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ORIGINAL ARTICLE

Comparative Study of Vitamin D Status in Late Preterm and Term Neonates with Sepsis and Healthy Neonates in a Tertiary Care Hospital in Northern India

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Abstract

Objective: Comparative study of vitamin D status in late preterm and term neonates with sepsis and healthy neonates in a tertiary care hospital in Northern India. **Methods:** In this hospital-based case control study conducted over 12 months, a total of 70 late preterm and term neonates were included, with 35 neonates each in the sepsis group and non-sepsis group. The difference between the mean vitamin D level of the neonates with sepsis and neonates without sepsis group was calculated. The association of VDD with neonatal sepsis was determined by multivariate logistic regression analysis. P value of 0.05 was considered statistically significant. **Results:** The study found that about 80% of neonates in the septic group and 52% of neonates in the non-septic group had vitamin D deficiency. The mean Cholecalciferol in the septic group (13.51 ng/ml ± 6.56 ng/ml) was significantly lower (p value 0.02) than in the non-septic group (24.47 n/ml ± 8.73 ng/ml). There was a positive correlation between degree of vitamin D deficiency (severe VDD and insufficiency) and sepsis ($p = 0.005$). Prolonged hospital stay and antibiotic treatment were observed in the septic group ($p < 0.001$) with significant mortality ($p < 0.001$). Logistic regression analysis showed that lower vitamin D levels, maternal hypertension, premature birth, and lower birth weight significantly increased the odds of sepsis, highlighting these as predictive factors for adverse outcomes. **Conclusion:** The study showed a significant association of neonatal sepsis with vitamin D. The study highlights extended hospital stays and increased mortality among septic neonates with vitamin D deficiency.

Keywords: Vitamin D; Sepsis; Late Preterm; Term; Hospital stay

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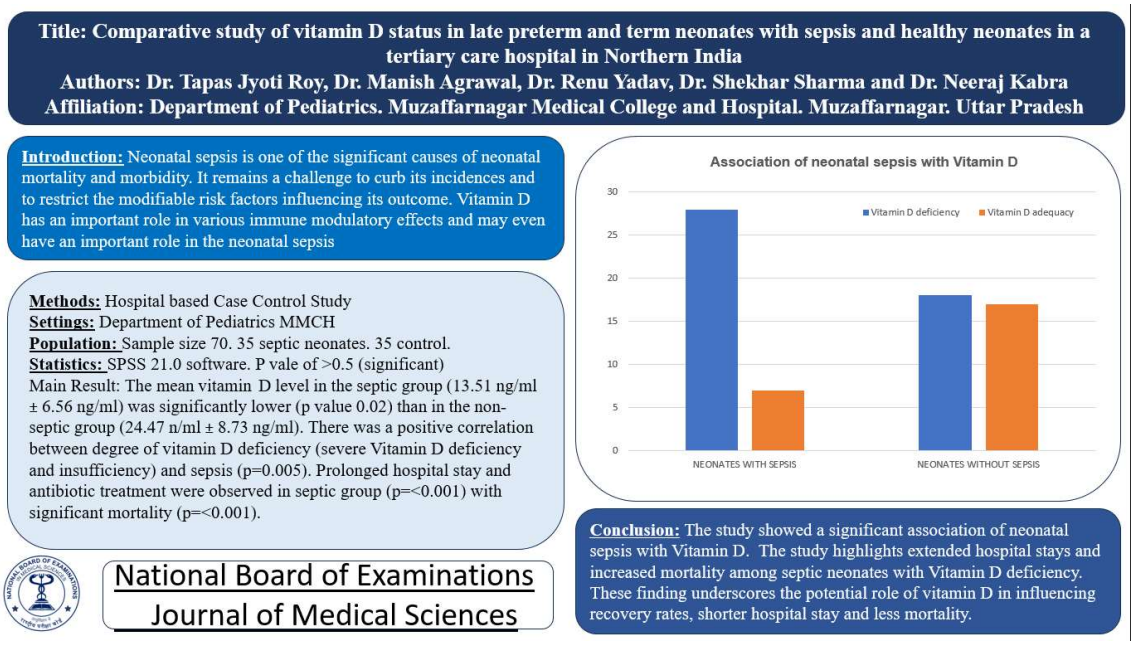
Graphical Abstract



