Vitamin D deficiency and its associated comorbidities in very low birth weight neonates

Suresh Kumar Tripathy[1], **Soumini Rath**[1], **Manas Kumar Nayak**[1], ***Santosh Kumar Panda**[1]

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Abstract

Objectives: To assess the prevalence of vitamin D deficiency (VDD) and its associated co-morbidities among very low birth weight (VLBW) neonates.

Method: A prospective observational study was carried out at a tertiary care neonatal centre of Odisha, India, from February to November 2018. All VLBW infants admitted within the first 24 hours after birth were included in the study after parental consent. Infants with multiple congenital malformations were excluded. Initial blood samples were collected within the first 24 hours of life and vitamin D estimation was done by chemiluminescence immunoassay. Serum calcium and alkaline phosphatise (ALP) were measured by cresolphthalein complexone and para-nitro phenyl phosphate method respectively. Statistical Package for the Social Sciences version 16 was used for statistical analysis.

Results: Our study included 40 VLBW neonates with a mean birth weight of 1133.95±208.487g and a mean gestational age of 30.60±2.274 weeks**.** VDD prevalence was 67.5% and mean vitamin D level was 16.906±12.708ng/dl. However, the indirect laboratory parameters of VDD like mean serum calcium, phosphorous and ALP levels were within the normal range. There was higher incidence of respiratory distress syndrome (RDS) and sepsis in babies with VDD.

Conclusions: In this study VDD was found in 67.5% of VLBW babies. Incidences of RDS and neonatal sepsis were greater in babies with VDD.

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[1]*Kalinga Institute of Medical Sciences, KIIT University, Bhubaneswar, Odisha, India*
**Correspondence:* doc.sant@yahoo.co.in

https://orcid.org/0000-0003-0421-8747

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Introduction

Around 5-18% of all deliveries are completed before 37 weeks and 4-8% of neonates are very low birth 'weight (VLBW) babies[1]. Vitamin D deficiency (VDD) is a known risk factor for osteopenia of prematurity[2]. In northern India VDD prevalence in pregnant women is 93.5%, whereas in southern India VDD prevalence is 61%[3,4].

Objectives

To assess the prevalence of VDD and its associated co-morbidities among VLBW neonates.

Method

A prospective observational study was carried out in the neonatal intensive care unit (NICU) of Odisha, India, from February to November 2018. All VLBW infants admitted to the NICU in the first 24 hours after birth were included in the study. Infants with congenital malformations and infants whose parents denied consent for the study were excluded.

In all cases, a detailed history was taken giving due importance to maternal factors such as age, parity, history of gestational diabetes, hypertension, premature rupture of membranes, infection, medication like ante-natal corticosteroid therapy, obstetric complications like fetal distress, mode of delivery and natal history such as Apgar scores, resuscitation, birth weight and gestational age. Data were recorded in a predesigned proforma.

Within 24 hours of birth 3ml blood samples of the newborns were collected in two separate red topped vials (with clot activator), one for 25 hydroxy vitamin D [25(OH) D] and the other for alkaline phosphatase (ALP), total serum calcium and phosphorous. Vitamin D was estimated by the chemiluminescence immunoassay (CLIA) method. Serum calcium and ALP were measured by cresolphthalein complexone method and para-nitro phenyl phosphate method respectively. We considered 25(OH) D <20 ng/mL as VDD and 25(OH) D <10 ng/mL as severe VDD as per Holick MF, *et al*[5]; 25(OH) D level between 21-29 ng/mL was considered vitamin D insufficiency and 25(OH) D level ≥30 ng/mL was taken as vitamin D sufficiency.

Septic screen was done when there was a history of preterm premature rupture of membranes, maternal fever, foul smelling liquor, severe birth asphyxia with active resuscitation, prolonged labour or when babies presented with poor feeding, lethargy, respiratory distress, seizures, abdominal distension or bleeding. Appropriate culture specimens were obtained before initiating antibiotic therapy which was modified later on the basis of laboratory reports and response of the patient to treatment.

Under aseptic conditions about one ml of blood was drawn for septic screening and one ml for blood culture. One ml of blood was drawn and collected in culture bottle. Alcohol-Povidone Iodine-Alcohol was applied in consecutive steps. Povidone iodine was applied in concentric circles moving outwards from the centre and allowed to dry for at least 30 seconds. One ml of blood in 10-20 ml of broth or 0.5 ml of blood in 5-10ml broth was taken for blood culture. Leucopenia (<5000/cu mm) or absolute neutropenia (<1800/cu mm), band cell count of more than 20% or immature to total neutrophil (I/T) ratio >0.2 and C-reactive protein (CRP) of >10mg/dl constitute a useful septic screen for clinically doubtful cases. Two or more positive tests out of the above 5 were considered as a positive septic screen.

