



Vitamin D Levels in Early Onset Neonatal Sepsis without Maternal Risk Factors: A Case-Control Study

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Abstract

Objective: Vitamin D is an immune modulator affecting innate and cell mediated immunity. Neonates are susceptible to infections as both innate and adaptive immune systems are not developed. This prospective study compares serum vitamin D among neonates with and without early onset sepsis without maternal risk factors (EOSWMRF) and blood culture positivity.

Methods: This observational study included 60 neonates <24 hours of age, who were divided into cases with sepsis and controls. Cases were with possible, probable and highly probable sepsis as based on sepsis criterion and blood culture among neonates without maternal risk factors (EOSWMRF). On the third day of life serum vitamin D levels (ng/ml) were measured and neonates were subdivided into having severely deficient, inadequate and adequate levels.

Results: Possible, probable and highly probable sepsis was found among 14, 9 and 7 cases. Mean vitamin D levels (ng/ml) among the case and control group was 13.43 ± 3.43 and 21.53 ± 6.6 respectively ($p < 0.001$). Severe deficiency was found among 5/6 of culture positive neonates. Among neonates, who died during study, culture positive was 5/6 and severely vitamin D deficient were 5/9. Inadequate levels were found among 86% ($n=44$) of discharged neonates. Strong positive correlation was seen among Vitamin D deficient levels and deaths ($p < 0.001$).

Conclusion: Severe vitamin D deficiency was associated with culture positive sepsis ($p < 0.001$) and deaths ($p < 0.001$) among cases. Among controls 20% were having adequate and 80% were having inadequate and no patient had severely deficient vitamin D levels.

Keywords: Case; Control; Vitamin D; Neonate; EOSWMRF; Blood culture

Introduction

Vitamin D is a fat-soluble vitamin, potent immune modulator which affects innate and cell mediated immune system. Neonatal sepsis is characterized by signs and symptoms of infection with accompanying bacteraemia in first month of life and is an important cause of morbidity and mortality [1,2]. It includes pneumonia, meningitis, septicaemia, arthritis, osteomyelitis. Incidence of neonatal sepsis is between 1-8/1000 live births which constitutes 25% of neonatal death worldwide [3,4].

Neonatal sepsis is classified into: Early and late onset sepsis [3]. Early onset sepsis (EOS) presents within first 72 hours of life. Neonates can become symptomatic at birth and EOS usually presents as respiratory distress and pneumonia [1]. EOS is generally associated with organism transmitted via mother i.e., infected maternal genital tract [1-5] Maternal risk factors that are known to cause EOS includes, premature rupture of membranes (PROM), mother with chorioamnionitis, abnormal vaginal discharge, repeated per-vaginal examinations and delayed progression of the labour. Other risk factors include preterm, low birth weight (LBW), meconium stained liquor (MSL) and prolonged labour with perinatal birth asphyxia [1-6]. However, cases of EOS are known to occur, without maternal risk factors (EOSWMRF) therefore, we decided to study serum vitamin D as an independent risk factor for EOS in neonates without maternal risk factors (EOSWMRF).

Vitamin D contributes to maintain normal calcium metabolism and bony mineralization [7,8]. Vitamin D enhance innate response of immunity by inducing cathelicidin (LL-37), which is antimicrobial peptide synthesised by macrophages and neutrophils. Neonate is more susceptible to infections as both innate and adaptive immune systems are not well developed [9-12]. Studies have shown increased incidence of neonatal sepsis in babies with vitamin D deficiency [10,11]. Vitamin D supplementation during pregnancy can leads to higher maternal vitamin D levels is known entity, which can subsequently have

beneficial effects in prevention of neonatal morbidities [10]. So present study was planned among neonates admitted within first 24 hours of life in neonatal intensive care unit (NICU). Primary objective of this study was to assess the correlation between serum vitamin D and EOS among neonates without maternal risk factors (EOSWMRF).

