalcemia, infant, newborn

Introduction

Neonatal hypocalcemia is defined as a total serum calcium (Ca) level of less than 7.5 mg/dL and ionized calcium (Ca²⁺) level of less than 4.4 mg/dL in term newborns. Neonatal hypocalcemia may manifest as signs of neuromuscular hyperexcitability (irritability, jitteriness, tetany, laryngospasm, and seizures), apnea, cyanosis, feeding problems, and/or cardiac rhythm disturbances (1,2). The causes of neonatal hypocalcemia are classified by the time of onset. Early neonatal hypocalcemia occurs within the first three days after birth, with an exaggeration of normal decline in serum Ca concentration in the first two postnatal days (2). Late neonatal hypocalcemia (LNH) occurs after the third day of birth, with the main causes being a high phosphate intake, hypoparathyroidism, parathyroid hormone (PTH) resistance, hypomagnesemia, and perinatal vitamin D deficiency. Neonatal vitamin D levels depend on maternal vitamin D status, breast-feeding, and sunlight exposure (3,4). The risk factors for maternal vitamin D deficiency include habitation at high altitude, darker skin pigmentation, and excessive skin coverage with clothing. Most studies conducted in developing countries, including Turkey, have shown a high prevalence of hypovitaminosis D in newborns with a direct relationship with maternal vitamin D levels (5,6,7,8,9,10,11). Maternal vitamin D deficiency results in a poor transplacental transmission of vitamin D during pregnancy and reduced storage in newborns, leading to reduced intestinal Ca absorption, reduced ionized Ca concentrations, and secondary hyperparathyroidism, which in turn decreases urinary Ca loss, increases bone Ca reabsorption, normalizes serum Ca levels, but also decreases renal phosphate reabsorption (12,13). The reduced serum phosphate concentration is mainly responsible

for the development of rickets, by impairing apoptosis of hypertrophic chondrocytes in the growth plate. In newborns and early infancy, a high metabolic demand for Ca, PTH resistance, and/or inadequate PTH response may result in hypocalcemia before radiological evidence of rickets (14). There are reports, albeit few in number, reporting hypocalcemia as a manifestation of vitamin D deficiency (15,16).

The aim of this study was to describe the clinical and biochemical characteristics of neonates with LNH, and to determine the relationship between LNH and maternal vitamin D deficiency in Turkey.

Methods

This cross-sectional, nationwide study was carried out between December 2015 and 2016 with the participation of 61 centers, and involved term neonates with LNH and their mothers. The study was endorsed by the Turkish Neonatology Society.

A case recording form (CRF), designed by two pediatric endocrinologists and a neonatologist (G.S.K., S.H., A.G.), was used to collect demographic data: maternal history including maternal clothing, lifestyle, and use of vitamin D supplements during pregnancy; anthropometric and clinical findings of the neonates; and laboratory findings of the newborns and their mothers. The CRF was uploaded to the FAVOR Web Registry System website. The study data were entered into the system over a 12-month period (December 11, 2015-2016) by the participating researchers. Data entered into the registry were also checked for consistency by a research assistant (G.S.K.). A total of 98 newborns with LNH and their mothers were registered in the database.

The babies enrolled in our study were those who presented with hypocalcemia or who were hospitalized for other reasons and developed hypocalcemia during their follow-up. Hypocalcemia in asymptomatic babies was identified through routine biochemical tests during the hospital stay.

Inclusion criteria were term newborns born at 37-42 weeks of gestation and the presence of LNH, defined as total serum Ca level of less than 7.5 mg/dL after the first 72 hours of birth. Registered data were reviewed in terms of the following exclusion criteria: a birthweight of less than 2,000 g and the presence of maternal diabetes; neonatal asphyxia; sepsis; malabsorption; renal insufficiency; liver disease; or use of anticonvulsants. Neonates fed with breast milk and/or formula were enrolled in the study.

Maternal and newborn venous blood samples were drawn at presentation to measure serum levels of total Ca, phosphate (PO_4), magnesium (Mg), albumin, alkaline phosphatase (ALP), intact PTH (iPTH), and 25 hydroxyvitamin D [25(OH)D]. Serum Ca, P, ALP, and iPTH levels were measured on the same day; for 25(OH)D, the blood supernatant samples after centrifugation were stored at -80°C until the time of assay in a single laboratory using an enzyme immunoassay method (IDS Immunodiagnostic Systems Ltd, Boldon, UK).

