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Are Lower Vitamin D Levels Associated With Increased Risk Of Neonatal Sepsis In Term Infants?

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ABSTRACT

Background: Vitamin D enhances the anti-microbial response of monocytes, suggesting a protective role of vitamin D in infection. **Objectives:** The main objective of this study was to determine the role of maternal and neonatal vitamin D deficiency in the development of neonatal sepsis. **METHOD**: This prospective study was performed in term infants with clinical and laboratory findings of neonatal sepsis who were >37weeks of gestational age and were admitted to neonatal intensive care unit. **Results**: The findings of this study indicate that mean neonatal vitamin D levels were 12.90ng/ml in the study group and 25.99ng/ml in the control group. In our study group 65.2% of neonates had vitamin D deficiency, 26.1% insufficiency and 8.7% had adequate vitamin D levels where as in the control group 17.4% of neonates had vitamin D deficiency, 21.7% insufficiency and 60.9% had adequate vitamin D levels and significant positive correlation existed between maternal and neonatal 25-OHD levels in both study ($r=0.839, p<0.01$) and control groups ($r=0.880, p<0.01$). **Conclusions:** Our study showed very high prevalence of Vitamin D deficiency in septic neonates and maternal vitamin D status directly affects neonatal vitamin D status so there is need to increase vitamin D supplementation of mothers during pregnancy in order to decrease sepsis and vitamin D deficiency in newborns.

Keywords: Maternal vitamin D deficiency, Neonatal vitamin D deficiency**,** Neonatal Sepsis, Vitamin D supplementation.

INTRODUCTION

Neonatal sepsis is characterized by signs and symptoms of infection with or without accompanying bacteremia in the first month of life and is an important cause of morbidity and mortality. [(1,2)] The incidence of neonatal sepsis varies between 1 and 8 neonates per 1000 live births.[(3)]

Neonatal sepsis can be classified into two major categories depending up on the onset of symptoms.[(4)]

Early onset sepsis (EOS):

It presents within the first 72 hours of life. In severe cases, the neonate may be symptomatic at birth. Infants with EOS usually present with respiratory distress and pneumonia. The source of infection is generally the maternal genital tract. Some maternal/perinatal conditions have been associated with an increased risk of EOS. Knowledge about these potential risk factors would help in early diagnosis of sepsis. Based on the studies from India,

the following risk factors seem to be associated with an increased risk of early onset sepsis. [4,5]

1. Low birth weight (<2500 grams) or prematurity.
2. Febrile illness in the mother with evidence of bacterial infection within 2 weeks prior to delivery.
3. Foul smelling and/or meconium stained amniotic fluid.
4. Rupture of membranes >24 hours.
5. Single unclean or > 3 sterile vaginal examination(s) during labor.
6. Prolonged labor (sum of 1st and 2nd stage of labor> 24hrs).
7. Perinatal asphyxia (Apgar score <4 at 1 minute).

Presence of foul smelling liquor or three of the above mentioned risk factors warrant initiation of antibiotic treatment. Infants with two risk factors should be investigated and then treated accordingly.

Late onset sepsis (LOS):

It usually presents after 72 hours of age. The source of infection in LOS is either nosocomial (hospital acquired) or community-acquired and neonates usually present with septicaemia, pneumonia or meningitis.[6,7] Various factors that predispose to an increased risk of nosocomial sepsis include low birth weight, prematurity, admission in intensive care unit, mechanical ventilation, intensive procedures, administration of parenteral fluids, and use of stock solutions. Factors that might increase the risk of community-acquired LOS include poor hygiene, poor cord care, bottle-feeding, and prelacteal feeds. In contrast, breastfeeding helps in prevention of infections.

Vitamin D is a fat-soluble steroid hormone that contributes to the maintenance of normal calcium homeostasis and skeletal mineralization.[8] Vitamin D also has immunomodulatory effects on immune function.[9] 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.[8,9] The major cause of vitamin D deficiency is the lack of appreciation that sun exposure in moderation is the major source of vitamin D for most humans. Very few foods naturally contain vitamin D, and foods that are fortified with vitamin D are often inadequate to satisfy either a child's or an adult's vitamin D requirement. Vitamin D deficiency causes rickets in children and will precipitate and exacerbate osteopenia, osteoporosis, and fractures in adults. Vitamin D deficiency has been associated with increased risk of common cancers, autoimmune diseases, hypertension, and infectious diseases. A circulating level of 25-hydroxyvitaminD of >75nmol/L, or 30ng/mL, is required to maximize vitamin D's beneficial effects for health. In the absence of adequate sun exposure, at least 800–1000 IU(international units) vitamin D(cholecalciferol) /day may be needed to achieve this in children and adults. [10] Breast milk concentration of vitamin D is low (<20IU/L) and is inadequate for the needs of the growing infant.[11] Vitamin D in breast milk relates to mothers vitamin D intake, skin pigmentation and sunlight exposure.[12] To avoid developing a vitamin D deficiency, the American Academy of Pediatrics recommends breastfed and partially breastfed infants be supplemented with 400 IU per day of vitamin D beginning in the first few days of life. Vitamin D supplementation should be continued unless the infant is weaned to at least 1 liter per day of vitamin-D fortified formula. Any infant who receives <1 liter or 1 quart of formula per day needs an alternative way to get 400 IU/day of vitamin D, such as through vitamin D supplementation.