Materials and Methods

Setting

This observational study (case-control study) was carried out between Oct 2017 to Jan 2018, and July to Sept 2018 at Paediatrics department of tertiary care hospital as per posting roster of the invigilator. Out of 833 neonates admitted, 60 neonates were included in the study who were admitted on the first day of the life, who were >34 weeks gestational age, with major exclusion being healthy term neonates.

Inclusion criterion

Cases group ($n=30$): Neonates >34weeks admitted (intramural and extramural babies) in NICU within 24 hours of life with septic screen and clinical and laboratory findings suggestive of EOS. Sepsis criteria used in this study was defined by Gitto (Table 1) [8]. Control group ($n=30$): Neonates >34 weeks admitted in NICU within 24 hours of life

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who had negative septic screen and laboratory findings of EOS but had some signs of sepsis which according to septic criteria is 'No sepsis'. This study deals with serum levels of vitamin D of neonates, deaths in neonates associated with sepsis, and blood culture status. Maternal levels were not studied.

Exclusion criteria

Neonates <34week, presence of maternal chorioamnionitis, PROM in mother, admitted after >24 hours of life, Parents not willing to give consent, healthy neonates admitted.

Ethics

Approved by IRB- institutional review board of Government Medical College, Bhavnagar (Gujarat); trial registered under CTRI (ctri.nic.in/2018/03/012269) (clinical trial registration India).

Septic screen

Sixty neonates with signs of sepsis were included in study. Septic screen was sent for these neonates, which included total leucocyte count (TLC), absolute neutrophil count (ANC), platelet counts, and C-reactive protein and blood culture. Based on signs of sepsis and septic screen reports neonates were divided into possible, probable, highly probable and no sepsis according to sepsis criteria and blood culture positive or negative sepsis. Neonates with signs of sepsis and negative septic screen were included in control group (n=30) and neonates with possible, probable and highly probable sepsis were included in case group (n=30) in the present study.

Vitamin D levels

Venous blood was drawn on third day of life from case and control groups to measure vitamin D levels (ng/dl). Based on vitamin D levels, patients were divided into groups with adequate (32-100), insufficient (11-31) and severely deficient (<10) levels. Complete blood count was performed using an automatic counter. C-reactive protein was determined by latex slide method. Blood cultures were analysed using culture media. Neonates were grouped as cases and control and vitamin D levels in two groups were compared. The data was evaluated by using chi-square test and the SPSS for Windows 15.0.

Results

From Oct 2017 to Jan 2018 and July to Sept 2018, 843 neonates were admitted in paediatrics department, 383 were <34 weeks which were excluded. From rest 560 neonates 500 were excluded based of maternal risk factors, neonates who were admitted after 24 hours of life, healthy neonates admitted and neonates with major congenital malformations. As per Table 2, The study population included 60 neonates with signs of sepsis, after obtaining written informed consent from parents, 30 neonates with septic screen positive (case group) and 30 with septic screen negative (control group). Two groups were comparable with respect to demographic criterions like gestational age, gender, religion, mode of delivery, place of delivery and maternal age (Figure 1).

This study included 20 (66%) preterm and 10 (33%) full term among case group and among control group 19 (63.34%) were preterm and 11 (37.7%) were full term (p=0.78). Case group neonates were subdivided into the possible 14/30 (46.6%), probable 9/30 (30.3%) and highly probable sepsis 7/30 (22.3%) (Tables 3-5). Six cases (20%) among case group had culture positive sepsis. Sepsis was equally distributed among males and female neonates (p=0.99) among case group neonates.

As per Table 3, there was significant difference among levels of vitamin D between case and control group (p< 0.001) with severe

deficiency was seen among 9/30 (30%) cases. No neonate from case group had adequate vitamin D levels. 6/30 (20%) controls had adequate levels of vitamin D. Mean vitamin D levels among case and control group were 13.43/3.43 and 21.53/6.6 ng/ml respectively. Mean vitamin D level for possible probable and highly probable sepsis were 14.4/3.8, 13.46/2.9 and 10.7/1.8 ng/ml respectively.

