RESEARCH ARTICLE

MCBS
Mol Cell Biomed Sci. 2024; 8(2): 90-5
DOI: 10.21705/mcbs.v8i2.473

Vitamin D Deficiency is Associated with Hypocalcemia in Preterm Infants

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Background: Vitamin D deficiency results in various problems, like rickets, osteomalacia, heart problems, cancer, diabetes, and autoimmune diseases. Hypocalcemia is a common disorder among preterm infants, indicating vitamin D deficiency. This study was conducted to analyze the association of vitamin D deficiency with hypocalcemia in preterm infants.
Materials and methods: A cross-sectional study was performed in preterm infants born in our hospital from December 2022 to May 2023. Venous blood was collected within the first 24 hours to assess vitamin D and calcium levels. Chi-square test and logistic regression analysis were used to assess the association of gestational age, sex, birth weight, and vitamin D with the incidence of hypocalcemia. The significance was determined with $p<0.05$.
Results: There were 40 preterm newborns, comprising 37.5% moderately preterm, 20% very preterm, and 42.5% extremely preterm. Most subjects were female (52.5%). Low birth weight, very low birth weight, and extremely low birth weight occurred in 55%, 27.5%, and 17.5%, respectively. Vitamin D insufficiency and deficiency were observed in 20% and 80% subjects, respectively. Most subjects had hypocalcemia (62.5%). Chi-square test obtained a significant association of vitamin D deficiency with hypocalcemia ($p=0.029$).
Conclusion: Vitamin D deficiency is significantly associated with the incidence of hypocalcemia in preterm infants.

Keywords: *Vitamin D, hypocalcemia, preterm neonates*

Introduction

Vitamin D has many roles in the human body, from the womb to adulthood. It takes significant part in cardiovascular, immune, respiratory, behavioral, reproductive, digestive, endocrine and musculoskeletal systems. Additionally, vitamin D helps to prevent a number of degenerative illnesses, including diabetes mellitus, cancer, heart disease, hypertension, obesity, and stroke.[1] Primarily, vitamin D functions to regulate calcium and phosphorus balance and bone mineralization.[2,3] Vitamin D affects fetal bone development through calcium metabolism. It also supports bone development and stability.[4-6] In tropical regions like Indonesia with plenty of sunshine, vitamin D deficiency is prevalent even in healthy people. A lifestyle of sun avoiding and inadequate vitamin D consumption result in a high vitamin D deficiency.[7] Diabetes, hyperlipidemia, and hypertension have all been related to vitamin D deficiency.[8]

Submission: March 27, 2024
Last Revision: April 25, 2024
Accepted for Publication: April 26, 2024

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Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v8i2.473

Hypocalcemia is a prevalent disorder in newborns, both in full-term and preterm babies.[9] Hypocalcemia is found in about 1/3 of preterm babies, especially those with very low birth weight experienced hypocalcemia in the first 48 hours of life.[10] Hypocalcemia is one of the signs of vitamin D deficiency in neonates.[11] Hypocalcemia in neonates commonly does not show clinical symptoms, like neuromuscular disorders, jittering, seizures, or impaired cardiac contractility.[12]

Reports regarding the incidence of hypocalcemia varies among studies.[9] A study in Saudi Arabia reported the incidence of hypocalcemia as much as 76%.[13] In another study, hypocalcemia was observed in all babies born to mothers suffering from vitamin D deficiency.[14]

There are two mechanisms how vitamin D serves calcium absorption in the body, either actively or passively. In active mechanism, 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] regulates calcium absorption, impacting the entry of calcium through the apical calcium channel. Meanwhile, the tight connections between the apical and basolateral membranes of enterocytes facilitate diffusion in passive absorption.[15]

Severe hypocalcemic is a sign of vitamin D deficiency. It may appear in the very early infancy.[16] Up to date, there have been a few studies on the association between vitamin D deficiency and hypocalcemia in preterm neonates. Therefore, we carried out this study to assess the association between vitamin D deficiency and hypocalcemia among preterm neonates.

