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Study of serum vitamin d levels and its association with neonatal sepsis among newborns

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Introduction: Vitamin D plays an important role in immune function. It plays an important role in the neonatal period in fetal skeletal growth, prevention of rickets, neonatal sepsis, respiratory tract infections, cardiovascular diseases, diabetes, and other endocrine disorders. It has been found that newborns' low level of cholecalciferol is closely related to neonatal sepsis. **Aims and Objectives:** To determine the neonatal plasma cholecalciferol levels and severity of vitamin D deficiency in neonatal sepsis. **Material and Methods:** This was a prospective, observational study conducted at Neonatal Care Intensive Unit of R. D. Gardi Medical College, Ujjain. The cases included all babies >34 weeks of gestational age having postnatal age of 0-28 days with clinical signs and laboratory findings of neonatal sepsis. Statistical analysis was performed using STATA 10.0. **Results:** Total 70 newborns with sepsis were included in the study among which n=47 (67%) had deficient (less than 20ng/ml), n=16 (22.86%) had insufficient (between 20-30ng/ml) and n=7 (10.00%) of newborns had sufficient levels of cholecalciferol (more than 30ng/ml). The mean cholecalciferol levels among term were 18.53ng/ml (±4.8) and in preterm is 15.7ng/ml (±2.6) and this finding was statistically significant (p=0.04). The mean cholecalciferol in newborns with culture-positive sepsis was 14.8ng/ml (±6.04) and in culture-negative sepsis was 16.4ng/ml (±5.2) and this finding was statistically significant (p=0.02). **Conclusions:** More than 65% of newborns were deficient in 25-OH cholecalciferol and the mean 25-OH cholecalciferol levels were lower in preterm than in term newborns and lower in newborns with culture-positive sepsis than in newborns with culture-negative sepsis.

Keywords: Vitamin D, Cholecalciferol, Childhood

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Introduction

Vitamin D is a regulator of immune function and is implicated in predisposing to infection, autoimmune and cardiovascular diseases, mental illness, and cancer [1-3]. It was suggested that it might have a role in the optimal functioning of the innate immune system by inducing antimicrobial peptides in epithelial cells, neutrophils, and macrophages [4,5].

Cholecalciferol has a direct role in the production of antimicrobial peptides such as cathelicidin, which are produced upon activation of up-regulated vitamin receptors, require 25(OH)D as a substrate for production, and may play an important role in preventing infection during pregnancy or early childhood [4]. Vitamin D insufficiency is an important risk factor for neonatal sepsis and acquiring infections such as tuberculosis, acute lower respiratory tract infections, pneumonia, and influenza.

There are two forms of vitamin D. Vitamin D2 (ergocalciferol) is derived from the ultraviolet irradiation of plant ergosterol, and vitamin D3 (cholecalciferol) is found in fish oils and is made in the skin. Cholecalciferol is produced from 7-dehydrocholesterol in skin exposed to ultraviolet B (UVB) radiation, [6] and both vitamins D2 and D3 may be ingested through the diet or through supplementation [7]. In the present study, vitamin D will refer to both vitamin D2 and D3.

Vitamin D is hydroxylated in the liver and becomes 25(OH)D, or calcidiol, the primary circulating form of vitamin D. 25(OH)D may be converted to 1,25(OH)2D (calcitriol), the active form of vitamin D, by 1-alpha hydroxylase (CYP27B1) in the kidneys and other organs. The production of 1,25(OH)2D in the kidney is regulated by plasma parathyroid hormone levels as well as serum calcium and phosphorus levels [7]. 1,25(OH)2D is broken down into its inactive metabolite by 24-hydroxylase (CYP24). Negative feedback aids in regulating 1,25(OH)2D levels, as 1,25(OH)2D inhibits renal 1-alpha hydroxylase and stimulates 24-hydroxylase, and this maintains circulating levels within a limited range.

The present study aimed to estimate the prevalence of low neonatal levels of cholecalciferol, identify maternal risk factors for low neonatal cholecalciferol levels, and correlate the relationship between low neonatal levels of cholecalciferol and adverse neonatal outcomes.

Material and Methods

This is a prospective, observational study which is conducted from November 2017 to July 2019. The study is conducted at N.I.C.U of C.R. Gardi Hospital and associated hospitals of R. D. Gardi Medical College, Ujjain. Diurnal variation of 1, 25 OH cholecalciferol must be considered, i.e. blood sampling must be standardized according to the time of day. In the present study, all the samples were taken between 10 am to 5 pm.