Lumbar puncture (LP) was done in suspected cases of late-onset neonatal sepsis or those having staring look, seizures and bulging fontanelle except when the infant was too sick to undergo LP. Cerebrospinal fluid (CSF) was inoculated for cytology and gram staining, biochemical analysis, and culture/ sensitivity. CSF cytology showing >30 white blood cells with >60% polymorphs, glucose <50% of blood glucose or <40 mg/dl, protein >150mg/dL in term babies and 180 mg/dL in preterm babies were diagnosed as meningitis. Midstream or catheterized urine was collected in a sterile container for routine examination. In suspected cases of inborn errors of metabolism, reducing sugar was especially looked for. For culture, suprapubic specimen was collected especially in girls and sent for culture / sensitivity. In babies who presented with respiratory distress, chest x-ray was done to look for radiological evidence of respiratory distress syndrome (RDS).

Ethical issues: Ethical clearance for the study was obtained from the Institutional Ethics Committee of the Kalinga Institute of Medical Science Hospital, Kalinga Institute of Industrial Technology University, Bhubaneswar, Odisha, India (No. KIMS/KIIT/IEC/83/2017). Written informed consent was obtained from the mothers of the neonates involved in the study.

Statistical analysis*:* Statistical Package for Social Sciences (SPSS) version 16 (Chicago, IL, USA) was used for statistical analysis and Chi-square test and unpaired t-test were used for comparison of descriptive characteristics between the groups.

Results

During the study period there were 415 admissions to the NICU, of which 63 were VLBW babies. Among the VLBW babies, 40 parents gave consent for the study. Our study thus included 40 VLBW neonates. The demographic profile of the study population is shown in Table 1.

Table 1: ***Demographic profile of study population***

Characteristic	Number (%)
Mode of delivery	
Vaginal delivery	22 (55)
Caesarean section	18 (45)
Sex	
Male	17 (42.5)
Female	23 (57.5)
Birth weight	
<1000g	11 (27.5)
1000-1499g	29 (72.5)
Gestational age	
<28 weeks	05 (12.5)
28 to <32 weeks	21(52.5)
32 to 35 weeks	14 (35.0)
>35 weeks	0 (0)
Place of delivery	
Inborn	34 (85.0)
Outborn	06 (15.0)
Antenatal steroid	
Complete	25 (62.5)
Incomplete	10 (25.0)
None	05 (12.5)

In our study 14 (35%) neonates were born between 32 to 35 weeks of gestation. Of them 7 (50%) had VDD <20 ng/ml, 6 (42.9%) having severe VDD (<10 ng/ml). The other 7 (50%) had vitamin D levels ≥20 ng/ml. The clinical characteristics of study subjects are shown in Table 2.

Table 2: ***Clinical characteristics of study subjects***

Variables	Mean (SD)
Birth weight (g)	1133.95 (208.487)
Gestational age (weeks)	30.60 (2.274)
Serum vitamin D (ng/ml)	16.9055 (12.708)
Serum calcium (mg/dl)	8.5278 (0.807)
Serum phosphorous (mg/dl)	6.505 (1.374)
Serum alkaline phosphatase (U/L)	202.8308 (73.002)

The vitamin D status of the 40 neonates is shown in Table 3.

Table 3: ***Vitamin D status***

Vitamin D level	n (%)
Severe vitamin D deficiency (<10ng/ml)	18 (45.0)
Mild vitamin D deficiency (10-19ng/ml)	09 (22.5)
Vitamin D insufficiency (20-29ng/ml)	07 (17.5)
Vitamin D sufficiency (≥30ng/ml)	06 (15.0)

Table 4 is a comparison between infants with and without vitamin D deficiency. Though the mean serum vitamin D level was below normal value, the indirect biochemical parameters of vitamin D status, like mean serum calcium (8.5278±0.80713 mg/dL), mean serum phosphorous (6.505±1.3744 mg/dL) and mean serum ALP (202.8308±73.00187 U/L) were within the normal range (Table 2)**.** However, gestational age or birth weight of babies did not have a significant association with VDD. Also no significant difference in serum calcium, phosphorous, ALP level was found between infants with or without VDD (Table 4).