Materials and methods

Study Design and Research Subject

A cross-sectional study in preterm neonates born was conducted between September 2022 to March 2023. Preterm neonates whose parents agreed to have vitamin D and electrolyte level checked were included in this study. All neonates who had congenital abnormalities and typical syndrome were excluded from the study. The characteristic data recorded were gestational age, sex, and birth weight. The study approval was issued by The Ethical committee of Dr. Moewardi Hospital, Surakarta, Central Java, Indonesia with the ethical clearance number 1.286/X/HREC/2022.

Measurement of Vitamin D and Calcium Level

Five mL venous blood samples from each subject in the first 24 hours of life in which 4 mL was used for measuring vitamin D level and 1 mL for calcium level. The blood sample was centrifuged at a speed of 4000 rpm for 15 minutes, then the serum was separated and put into the apparatus for examination. The measurement of vitamin D and calcium levels were conducted with VIDAS® 25OH Vitamin D (BioMérieux SA, Marcy-l'Étoile, France) and Smartlyte electrolyte analyzer (Diamond Diagnostic, Holliston, USA), respectively.

Statistical Analysis

The data were statistically analyzed with SPSS version 26 (IBM, Armonk, NY, USA) using bivariate analysis with the Fisher's test and Chi-square to produce crude Odd Ratio (OR) values. Multivariate analysis with logistic regression was performed to obtain adjusted OR. In addition, 95% confidence interval (95% CI) with significance value of $p<0.05$ was used.

Results

Forty preterm infants were eligible and included in the study. The subjects were predominantly female (52.5%). Based on gestational age, 42.5% of the subjects were extremely preterm. Most subjects had very low birth weight (55%). Out of total subjects, 32 of them had vitamin D deficiency (80%) and 25 of them had hypocalcemia (62.5%). Death occurred in 67.5% subjects (Table 1).

Bivariate analysis obtained a significant relationship between vitamin D deficiency and hypocalcemia (OR=7.67; 95% CI=1.30-45.90; p=0.036). This finding indicated that neonates with vitamin D deficiency had 7.67 times greater risk to have hypocalcemia (Table 2). Multivariate analysis also showed that vitamin D deficiency (OR=7.51; 95% CI=1.22-46.08; p=0.029) was the dominant factor affecting hypocalcemia in preterm infants treated in the neonatal intensive care unit (NICU) and neonatal high care unit (NHCU) (Table 2).

Factors associated with the outcomes of preterm babies were also investigated, it was found that vitamin D deficiency did not correlate with mortality (OR=0.64; 95% CI=0.11-3.69; p=1.000). However, extremely preterm gestational age was the most influential factor for mortality (OR=11.25; 95% CI=1.86-68.13; p=0.008). In addition, hypocalcemia was not a risk factor for mortality in these neonates (OR=0.65; 95% CI=0.16-2.64; p=0.730) (Table 3).

We also obtained that gestational age did not associate with vitamin D level of preterm

Table 1. Baseline characteristics of subjects.

Parameter	n (%)
Gestational Age	
Moderately Preterm	15 (37.5)
Very Preterm	8 (20.0)
Extremely Preterm	17 (42.5)
Sex	
Female	21 (52.5)
Male	19 (47.5)
Birth Weight	
Low Birth Weight (LBW)	22 (55.0)
Very Low Birth Weight (VLBW)	11 (27.5)
Extremely Low Birth Weight (ELBW)	7 (17.5)
Vitamin D Level	
Insufficiency	8 (20.0)
Deficiency	32 (80.0)
Calcium Level	
Normal	15 (37.5)
Hypocalcemia	25 (62.5)
Outcome	
Live	13 (32.5)
Died	27 (67.5)

Gestational age: Moderately preterm (32-<37 weeks); Very preterm (28-<32 weeks); Extremely preterm (<28 weeks). Birth weight: LBW (1,500-2,499 grams); VLBW (1,000-1,499 grams); ELBW (<1,000 grams). Vitamin D level: Insufficiency (12-20 ng/mL); Deficiency (<12 ng/mL). Calcium level: Normal (>1 mmol/L); Hypocalcemia (>1 mmol/L).

babies (moderate, very, and extremely preterm), demonstrated by the OR of 0.75 (95% CI=0.10-5.77; *p*=0.782) for very preterm and 1.17 (95% CI=0.20-6.89; *p*=0.865) for extremely preterm. These OR also indicated that the smaller the gestational age, the more these preterm neonates susceptible to vitamin D deficiency was. Extremely preterm neonates may have a risk of vitamin D deficiency by 1.17 as compared to moderately preterm neonates (Table 4).