Material and Methods

Study Design

This prospective study was performed in term infants with clinical and laboratory findings of Neonatal Sepsis who were >37weeks of gestational age and were admitted to Neonatal Intensive Care Unit of Sheri-Kashmir Institute of Medical Sciences Soura, Srinagar between September 2016 and July 2018. This study was approved by the institutional ethical committee. Informed written consent from parents of the infants was obtained.

Participants, Case definitions: The study group consisted of breast feeding term neonates clinically suspected to have an infection within the first 28 postnatal days of life with no prior history of neonatal admission in hospital. Control group include all those babies who were admitted for neonatal

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hyperbilirubinemia. We used sepsis criteria for case definition as shown in table:

The sepsis criteria used in the study.

Groups	**Criteria**
highly probable sepsis	At least three sepsis-related clinical signs. CRP >1mg/dl. At least two other altered serumparameters in addition to CRP. Blood culture; positive or negative,
probable sepsis	Less than 3 sepsis-related clinical signs. CRP >1mg/dl. At least two other altered serum parameters in addition to CRP. Blood culture; negative.
possible sepsis	Less than 3 sepsis-related clinical signs CRP <1mg/dl. Less than 2 other altered serum parameters in addition to CRP. Blood culture; negative.
no sepsis	No sepsis-related clinical signs. CRP <1mg/dl. No altered serum parameters. Blood culture; negative.

Probable/possible/no sepsis groups were not included in the study group. Also excluded from our study group were neonates with major congenital abnormality, on formulae feeds, with prelacteal feeding history, Refusal of parental consent and maternal history of choroamnionitis and premature rupture of membranes.

Blood for neonatal and maternal vitamin D levels was obtained from all infants and their mothers at the postpartum period at the time of hospital admission and at first outpatient department visit for control group.

The maternal demographic features including age, educational level, socioeconomic status, presence of disease, mother's outdoor clothing status was recorded. Gestational age, birth weight, sex, mode of delivery, Apgar scores, birth season of all infants was also recorded.

Vitamin D deficiency was staged as

- Deficiency (serum 25-OHD<2Ong/ml)
- Insufficiency (serum 25-OHD between 20and 30ng/ml)and
- Adequate (serum 25-OHD>30ng/ml). [13]

A septic screen including total leukocyte count, absolute neutrophil count, immature to total neutrophil count, blood smear evaluation and C-reactive protein (CRP) was performed in all neonates

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with suspected sepsis to corroborate diagnosis of Neonatal Sepsis. Blood samples for whole blood count, CRP and culture were obtained before initiating antimicrobial therapy. Plasmas of both maternal and neonatal blood samples were Separated and stored at -20oC Levels of 25-OHD was determined using Chemiluminicense method. Whole blood count was performed using an automatic Beckman Coulter CBC analyzer. CRP was determined by LATEX agglutination method. Blood cultures were analyzed using fully automatic BACTEC Method. The main objective of this study was to determine the role of maternal and neonatal vitamin D deficiency in the development of neonatal sepsis, with secondary objectives being to determine prevalence of vitamin D deficiency in mothers and neonates and to identify any correlation between maternal and neonatal vitamin D levels.

STATISTICAL ANALYSIS

The sample for study was calculated using https://www.openepi.com/SampleSize/SSCC.htm. The estimated minimum sample sizes were 40 pairs, and our study is based on 46 cases & controls, mother-neonatal pairs.

The recorded data was compiled and entered in a spreadsheet (Microsoft Excel) and then exported to data editor of SPSS Version 20.0 (SPSS Inc., Chicago, Illinois, USA). Continuous variables were expressed as Mean±SD and categorical variables were summarized as frequencies and percentages. Frequency distribution tables, bar and pie charts were used for data presentation. Karl Pearson's correlation coefficient and Scatter Plots were employed to establish correlation between various variables. Student's independent t-test and ANOVA were used for comparing various parametric data. A P-value of less than 0.05 was considered statistically significant. All P-values were two tailed.