According to the Global Consensus Recommendations, maternal and neonatal 25(OH)D levels of less than 12 ng/mL (30 nmol/L) were considered to be indicative of vitamin D deficiency (17). The inter- and intra-assay coefficients of variation were 6.7% and 8.7%, respectively. The reference range for total Ca was 8.7-10.4 mg/dL in mothers (adult women). The normal range of inorganic phosphate was 5.2 to 8.4 mg/dL for infants 0-5 months of age and 2.5 to 4.5 mg/dL for adult women. The upper normal limits for ALP were 420 IU/L and 130 IU/L for infants and non-pregnant women, respectively (18). The normal range for iPTH was defined as 5-65 pg/mL.

Anterior fontanelle evaluation was performed using the following technique. The anteroposterior diameter (AB) was determined as the length and the transverse diameter (CD) as the width. The average of the longitudinal and transverse dimensions was recorded as the mean fontanelle size ($[\text{AB} + \text{CD}]/2$). Neonates with mean fontanelle size above 97th percentile were determined as having large fontanelle (19).

Regular vitamin D intake during pregnancy was accepted as 1,200 IU/day vitamin D starting from 12th week of pregnancy, irrespective of vitamin D status, as stated in Turkish Ministry of Health Support Program (20).

The consistency and appropriateness of the diagnoses and enrolment were reviewed by two of the authors (G.S.K,

A.G.). After exclusion of two neonates and five mothers due to the lack of centrifuged serum samples for the 25(OH)D measurement, the final analyses included 96 newborns with newly-diagnosed late hypocalcemia and their 93 mothers.

Written informed consent was obtained from parents of the newborns, and the study protocol was approved by the Kocaeli University Ethics Committee (report number: KOU KAEK 2015/322).

Statistical Analysis

Data were processed using Statistical Package for the Social Sciences, version 20 (IBM Inc., Armonk, NY, USA). The data for normality of distribution was assessed. Numerical variables were expressed as medians and are presented as interquartile range (IQR) and frequencies (percentages). The association between the maternal and neonatal 25(OH)D levels was evaluated by the Spearman's correlation coefficient. A p value of less than 0.05 was considered to be significant.

Results

Among the 96 neonates, which included 38 (39.6%) girls and 58 (60.4%) boys, with newly diagnosed late hypocalcemia, the median (IQR) age of onset was 5.0 (4.0-8.0) days (Table 1). Thirty-three (34.4%) neonates presented with the symptoms of hypocalcemia, with convulsion being the most common symptom ($n = 18$). There was a seasonal pattern of presentation, with increased incidence in winter and spring months (65.6%). The majority of mothers ($n = 74$, 77.1%) used covered clothing and spent most of their time indoors. Only 20.4% of the mothers received regular vitamin D supplements during pregnancy.

Table 2 shows the laboratory findings of neonates with LNH. The median (IQR) serum Ca level was 6.9 (6.4-7.2) mg/dL, serum phosphorus 7.2 (6.1-8.5) mg/dL, serum ALP 197 (142.5-279.2) IU/L, and serum iPTH 69.1 (37.4-106) pg/mL. The median (IQR) serum vitamin D level was 6.3 (4.1-9.05) ng/mL, with 83 (86.5%) neonates having vitamin D deficiency, defined as a serum 25(OH)D level < 12 ng/mL. Serum phosphorus levels were normal/high in all but six neonates, and iPTH levels were high in 44 neonates (45.8%).

The maternal median (IQR) serum vitamin D level was 5.2 (4.7-8.8) ng/mL, and 93% of the mothers were vitamin D deficient (Table 3).

All but three mothers with vitamin D deficiency had neonates who also had vitamin D deficiency. A strong positive correlation was found between maternal and neonate serum 25(OH)D levels ($r = 0.513$, $p < 0.001$) (Figure 1).

Table 1. Demographic and clinical characteristics of 96 neonates with late neonatal hypocalcemia

Characteristics (n = 96)	Value*
Age at onset of hypocalcemia, (days)	5 (4-8)
Sex	
Male	58 (60.4)
Female	38 (39.6)
Season of birth time	
Winter (December-February)	27 (28.1)
Spring (March-May)	36 (37.5)
Summer (June-August)	14 (14.6)
Autumn (September- November)	19 (19.8)
Living area	
Urban	49 (51)
Rural	47 (49)
Maternal clothing	
Covered	74 (77.1)
Not covered	22 (22.9)
Maternal vitamin D supplementation during pregnancy	
Regular	4 (4.3)
Irregular	70 (75.3)
None	
Symptom (+)	33 (34.4)
Convulsion	18 (18.8)
Poor sucking reflex	17 (17.7)
Exaggerated startle	11 (11.5)
Lethargy	7 (7.3)
Vomiting	3 (3.1)
Cyanosis	2 (2.1)
Apnea	1 (1)
Large anterior fontanelle	6 (6.3)
Gestational age (weeks)	38 (37-39)
Birth weight (g)	3305 (2976-3640)
Apgar score	
1-minute	8 (7-8)
5-minute	9 (9-10)

*Values are presented as number (%), median (interquartile range), or number only.

