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Original article

Role of Vitamin-D Deficiency in Term Neonates with Late-Onset Sepsis at Benha University Hospitals

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Abstract:

Background: Neonatal In both full-term and premature newborns, sepsis is the leading cause of death and illness. Aim: The purpose of this research was to determine whether or not there is a correlation between vitamin-D status and LOS in full-term infants. **Methods:** Eighty participants were randomly assigned to one of two groups: Group I (LOS group) consisted of 40 newborns diagnosed with LOS at presentation between 72 and 28 days of age. Forty full-term, healthy newborns made comprised Group II, the control group. All of the newborns involved in the study had a thorough history taken, a comprehensive clinical examination performed, and several laboratory tests performed, including a complete blood count, C-reactive protein, Random blood sugar, blood culture, urine culture, and CSF analysis and culture. The 25-hydroxyvitamin D (25-OH-D) levels were measured quantitatively using an auto chemiluminescence immunoassay instrument (Maccura i1000). **Results:** The results showed that the LOS group had significantly lower vitamin D levels than the control group, as well as a significantly greater prevalence of vitamin D insufficiency and severe deficiency. Patients with a maternal history of diabetes, hypertension, or recurrent urinary tract infections also had lower vitamin D levels than their rural counterparts. Vitamin D levels were positively correlated with the APGAR score, while total bilirubin levels were inversely correlated with Vitamin D levels. **conclusion:** In conclusion, term newborns with LOS had lower levels of 25-hydroxyvitamin D than the control group. Patients from metropolitan regions, as well as those with a maternal history of diabetes, hypertension, or recurrent urinary tract infections, had significantly lower vitamin D levels.

Keywords: Vitamin-D; Deficiency; Term; Neonates; late-onset sepsis.

Introduction

Neonatal In both full-term and premature newborns, sepsis is the leading cause of death and illness [(1)]. Blood, urine, cerebrospinal fluid, peritoneal fluid, and pleural fluid are all considered to be sterile, making the presence of germs in these fluids' diagnostic of neonatal sepsis. Time of onset after neonatal delivery has led to its categorization as Early Onset Sepsis (EOS) or Late Oset Sepsis (LOS) [(2)].

Years of research have shown vitamin D as a fat-soluble steroid hormone essential for calcium homeostasis and normal bone mineralization. Over the last decade, it has been clear that organs and tissues other than the kidneys, including immune system cells, the intestines, the pancreas, and the prostate, express the vitamin D receptor (VDR) and the vitamin D-activating enzyme 1-alpha hydroxylase (CYP27B). Thus, it has been proposed that particularly active vitamin D has features with locally active cytokines, in addition to its well-established classical effects [(3)].

Due to the immaturity of their adaptive immune systems, infants rely heavily on their innate immune systems for defense against infections. Vitamin D boosts both the adaptive and innate immune systems. Vitamin D is a key factor in avoiding infections in pregnant women and infants because it is a substrate for the creation of antimicrobial peptides like cathelicidin [(4)].

The prevalence of vitamin D insufficiency among newborns, which has been estimated to be between 73% and 94%, indicates a significant public health issue for infants just entering the world [(5)].

Vitamin D insufficiency has been linked to a lower number of lymphocyte subsets and different patterns of T-lymphocyte activation, both of which might increase the risk of infection in infants [(6)].

Serum 25-(OH) D3 levels (defined as the sum of 25-(OH)D2 and 25-(OH)D3) are used to represent clinically meaningful reserves of vitamin D since 1,25-(OH)2D3 is homeostatically controlled and has a short half-life (4-8 h) [(7)].

Several studies have demonstrated that term newborns with low vitamin-D levels are more likely to develop neonatal sepsis [(8)]. Only a small number of research have looked at the effects of vitamin-D on neonates with LOS, therefore the significance of vitamin-D is still unclear [(9)].

This study aims to elucidate the function of vitamin d insufficiency in propensity to late onset newborn sepsis by determining the correlation between vitamin-D levels and LOS in full-term infants' sepsis.