Discussion

The present study obtained a significant association between vitamin D deficiency and hypocalcemia in preterm neonates admitted to the NICU and NHCU. Ninety two percent neonates with vitamin D deficiency had hypocalcemia. A significant correlation of vitamin D deficiency with hypocalcemia was also observed in a study conducted in Iranians. In addition, these findings are in accordance with the meta-analysis performed in China.[17,18] The relationship between vitamin D deficiency and hypocalcemia is described by the physiological and pathological mechanisms. Vitamin D plays a crucial role in absorbing calcium and phosphor in the gut. Clinical studies have shown that vitamin D deficiency leads to a decrease in the effective absorption of calcium and phosphor by up to 50%. The accumulation of a continuous decrease in calcium absorption results in low calcium in the blood.[19] The requirement and absorption of prominent elements are massive during growth and development phases. Infants with vitamin D deficiency will be more susceptible to hypocalcemia which is important for homeostasis and bone mineralization.[20] In our study, hypocalcemia occurred in 62.5% subjects. This is in accordance with studies in Saudi Arabia and Iran which revealed the prevalence of hypocalcemia was 76% and 100%, respectively.[13]

Some studies reported that the deficiency or insufficiency of vitamin D is not always followed by hypocalcemia, but the mechanism of this relationship is unclear.[21] Phosphate levels, calcium intake, and foods high in calcium are some additional factors that also affects calcium levels.[7] Several studies demonstrated no significant relationship between vitamin D deficiency and hypocalcemia. This finding is in discrepancy with the result of the present study. It is probably because they used full-term babies for their study subjects and the blood sampling time is also different.[13,22]

There has not been any standard for determining the cut-off point of vitamin D level. A study proposed >30 ng/mL as normal vitamin D level; 21-29 ng/mL as vitamin D insufficiency, and <20ng/mL as vitamin D deficiency.[23] Based on Global Consensus recommendation, the vitamin D levels of >20 ng/mL, 12-20 ng/mL, and <12 ng/mL are considered normal, insufficiency, and deficiency, respectively.[24] In this study, none of preterm neonates had normal vitamin D level, 80% of them had vitamin D deficiency and the remaining

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v8i2.473

Table 2. The association of gestational age, sex, birth weight, and vitamin D level with hypocalcemia.

Parameter	Calcium Level n (%)		Bivariate		Multivariate	
	Normal (n-15)	Hypocalcemia (n=25)	OR (95% CI)	p-value	OR (95% CI)	*p*-value
Gestational Age						
Moderately Preterm	5 (33.3)	10 (40.0)				
Very Preterm	4 (26.7)	4 (16.0)		0.438		
Extremely Preterm	6 (40.0)	11 (44.0)		0.907		
Sex						
Female	10 (66.7)	11 (44.0)			Ref.	
Male	5 (33.3)	14 (56.0)		0.165	2.47 (0.58-10.43)	0.219
Birth Weight						
LBW	7 (46.7)	15 (60.0)				
VLBW	5 (33.3)	6 (24.0)		0.445		
ELBW	3 (20.0)	4 (16.0)		0.594		
Vitamin D Level						
Insufficiency	6 (40.0)	2 (8.0)	Ref.		Ref.	
Deficiency	9 (60.0)	23 (92.0)	7.67 (1.30-45.90)	0.036*	7.51 (1.22-46.08)	0.029*

*Significant if $p<0.05$.

subjects had vitamin D insufficiency. This is in line with a study in Turkey that vitamin D deficiency was common in preterm babies with gestational age 29-35 weeks.[25] Another study suggests that fetal vitamin D level is not affected by gestational age, but it is associated with maternal vitamin D level.[26]