Discussion

This is the first cross-sectional, multi-center study of term neonates with late hypocalcemia to investigate the relationship between maternal vitamin D status and LNH in Turkey. Maternal and neonatal vitamin D deficiency was found to be related with neonatal late hypocalcemia. Although infants can be protected from severe hypocalcemia thanks to a nationwide program of free supplementation of 400 U/day vitamin D for infants, the problem of LNH will continue unless pregnant women are provided with and consistently use vitamin D supplementation (21).

The prevalence of inadequate vitamin D status is high among pregnant and lactating women around the world (22,23,24). Women who wear covered clothing, live at high altitudes, and do not have an adequate exposure to sunlight are especially at risk for vitamin D deficiency (14).

Table 2. Biochemical parameters of 96 neonates with late neonatal hypocalcemia

Variable	Median (IQR)	n (%)
Calcium (mg/dL)	6.9 (0.9)	
Phosphorus (mg/dL) (normal: 5.2-8.4)	7.2 (2.4)	Low 6 (6.3) Normal 63 (65.6) High 27 (28.1)
ALP (IU/L) (normal: 145-420)	197 (136.7)	Normal 87 (90.6) High 9 (9.4)
iPTH (pg/mL) (normal: 5-65)	69.1 (68.6)	Low 2 (2.1) Normal 50 (52.1) High 44 (45.8)
25(OH)D (ng/mL) (deficiency < 12 insufficiency 12-20 sufficiency > 20)	6.3 (4.9)	Deficiency 83 (86.5) Insufficiency 5 (5.2) Sufficiency 8 (8.3)

ALP: alkaline phosphatase, iPTH: intact parathyroid hormone, 25(OH)D: 25 hydroxyvitamin D, IQR: interquartile range, n: number

Table 3. Biochemical parameters in mothers of term neonates with late neonatal hypocalcemia

Variable	Median (IQR)	n (%)
Calcium (mg/dL)	8.9 (0.9)	
Phosphorus (mg/dL) (normal: 2.5-4.5)	3.9 (0.87)	Normal 71 (88.8) High 9 (11.3)
ALP (IU/L) (normal: < 150)	126 (60.1)	Normal 57 (70.4) High 24 (29.6)
iPTH (pg/mL) (normal: 10-65)	65.5 (56.9)	Low 1 (1.1) Normal 43 (49.4) High 43 (49.4)
25(OH)D (ng/mL) (deficiency < 12 insufficiency 12-20 sufficiency > 20)	5.2 (4.1)	Deficiency 80 (93) Insufficiency 4 (4.7) Sufficiency 2 (2.3)

ALP: alkaline phosphatase, iPTH: intact parathyroid hormone, 25(OH)D: 25 hydroxyvitamin D, IQR: interquartile range, n: number

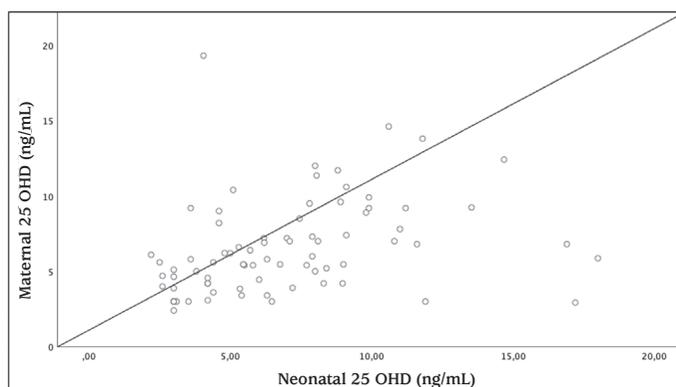


Figure 1. Correlation between maternal and neonatal serum 25 hydroxyvitamin D levels

In a study from Turkey, in lactating women and their babies a serum 25(OH)D level below 11 ng/mL was accepted as severe vitamin D deficiency, 11-25 ng/mL as moderate

deficiency, and a value >25 ng/mL as normal. Severe vitamin D deficiency was found in 27% of the mothers, and moderate deficiency in 54.3%. Severe vitamin D deficiency was detected in 64.3% of the neonates, and moderate deficiency in 32.9% (24).