Patients and methods

- This type of study From February 2023 to September 2023, 80 neonates were split into two groups for case-control research in the Neonatal Intensive Care Unit (NICU) of the Pediatrics Department at Benha University Hospital in Benha, Egypt.
- **Group I** (LOS group): consisted of 40 neonates, 23 (57.5% male) and 17 (42.5% female), who presented between 72 hours and 28 days of age with culture-proven LOS.
- Babies that were born at or after 37 weeks of gestation were considered full term.
- Each gender was represented.

Exclusion criteria:

- Awaiting the sixth day after birth or later.
- Neonates born before 37 weeks are not eligible.
- Transient tachypnea of infant, hyaline membrane illness, and perinatal hypoxia are examples of co-morbidities.
- Major birth defects.
- Newborns whose moms had chorioamnionitis or whose membranes ruptured prematurely.
- Infants born to moms who have taken prenatal vitamin D supplements or infants who have received vitamin D

supplements shortly after birth are considered vitamin D supplements.

- **Group II** (control group) consisted of 40 full-term, healthy newborns who remained in the postnatal unit with their mothers for a variety of reasons. There were 21 men (52.5% of the total) and 19 girls (47.5%).
- Each participant's legal guardian provided written informed permission after hearing about the study's importance and the procedures that will be carried out. Ethics for medical research involving human participants were considered, and the study was conducted in accordance with the Declaration of Helsinki. The Benha University School of Medicine's local ethics committee gave its stamp of approval to the whole research plan. At each stage of the research, participants' anonymity and confidentiality were protected. There were no penalties for withdrawal from the study at any point for the legal guardians. The information gathered was not, and will not be, utilised for any other purpose.
- All of the newborns involved in the study had a thorough history taken, a comprehensive clinical examination performed, and several laboratory tests performed, including a complete blood count, C- reactive protein, Random blood sugar, blood culture, urine culture, and CSF analysis and culture.
- The 25-hydroxyvitamin D (25-OH-D) levels were measured quantitatively using an auto chemiluminescence immunoassay instrument (Maccura i1000). Amounts in nanograms per millilitre were reported. Each participant's serum 25-hydroxyvitamin D (25-OH-D) levels were measured. Deficits were classified as serum 25-OH-D levels 15 ng/ml, sufficiency as >20 ng/ml, and severe deficiencies as 5 ng/ml [(10)]. After that, people's vitamin D levels were classified into 4 groups Reference:
- Vit. D3 levels adequate (>20 ng/ml)
- 15-20 ng/ml Vitamin D deficiency
- 5-15 ng/ml is considered low for vitamin D.
- Vitamin Critical shortfall: <5 ng/ml

Statistical analysis:

The SPSS version 16 software was used to tabulate and analyze the gathered data (SpssInc, Chicago, ILL Company). Quantitative data were reported as a mean standard deviation and range, whereas categorical data were provided as raw numbers. The Chi-Square (x2) test was performed to examine the distribution of the categories. Numbers and statistics. The st, t test was performed to compare normally distributed variables between the two groups. Non-parametric variables were tested for correlation using Spearman's correlation (rho). The optimal sensitivity and specificity cutoff values for the examined markers were identified using ROC curve analysis. In this study, a probability of less than 0.05 was deemed statistically significant).

Research ethics committee: Ms.4.7.2022

Results

The most common symptom reported was difficulty breathing (62.5%), followed by issues with suckling (50%) and activity (52.5%), then irritability (37.5%), then hypothermia (20%), then fever (17.5%), then vomiting (17.5%), then paleness (10%), then and finally skin pigmentation (2.5%), (Figure 1).

Vitamin D levels were significantly lower in the LOS group compared to the control group, and the prevalence of vitamin D insufficiency and severe deficiency was significantly greater in the LOS group, p value (Table 1).