As per Table 4, culture positive sepsis was common among neonates with lower levels of vitamin D. 66% (4/6) of culture positive septic neonates had severe vitamin D deficiency (p<0.001). Among those who were discharged in both the groups, 80% (n=44) of neonates had inadequate levels. All patients with adequate levels were discharged (p<0.001).

As per Table 4, severe vitamin D deficiency was seen among all case group neonates who died (n=5) (55.6%) (p<0.001). 5/6 (83%) of blood culture positive neonates died. There was strong positive correlation between deficiency of vitamin D and deaths, culture positivity and highly probable sepsis (p < 0.001). In this study we found that lower levels of vitamin D are found among case group as compared to control group. Neonates with culture positive sepsis has severe deficiency of vitamin D as compared to the culture negative sepsis and deaths were

Groups/parameters	Highly probable Sepsis	Probable sepsis	Possible sepsis	No sepsis
Altered CBC#	>2	=2	<2	-
C-reactive protein	+++	++	+	+/-
Blood culture	+/-	+/-	+/-	-
Clinical*	3	3	3	3

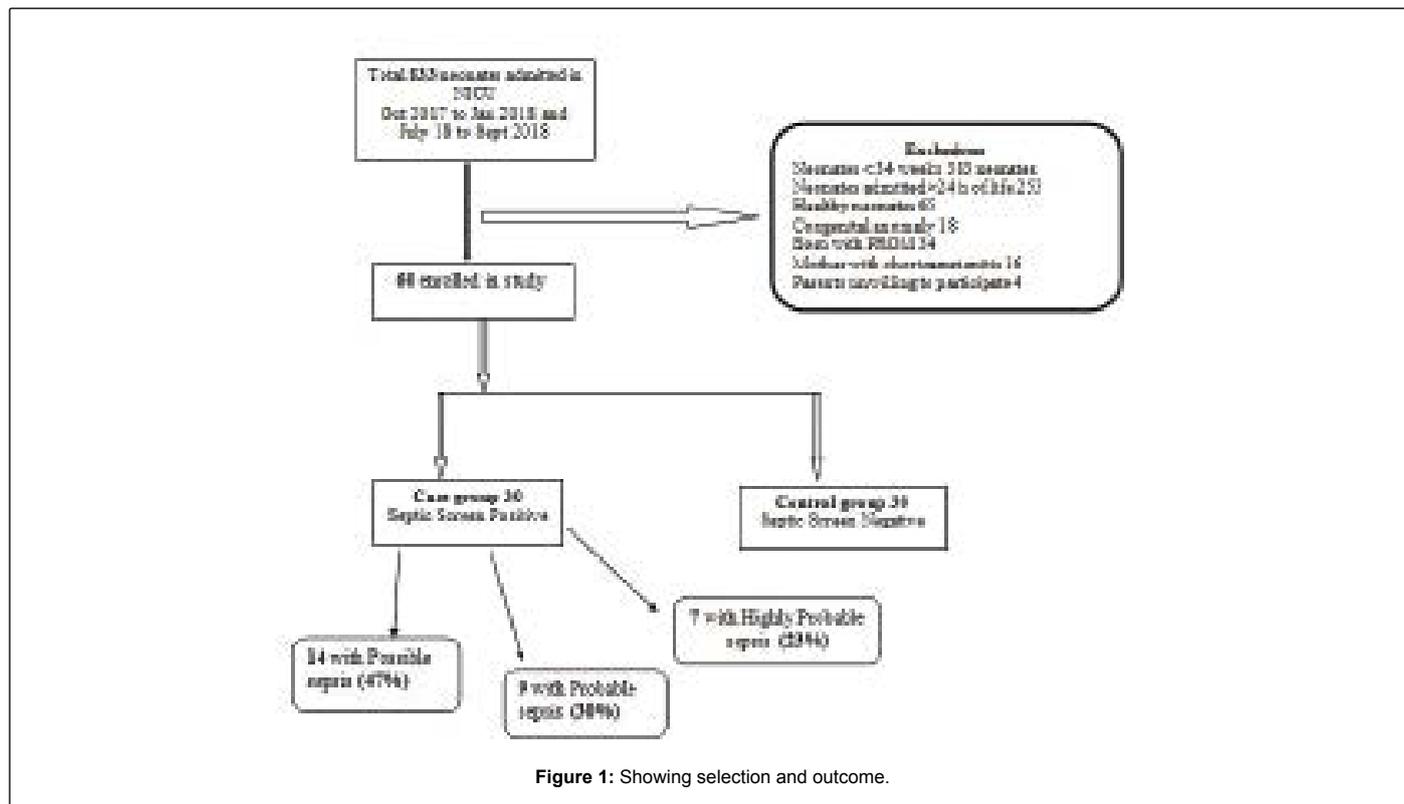
*Temperature instability, apnoea, need for ventilation or supplemental oxygen, tachycardia, bradycardia, hypotension, feeding intolerance, abdominal distention.
#Total leucocyte count, absolute neutrophil count, platelet count.