Introduction

Mortality in newborn periods accounts for 47% of under-5 mortalities [1]. The neonatal mortality rate worldwide is 19 per thousand live births [2]. In India, neonatal mortality is higher, at 20 per 1000 live births, and it constitutes 62.5% of all under-5 deaths [3]. Neonatal sepsis accounts for about 25% of the total neonatal deaths [4]. Sepsis in newborns manifests with various clinical features of infection, with or without bacteremia, in the initial 4 weeks of life. It includes septicaemia, pneumonia, meningitis, UTI, osteomyelitis, and arthritis but excludes superficial infections [5]. Early-onset sepsis manifests within the first 3 days of a newborn's life [6]. It primarily results from infections transmitted while the baby passes through the genital tract of the mother during childbirth. Late-onset sepsis typically manifests itself after the initial 72 hours of a newborn's life and is often presumed to be the result of infections transmitted horizontally, predominantly through the hands of those providing care [6]. Management of sepsis in newborns is challenging due to its subtle and confusing clinical features, absence of definite and specific biomarkers, judicious requirement for starting empirical and high-cost antibiotics, and emergence of resistance to antibiotics. Thus, preventive approaches, including the identification of risk factors and various modifiable adjuvant therapies, have become areas of newer research interest in the battle against sepsis in newborns.

The function of vitamin D extends beyond its well-known roles in calcium and phosphorus metabolism affecting the skeletal system [7]; it has a role in certain systemic conditions like various autoimmune disorders, certain cancers, cardiac diseases, and metabolic syndromes. It exerts its immunological action mainly by binding with the vitamin D receptor on the cells of monocytes, macrophages, and neutrophils, leading to augmented chemotactic, phagocytic, and bactericidal

activities [8]. Low levels of vitamin D were seen in pregnant mothers and neonates worldwide [9]. The immune modulator role of vitamin D and the increasing global pandemic of vitamin D deficiency have stoked the speculation of a possible relation of VDD with sepsis in newborns [10].

The rationale for such a comparative study of the status of cholecalciferol in newborns with sepsis and healthy newborns in a tertiary care hospital in northern India stems from the fact that the identification and proper timely management of sepsis and VDD in such newborns may have a short-term as well as a long-term prognostication value.

Material and Methods

This hospital-based case control study was conducted from September 2022 to August 2023 in the Department of Paediatrics, Muzaffarnagar Medical College and Hospital, Muzaffarnagar, UP. A total of 70 late preterm and term neonates were included, with 35 neonates each in the sepsis group and non-sepsis group. Cases included all neonates over 34 weeks of gestational age with sepsis within days of life 28 who were admitted to the NICU. Cases also included those with probable sepsis, culture-positive sepsis, and clinical sepsis. Healthy newborns in the postnatal ward roomed in with mothers over 34 weeks of gestation without any sepsis were taken as control. Neonates with major congenital anomalies, receiving formula feed, starting on supplementation of vitamin D, or less than 34 weeks of gestation were excluded.

A written informed consent was obtained from parents or guardians before enrolment of each baby in the study. Demographic details of the mother and the newborn were collected. In a predesignated validated pro forma, all clinical findings and relevant investigations were documented. Gestational age was assessed by the LMP and the new Ballard score. Haemoglobin, TLC, C-Reactive Protein, ESR, I/T Ratio, ANC, and blood culture, which were noted.