Patients living in the country had much greater vitamin D levels than city dwellers. Patients with a family history of diabetes, hypertension, and recurrent urinary tract infections had significantly lower vitamin D levels. A positive link was found between vitamin D and APGAR score p value and a negative correlation was found between vitamin D and total bilirubin level

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(Table 2), but there was no statistically significant variation in vitamin D levels by sex, maternal anaemia, or antepartum haemorrhage. Even if vitamin D levels weren't linked to any other clinical metrics (Table 3).

AUC in letters for vitamin D in predicting late-onset newborn sepsis was 0.702 (95% CI, 0.588-0.817), with a significance level of 0.002. The sensitivity was 70% and the specificity was 73.3% at a cutoff threshold of 15.4 ng/ml. (Figure 2).

Table 1: Comparison between LOS and control groups regarding 25-hydroxy vitamin D level.

		LOS group (N=40)		Control group (N=40)		Test	P value
		N	%	N	%		
25-hydroxy vitamin-D (ng/ml)	**Mean ±SD**	15.4±7.9		20.6±7.6		t=2.9	**0.004***
	Range	2.3-29.4		5.1-31.5			
Vitamin-D status	**Sufficiency**	9	22.5%	21	52.5%	X^2=9.3	**0.025***
	Insufficiency	9	22.5%	9	22.5%		
	Deficiency	17	42.5%	8	20.0%		
	Severe deficiency	5	12.5%	2	5.0%		

t: Student t-test; X^2: Chi square test; *: significant, LOS: late onset sepsis

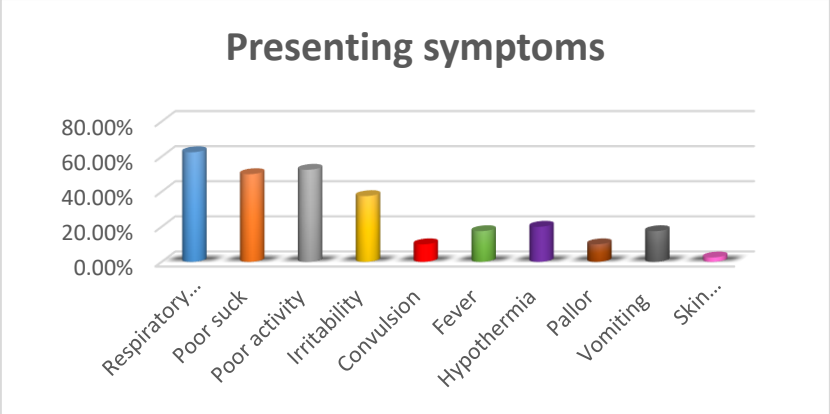

Figure 1: Presenting symptoms in LOS group.

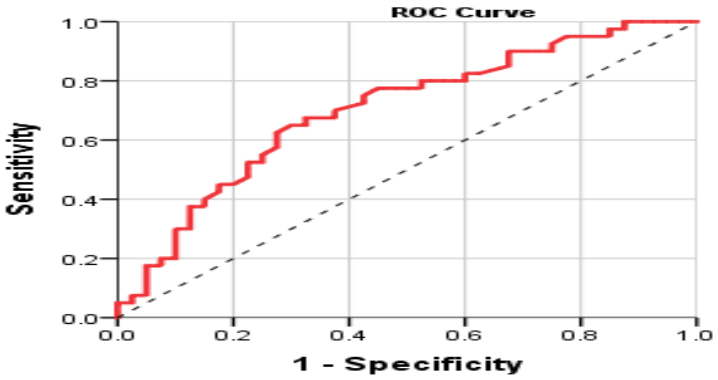

Figure 2: ROC curve of performance of vitamin D to predict late onset neonate

Table 2: Vitamin D level according to sociodemographic data and perinatal history in LOS group.