Results

The total of 92 (46 cases & 46 controls) mother-neonatal pairs, were enrolled in our study for a period of 2 years. The mean age of neonates was 7.13 days and 6.59 days, birth weight of 2.53kgs and 3kgs and gestational age was 38.67 weeks and 39.13 weeks respectively for the study group and control group, the difference was statistically insignificant. Majority (41.3%) of neonates in study group were of 2nd birth order while majority(43.5%) in the control group were of 1st birth order. In both groups LSCS (lower segment Cesarian section) was the most common mode of delivery (58.7% vs 63%).

Maternal 25-hydroxyvitamin D (25-OHD) levels: Of the total 58.5% of mothers was either vitamin D deficient or insufficient. Mean maternal vitamin D levels were 22.98ng/ml in the study group and 37ng/ml in the control group. In the study group 69.6% of mothers had vitamin D deficiency, 21.7% had vitamin D insufficiency and 8.7% had adequate vitamin D levels where as in the control group 13% of mothers had vitamin D deficiency and insufficiency each and 74% had adequate vitamin D levels (Table 1).

Table 1. Maternal 25-OHD status in the two groups

Maternal 25-OHD Levels	Frequency		Percentage	
	Cases (n=46)	Controls (n=46)	Cases	Controls
Deficiency (<20ng/ml)	32	6	69.6	13
Insufficiency (20-30ng/ml)	10	6	21.7	13
Adequate (>30ng/ml)	4	34	8.7	74

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Neonatal 25-hydroxyvitamin D (25-OHD) levels: Of the total 65% of neonates was either vitamin D deficient or insufficient. Mean neonatal vitamin D levels were 12.90ng/ml in the study group and 25.99ng/ml in the control group. In our study group 65.2% of neonates had vitamin D deficiency, 26.1% insufficiency and 8.7% had adequate vitamin D levels where as in the control group 17.4% of neonates had vitamin D deficiency, 21.7% insufficiency and 60.9% had adequate vitamin D levels (table 2).

Table 2. Neonatal 25-OHD status in the two groups

Neonatal 25-OHD Levels	Frequency		Percentage	
	Cases (n=46)	Controls (n=46)	Cases	Controls
Deficiency (<20ng/ml)	30	8	65.2	17.4
Insufficiency (20-30ng/ml)	12	10	26.1	21.7
Adequate (>30ng/ml)	4	28	8.7	60.9

Correlation of neonatal vitamin D levels with various variables:

1. *Maternal vitamin D levels:* A positive significant correlation was seen in both study ($r=0.839$, $p<0.01$) and control ($r=0.880$, $p<0.01$) groups. (table 3, figure 1a, 1b)
2. *Age of mother*: A statistically insignificant Correlation was seen between Age of mother and Neonatal Vitamin D in both study and control groups.(Fig.2a,2b)
3. *Gestational age of neonate:* A statistically insignificant Correlation was seen between Gestational age and Neonatal Vitamin D in both study and control groups.(Fig.3a,3b)
4. *Neonatal Birth Weight:* Insignificant correlation was seen between Neonatal Birth Weight and Neonatal Vitamin D in both study and control groups (Fig. 4a, 4b).
5. *Culture positives case:* In our study 11 out of 46 (23.9%) cases had positive blood culture. Mean neonatal 25-OHD levels in culture positive and culture negative cases were 11.40ng/ml and 13.37ng/ml respectively, the difference again being statistically insignificant (p=0.49).

