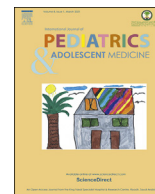


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Original article

## Correlation of 25-hydroxy vitamin D level with neonatal hyperbilirubinemia in term healthy newborn: A prospective hospital-based observation study

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### ABSTRACT

**Background:** Neonatal hyperbilirubinemia is one of the common entities that lead to frequent hospital admission of newborn. There are many risk factors that, when present, can lead to increased chances of neonatal hyperbilirubinemia. It is essential to identify these risk factors that are involved in the increased incidence of neonatal hyperbilirubinemia because its incidence can be decreased by modifying them.

**Objective:** To compare and determine any correlation of 25-hydroxy vitamin D levels of newborn and their mothers with the serum bilirubin level of newborn.

**Methodology:** This was a case–control study conducted in a tertiary care hospital on 100 newborn who were divided into two groups (50 in each group) on the basis of their serum bilirubin level on the 5th day of life. Newborns having serum bilirubin levels in the physiological range were included in the control group, and newborns having serum bilirubin levels out of the physiological range and who needed treatment were included in the case group. The blood samples of both newborns and their mothers were collected on the 5th day and sent to the laboratory for serum bilirubin and 25-hydroxy vitamin D estimation.

**Results:** The mean serum vitamin D level of cases was found to be lower than that of controls in both newborn and their mothers. A statistically significant difference was noted only between the vitamin D level of newborn but not in mothers when cases and controls were compared. Negative, statistically insignificant correlation was seen between vitamin D level and serum bilirubin in cases and controls. However, correlation of the vitamin D level of cases and their serum bilirubin was statistically significant, with a correlation coefficient of  $-0.335$  and a  $p$  value of  $0.0172$ .

**Conclusion:** Term healthy newborn having hyperbilirubinemia, with serum bilirubin levels out of the physiological range, have significantly low vitamin D levels and show a statistically negatively correlation with neonatal hyperbilirubinemia (which is out of the physiological range). Thus, decreased vitamin D can be included in the list of risk factors for neonatal jaundice.

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## 1. Introduction

Neonatal hyperbilirubinemia is one of the common entities that lead to frequent hospital admission of newborn. A peak serum

bilirubin level of  $>12.9$  occurs in 6.1% of healthy term newborn, and 3% develop a peak level of  $>15$  mg/dl [1]. Although the serum bilirubin level in most cases of neonatal hyperbilirubinemia is in the physiological range, which needs no treatment, some cases develop a peak serum bilirubin level, which, if not treated properly, may have devastating consequences on neonatal life. Kernicterus is one such complication in which a distinct yellowish pattern staining the brainstem, hippocampus, cerebellum, and certain brainstem nuclei (particularly the globus pallidus and subthalamic nucleus) is seen at autopsy in infants who die due to acute bilirubin toxicity. Most of sequelae of this disease arises from damage to

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these brain structures [2,3].

Vitamin D is one of the fat-soluble vitamins technically considered a hormone. Many functions of vitamin D have been deciphered in medical research, where derangement can lead to cardiovascular, pulmonary, obesity, diabetes, and neoplastic diseases such as breast and colorectal carcinoma [4]. Increased incidence of many diseases including wheezing and asthma [5], acute disseminated encephalomyelitis (ADEM) and future multiple sclerosis [6], schizophrenia [7], irregular neurocognitive result [8], type 1 diabetes mellitus, and insulin resistance [9] has been correlated with decreased vitamin D concentration in pregnant women and their offspring. Advanced research has revealed the occurrence of 25-hydroxy vitamin D receptors on cells that have their actual origin from hepatic, neural, pancreatic, and genitourinary (prostate) systems. Immune system components such as lymphocytes and macrophages also contain vitamin D receptors [10]. The major sources of vitamin D are through the skin and diet. Both these sources contain the inactive form of vitamin D. Its activation occurs in the liver and kidney by hydroxylation [11]. Other cells that can synthesize vitamin D are monocytes and placenta during pregnancy [12]. A hypothetical relationship between vitamin D and bilirubin can be explained by the synthesis of both entities in the liver [13]. Although the metabolism of both compounds occurs through different pathways in the liver, they can affect each other's metabolism, which remains to be proven [14].

Plenty of research is available on risk factors for neonatal hyperbilirubinemia, such as excessive hemolysis due to immune (ABO/Rh incompatibility) causes, nonimmune (hereditary spherocytosis, G6PD deficiency) causes, trauma (cephalohematoma), oxytocin, and diabetes in mothers. Limited number of studies is available on the relationship between hyperbilirubinemia and neonatal serum vitamin D [15]. Keeping in view the paucity of literature on the correlation of vitamin D with hyperbilirubinemia, the main aim of this study was designed, which was to find any association between serum bilirubin and vitamin D; thus, if results are significant, then we can decrease the incidence of neonatal hyperbilirubinemia by modifying the risk factors by supplementation of vitamin D.