A Practical Sepsis Screen

Components	Abnormal Value
Total leukocyte count	<5000/mm^3
Absolute neutrophil count	Low counts as per Manroe Chart[21] for term and Mouzinho's chart[22] for VLBW infants
Immature/ total neutrophil	>0.2
Micro-ESR	>15mm in 1st hour
C reactive protein (CRP)	>1mg/dL

Before initiating antimicrobial therapy, blood cultures were taken. Aseptic measures were taken before collecting blood culture samples. 1 ml of blood in 10 ml of broth for blood culture was collected. Colonies were examined after 24, 48, 72, and 120 hours.3 ml of blood collected under aseptic precautions was sent for routine examinations. CBC was done using a blood count analyser (Bachman Coulter). Serum Cholecalciferol assay was done in healthy newborns (control) within DOL 3 and in cases of newborns with culture-positive sepsis or with suspicion of sepsis done before the antibiotic was started. Serum cholecalciferol was assessed by an immunoassay system (detects concentration of vitamin D using an antibody as reagent) - Beckman Coulter Access 2 Immunoassay System. Cholecalciferol levels were assessed in ng/mL.Serum vitamin D levels less than 12 ng/ml indicated severe deficiency; insufficiency was 12 to 20 ng/ml, and 20 to 100 ng/ml indicated adequacy.

CRP levels were quantified using the immunoturbidimetric method. The detection limit for CRP was 1 mg/dl.

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS), version 21. Continuous variables have been represented as mean ± SD. Category variables have been displayed as frequencies and percentages. The independent sample t-test was used while comparing vitamin D levels in two groups. A chi-square test or Fisher's exact test was used to compare between two groups. The association of VDD with neonatal sepsis was determined by logistic regression analysis and expressed as an odds ratio. A P value <0.05 was considered statistically significant for this study.

Table 1. Maternal sociodemographic status

Variables	**Cases**	**Controls**	**p-Value**
Age (yrs)			
<20	7(20%)	2(6%)	**0.02**
20-30	18 (51%)	26 (74%)	
>30	10 (29%)	7 (20%)	
Education			
Illiterate	10 (29%)	2 (6%)	**0.04**
Primary	10 (29%)	8 (23%)	
Secondary	10 (29%)	15 (43%)	
Higher	5 (14%)	10 (29%)	
SES			
Lower	20 (57%)	8 (23%)	**0.003**
Middle	15 (43%)	27 (63%)	
Residence			
Urban	20 (57%)	30 (86%)	**0.03**
Rural	15 (43%)	5 (14%)	
Children			
1	10 (29%)	20 (57%)	**0.015**
>1	25 (71%)	15 (53%)	

Results and Discussion

Regarding socio-demographic characteristics of the mothers (Table 1), it was observed that there were a greater number of mothers aged less than 20 years, in the sepsis group (20% vs. 6%). In the control group, 74% of the mothers were in the 20-30 year group, compared to 50% in cases. It suggested that lower age at conception had a direct correlation (p = 0.02) with sepsis and VDD; this finding was similar to the study by Bitew et al. [11] which showed a higher proportion of women who bore children before the age of 20 had higher incidences of sepsis and VDD. Maternal education exhibits a notable distinction, with cases showing higher percentages of mothers with no education (29% vs. 6%, p = 0.04) and primary education (29% vs. 23%) compared to controls. In terms of socioeconomic status, statistically significant differences are observed between cases and controls, with sepsis cases being more predominant among lower socioeconomic groups. Notably, location of residence shows a significant disparity, with cases more likely to reside in

rural areas compared to controls (43% vs. 14%, p = 0.03), suggesting environmental or resource-based factors could influence health outcomes. Regarding the number of children in the household, there is a significant trend towards smaller households (single child) among controls, while neonates with sepsis belonged more to larger households. (p= 0.015). This suggests that with multiple children and with a decreased gap between 2 pregnancies, mothers may have poor nutritional status as well as low vitamin D levels, causing them to have children who are prone to VDD and sepsis. Odabasi et al. [12] study on sepsis also showed greater incidences of sepsis and VDD in rural, illiterate, and multiparous mother.