Table 3. Showing correlation between Maternal and Neonatal vitamin D levels

Parameter	Pearson Correlation (r)		P-value	
	Cases	Controls	Cases	Controls

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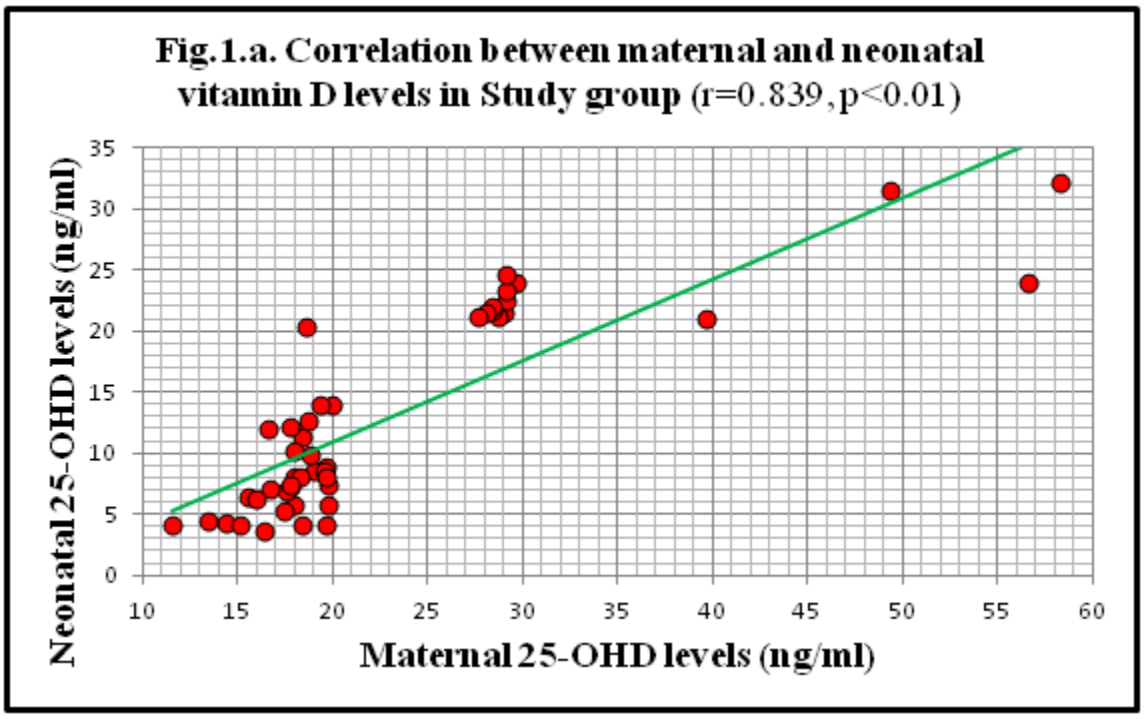
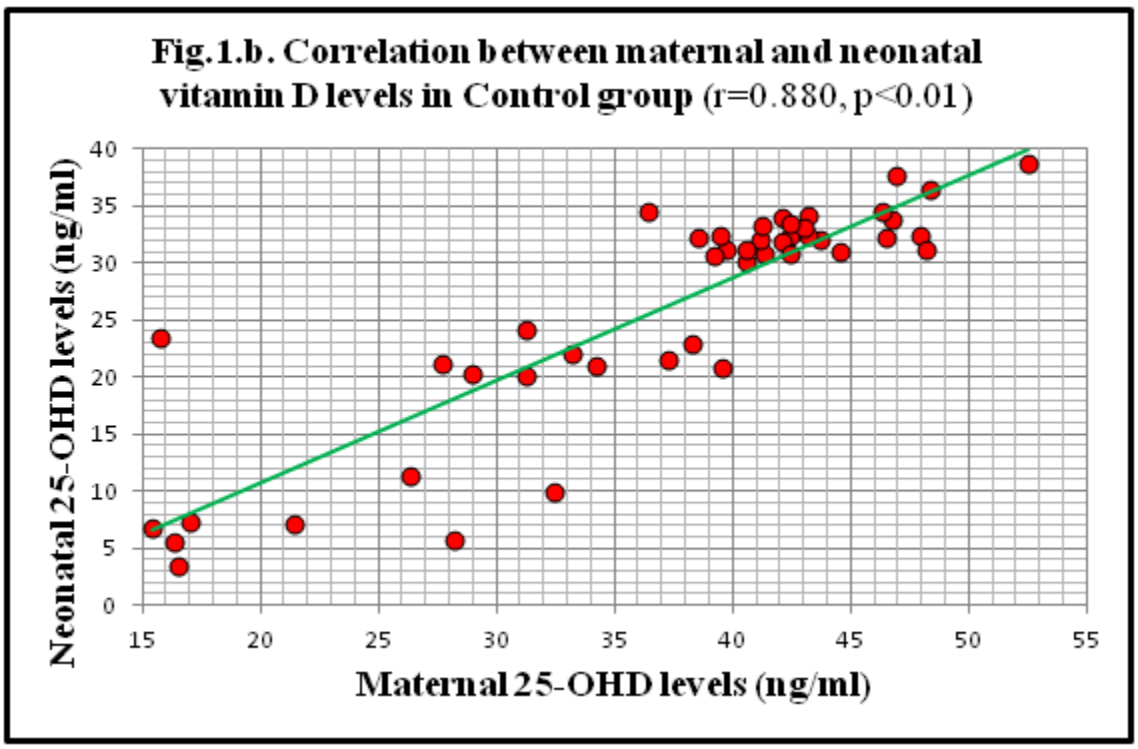
Maternal vitamin D-Neonatal vitamin D	0.839	0.880	<0.001	<0.001