## 2. Methodology

This was a prospective observational case–control study conducted in World College of Medical Sciences, Jhajjar, Haryana, India, from June 2017 to May 2018. The total number of newborn enrolled in our study was 100, i.e., 50 as cases and 50 as controls. Most of the guardians of the newborns refused to take part in the study because of poor socioeconomic status, and a less percentage of newborn returned for follow-up. These two were the main factors that limited the sample size to only 50 in each group. Cases and controls were chosen on the basis of serum bilirubin levels. Newborn in the cases group had their bilirubin level in the physiological range and did not need any treatment, and newborn in the control group had their serum bilirubin level in the range of intervention such as phototherapy, exchange transfusion, or other forms of treatment as per AAP guidelines.

Newborns were included in the study on the basis of the inclusion and exclusion criteria.

## 3. Inclusion criteria

- Exclusive breast-fed babies [16].
- In-born hospital-delivered babies
- Term healthy newborn babies > 37 weeks of gestation.

- Babies of RH-negative mothers and O-blood group mothers after confirmation of the DCT status. They were included only if DCT was negative.

## 4. Exclusion criteria

- Newborn with major congenital abnormalities.
- Rh/ABO incompatibility
- Newborn with a history of perinatal asphyxia, meconium aspiration syndrome, pneumonia, sepsis, and conjugated hyperbilirubinemia.
- Newborn with life-threatening abnormalities such as TEF, CDH, pulmonary sequestration, or anorectal malformation.

Before discharge, all mothers and caretakers were counseled about the breastfeeding, and a registered breastfeeding counselor was available to counsel and teach mothers about breast-feeding practice. According to our hospital protocol, all newborn are advised to come for follow-up for reviewing newborn examination on the 5th day, and relevant tests such as thyroid function tests, serum bilirubin, and blood group determination are routinely performed on every newborn. Parents were counseled about this research during this time. Determination of the 25-hydroxy vitamin D level of both the mother and the neonate along with serum bilirubin, thyroid profile, and blood group was done simultaneously on the 5th day of newborn life. Newborn having neonatal hyperbilirubinemia, which fall into the treatment zone according to the AAP nomogram, were shifted to NICU for further care.

In this study, all the 50 newborn were managed by phototherapy. One newborn needed exchange transfusion, but the guardians did not provide consent for inclusion in this research.

Serum bilirubin was estimated by micro bilirubin (Jendrassik and Grof method) and 25-hydroxy vitamin D by chemiluminescent immunoassay.

In this study, the status of levels of vitamin D was defined as [17].

Deficiency: <20 ng/ml.

Suboptimal range: 5–10 ng/ml.

Optimal vitamin D level = 30–50 ng/ml.

## 5. Statistical analysis

SPSS version 24 and MedCalc 18.11 were used for data analysis. Serum bilirubin and vitamin D level were analyzed for their mean and standard deviation. Correlation among groups was determined by Pearson's correlation. Difference in mean values between groups was analyzed by Student *t*-test. *P* value of <0.05 was considered significant.

## 6. Results

In this study, a total of 100 cases were included, i.e., 50 to the case group and 50 to the control group. Mothers' age, gestational age, mode of delivery, birth weight, and gender were all comparable in both groups, as proven by their mean values, percentage, and comparison *P*-value of >.05, thus not significant as shown in Table 1. Socioeconomic status was not formally compared in cases and controls.

Table 2 shows the mean and standard deviation of serum bilirubin and vitamin D status of both mothers and newborn of both groups. Mean serum bilirubin of cases was 18.056 mg/dl with the standard deviation of 1.099, which was quite high as compared to that in the control group (mean serum bilirubin of 8.05 mg/dl and standard deviation of 1.09). Vitamin D status in mothers of cases and controls was 22.59 ng/ml and 26.33 ng/ml, with standard

**Table 1**  
Demographic characteristics of the cases [mean  $\pm$  SD].

	Study group (n = 50)	Control group (n = 50)	P value
Mothers' age, in years	25.3 (6.1)	25.7 (4.9)	.15
Gestational age, in weeks	38.6 (1.5)	38.0 (1.7)	.09
Delivery type	Vaginal delivery	31 (62%)	.35
	Cesarean section	19 (38%)	.75
Male/female	30/20	28/22	.18
Birth weight, in kg	2.75 (0.35)	2.82 (0.43)	.08
Postnatal age, days	4.7 (1.6)	4.9 (1.6)	.65

