Microbes and Infectious Diseases 2021; 2 (2): 361-371

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

The relation between neonatal vitamin D deficiency and early onset sepsis in term infants

Doaa Y. Ali *[1], Ahmed M. Abd EL-moktader [2], Riham Makhlof Abd-El Baky [3], Sherin Khamis Hussein [2]

1- *Department of Clinical and Chemical Pathology, Faculty of Medicine, Fayoum University, Al Fayoum, Egypt.*
2- *Department of Pediatrics, Faculty of Medicine, Fayoum University, Al Fayoum, Egypt.*
3- *Department of Pediatrics, Fayoum general hospital, Al Fayoum, Egypt.*

ARTICLE INFO

Article history:
Received 13 February 2021
Received in revised form 17 March 2021
Accepted 26 March 2021

Keywords:
Early onset neonatal sepsis
Serum 25(OH)D level
Term infants

ABSTRACT

Background: Vitamin D had an important influence on the innate and adaptive immune system. 25-hydroxyvitamin D (25(OH)D) serum level is known to be the best predictor of vitamin D level. **Objectives**: To determine the relationship between neonatal 25(OH)D level in early-onset sepsis (EOS) and its severity in term infants. **Methods**: This case-control study was performed on 50 septic neonates admitted to Fayoum University Hospitals' Neonatal Intensive Care Unit as a case group, and 50 healthy neonates as a control group. Each subject was subjected to a detailed history and meticulous general & systemic examinations. Laboratory assessment in form of complete blood count with differential counts, C-reactive protein (CRP), blood culture, and serum 25(OH)D level in infants was done within 72 hours of life. **Results:** 25(OH)D levels in cases (4.91 ng/ml) were significantly lowered than those in controls (13.0 ng/ml) (*p <0.0001*). The number and percentage of mothers with no history of vitamin D supplements throughout pregnancy was statistically significantly higher in cases (n=48;96.0%) than in controls (n=42;84.0%; *p=0.046*). Blood culture was positive in 34 neonates and negative in 16 neonates in the case group. Twenty-eight percent of the isolated organisms were Gram-negative bacilli. Vitamin D mean level was lowered in the case with positive blood culture compared to negative blood culture (4.8ng/ml vs. 5.02 ng/ml) respectively with no statistical significance (*p=0. 876*). **Conclusions**: Early-onset sepsis in term infant occurs more frequently in the presence of Vit-D deficiency.

Introduction

Neonatal sepsis is characterized as a systemic inflammatory response occurring as a result of a suspected or confirmed infection in the first four weeks of life [1]. It's a common condition that affects 1.1% to 2.7% of all newborns. Therefore, in early human life, the most important cause of morbidity and mortality is bacterial infections. [2]. Approximately 2,202 neonatal sepsis cases per 100,000 live births worldwide each year. The prevalence of neonatal mortality for sepsis was (1% to 5%) and for severe sepsis was (9% to 20%) [3]. Early onset neonatal sepsis (EOS) refers to neonatal sepsis at or before 72 hours of birth, and late onset neonatal sepsis (LOS) refers to sepsis at or after 72 hours of life [4]. Vitamin D is considered a steroidal prohormone, the active form plays a critical role in phosphate and calcium absorption [5].

Vitamin D also modifies Toll-like receptor (TLR) expression, and co-receptor CD14 on large

DOI: 10.21608/MID.2021.63684.1125
* *Corresponding author:* Doaa Y. Ali
E-mail address: younisdoaa@yahoo.com

innate immune cells enables the transformation of 25 (OH) D3 to its active type (1,25 (OH) 2D3) and induces the synthesis of anti-microbial peptides such as cathelicidin, which inhibit both gram-positive and gram-negative bacterial growth [6].

In infants and children, an intense relationship between the deficiency of vitamin D and respiratory tract infections has also been reported [7,8]. Our objectives are to determine the relationship between neonatal vitamin D levels in EOS, and its severity in term infants.

Material and method

Study design and setting

This case-control study was conducted at Neonatal Intensive Care Units (NICUs) of the Pediatric Department at Fayoum University hospital from May 2018 to February 2019. The study was conducted on 100 term neonates. The case group composed of 50 term neonates who admitted to NICU within the first three postnatal days with laboratory findings and clinical features suggesting EOS according to Griffin Neonatal Sepsis Score [9]. The control group composed of 50 term neonates without any clinical, and laboratory signs of infection.