		25-hydroxy vitamin D (ng/ml)			Test	P value
		Mean±SD	Min.	Max.		
Sex	Male	14.3±8.5	2.30	29.40	t=0.79	0.44
	Female	15.8±6.6	3.40	27.30		
Residence	Rural	16.9±8.3	2.30	29.40	t=1.97	0.048*
	Urban	13.8±6.5	2.90	26.30		
Maternal anemia	No	15.9±7.7	2.30	29.40	t=0.75	0.33
	Yes	14.7±7.8	4.60	26.30		
Maternal DM	No	21.5±4.4	15.4	29.40	t=2.6	<0.001*
	Yes	14.4±7.6	2.3	26.10		
Maternal HTN	No	18.6±7.2	2.30	28.30	t=2.15	0.032*
	Yes	14.3±9.9	2.90	29.40		
Recurrent UTI	No	17.8±9.1	2.30	28.30	t=2.04	0.042*
	Yes	14.3±7.4	2.90	29.40		
Antepartum hemorrhage	No	15.1±8.1	2.30	29.40	t=1.1	0.13
	Yes	13.8±4.7	5.20	15.20		

t: Student t-test; *: significant, DM: diabetes mellitus, HTN: hypertension, UTI: urinary tract infection. LOS: late onset sepsis.

Table 3: Correlation between vitamin D and other clinical data in LOS group.

	Vitamin D (ng/ml)	
	r	P value
Gestational age (weeks)	-0.133	0.241
Age on admission (days)	0.153	0.175
Mother age (years)	-0.059	0.604
APGAR score	0.239	**0.033***
weight (kg)	-0.011	0.922
Height (cm)	0.035	0.759
Head circumference (cm)	0.023	0.836
Random blood sugar	-0.006	0.960
Hemoglobin	0.180	0.109
WBCs	0.159	0.160
Neutrophils	0.102	0.366
Lymphocytes	-0.144	0.203
Platelets	0.111	0.326
Total bilirubin	-0.373	**<0.001***
Sodium	0.061	0.588
Potassium	-0.047	0.681
Calcium	0.225	0.054
C-reactive protein	-0.117	0.302
Töllner sepsis score	-0.024	0.884
Hospital stays (days)	0.223	0.066

r: correlation coefficient, *: significant,

Discussion

This study sought to elucidate the function of vitamin d insufficiency in propensity of late onset newborn sepsis by determining the correlation between vitamin-D levels and LOS in term neonates. Vitamin D levels were significantly lower in the LOS group compared to the control group (mean: 15.47.9 ng/ml vs. 20.67.6 ng/ml, p=0.004). Furthermore, vitamin D insufficiency (42.5%) and severe deficiency (12.5%) were significantly more common in the LOS group than in

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the control group (20% & 5%, respectively, p=0.025).

Similar findings were found by Dhandai et al. [11], who found that the blood 25(OH) vitamin D level in the sepsis (case) group was 15.4 10.0, which was considerably lower than in controls 21.4 9.5 (p = 0.001). Only 8% of cases and 13% of controls had adequate vitamin D levels. Vitamin D deficiency was found in almost two-thirds of septic neonates and in half of the controls. In a similar vein, Bilgin & Gonulal [12] showed that babies with LOS had lower blood 25(OH)D levels than the control group (12.9 6.36 ng/ml vs. 21 6.38 ng/ml; p 0.001).

Agrawal et al. [9] found that vitamin D insufficiency was more common than previously thought, reporting that 151 out of 175 patients (86.28 percent) and 37 out of 50 neonates (74 percent) were deficient. Vitamin D levels were considerably lower in the patients (12.28 6.11 ng/ml) compared to the controls (14.88 7.2 ng/ml; p = 0.002). There were 36 instances of severe vitamin-D deficiency (20.57%), 86 cases of vitamin-D deficiency (49.14%), 29 cases of vitamin-D insufficiency (16.57%), and only 24 cases (13.7%) with vitamin-D levels >20 ng/ml. Seven (14% of the total) of the controls were severely vitamin-D deficient, fifteen (30%) were vitamin-D deficient, fifteen (30%) had vitamin-D insufficiency, and thirteen (26% of the total) were vitamin-D sufficient.