Table 2. Neonatal Baseline Characteristics

Variables	**Cases**	**Controls**	**p-Value**
Gestational Age (wks)	**36.77 ±1.26**	**36.60 ± 1.75**	
Late Preterm	15(42%)	16(45%)	0.8
Term	20 (48%)	19(55%)	
SGA	18 (51%)	9 (25%)	0.27
AGA	17 (49%)	26 (71%)	
Male	16 (49%)	17 (52%)	0.8
Female	19 (51%)	18 (48%)	
LSCS	21 (54%)	25 (71%)	0.3
VD	14 (46%)	10 (29%)	
Inborn	8 (23%)	33 (94%)	**<0.001**
Outborn	27 (77%)	2 (6%)	

Regarding baseline characteristics of neonates in the study group (Table 2), the mean gestational age in cases was 36.77 ± 1.26 weeks and in healthy neonates was 36.60 ± 1.75 weeks. Among late preterm newborns, there were 15 septic cases and 16 non-septic cases with a non-significant p-value (0.8). 51% of the sepsis cases were SGA, while the rest 49% were AGA (p value 0.27). No significant association of gender with sepsis (p = 0.8) was observed. Mode of delivery had no significant correlation with neonatal sepsis, as evident by a p value of 0.3. A statistical significance was observed between the place of delivery and sepsis (p value of less than 0.001). 77%

of the septic babies were outborn, which shows that there is a particular need to have a proper segregation between inborn and outborn babies, and there is a particular need to have a proper screening in place for outborn babies. These findings resemble a study by Coggins et al. (2023) [13].

Table 3. Comparison of septic and non-septic group according to vitamin D level

Characteristics	**VDD**	**Adequacy**	χ^2	**P-value**
Culture positive sepsis	21	1	5.6	0.01
Probable sepsis	5	3		
Clinical sepsis	2	2		
EOS	24	3	5.8	0.01
LOS	4	4		
Neonates with sepsis	28	7	6.34	0.02
Neonates without sepsis	18	17		

Regarding the association of vitamin D and neonatal sepsis (Table 3), in our study, 28 neonates with sepsis (80%) were found to have VDD (<20 ng/ml), and the rest, 7 (20%) neonates with sepsis, were found to have adequate vitamin D. A definite correlation (P = 0.02) was found between neonatal sepsis and VDD. There were 22 cases of culture-positive sepsis, out of which 21 cases showed VDD and 2 cases had adequate levels of vitamin D. Among 8 probable sepsis cases, 5 neonates were found to have vitamin D deficiency, and 3 had adequate vitamin D. There were 4 cases of clinical sepsis, out of which 2 cases showed VDD and 2 neonates had adequate levels of cholecalciferol. Among 27 newborns with EOS, 24 neonates had vitamin D deficiency and 3 had adequate vitamin D, while in LOS equal numbers (4) had VDD and adequacy. Among all the kinds of sepsis, culture-positive sepsis (63%) and early-onset sepsis (80% vs. 20% in LOS) were predominant. We found an association of VDD with EOS, which was in line with a study undertaken by Cizmeci et al. [14], where association of hypovitaminosis D with EOS was evident by significantly lower vitamin D levels (median 12.6 ng/mL (3.1-78.9) in the septic neonates than in their non-septic counterparts (median 21 ng/ml with a p value of 0.038).

A significant correlation was established between EOS and VDD. Our study showed that early-onset sepsis had a significant correlation (p = 0.02) with vitamin D deficiency compared to LOS, which was different from a study by Dhandai et al. that showed the case group had significantly lower mean (SD) vitamin D levels [15.37 ng/ml (10.0)] than the control group [21.37 ng/ml (9.53)] (p = 0.001) [53]. The results are parallel with the studies by Terek et al. [19] and Abdelmaksoud et al. [20].