Inclusion criteria

- The cases included all babies >34 weeks of gestational age having postnatal age of 0-28 days with clinical signs and laboratory findings of neonatal sepsis admitted to NICU at Department of Pediatrics CRGH Hospital, Surasa Ujjain

Exclusion criteria

- Neonates less than 34 weeks gestation.
- Presence of major congenital anomalies.
- Severe birth asphyxia.
- Infants who have received vitamin D supplementation after birth.
- Infants who have already received Antibiotics.
- If parents were not available to give informed consent or refused to give consent.

The definition used for study purposes: Neonatal septicemia refers to a clinical syndrome characterized by systemic signs and symptoms due to generalized bacterial infection with positive blood culture in the first 4 weeks of life [8,9].

Neonatal septicemia is further divided into [10]:

- Early-onset septicemia is defined as septicemia within the first 72 hours of life.
- Late-onset septicemia is defined as septicemia after 72 hours of life.

New Ballard scoring to calculate the gestational age of newborns after birth [11,12].

The babies will be classified according to Lubchenco and Battaglia charts as [12]: Appropriate for the gestational age (AGA), Small for the gestational age (SGA), and Large for the gestational age (LGA). The birth weight of the neonates is categorized as 1501 - 2000 g, 2001 - 2500 g, and More than 2500 g.

Serum levels of vitamin D is classified as: [6] Sufficient = > 30 ng/ml, Insufficient =>20-30ng/ml

And Deficient = <20 ng/ml.

Suspected neonatal sepsis will be considered if the neonate has features of perinatal risk factors:- Maternal pyrexia (within 1 week prenatal and/or 48 hours postnatal), Prolonged rupture of membranes (more than 24 hours), Foul-smelling vaginal discharge or/and Maternal urinary tract infection diagnosed in last month.

Neonates having clinical features suggestive of sepsis are included such as- unexplained hypothermia/hyperthermia, lethargy, irritability, poor feeding or milk intolerance, respiratory dysfunction, cardiovascular dysfunction, hypotonia, circumoral cyanosis or pallor are included.

Sample size: The sample size based on proportion with a 90% confidence level was 63 cases [13]. The total study sample taken was 70 cases.

Surveillance: All the neonates who fulfill the inclusion criteria were taken in the study after taking written consent from the patient.

A pretested written Proforma was used to record the detailed history, clinical findings, and investigations. A detailed antenatal history including maternal age, religion, socio-economic status, last menstrual period, risk factors, and drug intake was obtained from the mother/legal guardians of the baby and from mother's medical records.

Investigation as and when required: Vitamin D levels, Hemoglobin, Total leukocyte count, Direct leukocyte count, CRP, Electrolytes, Random blood sugar, CSF, Blood culture.

Blood Culture Sampling and Processing: Blood was collected using aseptic technique as per the standard procedure. 0.5ml of blood was mixed with 10ml of citrated glucose broth and inoculated as per standard procedure on blood and MacConkey agar. The colonies were examined after 24, 48, 72, and 120 hours.

Vitamin- D Testing: Cholecalciferol estimation was done using MAGLUMI 25-OH VITAMIN D Kit manufactured by Snibe diagnostics. The test was performed on MAGLUMI fully-auto chemiluminescence immunoassay (CLIA) analyzer.

The MAGLUMI 25-OH cholecalciferol assay uses a two incubation chemiluminescence immunoassay for the quantitative estimation of cholecalciferol in human serum. First incubation: 25-OHD is dissociated from its binding protein by a displacing Reagent and binds to 25-OHD antibody-forming an antigen-antibody complex. Second incubation: 25-OHD coated microbeads are added, and then the solid phase binds to the unbound 25-OHD antibodies. The unbound material is removed during a wash cycle.

Subsequently, a starter was added to initiate a flash reaction. The resultant chemiluminescent reaction is measured as relative light units (RLUs).

An inverse relationship exists between the amount of 25-OH cholecalciferol and RLUs. The analyzer automatically calculates cholecalciferol levels in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in ng/ml.

Ethical Approval: The study was approved by the institutional ethical board (IEC ref no. 170).