**Table 2**  
Mean and standard deviation of maternal and baby Vit D and serum bilirubin.

	Minimum	Maximum	Mean	Std. deviation	
Case number = 50	Maternal vit D ng/ml	10.300	47.000	22.59000	9.152010
	Baby vit D ng/ml	6.900	32.000	12.02400	5.067164
	Sr. bilirubin ng/dl	15.000	20.000	18.05600	1.099195
Control number = 50	Maternal vit D ng/dl	14.900	43.000	26.33200	6.538877
	Baby vit D ng/ml	13.00	29.00	20.9136	3.20662

deviations of 9.15 and 6.54, respectively. Vitamin D levels of newborn were 12.02 ng/ml and 20.91 ng/ml with standard deviations of 5.06 and 3.02 in cases and controls, respectively.

When the correlation of groups was analyzed, only the vitamin D level of cases showed a significant correlation with their serum bilirubin, with a correlation coefficient ( $r$ ) of  $-0.3355$  and a significant  $P$  value of  $.0172$  ( $<.05$ ). All the remaining correlations between groups were not significant as shown by their correlation coefficients listed in Table 3. The negative correlation of serum bilirubin with vitamin D levels of both newborn and their mothers in both cases and controls is to be noted as shown in Fig. 1.

As shown in Table 2, our study revealed mean vitamin D levels of 22.59 ng/ml and 26.33 ng/ml with a mean difference of  $-6.96$  ng/ml in the mothers of newborn of cases and controls with a statistically insignificant  $p$  value of 0.082, respectively (Table 4). However, a statistically significant difference was noted between the vitamin D levels of cases and controls ( $P$  value 0.00) with a mean vitamin D level of 12.02 ng/ml and 20.9136 ng/ml, respectively, and a mean difference of  $-8.889$ .

## 7. Discussion

In this study, the mean 25-hydroxyvitamin D level in the mothers of both cases and controls was in the suboptimal range, i.e., 20–30 ng/ml. Garg R et al. [18] reported that 98.75% of women in the Indian population were having vitamin D  $< 30$  ng/ml. The low normal range of vitamin D in both studies can be explained by the particular dress code in our country. The mean vitamin D level of newborn was in the normal range in controls but significantly decreased in cases, and their difference was statistically significant. Mutlu M et al. [19] in their study showed 83% of newborn who developed jaundice with serum bilirubin in the physiological range

had vitamin D level in the range of 5–14.9 ng/ml. Aletayeb SMH et al. [15] showed that the mean vitamin D level of cases (cases in their study mean jaundiced infants which are same is our case criteria) was 84.38 nmol/l (18.75 ng/ml) which is below the normal range as shown in our study.

Our study reveals a negative but insignificant correlation (negative correlation means as the vitamin D level decreases between Vitamin D and serum bilirubin except in cases where correlation was statistically significant). Mutlu M et al. [19] and Aletayeb SMH et al. [15] also showed in their significant correlation between the vitamin D level of newborn who developed hyperbilirubinemia, which was out of physiological range, and their serum bilirubin levels.

To conclude, our study revealed low vitamin D level in newborn who developed jaundice, which was out of physiological range, and its significant negative correlation with their serum bilirubin. The main lacuna of our research is the small study sample and in a particular region of our country. To declare low vitamin D level as a risk factor for hyperbilirubinemia, we need more extensive research studies from different regions of the world, so that the future incidence of hyperbilirubinemia can be decreased by modifying this risk factor with vitamin D therapy.

## 8. What is known

Only two or three research articles are available in neonatology literature on the correlation of 25-hydroxy vitamin D with neonatal hyperbilirubinemia.

## 9. What is new in our study

A decreased level of 25-hydroxy vitamin D in term healthy

**Table 3**  
Pearson correlation analysis among various groups.

Correlation	$r$	CI for $r$	Sig. ( $P$ value)	
Case	Maternal vit D vs. baby vit D	0.01758	$-0.2621$ to $0.2945$	.9036
	Maternal vit D vs. sr. bilirubin	$-0.03473$	$-0.2460$ to $0.3101$	.8107
	Baby vit D vs. sr. bilirubin	$-0.3355$	$-0.4679$ to $0.06436$	.0172
Control	Maternal vit D vs. baby vit D	0.2163	$-0.06607$ to $0.4665$	.1314
	Maternal vit D vs. sr. bilirubin	$-0.09305$	$-0.3620$ to $0.1902$	.5204
	Baby vit D vs. sr. bilirubin	$-0.06546$	$-0.3377$ to $0.2168$	.6515

$r$  = Pearson correlation coefficient, CI = Confidence interval, Sig = Two-tailed significance.