Our findings also did not match those of Ozdemir and Cag [8], who looked at the correlation between low vitamin D levels in neonates and an increased risk of sepsis. Of the 51 infants in their study, 39 (76.5% of the total) were diagnosed with EOS and 12 (23.5% of the total) were diagnosed with LOS. Vitamin D levels were lower in infants with EOS (10.45.7 ng/ml) compared to those with LOS (12.84.3 ng/ml), although the difference was not significant (p=0.075). While there was no significant difference between the mean vitamin D levels of infants with LOS and the control group (p=0.7), there was a significant difference between the mean vitamin D levels of infants with EOS and the control group (p=0.02). Patients with positive (n=11; 10.45.4 ng/ml) and negative (n=40; 115.3 ng/ml) blood cultures and the infants in the control group had comparable mean vitamin D levels (p=0.7, p=0.2).

In the current research, we found that total bilirubin levels were negatively correlated with vitamin D and that APGAR scores were positively correlated with vitamin D p values. In spite of this, neither C-reactive protein nor haemoglobin nor platelets were significantly correlated with vitamin D intake.

Similar findings were reported by Agrawal et al. [9], who observed no connection between CRP and vitamin-D levels. Furthermore, the bacteriological culture profile of patients is unrelated to vitamin D insufficiency. Serum vitamin D levels were shown to have a substantial negative link with CRP; significant positive relationships with Apgar score, haemoglobin concentration, and platelet count in a study of term newborns with early onset sepsis by Soliman et al. [13].

Our findings were consistent with those of Mutlu et al. [14], who compared 25-hydroxy Vitamin D levels in babies with pathologic and physiological hyperbilirubinemia and discovered that there are substantial disparities between the case and control groups. 25-hydroxyvitamin d, serum bilirubin, and PTH levels were all substantially negatively correlated in the sampled newborns. Previous studies [15,16] similarly found significantly lower levels of vitamin D in jaundiced patients compared to controls (p0.05), which is consistent with our findings.

While it is true that vitamin D is essential for bone metabolism, it also has several other functions in the body and is called a steroid with multi system effects [17]. The mechanism by which vitamin D affects newborn hyperbilirubinemia is unknown at this time. The following are the primary

reported biological viewpoints. Indirect bilirubin is mostly broken down by red blood cells. The hormone erythropoietin plays a crucial role in stimulating the body to produce new RBCs. Vitamin D insufficiency has been linked to an increased risk of newborn jaundice because of previous research showing that vitamin D may lower erythropoietin levels [(18)].

AUC for vitamin D in predicting late-onset newborn sepsis was 0.702 (95% CI, 0.588-0.817), p=0.002 in the present study's ROC analysis. The sensitivity was 70% and the specificity was 73.3% at a cutoff threshold of 15.4 ng/ml.

The optimal cut off value of 25(OH)D in ROC analysis was revealed to be 15.45 ng/ml with 91.3% sensitivity and 71.7% specificity (AUC = 0.824, 95% confidence range 0.7370.912, p 0.001) for predicting LOS, which is consistent with the findings of Bilgin & Gonulal [(12)].

The ROC curve of blood vitamin D was also used to differentiate between septic and control groups in research by Soliman et al. [(13)]. At a cutoff value of 18.75ng/ml, the AUC was 0.907, the sensitivity was 100%, the specificity was 80%, the PPV was 83.3%, the NPV was 100%, and the accuracy was 100%.90%.

Conclusion

This Term neonates with late-onset sepsis had decreased levels of 25-hydroxyvitamin D compared to the control group. Vitamin D levels were positively correlated with the APGAR score, while total bilirubin levels were inversely correlated with Vitamin D levels. Patients with a maternal history of diabetes, hypertension, or recurrent urinary tract infections also had lower vitamin D levels than their rural counterparts. Therefore, further evaluation paid for them.

Conflict of interest:

The authors declare no conflict of interest.

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