Sample size estimation

Sample size was adjusted using (G power version 3.0.10). The sample of 42 in each group were calculated in order to achieve a statistical power of 90%, the case/control ratio of 1, and an alpha error of 5% assuming 30% (80% vs. 50%) as a difference in vitamin D deficiency between case and control [10]. Finally, sample size was increased by 20% to overcome problem of missing data to reach 50 in each group.

The research has been reviewed and accepted by The Ethics Committee of Faculty of Medicine, Fayoum University (ethical approval number:2017-M262). The research was performed according to the Declaration of Helsinki guideline and written, informed consent was obtained from mothers of both cases and controls.

The study was conducted to support the hypothesis that there was a relation between neonatal vitamin D levels and EOS, also with EOS severity in term infants.

The exposure, and the outcome of the study

The exposure of the study is neonatal vitamin D deficiency. Also, insufficiency maternal vitamin D intake during pregnancy, insufficiency maternal exposure to sunlight especially in winter seasons [11], maternal illness during pregnancy as (preeclampsia and placental insufficiency) [12] are confounding factors that affect neonatal vitamin D state especially those with EOS.

The prevalence of having adverse clinical outcomes especially neonatal bloodstream infection or sepsis between neonates with 25(OH)D deficiency and control group is the primary outcome of our study. While the secondary outcome is to measure the difference in the mean, and the prevalence of 25(OH)D levels between the case group and the control group.

The inclusion criteria of the cases

- Clinical signs related to sepsis: temperature instability, apnea, tachycardia/ bradycardia, hypotension, feeding intolerance, and abdominal distension, the requirement for supplemental oxygen, need for mechanical ventilation
- Neonates with score ≥2 on Griffin Neonatal Sepsis [9].
- Full term neonates >37 weeks gestational age, admitted to NICU before 72 hours of life.

The exclusion criteria of the cases

- Neonates with significant congenital abnormalities
- Neonates with a Griffin Neonatal Sepsis Score < 2 score [9].
- Mothers with some risk factors that could predispose to developing of EOS; chorioamnionitis, premature rupture of membrane, intrapartum fever or urinary tract infection
- Preterm neonates or neonates admitted to NICU after 72 hours of life.

The following laboratory assessments were done for each neonate

- Complete blood count (CBC) with differential counts [(hemoglobin (HB), platelet count, total WBC count, Polymorphonuclear cell (PMN), Immature: Total PMN ratio (I: T), Immature: Mature PMN ratio (I: M)] was assessed by (Horiba ABX Micros 60 Hematology Analyzer). Results of CBC were interpreted using HSS Hematological Scoring System [13].
- Blood glucose level was measured by (Beckman Coulter AU 480/JAPAN).

- Quantitative C-reactive protein (CRP) was assessed by an automated CRP analyzer (HEALERS QR-1000).
- Automated blood culture (BC) was done, BC bottles were inoculated with patients' blood (Peds Plus; Becton Dickinson) using BACTEC 9050 instrument (Becton Dickinson, Sparks, MD). Before being interpreted as negative, the bottles were incubated for five days in the instrument. After reporting a positive signal, a gram staining and a subculture on MacConkey agar and blood agar plates (Oxoid Ltd, UK) were performed from each presumed positive vial. The plates were incubated overnight at 35°C- 37°C, the bacterial isolates were recognized from Gram staining, colony morphology, and biochemical reaction. Also, analytic profile index (API-BioMereurx) was used to identify the organisms, and susceptibility testing was performed according to standards [14].
- Serum 25-hydroxyvitamin D (25(OH)D) level was assayed within 72 hours of life using a direct enzyme-linked immunosorbent assay (ELISA) kit (SHANGHAIKORAIN biotech CO, LTD; cat. no: E1981Hu). The Endocrine Society guidelines reported that serum (25-OHD) level was graded into vitamin D sufficient for more than 20 ng/ml (>50 nmol/l), vitamin D insufficiency for 12-20 ng/ml (30-50 nmol/l), and vitamin D deficiency for <12 ng/ml (30 nmol/l). The studied groups were classified into 3 groups accordingly [15].