Table 1: The sepsis criteria [8].

Neonatal Profile	Cases n=30	Controls n=30	p-value
Gestational age			
Preterm	20	19	0.787
Full term	10	11	
Gender			
Male	18	15	0.496
Female	12	15	
Religion			
Muslim	14	12	0.61
Hindu	16	18	
Mode of delivery			
Normal vaginal	19	23	0.260
LSCS	11	7	
Place of delivery			
Intramural	12	22	0.09
Extramural	18	8	
Maternal age			
<20 years	5	3	0.072
21-30 years	21	27	
>30 years	4	0	
	Mean /SD	Mean /SD	
White blood count (× 10 ⁶ /l)	14.5/ 5.2	12.04/ 4.3	0.312
ANC (× 10 ⁶ /l)	7.9/ 3.71	6.9/ 3.0	0.257
Platelet count (10 ⁹ /l)	1.64/ 0.77	1.92/ 0.74	0.159

LSCS: Lower Segment Caesarian Section, ANC: Absolute Neutrophil Count

Table 2: Demographic data.



Vitamin D levels (ng/ml)	Case	Control	Blood culture Positive	Blood culture Negative	Total	p
<11	9	0	5	4	9	<0.001
11.1-30	21	24	43	2	45	
>30	0	6	6	0	6	

Table 3: Comparison of neonatal 25-hydroxy vitamin D levels and blood culture.

Comparison	Parameters n (%)	Vitamin D deficiency (25OHD<11 ng/ml)	Vitamin D inadequacy (25OHD 11-30 ng/ml)	Vitamin D adequacy (25OHD>30 ng/ml)	p
	Deaths	4 (80)	1 (20)	0	<0.0001
	Discharge	5 (9.8)	44 (86)	6 (11.7)	
	Culture positive sepsis	4 (66.6)	2 (33.3)	0	<0.001
	Culture positive sepsis	5 (9.2)	43 (79.6)	6 (11.1)	
Cases	Sepsis				<0.001
	Possible	2 (6.6)	11 (36.7)	0	
	Probable	2 (6.6)	8 (26.7)	0	
	Highly probable	5 (16.6)	2 (6.6)	0	
Control	No sepsis	0	24 (80)	6 (20)	

Table 4: Correlation of sepsis with vitamin D deficiency n (%).

common among neonates with culture positivity and with severe vitamin D deficiency. Also, patients with highly probable sepsis have lower levels of vitamin D as compared to probable and possible sepsis.

Discussion

As per Table 5, mean weight in present study was markedly less than that in study by Cetinkaya [10] as later was performed in developed country among term neonates. Kanth [11] had included preterm and full-term neonates for study. Weight in Kanth [11] study is comparable

with present study as both are performed in developing countries and included both term and preterm neonates. Study done by Yu [13] has included only full-term neonates.