Statistical Analysis: Data was entered in EpiData Entry (Version 3.1, EpiData Software Association, Odense, Denmark) and statistical analyses were performed using Stata (Version 13.0, StataCorp. Texas, USA). Analyses of 25-OH cholecalciferol levels in the study group were (mean ± s.d.) for continuous data with normal distribution, median and interquartile range (median (IQR)) for continuous data with non-normal distribution and frequencies and percentages for quantitative data. The differences between groups were evaluated using X2 tests for qualitative data and t-test for independent samples for continuous data with the normal distribution.

Pearson correlation was used to evaluate the relationship between maternal and neonatal 25-OHD. Continuous variables were reported as meanwhile categorical variables were given as the number or the percentage of newborns with the characteristic of interest. A p-value < 0.05 was considered statistically significant. Statistical analysis was performed using STATA 10.0.

Results

A total of 70 neonatal cases were studied and the newborns were divided into 3 groups on the basis of 25-OH cholecalciferol deficiency into Group1, Group 2, and Group3 as deficient, insufficient, and sufficient respectively.

The male and female ratio in the study group was almost equal (48.57% vs 51.53%). The majority of the population in the present study were Hindu 59

(84.29%), as it is the Hindu predominant area.

Maximum number of newborns 48.57% had birth weight more than 2.5kg followed by 35.71% having a weight between 2-2.5kg. Only 15.7% of newborns had birthweight between 1.5-2kg. Low birth weight was found in 51.42% newborns. 50.00% were term AGA, followed by term SGA (22.86%), preterm SGA (14.29%) and preterm AGA (12.86%) and the Mean Gestational age was 39.48 weeks.68.57% of newborns had the age of their mothers between 20-30yrs and mean maternal age in the study group is 26.6 years and 67.14% mothers in the study group were Primi gravida.

38.57% mothers did not have any risk factors while 61.43% mothers had risk factors in which PROM (15.71%) was the most common risk factor followed by fever (14.29%), anemia (12.86%), pre-eclampsia (10.00%) and hypertension (8.57%) Respectively.

Table-1: Distribution of newborns according to Maternal risk factors.

Maternal risk factors	Cases(n=70)
Fever	10 (14.29%)
PROM	11 (15.71%)
Pre-eclampsia	7 (10.00%)
Hypertension	6 (8.57%)
APH	0
Anemia	9 (12.86%)
None	27 (38.57%)
Total	55

In the present study, the most common clinical feature among newborns was respiratory distress (47.14%), followed by apnea (14.28%) and hypothermia (7.14%). The most common system involved was the respiratory system (61.43%).

Table-2: Distribution of newborns according to Clinical features.

System	Symptoms	A study group with specified symptoms	A study group with specified system involvement
Respiratory system	Respiratory distress	33 (47.14%)	43(61.43%)
	Apnea	10 (14.28%)	
Central nervous system	Dullness	4 (5.71%)	9 (12.86%)
	Excessive crying	3 (4.28%)	
	Neonatal seizures	2 (2.85%)	
Gastrointestinal Tract	Vomiting	4 (5.71%)	6 (8.57%)
	Abdominal distention	2 (2.85%)	
Miscellaneous	Not accepting feed	3 (4.28%)	12(17.14%)
	Fever	2 (2.85%)	
	Hypothermia	5 (7.14%)	
	Icterus	1 (1.42%)	
	Pustules>10	1 (1.42%)	

67.14 % newborns were deficient in 25-OH cholecalciferol, 22.86% newborns had insufficient levels, while 10.00% of newborns had sufficient levels of 25-OH cholecalciferol.

Table-3: Distribution of newborns according to 25-hydroxy Vitamin D level.

25-hydroxy VitaminD level (ng/ml)	Cases(n=70)
Deficient (less than 20)	47 (67.14%)
Insufficient (20-30)	16 (22.86%)
Sufficient (more than 30)	7 (10.00%)
Total	70

The mean 25-OH cholecalciferol levels among term were 18.53ng/ml (±4.8) while in preterm is 15.7ng/ml (±2.9) and this data is statistically highly significant(p-0.04).

Table-4: Distribution according to Mean 25-OH cholecalciferol in newborns with culture-positive and in culture-negative sepsis group.

Mean 25-OH cholecalciferol level	Culture positive	Culture negative	P-value
	14.8ng/ml (±6.04)	16.4ng/ml (±5.2)	p = 0.02

The mean 25-OH cholecalciferol in newborns with culture-positive sepsis was 14.8ng/ml (±6.04) (p-0.02). 90% (n-63) newborns had a positive blood culture and the most common organism grown was Burkholderia (40.85%).