An Indian study by Mehrotra et al (5) in 2010 found that 90% of neonates with hypocalcemic convulsions and 89% of their mothers had vitamin D deficiency. Furthermore, a significant correlation was found between the serum 25(OH)D levels of the mothers and infants.

Hatun et al (15) examined the medical records of infants with vitamin D deficiency and/or nutritional rickets in Turkey and found that 79% of the infants whose mothers were also vitamin D deficient had presented with hypocalcemic seizures. In the present study, vitamin D deficiency was diagnosed in 86.5% of the neonates with late hypocalcemia and in 93% of their mothers and, consistent with many previous reports, there was a significant correlation between maternal and newborn 25(OH)D levels (5,10).

As a known risk factor for vitamin D deficiency, covered clothing was seen in 77.1% of the mothers. Another study conducted in Turkey reported similar results (15). In a study carried out in Iran, where the vast majority of the population are Muslim and where women are required to veil themselves, among 100 neonates diagnosed with hypocalcemia, the prevalence of maternal vitamin D deficiency was 74% (9).

According to the Global Consensus Recommendations, the basic approach to prevent LNH is to supplement women with vitamin D of at least 600 U/day particularly over the last three months of pregnancy. However, data from an earlier national study showed that 25(OH)D levels can be normalized only by the administration of 2000 U of vitamin D per day during pregnancy (25).

In the current study, the majority of late hypocalcemic babies were born in the winter and spring months, which is consistent with a previous study from Korea that included 17 term newborns with LNH (26). This may be explained by limited sunlight exposure for mothers whose late pregnancy periods take place during the winter and spring months, leading to low maternal and neonatal vitamin D levels. We observed a male preponderance, which was previously reported by an American study of 78 full-term neonates with transient hypocalcemia, as well as by a study from the United Kingdom involving hypocalcemic neonates and children due to vitamin D deficiency (27,28).

In our study, despite the high prevalence of vitamin D deficiency (86.5%), large anterior fontanelle was observed

in a small percentage of neonates (6.3%). Since reduced serum phosphate concentration is mainly responsible for the skeletal findings of rickets, we can speculate that, regardless of the amount, continuous transplacental transfer of Ca and phosphorus to the fetus might have limited the clinical and biochemical manifestations of vitamin D deficiency in these neonates. Elevated PTH level is an important cause of decreased phosphorus reabsorption. However, in neonates and young infants, PTH resistance and/or inadequate PTH response may result in hypocalcemia before skeletal findings occur.

Vitamin D deficiency is associated with biochemical disruption to Ca homeostasis, resulting in a typical constellation of hypocalcaemia, hypophosphatemia, and elevated levels of alkaline phosphatase and PTH. Other studies have documented that vitamin D deficiency triggers PTH release in adults, children, and older infants. However, in newborns and young infants, this feedback loop appears not to occur, and hypovitaminosis D coexisted with blunted PTH response and/or PTH resistance (29,30,31,32,33). In our study, baseline iPTH concentrations were increased in only 45.8% of neonates, and serum phosphorus levels were normal/high in all but five patients. Do et al (26) reported that, in 17 neonates with late-onset hypocalcemia secondary to vitamin D deficiency, iPTH levels were not remarkably elevated except in one case. Similar results in neonates were described by Maghbooli et al (34), who reported elevated serum iPTH levels in only 10% of vitamin D deficient neonates at birth. Late maturation of the parathyroid axis is thought to be a main cause of transient neonatal hypocalcemia, as suggested by the low or inappropriately normal PTH levels and high phosphorus levels in these infants. We also observed biochemically normal/high phosphorus levels in hypocalcemic neonates, although hyperparathyroidism is present, reflecting a possible PTH resistance. In the literature, some studies report PTH resistance in bone, secondary to vitamin D deficiency (15).