The relationship of infant outcomes with several risk factors was also assessed in the present study. It was found that the most influential factor was gestational age, whereas extremely preterm neonates had a risk of death 11.25 times when compared to moderate preterm ones. This is because extremely preterm infants are riskier to have various complications such as sepsis, asphyxia, respiratory distress syndrome, necrotizing enterocolitis, and hypothermia.[27]

The present study revealed that vitamin D and calcium levels had no association with mortality in preterm neonates. A study conducted in 2021 also revealed that there is no relationship between vitamin D level and mortality in neonates. Similar findings were obtained in a study performed in Turkey showing that vitamin D deficiency has no correlation with neonatal sepsis, respiratory distress, necrotizing enterocolitis, intraventricular hemorrhage, and mortality.[18,28] Some experts stated that hypocalcemia is not an independent predictor for mortality; hypocalcemia is most likely a marker of disease severity.[29,30]

This study demonstrates the associations between hypocalcemia and vitamin D deficiency in premature neonates. The strength of our study is that we only included premature neonates. However, this study has several limitations. Firstly, we did not measure maternal vitamin D, magnesium, and parathyroid hormone (PTH) levels. Secondly, this is a single center study so that the subjects do not represent general population. Lastly, the short study period limited the sample size. Thus, future study may require maternal vitamin D level measurement, along with magnesium and PTH levels. In addition, longer period and multi centers involvement may give more robust outcome.

Conclusion

In preterm neonates, vitamin D deficiency is significantly associated with hypocalcemia. Preterm neonates with vitamin D deficiency have eight times

Table 3. The association of gestational age, sex, birth weight, vitamin D level, and calcium level with the outcomes.

Parameter	Outcome n (%)		Bivariate	
	Live (n=13)	Died (n=27)	OR (95%CI)	*p*-value
Gestational Age				
Moderately Preterm	9 (69.2)	6 (22.2)	Ref.	
Very Preterm	2 (15.4)	6 (22.2)	4.50 (0.67-30.23)	0.122
Extremely Preterm	2 (15.4)	15 (55.6)	11.25 (1.86-68.13)	0.008*
Sex				
Female	7 (53.8)	14 (51.9)	Ref.	
Male	6 (46.2)	13 (48.1)	1.06 (0.43-2.59)	0.906
Birth Weight				
LBW	9 (69.2)	13 (48.1)	Ref.	
VLBW	1 (77.7)	10 (38.0)	6.92 (0.75-64.02)	0.088
ELBW	3 (23.1)	4 (14.8)	0.92 (0.16-5.16)	0.927
Vitamin D Level				
Insufficiency	2 (15.4)	6 (22.2)	Ref.	
Deficiency	11 (84.6)	21 (77.8)	0.64 (0.11-3.69)	1.000
Calcium Level				
Normal	4 (30.8)	11 (40.7)	Ref.	
Hypocalcemia	9 (69.2)	16 (59.3)	0.65 (0.16-2.64)	0.730

*Significant if $p<0.05$.

risk of hypocalcemia. Therefore, vitamin D screening can be considered for premature neonates in order to give early intervention to prevent serious complication due to vitamin D deficiency.

Acknowledgements

The author gratefully acknowledged the study participants who had volunteered to participate in this study.

Table 4. The relationship between gestational age and vitamin D level in preterm neonates.

Parameter	Vitamin D Level n (%)		Bivariate	
	Insufficiency (n=8)	Deficiency (n=32)	OR (95%CI)	*p*-value
Gestational Age				
Moderately Preterm	3 (37.5)	12 (37.5)	Ref.	
Very Preterm	2 (25.0)	6 (18.8)	0.75 (0.10-5.77)	0.782
Extremely Preterm	3 (37.5)	14 (43.8)	1.17 (0.20-6.89)	0.865

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v8i2.473

Authors Contribution

N, HWM, and AGM were involved in conceptualizing and planning the research. N performed the data acquisition and collection, as well as calculated the experimental data. N also designed the figures. N, HWM, and AGM performed the data analysis, drafted the manuscript, interpreted the results, and took part in giving critical revision of the manuscript.

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