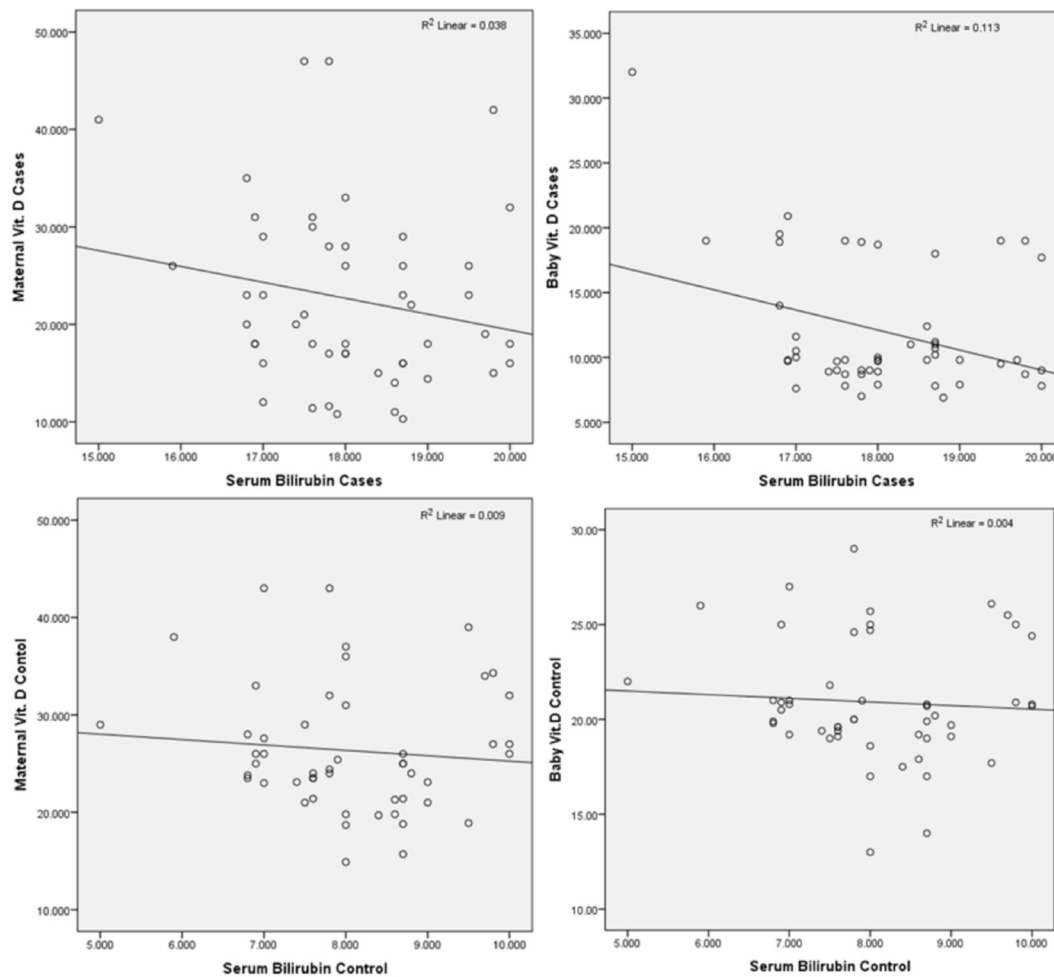


Fig. 1.

**Table 4**  
Student *t*-test difference among various groups.

	Paired differences					t	df	Sig.
	Mean	Std. deviation	Std. error of the mean	95% Confidence interval of the difference				
				Lower	Upper			
Maternal vit D case vs. maternal vit D control	-1.7420	6.93780	0.981153	-3.713	.229702	-1.775	49	.082
Baby vit D case vs. baby vit D control	-8.889	6.03780	0.859601	-10.617034	-7.162166	-10.34	49	0.00

newborn has strong correlation with neonatal hyperbilirubinemia. Thus, this can be included in the list of risk factors for neonatal hyperbilirubinemia, provided more research in the future supports our study.

#### 10. Limitations of our study

The major limitation of our study was the small sample size. This is due to the poor economic status of the people, and the study was conducted in private medical college where charges for the determination of the serum bilirubin + vit D<sub>3</sub> level are 1500 INR. Although we discussed with the laboratory department about this research project and persuaded them to decrease the cost to 500 INR, it was not acceptable to the local population to pay this amount for these two tests.

#### Ethical approval

Proper ethical approval was obtained from hospital ethical committee, and proper consent was taken from parents of newborn.

#### Funding

None.

#### Declaration of competing interest

The authors declare that they have no conflict of interest.

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