Discussion

Vitamin D improves survival in acute illness by

Boosting innate immunity. It acts as an immune system modulator. Cholecalciferol has systemic antimicrobial effects that may be crucial in a variety of both acute and chronic illnesses. A major component of the antimicrobial action of cholecalciferol is through the production of antimicrobial peptides.

Maternal 25(OH)D, is thought to freely cross the human placenta as it does in rats. The placenta expresses vitamin D receptors (VDR) and also produces the enzyme CYP27B1 to convert 25(OH)D to its active form Adequate cholecalciferol status is critically important for the neonates.

At 4 weeks of gestation, the placenta is formed, allowing nutrients to be transferred from the mother to the fetus. From 4 weeks of gestation to term, 25(OH)D easily diffuses across the placenta, allowing the 25(OH)D concentrations in fetal cord blood to reach 87% to that of the mother's concentrations.

The physiologically active metabolite 1,25(OH)2D, does not readily cross the placenta. However, the placenta and the fetal kidney express the enzyme 1α-hydroxylase that converts 25(OH)D to 1,25(OH)2D in these tissues, which may contribute to fetal circulating levels of 1,25(OH)2 D.

Vitamin D deficiency is considered when 25-OH cholecalciferol levels are less than 20ng/ml, insufficient levels are between 20-30 ng/ml and sufficient levels are above 30ng / ml according to AAP.

38.57% mothers did not have any risk factors while 61.43% mothers had risk factors in which PROM (15.71%) was the most common risk factor followed by fever (14.29%), anemia (12.86%), pre-eclampsia (10.00%) and hypertension (8.57%) respectively. A similar study was conducted by Taha Soliman [20] he found that 52% of mothers had associated risk factors for early-onset sepsis among study group and 20% in the control group.

In a previously conducted study in which 42.8% of mothers have maternal risk factors among the study group. Risk factors in the control group in the present study have considerably reduced from the previous study (23.7% vs 38.4%) because of early prevention, detection of risk factors, and early referral of pregnant mothers for institutional deliveries.

In the present study, the most common clinical Feature among newborns was respiratory distress (47.14%), followed by apnea (14.28%) and hypothermia (7.14%). The most common system involved was the respiratory system (61.43%).

67.14 % of newborns in the study group were deficient in 25-OH cholecalciferol (less than 20ng/ml), 22.86% of newborns in the study group had insufficient levels of 25-OH cholecalciferol (between 20-30ng/ml), 10.00% of newborns in the study group had sufficient levels of 25-OH cholecalciferol (more than 30ng/ml).

Similarly study conducted previously found that 63% newborns had a deficiency, 24% had insufficient levels and 13% had sufficient levels of 25 OD cholecalciferol [10,11,12].

In the present study, the mean 25-OH cholecalciferol levels among the term are 18.53ng/ml (±4.8) and in preterm is 15.7ng/ml (±2.6) and this data is statistically highly significant(p-0.04).

In the present study, 88.57% of newborns had a positive blood culture and the most common organism in the present study came out to be Burkholderia (40.85%) followed by Pseudomonas (23.94%) and MRSA (8.45%). Similarly, a study conducted previously, found an increased incidence of Burkholderia in neonatal sepsis, although the prevalence of the organism was not stated.

The mean 25-OH cholecalciferol in newborns with culture-positive sepsis was 14.8ng/ml (±6.04) and in culture-negative sepsis group, it was found to be 16.4ng/ml (±5.2). The data is statistically highly significant in the studies group (p-0.02) and the level of mean 25-OH cholecalciferol is found to be lower in the culture-positive group than in a culture-negative group.

Similarly, a study done by Cetinkaya et al [13] found that mean 25-OH cholecalciferol in culture-positive sepsis is 10.1ng/ml(±1.8) while in culture-negative sepsis group is 8.4ng/ml(±3.2). (p-0.25) showing that the data is insignificant.

Conclusion

The present study strongly recommends the assessment of 25-OH cholecalciferol levels in both term and preterm newborns as a sensitive marker for neonatal sepsis.

What does the study add to the existing knowledge?

The study strongly recommends early supplementation of 25-OH cholecalciferol to all preterm and term newborns who are found deficient or insufficient levels of 25-OH cholecalciferol.

Author's contribution

Dr. Vijay Kumar Gupta: Concept, study design

Dr. (Mrs) Mamta Dhaneria: Manuscript preparation

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