Statistical analysis

Data were statistically analyzed using SPSS version 22 (SPSS Inc, USA). The mean, median, standard deviation (SD), and range were measured, for quantitative data. Kolmogorov-Smirnov test (KS) test was performed as a test of normality; if variables were normally distributed independent t-test was used. If not-normally distributed variables, Mann- Whitney-U test was used to compare between two groups, chi-square ($\chi 2$) test for qualitative data. *p-value <0.05* was considered to be significant.

Results

General characteristics of the studied groups

We classified the patients into 2 groups: Case group including 50 neonates [26 (52.0%) females, 24 (48.0%) males] with evidence of sepsis, and control group including 50 healthy stable full-term neonates [20(40.0%) females, 30(60.0%) males]. The mean age in the cases and controls was (3 days, and 1.7 days respectively). There was a statistically significant difference between case and control groups as regard age, history of maternal intake of vitamin D, mode of delivery and birth season ($p < 0.05$). On the other hand, there was no statistically significance difference between case and control as regard weight, gender, and history of maternal illness during pregnancy especially preeclampsia, and placental dysfunction ($p > 0.05$). Details of general characteristics of the studied group were discussed in **Table (1)**.

Clinical and laboratory data of the studied groups

There was a statistically significant difference between cases and controls as regard temperature instability, hypotonia or lethargy, intolerance to food, hypotension, and poor perfusion ($p < 0.0001$). As regards respiratory distress, we didn't report a statistically significant difference between cases and controls ($p > 0.05$). Details of clinical data of the studied groups were shown in **Table (2).**

As regard the laboratory data, the number and percentage of anemia, thrombocytopenia, leucocytosis, increased I/T ratio, hypoglycemia, and positive CRP among cases were higher than among controls with a statistically significant difference ($p < 0.05$). A significantly lower serum vitamin D mean level was observed in case group in relation to control group (4.91 ng/ml ,13.0 ng/ml respectively; $p < 0,0001$) **(Figure 1)**. The number and percentage of the newborns with vitamin D deficiency was significantly higher in the cases (49, 98.0%) than in the controls (24, 48.0%; $p<0.0001$). Details of laboratory result were shown in **Table (3)**.

The sepsis workup between the studied groups was shown in **Table (4).**

Blood culture was positive in 34 neonates, and negative in 16 neonates in the case group, while all control group showed negative blood culture with a statistically significant difference ($p<0.0001$). Gram-negative bacilli represented 28% of the isolated microorganisms mainly *Klebsiella pneumonia* then *Escherichia coli*, and *Pseudomonas aeruginosa*. While *candida non albicans* represented (14%), and Gram-positive cocci represented (26%) mainly S*taphylococcus aureus* followed by *Staphylococcus epidermidis*. *Staph epidermidis* was detected in 2 discrete blood culture sets from two different sites, it was reported as a pathogenic organism rather than a skin contaminant.

The median vitamin D level was lowered in cases with positive blood culture (4.8ng/ml) in comparison with those with negative blood culture(5.02ng/ml) with no statistically significance (*p=0. 876*). While, the number and percent of vitamin D deficiency was higher in cases with positive blood culture (n=44; 97.2%) than those with negative blood culture (n=26; 52.0%) with no statistically significance *(p=0.588*) **Table (5)**. As regard disease outcome, (78%) of cases discharged from hospital while (22%) of cases died.

By logistic regression analysis model to determine the predictor factors of EOS, there was a significant increase in the odds ratio for neonatal vitamin D deficiency as a risk factor for EOS (OR= 53.083; 95% CI:6.792-414.881, *p<0.0001*). Also, we assessed the odds ratios of some confounder factors that associated with 25(OH) D deficiency in EOS, there was a significant relationship between some maternal factors during pregnancy as: insufficiency maternal vitamin D intake (*p= 0.046),* insufficiency maternal exposure to sunlight especially in winter seasons (*p <0.0001*), maternal illness as (preeclampsia and placental insufficiency) (*p <0.0001*) **Table (6)**.

Table 1. General characteristics of the studied groups.

#Man-Whitney U test ##chi square ($\chi 2$) test; * *P-value < 0.05* was considered statistically significant; C.S: cesarean section.