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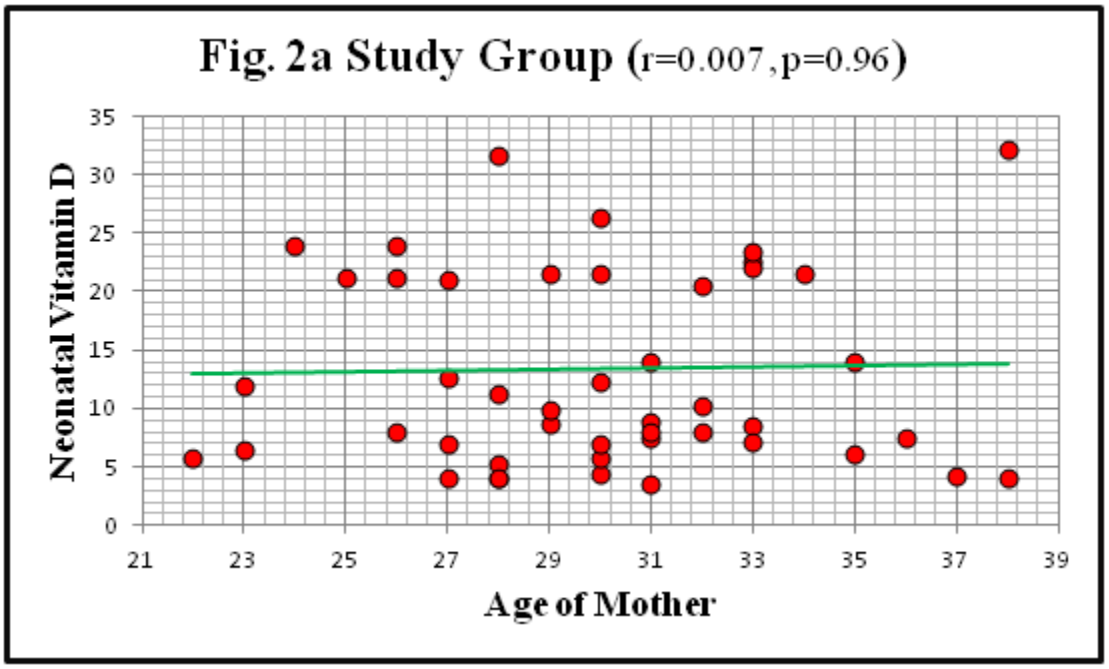