Case and control group are comparable with respect to gender among all four studies. LSCS rates were similar among all four studies between case and control group. Mean maternal age (years/SD) in years in present study was 24.9/5.0 among case group and 24.2/2.6 among control group, which was 26.8/6.6 and 28.4/5.2 years in study by Cetinkaya [10], 27.0/3.8 and 25.4/5.0 in case and control group in study

Demographic data		Cetinkaya M [10]	S Uday Kanth [11]	Present study
Birth weight (gm)	Case	3454/460	2140/660	2160/526
	Control	3223/460	2410/590	2166/477
Gender (M/F)	Case	26/24	18/21	18/12
	Control	29/21	20/19	15/15
Caesarean section	Case	20	31	11
	Control	28	25	7
Maternal age (years)	Case	26.8/6.6	27.03/3.75	24.93/4.96
	Control	28.4/5.2	25.44/5.01	24.20/2.61
White blood cell ($\times 10^9/L$)	Case	17.3/1.3	12.4/4.0	14.5/5.2
	Control	12.0/0.7	12.33/2.8	12.04/4.3
Platelet count (lac/ml)	Case	2.09/0.17	2.54/1.02	1.67/0.77
	Control	3.0/0.17	3.08/1.47	1.92/0.74

Table 5: Comparison of demographic data with reference studies, mean/SD.

Comparison		M Centinkaya [10]	Yu YK [13]	S. Uday Kanth [11]	Present study
Mean Vit. D level (ng/ml)	Case	8.6/3.1	9.82/2.65	14.69/4.45	13.43/3.43
	Control	19.0/4.8	18.45/4.37	26.46/2.20	21.53/6.6
Severe deficiency %	Case	42	40	14	9
	Control	1	26	5	0
Inadequate levels %	Case	8	0	25	21
	Control	49	13	34	24
Culture-positive	Case	10.1/1.8, n=5*	-	-	11.2/1.5, n=6#
Culture-negative	Case	8.4/3.2, n=45*	-	-	18.18/6.6, n=24#

* p=0.25; # p<0.001

Table 6: Comparison of vitamin D levels among different studies, mean/SD.