Variable	**Cases (N=50)**		**Controls (N=50)**		***P-value***
	Mean	**SD**	**Mean**	**SD**	
Age (days)	3	0.6	1.7	0.8	*<0.0001#**
Weight (kg)	3.2	0.3	3.2	0.3	*0.797#*
Variable	**N**	**%**	**N**	**%**	***P-value***
Gender					
Male	24	48.0%	30	60.0%	*0.229##*
Female	26	52.0%	20	40.0%	
Maternal illness during pregnancy					
Yes	4	8.0%	4	8.0%	*1.000##*
No	46	92.0%	46	92.0%	
Maternal taken vitamin D during pregnancy					
Yes	2	4.0%	8	16.0%	*0.046##**
No	48	96.0%	42	84.0%	
Mode of delivery					
Vaginal	33	66.0%	23	46.0%	*0.044##**
C.S	17	34.0%	27	54.0%	
Birth season					
Winter	30	60.0%	8	16.0%	*<0.0001##**
Summer	10	20.0%	28	56.0%	
Spring	10	20.0%	14	28.0%	

Table 2. Clinical data of cases and controls

	Cases (N=50)	**Controls (N=50)**	***P-value#***

	N	%	N	%	
RD					
Present	31	62.0%	22	44.0%	*0.071*
Absent	19	38.0%	28	56.0%	
Temperature instability					
Present	18	36.0%	0	0.0%	*<0.0001**
Absent	32	64.0%	50	100.0%	
Hypotonia or lethargy					
Present	34	68.0%	0	0.0%	*<0.0001**
Absent	16	32.0%	50	100.0%	
Feeding intolerance					
Present	42	84.0%	0	0.0%	*<0.0001**
Absent	8	16.0%	50	100.0%	
Hypotension					
Present	17	34.0%	0	0.0%	*<0.0001**
Absent	33	66.0%	50	100.0%	
Poor perfusion					
Present	4	8.0%	0	0.0%	<0.0001*
Absent	46	92.0%	50	100.0%	

Chi square ($\chi 2$) test; * P-value < 0.05 was considered statistically significant, RD; respiratory distress.

Table 3. Laboratory results of cases and controls.

	Cases (N=50)		**Controls (N=50)**		***P-value***
	N	**%**	**N**	**%**	
H.B					
Anemia	26	52.0%	12	24.0%	*0.004#**
Normal	24	48.0%	38	76.0%	
Platelets					
Thrombocytopenia	19	38.0%	2	4.0%	*<0.0001#**
Normal	31	62.0%	48	96.0%	
WBCs					
Leucopenia	6	23.0%	3	5.0%	*0.044#**
Normal	32	63.0%	43	85.0%	
Leucocytosis	13	26.0%	6	12.0%	
I/T					

Normal	38	76.0%	100	100.0%	*<0.001#**
Increased	12	24.0%	0	0.0%	
Blood glucose					*<0.0001#**
Normal	9	8.0%	41	82.0%	
Hypoglycemia	25	25.0%	9	18.0%	*<0.0001#**
Hyperglycemia	16	32.0%	0	0.0%	
Vitamin D					
Deficiency	49	98.0%	24	48.0%	<0.0001#*
Insufficiency	1	2.0%	12	24.0%	
Sufficient	0	0.0%	14	28.0%	
CRP					
Positive	48	96.0%	9	18.0%	*<0.0001#**
Negative	2	4.0%	41	82.0%	
	Median	**Range**	**Median**	**Range**	***P-value***
Serum vitamin D (ng/mL)	4.91	(0.4-13.3)	13.0	(0.8-50)	*<0.0001##**

#Chi square ($\chi 2$) test, and ## Man-Whitney U test; * P-value < 0.05 was considered statistically significant, H.B: hemoglobin; WBCs: white blood cells, I/T; Immature: Total PMN ratio, CRP; C-reactive protein.

Table 4. Sepsis workup between cases and controls.

Variable	**Cases (N=50)**		**Controls (N=50)**		***P-value***
	Median	**Range**	**Median**	**Range**	
Griffin score	4.5	(2-8)	0	(0-1)	*<0.0001#**
Variable	**N**	**%**	**N**	**%**	***P-value***
CRP					
Positive	48	96.0%	9	18.0%	*<0.0001##**
Negative	2	4.0%	41	82.0%	
Blood Culture					
Positive	34	68.0%	0	0.0%	*<0.0001##**
Negative	16	32.0%	50	100.0%	

#Man-Whitney U test ##chi square ($\chi 2$) test; * *P-value < 0.05* was considered statistically significant, CRP; C-reactive protein.