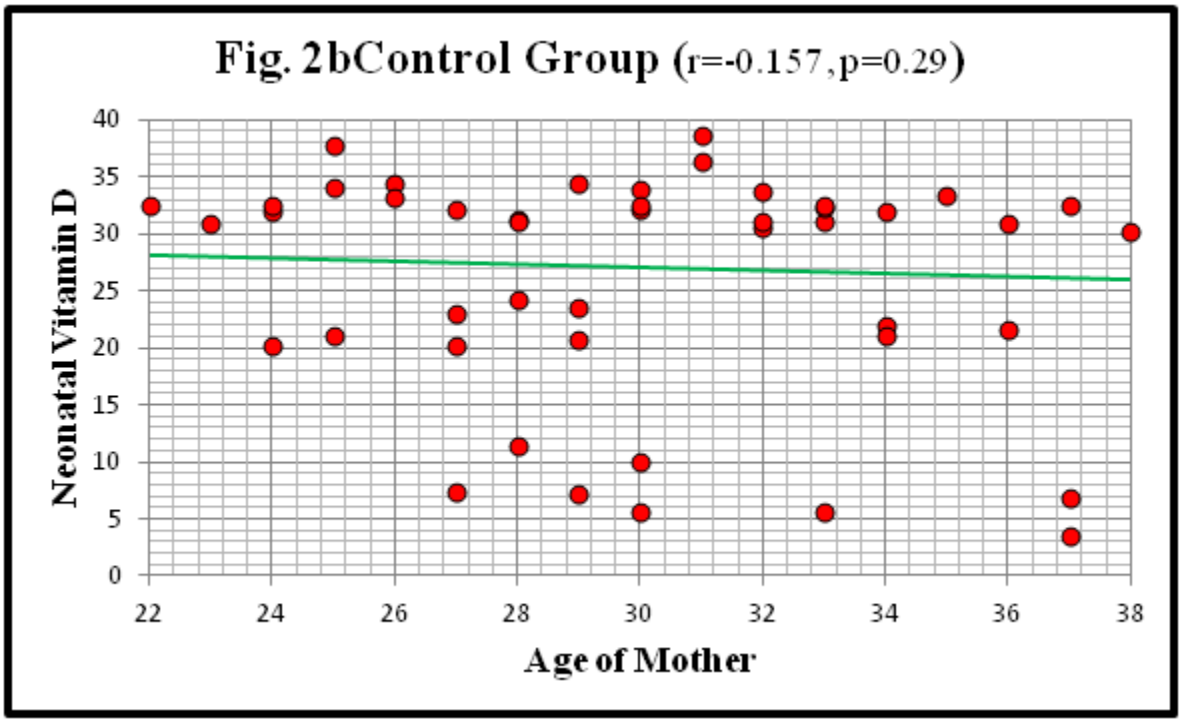

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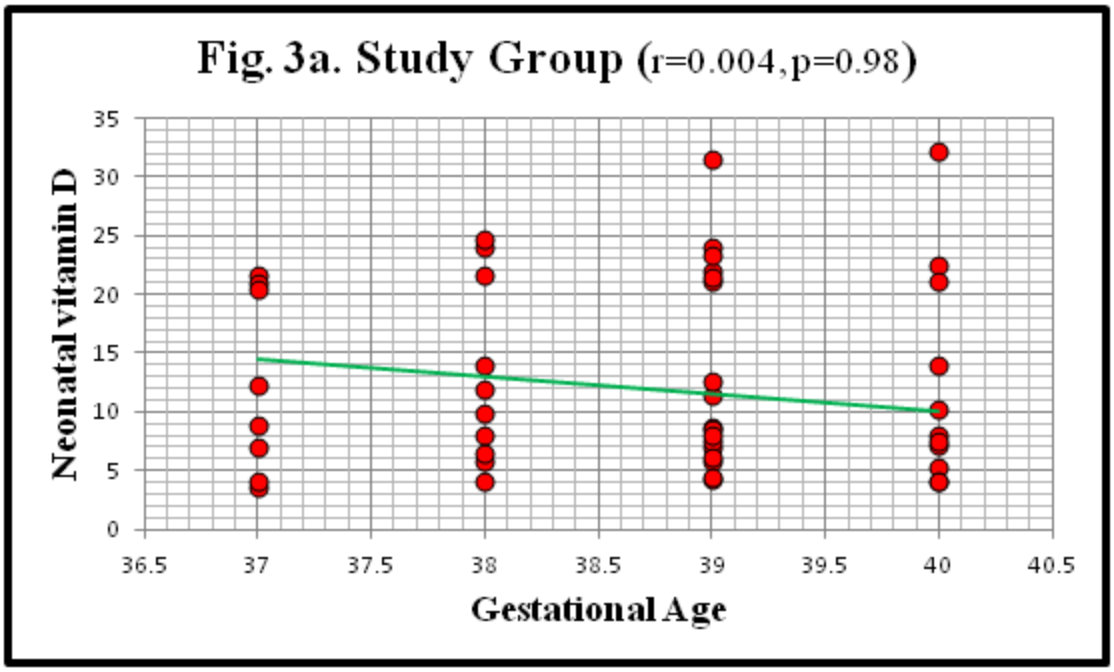