Table 4: ***Comparison between infants with and without vitamin D deficiency***

Variables	Vitamin D deficiency		p-value
	No	Yes	
	Mean ±SD	Mean ±SD	
Gestational age (weeks)	31.15 ±1.95	30.33 ±2.40	0.29
Birth weight (g)	1140.31±163.50	1128.30 ±225.64	0.86
Serum calcium (mg/dl)	8.6015 ±0.7335	8.4922 ±0.8514	0.47
Serum phosphorous (mg/dl)	6.575 ±1.809	6.474 ±1.172	0.83
Serum alkaline phosphatase (U/L)	191.5800 ±68.1182	207.8311 ±75.7723	0.51

The relationship of VDD with neonatal morbidities is shown in Table 5. Neonates with VDD had significantly higher incidence of RDS and neonatal sepsis. There was no significant association between the incidence of necrotising enterocolitis, broncho-pulmonary dysplasia, retinopathy of prematurity and osteopenia of prematurity and neonatal vitamin D status at birth (Table 5).

Table 5: ***Relationship of vitamin D deficiency with neonatal morbidities***

Variables	Vitamin D deficiency		p-value
	No *(n=13)*	Yes *(n=27)*	
Respiratory distress syndrome	05	15	0.0253
Sepsis	02	10	0.0209
Necrotising enterocolitis	03	05	0.4795
Broncho-pulmonary dysplasia	06	09	0.4386
Retinopathy of prematurity	04	07	0.3657
Osteopenia of prematurity	06	11	0.1452

Discussion

This study was conducted to assess the prevalence of VDD among VLBW neonates and its associated co-morbidities. Low neonatal vitamin D was associated with low maternal vitamin D level in various studies[6,7]. In our study, 67% of VLBW neonates had VDD and 45% of babies had severe VDD. The mean birth weight and gestational age of VLBW neonates in our study were 1133.95±208.487g and 30.60±.274 weeks respectively and the mean vitamin D level was 16.9055±12.70802 ng/ml. Similar to our study, Chhina AS, *et al*[8] analysed the 25(OH) D levels of preterm infants <32 weeks at 48-72 hours, in Bangalore, India. The mean 25(OH) D level was 14.8±7 ng/mL and neonates born at<28 weeks had low vitamin D levels.

The prevalence of VDD in VLBW neonate at birth varied in many studies. The mean vitamin D level of VLBW neonate at birth was 16.906±12.708 ng/ml and the prevalence of VDD 98.9% and severe VDD 51.5% in the study by Park SH, *et al*[6]. In an Indian study by Jain V, *et al*[7]*,* prevalence of VDD, severe VDD and vitamin D insufficiency were 66.7%, 27.1% and 19.8% respectively[7]. Naik KD, *et al*[9] found the mean cord blood 25(OH) D level of term healthy neonates to be 11.36±4.75 ng/mL in Kerala, India[9]. The above study showed that neonatal VDD was also found in tropical climates. Due to financial constraints we could not evaluate the vitamin D status of mothers.

In this study, the mean serum calcium, phosphorous and ALP levels were comparable in infants with or without VDD at birth. In our study population, majority of neonates had VDD but the indirect biochemical parameters of vitamin D status like mean serum calcium, phosphorous, ALP were within the normal range. Hence indirect biochemical marker could not suggest the vitamin D status at birth. We found no positive correlation between VDD and birth weight. With subgroup analysis of our study, higher VDD was found among extremely premature (<28 weeks) infants. Several studies found that there was no correlation between vitamin D level and birth weight or gestational age[7,10,11].

We found that VDD was significantly associated with RDS and neonatal sepsis but had no significant association with bronchopulmonary dysplasia, osteopenia of prematurity, necrotizing enterocolitis

or retinopathy of prematurity. Lung maturation is enhanced by vitamin D. Lipopolysaccharide induced alveolar inflammation and epithelial damage are aggravated by VDD. Boskabadi H, *et al*[12] and Gatera VA, *et al*[13] also reported a significant correlation between VDD and RDS. Vitamin D plays an important role in neonatal immunity. It regulates inflammation, chemokine production and immunomodulation. Nearly all immune cells like lymphocytes, monocytes and dendritic cells have vitamin D receptor (VDR). Vitamin D regulates neutrophil chemotaxis and phagocytic function. Vitamin D helps in improvement of immune function by production of antimicrobial peptides like cathelicidin and β defensin from lymphocytes, monocytes and macrophages. Cathelicidin defends against micro-organism like bacteria, fungi and mycobacteria entry at various sites including skin, respiratory mucosa and gastrointestinal systems. Both peptides killed microbes by disrupting their cell membrane. Cetinkaya M, *et al*[14] and Cizmeci MN, *et al*[15] found early onset neonatal sepsis was higher in babies with VDD.

Conclusions

In this study VDD was found in 67.5% of VLBW babies. Incidences of RDS and neonatal sepsis were greater in babies with VDD.

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