Table 5. Vitamin D in relation to blood culture among cases.

	Positive blood culture (N=34)		**Negative blood culture (N=16)**		***P-value****
	Median	**Range**	**Median**	**Range**	
Serum vitamin D (ng/mL)	4.8	(0.4-13.3)	5.02	(0.9-9.6)	*0. 876*[#]
Variable	**N**	**%**	**N**	**%**	***P-value***
Vitamin D					
Deficiency	44	97.2%	26	52.0%	*0. 588*[##]
Insufficiency	1	2.9%	1	1.0%	

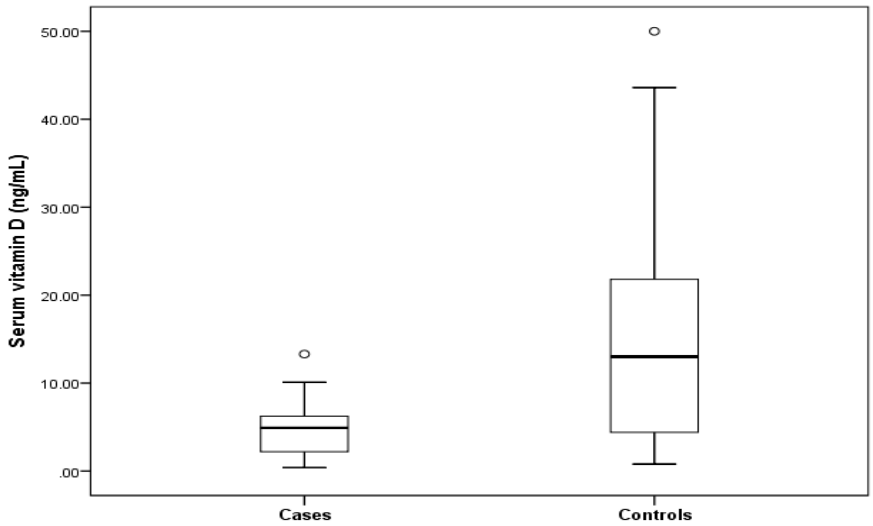
#Man-Whitney U test ##chi square ($\chi 2$) test; * *P-value < 0.05* was considered statistically significant.

Table 6. The predictors of EOS by logistic regression analysis model.

	OR	**95.0 % CI**	***P-value***
Neonatal vitamin D deficiency	53.083	(6.792-414.881)	*<0.0001**
Inadequate maternal vitamin D intake during pregnancy	4.571	(0.919-22.730)	*0.046**
Maternal illness during pregnancy	9.935	(4.257-23.189)	*<0.0001**
Birth season (winter)	7.875	(3.063-20.247)	*<0.0001**

Logistic regression analysis test; OR: odds ratio; CI: confidence interval;* *P-value < 0.05* was considered statistically significant.

Figure 1. Median of serum vitamin D between cases and controls



Discussion

Our aim is to evaluate the relation between deficiency of vitamin D and EOS in term infants. Regarding the demographic features and birth-related factors, there was no statistically significant difference between cases and controls regarding gender. This was matched with **Oncel et al,** [16]. while was disagreed with **Makkar et al.** [17]. This difference may be due to the larger number of cases in their study. There was no statistically significant difference between cases and controls as regard weight as the average weight of both groups was 3.2 kg. This result was in disagreement with **Hornik et al.** [18] who reported that the risk of sepsis increases as the birth weight decrease.

We also confirmed that the neonates born by normal vaginal delivery were significantly associated with an increased frequency of sepsis. This result disagreed with **Oncel et al**. [16]. The reduced probability of neonatal sepsis whom delivered by cesarean section may be due to effective sterilization and intrapartum chemoprophylaxis.

We reported a statistically significant difference between the cases and controls regarding the maternal history of vitamin D intake during pregnancy. As (n=48; 96.0%) of mothers of cases did not intake vitamin D during pregnancy. This comes in agreement with previous studies [10,19,20].