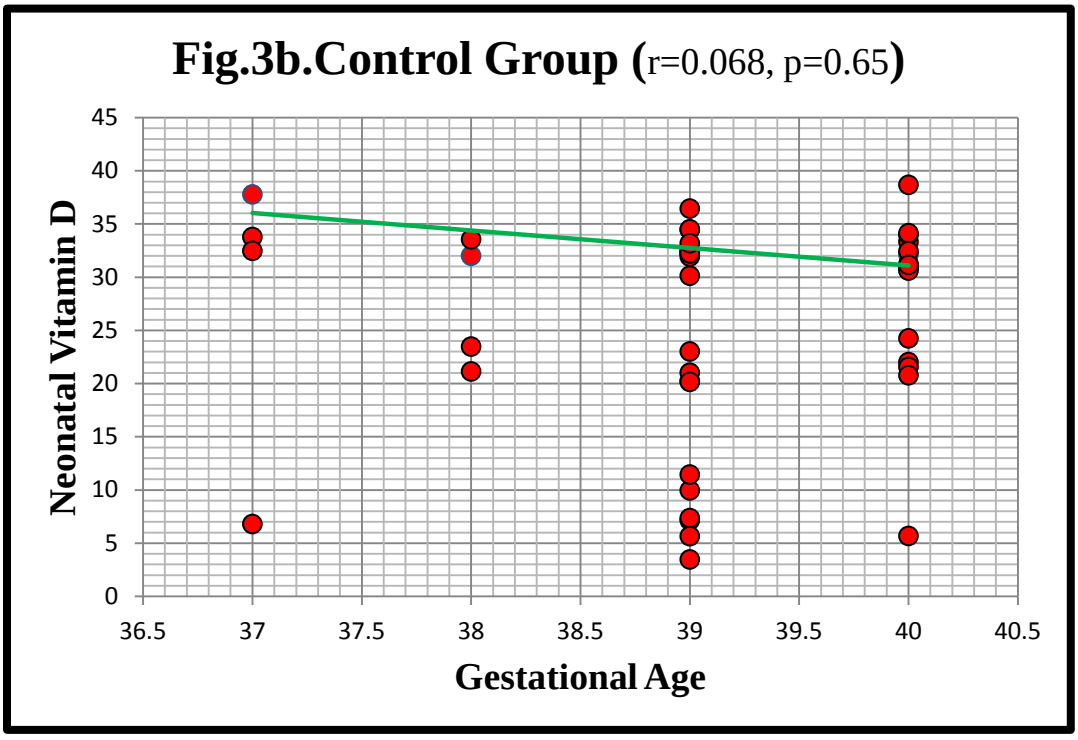

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Fig. 4a. Study Group (r=0.168, p=0.26)

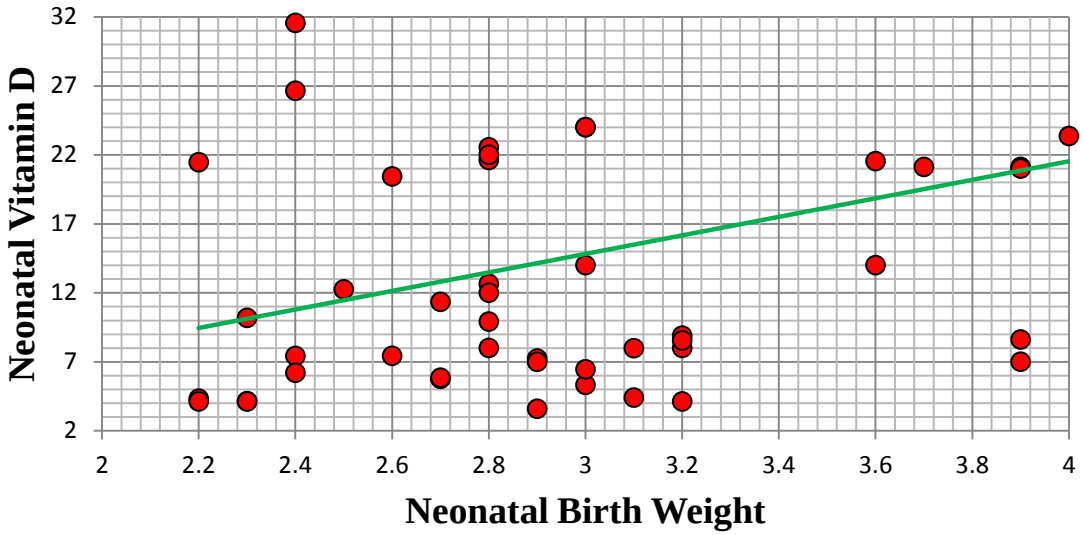
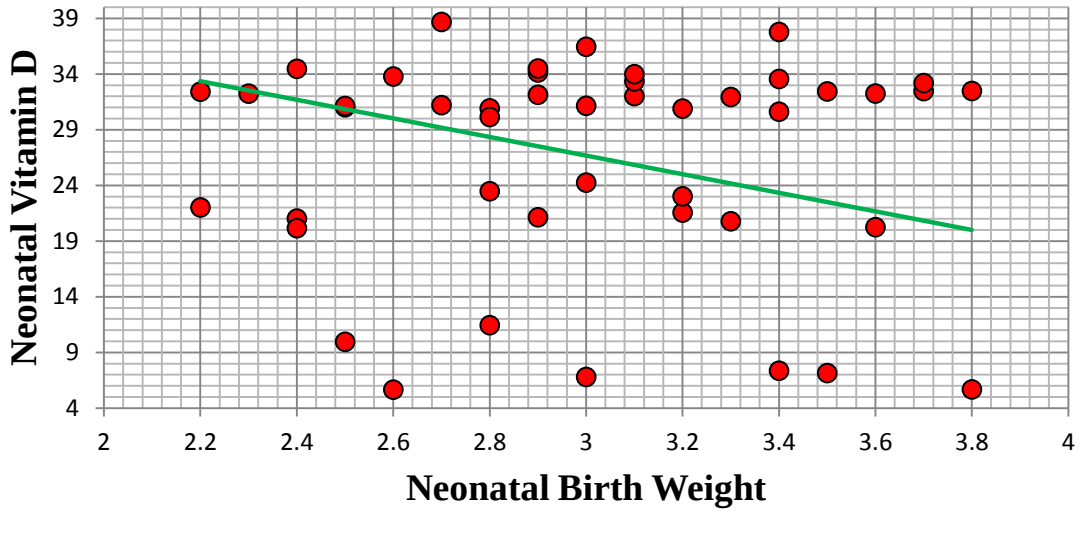