One of the basic points that we would like to emphasize is that a secondary increase in PTH level as a response to hypocalcemia (progress from stage 1 to stage 2) may not take place in vitamin D deficiency in early infancy. Thus, a decreased Ca level and normal or elevated serum phosphorus level in the presence of low and/or inappropriately normal iPTH level may be detected, mimicking hypoparathyroidism. This puzzles clinicians, and pseudohypoparathyroidism is considered for cases where PTH level is high. We would also like to stress that, in some vitamin D deficient cases, serum phosphorus levels may be normal or high, despite an increase in PTH level, due to secondary PTH resistance. Thus vitamin

D deficiency should be kept in mind in the differential diagnosis of pseudohypoparathyroidism. Elevated serum PTH concentration in the face of hypocalcemia and normal/high serum phosphate indicates an element of end organ resistance to PTH, mimicking pseudoparathyroidism. It was postulated in experimental rats that vitamin D depletion made them unresponsive to PTH. The vitamin D-depleted hypocalcemic rats failed to show elevation of serum Ca or a phosphaturic effect to injected PTH extracts, which could be corrected by addition of a small dose of vitamin D. The end organ resistance observed in vitamin D deficiency could result from down regulation of PTH/PTHrP receptor (35). Rao et al (36) observed an impaired phosphaturic response but normal urinary cAMP excretion to PTH in vitamin D deficient infants, similar to the picture in pseudohypoparathyroidism. The response to PTH was restored to normal following vitamin D and Ca supplementation, suggesting that in the presence of vitamin D deficiency and/or hypocalcemia, the renal tubules are resistant to the action of PTH.

The aim of our study was to determine the prevalence of vitamin D deficiency in babies with LNH, to draw attention to the importance of checking perinatal vitamin D levels when investigating the cause of LNH and to highlight that vitamin D deficiency may have biochemical findings leading to clinical confusion with other etiologies. Although the majority of the babies and their mothers included in our study was vitamin D deficient, we tried to be cautious not to indicate vitamin D deficiency as the sole reason for hypocalcemia in these babies. We wanted to emphasize that the prevalence of vitamin D deficiency is high in babies with hypocalcemia and it should be kept in mind as one of the leading possible causes. Cases of pseudohypoparathyroidism due to G protein receptor defects are known to present with symptoms after the newborn and infancy period (37). The cases that may be confused with vitamin D deficiency in the neonatal period are mainly of transient pseudohypoparathyroidism due to PTH receptor immaturity. In these cases, serum 25-OHD levels are not expected to be low. In addition, delay in both PTH release and the maturation in PTH receptors may be associated with neonatal vitamin D deficiency, which leads to biochemical findings, such as hyperphosphatemia and symptomatic hypocalcemia (15).

Study Limitations

A potential limitation of the study was the small number of the patients despite the participation of many centres. It is, however, difficult to recruit a large number of neonates with hypocalcemia in intensive care units due to the exclusion criteria including the presence of maternal diabetes,

neonatal asphyxia, malabsorption, renal insufficiency, liver disease, or use of anticonvulsants and the concerns of parents about giving approval for taking blood samples from their babies. In addition, ELISA which was used in this study to measure vitamin D levels is not the gold standard test because of interference with vitamin D metabolites.

Conclusion

This study provides further evidence highlighting the need to maintain adequate vitamin D status in pregnancy. LNH has a close association with perinatal vitamin D deficiency. Thus the first step of a diagnostic work-up for LNH should be to measure serum 25(OH)D levels.

Ethics

Ethics Committee Approval: The study were approved by the Kocaeli University Ethics Committee (report number: KOU KA EK 2015/322).

Informed Consent: Written informed consent was obtained from parents of the newborns.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Gülcan Seymen-Karabulut, Ayla Günlemez, Ayşe Sevim Gökalp, Şükrü Hatun, Design: Gülcan Seymen-Karabulut, Ayla Günlemez, Ayşe Sevim Gökalp, Şükrü Hatun, Data Collection or Processing: Fatma Kaya Narter, Mehmet Mutlu, Şebnem Kader, Demet Terek, Deniz Hanta, Emel Okulu, Leyla Karadeniz, H. Gözde Kanmaz Kutman, Ayşegül Zenciroğlu, Özmert M. A. Özdemir, Dilek Sarıcı, Muhittin Çelik, Nihat Demir, Özden Turan, Kıymet Çelik, Fatih Kılıçbay, Sinan Uslu, Sara Erol, Sabahattin Ertuğrul, İlkey Er, Hasan Tolga Çelik, Merih Çetinkaya, Filiz Aktürk-Acar, Yakup Aslan, Gaffari Tunç, Ömer Güran, Ayşe Engin Arısoy, Analysis or Interpretation: Gülcan Seymen-Karabulut, Ayla Günlemez, Literature Search: Gülcan Seymen-Karabulut, Ayla Günlemez, Şükrü Hatun, Writing: Gülcan Seymen-Karabulut, Ayla Günlemez, Şükrü Hatun.

Financial Disclosure: This study was supported by Kocaeli University Scientific Research Fund.

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