Our findings indicate that the newborn's vitamin D level at birth depends primarily on the mother's vitamin D levels throughout pregnancy. Sufficient vitamin D intake for mothers should be insistence throughout pregnancy and lactation [21,22].

We reported a statistically significant difference between cases and controls regarding the season of birth. We found that (60%) of our cases were born in winter. As there were no other risk factors for the occurrence of sepsis in our cases, the high incidence may be referred to as vitamin D deficiency in this season. This comes in agreement with a previous study [10]. That suggested the circulating vitamin D in the mothers is mainly originated from the synthesis in the skin under the effect of sunlight, relying on the season.

As regards, the laboratory finding., the mean platelet count in cases was significantly lower than that of controls (*p <0.0001*). Thrombocytopenia is due to platelets' direct toxic injury, megakaryocyte suppression, and increased peripheral consumption as in disseminated intravascular coagulopathy or the presence of an immune component due to increased level of platelet associated immunoglobulins [23].

In our study, we noticed that (25%) had hypoglycemia while (32%) of cases had hyperglycemia. The increased metabolic demand and hypothermia caused by sepsis can reduce glucose levels. Sepsis had been reported to be the cause of (9.6%) neonatal hypoglycemia cases [24]. The high glucose level in neonatal sepsis patients may be due to a rise in stress hormone syntheses such as adrenaline, glucagon, and cortisol.

Sepsis is a frequent cause of serious disease in the pediatric age group, such as hyperglycemia [25].

C-reactive protein (CRP) was significantly higher in case group than control group. This was agreed with **Rotshenker-Olshinka et al.** [26]. On the contrary, **Streimish et al.** [27] noted that serial measurement of CRP is more beneficial for assisting the antibiotic therapy duration, rather than sepsis diagnosis.

Serum vitamin D median level was higher in the control group than the case group with a significant difference (*p<0.0001*). Also, the percentage of vitamin D deficiency was lower in control group than in case group (n=24; 48%, n= 49; 98%) respectively. Our findings found that the seriousness of vitamin D deficiency enhanced EOS risk. That was matched with previous studies [10,20]. Severe vitamin D deficiency has also been related to an elevated risk of pediatrics' lower respiratory tract infections [7].

In the present study, we assessed the association between culture-proven EOS and neonatal 25(OH) D deficiency. There were 34 (68.0%), neonates, with sepsis who were culture positive. This disagreed with **Procianoy and Silveira** [28], who found that culture-proven sepsis occurred in only 21% of cases with sepsis.

In the present study, the most common organisms were Gram-negative bacilli that represented about (28%) mainly *Klebsiella pneumonia*. While, Gram-positive cocci, and *candida* species represented (26%,14% respectively.) That agreed with **Cetinkaya et al.** [10]. However, the previous study [18] reported that Gram-positive bacteria mainly *Staphylococcus aureus* accounts for the majority of the culture growth. The difference in isolated organisms shows that every neonatal unit has its own pattern of microorganisms, and antimicrobial combinations should be altered according to culture results. In the present study, the percentage of vitamin D deficiency was lowered in cases with negative blood culture than those with positive blood culture with no statistically significant difference (n=26;52.0% versus n=44;97.2%; *p=0. 588*). That agreed with the previous study [10]. Also, **Sarwade et al.** [29] proofed that significant deficiency in vitamin D was related to culture-positive sepsis (*p <0.001*).

In our study, we noticed that (22%) of the cases died. Our result proofed that serum (25(OH)D) deficiency was related to adverse clinical outcomes.

This agrees with **Rech et al.** [30] who found that 25(OH)D deficiency is a significant indicator of 30-day mortality in patients with sepsis. The low maternal vitamin D level throughout pregnancy is linked with adverse neonatal consequences as small for gestational age, preterm births, harmful influence on teeth and bone, offspring development, and increasing infectious diseases risk [31].

Conclusion

This study concludes that the significant decreased level of 25(OH)D level in EOS in term infant relative to control suggests effective implementation of vitamin D supplementation programs for pregnant female in Egypt to minimize health issues accompanied by its deficiency**.**

Conflict of interest

No conflict of interests was reported from all author.

Funding

No funding was obtained to perform this study.

Author's contribution

All authors equally contributed to this work

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