Table 4. Correlation Between Vitamin D Levels and Hospital Stay

Vitamin D Status at Admission	Hospital Stay (Days)	Correlation Coefficient (r)
Sepsis Cases		
Severe Deficiency (<12 ng/ml)	18 ± 5	-0.60
Insufficiency (12-20 ng/ml)	14 ± 4	-0.45
Adequacy (>20 ng/ml)	10 ± 3	-0.30
Controls		
Severe Deficiency (<12 ng/ml)	5 ± 2	-0.20
Insufficiency (12-20 ng/ml)	3 ± 1	-0.15
Adequacy (>20 ng/ml)	2 ± 1	-0.10

In our study, the relationship between vitamin D levels at admission and the duration of hospital stay for both sepsis cases and controls (Table 4) showed that sepsis cases with severe deficiency (<12 ng/ml) had an average hospital stay of 18 ± 5 days, showing a strong negative correlation coefficient (r = -0.60). Similarly, those with insufficiency (12-20 ng/ml) and adequacy (>20 ng/ml) had shorter stays with increasing Vitamin D levels, with correlation coefficients of -0.45 and -0.30, respectively. In contrast, control subjects exhibited shorter hospital stays overall, with severe deficiency, insufficiency, and adequacy displaying progressively shorter stays (5 ± 2 days, 3 ± 1 days, and 2 ± 1 days, respectively), albeit with weaker negative correlation coefficients (-0.20, -0.15, and -0.10, respectively). These findings suggest a potential association between vitamin D status and duration of hospitalisation in both cases of sepsis and controls, with stronger correlations observed in the sepsis cohort. These findings were similar to a study done by Atif et al. (2020) [14], where a positive relation was obtained between the duration of hospital stay in septic patients and VDD. Studies by Dutta et al. (2021) [15] gave a pattern of VDD and type of sepsis in prolonged use. Similar findings were also observed in a study by Madden et al. [17] examining the admission levels of vitamin D in critically ill children, where they discovered a significant prevalence of VDD (40% children). It showed heightened disease severity in children with VDD upon

admission [18] with a longer duration of hospital stay in the sepsis group.

Our findings on duration of hospital stay and antibiotic treatment duration further underline the health disparities between cases and controls, especially noted in the longer stays and more extended treatment durations among cases and the associated vitamin D deficiencies in sepsis cases when the duration of hospital stay or the duration of antibiotic treatment is longer. This highlights the severity of the conditions impacting cases compared to controls.

Table 5. Logistic Regression Analysis Predicting Sepsis based on Vitamin D Levels and Other Clinical Factors

Variables	**coefficient**	**Odds ratio**	**p-Value**
Vitamin D level (per 10ng/ml increase)	0.25	0.78	0.03
Maternal HTN	0.60	1.82	0.05
Premature Birth	1.25	3.49	**<0.001**
Birth weight	0.90	2.46	**0.01**
Male	0.35	1.42	0.10

Logistic regression analysis (Table 5) predicted the likelihood of sepsis based on initial vitamin D levels and other clinical factors. Higher vitamin D levels, with each 10 ng/mL increase, show a significant negative association ($\beta = -0.25$, odds ratio = 0.78, $p = 0.03$) with the outcome. Maternal hypertension ($\beta = 0.60$, odds ratio = 1.82, $p = 0.05$) and premature birth ($\beta = 1.25$, odds ratio = 3.49, $p < 0.001$) are positively associated, indicating increased odds. Similarly, lower birth weight (<2500g) ($\beta = 0.90$, Odds Ratio = 2.46, $p = 0.01$) and male gender ($\beta = 0.35$, Odds Ratio = 1.42, $p = 0.10$) are associated with higher odds of the outcome, though the gender association is not statistically significant at conventional levels.

Conclusion

The study showed a significant association of neonatal sepsis with vitamin D. The study highlights extended hospital stays and increased mortality among septic neonates with vitamin D deficiency. These findings underscore the potential role of vitamin D in influencing recovery rates, shorter hospital stays, and less mortality. Further large-scale studies are required to determine the direction of this association.

Conflict of interest

The authors declares that they do not have conflict of interest.

Ethical Approval

The study was approved by the institutional ethics committee.

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