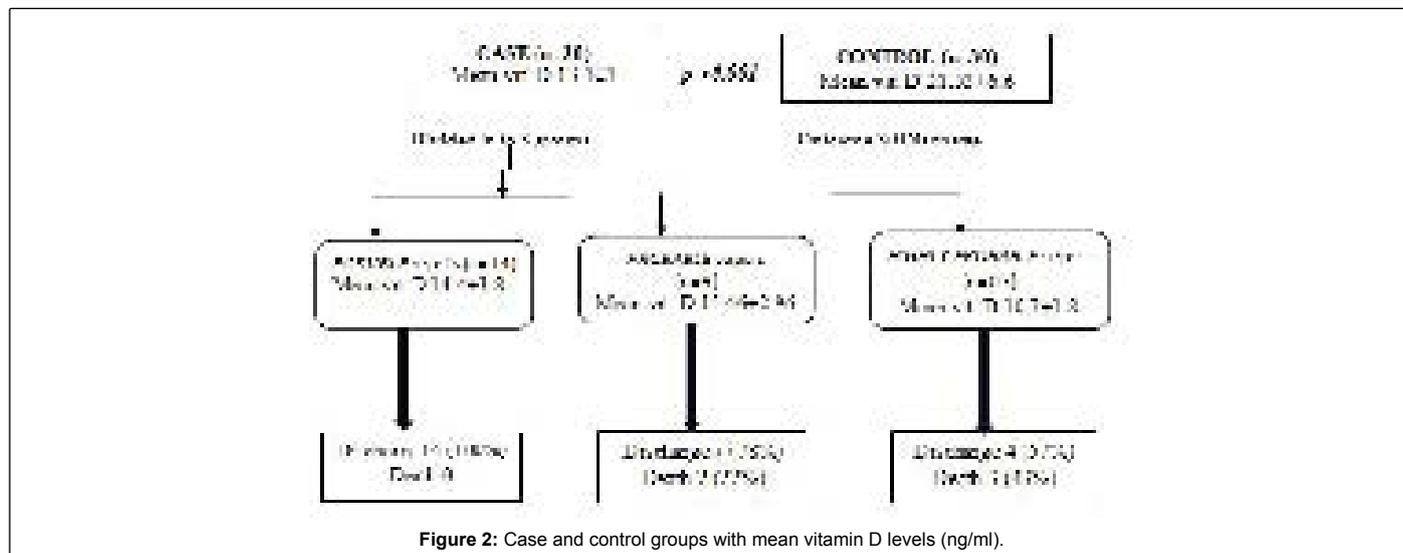


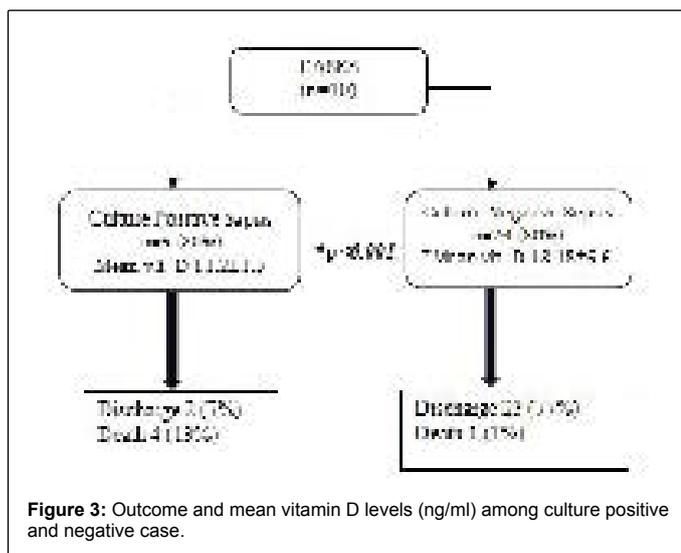
Figure 2: Case and control groups with mean vitamin D levels (ng/ml).

by Kanth [11] ($p=0.07$). On comparing the laboratory parameters, higher WBC counts, low platelet counts were observed among cases as compared to control group neonates in all three studies (Figures 2 and 3).

As per Table 6, present study had severe vitamin D deficiency among 9 (30%) cases and zero control group neonates. 21 (70%) cases and 24 (80%) of control group neonates had inadequate levels of vitamin D. 6 (20%) control group neonates have adequate vitamin D levels. In study done by Cetinkaya [10] severe vitamin d deficiency and inadequacy was seen among 42 (82%) and 8 (16%) case group neonates respectively. (2%) control had severe deficiency and 49 (98%) had inadequacy. In

study by Kanth [11-16] (35.9%) cases and 5 (12%) Control had severe deficiency of vitamin D. Inadequacy was seen in the 25 (64%) of cases and 34 (87%) of control cases. Present study was comparable to the study performed by Kanth [11]. As per study performed by Yu YK13 severe deficiency was seen among all cases $n=40$ and 26 controls group patients with 13 cases having inadequate levels of vitamin D.

As per Table 6, the lower mean vitamin D levels and severe vitamin D deficiency was found common among cases than controls which is similar to both other studies. Study done by Centinkaya [10] showed that higher values of vitamin D were seen in patients with the culture suggests lower levels of vitamin D in the culture proven septic neonates as compared to culture negative septic neonates ($p<0.0001$).



Conclusion

Severe vitamin D deficiency (<10 ng/ml) among preterm and full-term sick neonates without maternal risk factors was significantly associated with culture positive early onset sepsis in neonates in first three days of life ($p < 0.001$).

What was already known?

Two previous studies involving neonates have found that low level of serum vitamin D at birth in sick neonates was associated with higher risk of early onset culture positive sepsis and death.

What this study adds?

This study excludes neonates with maternal risk factors while studying relation between vitamin D (deficiency, <10 ng/ml) and early onset sepsis and death. Among neonates, without maternal risk factors and less than three days of age, deficiency of vitamin D is a risk factor for early onset culture positive sepsis and death.

Limitation

Demographic factors like socio economic status, education of parents, parental addictions like tobacco, alcohol or others, Gravidia, Para, Living, Abortion status of mother, and consanguinity of marriage was not considered.

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