Fig. 4b. Control Group (r=-0.03, p=0.84)



Discussion

The main objectives of this study were to determine prevalence of vitamin D deficiency in mothers and neonates, the role of maternal and neonatal vitamin D deficiency on the development of neonatal sepsis and to determine the correlation between maternal and neonatal vitamin D levels. In this study, we in brief assessed the serum level of 25-hydroxy vitamin D in newborns and mothers and intended to compare vitamin D level in septic and nonseptic neonate–mother pairs.

The findings of this study indicate that vitamin D insufficiency is present in more than half of the mothers and neonates (58.5% of mothers and 65% of neonates). Our results are consistent with other Indian study by Mehrotra P et al who in 2010 reported prevalence of hypovitaminosis D of 67.5% in mothers and 65.5% in neonates. Our prevalence of hypovitaminosis D was marked lower than that, reported from Pakistan (Nazli Hossien etal 2010 reported prevalence of 89% of hypovitaminosis D in both mother and newborns while Atiq M et al reported 85% of neonates and 74% of mothers with

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vitamin D deficiency.) Various researches from developed countries show somewhat lower prevalence of vitamin D deficiency ranging 5-50% in mothers and 10-56% in neonates (Kovacs, 2001; Vandevijvere et al., 2012; Choi, Kim et al., 2015; Soltirovska Salamon et al., 2015; Vinkhuyzen et al., 2015). The higher prevalence of hypovitaminosis D in our study population could be explained by the fact of Kashmir being a Muslim dominated state; women have limited exposure to sunlight due to religious obligation of covering whole body. This deficiency is compounded by long winters and non availability of fortified foods.

Clancy N et al in 2013 suggested that vitamin D might have a role in the optimal functioning of the innate immune system by inducing antimicrobial peptides in epithelial cells, neutrophils and macrophages. This proposal was further strengthen by our study demonstrating significant difference in both mean maternal and neonatal 25-OHD levels between septic and non septic groups. *M Cetinkaya et al* in 2014, *Kanth SU et al* in 2016 and *Aye AM, et al* in 2018 in their respective studies reported similar statistically significant difference in mean maternal and neonatal vitamin D levels between study and control groups.

Pehlivan I et al (2003) reported vitamin D reserves in neonates are directly related to maternal vitamin D supply. As a consequence, if mother suffers from vitamin D deficiency, the neonate encounters this deficiency due to reduced placental vitamin D transmission which was strongly confirmed in this study too. In our study we found significant positive correlation between maternal and neonatal 25-OHD levels in both study ($r=0.839, p<0.01$) and control groups ($r=0.880, p<0.01$). Significant positive correlation consistent with our results was also reported by M *Cetinkaya et al, Marwa Ahmad et al a Fallahi M et al*.

Similar to the M *Cetinkaya et al and Aye AM et al* our study demonstrated no significant difference between mean maternal and neonatal vitamin D levels between culture positive and culture negative neonates however contrarily to our results Marwa Ahmad et al reported significant difference. The possible reason for such difference could not be ascertained.

Furthermore no significant correlation was seen between neonatal vitamin D levels and maternal age, birth weight of a baby and gestational age of neonate.

Our study has limitations, first our data is based on small number of patients from a single medical institute, so the findings may not be generalized. Second we did not consider seasonal variations in vitamin D levels during selection of patients.

In conclusion our study demonstrates a high prevalence of vitamin D deficiency in both mothers and neonates, significant positive correlation between maternal and neonatal vitamin D levels and significant mean difference of maternal and neonatal vitamin D levels between septic and non septic group. So there is need to increase vitamin D supplementation of mothers during pregnancy in order to decrease sepsis and vitamin D deficiency in newborns.

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