ISSN: 0975-3583,0976-2833 VOL15, ISSUE 04, 2024

ORIGINAL RESEARCH

To investigate the relationship between levels of 25-hydroxy vitamin D and neonatal hyperbilirubinemia in healthy term newborns

[1]Dr. Aanshul Rai Gupta, [2]Dr. Nikhil Arora, [3]Dr. Amandeep Kaur

[1]Junior Resident, [2]Senior Resident, Department of Pediatrics, Government Medical College, Patiala, Punjab, India
[3]Assistant Professor, Government Medical College, Patiala, Punjab, India

Corresponding author: Dr. Amandeep Kaur
Email: amandeepk441@gmail.com

Received date: 20 February, 2024 Acceptance date: 22 March, 2024

Abstract
Aim: To investigate the relationship between levels of 25-hydroxy vitamin D and neonatal hyperbilirubinemia in healthy-term newborns. Material and methods: The research included 200 babies, 100 classifieds as cases, and 100 as controls. Subjects were selected as cases or controls based on their serum bilirubin levels. The newborns in the cases group had bilirubin levels within the normal physiological range and did not need any treatment. In contrast, the newborns in the control group had serum bilirubin levels that fell within the range requiring intervention, such as phototherapy, exchange transfusion, or other treatment types, as the American Academy of Pediatrics recommended. The research characterized the state of vitamin D levels as follows: Insufficiency: <20 ng/ml. The range is suboptimal, with a 5-10 ng/ml value. The optimal amount of vitamin D is often between 30 to 50 ng/ml. Results: The mean serum bilirubin level in the cases was 18.47 mg/dl, with a standard deviation of 2.45. This was significantly higher than the mean serum bilirubin level in the control group, which was 8.45 mg/dl with a standard deviation of 0.36. The vitamin D levels in the mothers of the cases and controls were 22.67ng/ml and 26.82 ng/ml, respectively. The standard deviations for these levels were 3.89 and 2.95, respectively. The newborns in the cases had a vitamin D level of 12.56 ng/ml with a standard deviation of 2.11.
In contrast, the newborns in the control group had a vitamin D level of 21.35 ng/ml with a standard deviation of 2.17. Our research found that the average vitamin D levels in the mothers of newborns were 22.67 ng/ml for cases and 26.82 ng/ml for controls. The mean difference between the two groups was -6.59 ng/ml. However, the p-value of 0.07 indicates this difference is not statistically significant. Nevertheless, an essential statistical distinction was seen in the vitamin D levels between the cases and controls (P value 0.01). The patients had a mean vitamin D level of 12.56 ng/ml, while the controls had a mean vitamin D level of 21.35 ng/ml, resulting in a mean difference of -8.79. Conclusion: We found that newborns who experienced jaundice had a low vitamin D level that fell beyond the normal range. Furthermore, their blood bilirubin and vitamin D levels had a strong negative association.
Keywords: Bilirubin, Vitamin D, Jaundice

Introduction
Neonatal hyperbilirubinemia, often known as neonatal jaundice, is a prevalent condition in infants that occurs shortly after birth. Jaundice occurs in around 60%-80% of neonates during the first week following delivery [1,2]. The primary indication is the presence of yellowish discoloration in the sclera and skin, which occurs when the bilirubin levels are beyond the

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 04, 2024

normal range, particularly when the total bilirubin level reaches 5 mg/dl [3]. Bilirubin undergoes decomposition by red blood cells and is then released either directly or generated from hemoglobin obtained from red blood cell progenitors in the liver, bone marrow, and other organs. Hemoglobin undergoes metabolism by the enzyme heme oxygenase, resulting in the production of biliverdin. Biliverdin is subsequently transformed into bilirubin by the enzyme biliverdin reductase. The unconjugated bilirubin is liberated into the bloodstream and strongly associated with albumin to create a complex known as bilirubin albumin. Upon transportation to the liver, the complex binds with glucuronidase in hepatic cells, producing mono-bilirubin and glucuronic acid. These substances are then eliminated via the bile and intestinal system. UGT1A1 catalyzes the binding process. In neonates, most of the conjugated bilirubin in the intestines is converted back to unbound bilirubin. This conversion process is facilitated by the enzyme β-glucuronidase, which is found in the intestinal mucosa. Indirect bilirubin undergoes reabsorption into the circulation via the small intestine, so it participates in the enterohepatic cycle. Unbound bilirubin, being soluble in fat, can cross the blood-brain barrier. During the neonatal period, the blood-brain barrier is not fully developed, making it easier for bilirubin to build up in brain cells. This accumulation can result in bilirubin encephalopathy and insufficient central nervous system functioning. Such conditions can cause irreversible damage, particularly when the serum total bilirubin level exceeds 20 mg/dl or increases by more than 0.5 mg/dl. Hyperbilirubinemia is a significant condition that affects neonates. Doctors must be attentive and implement early therapies [4–6]. Currently, it is widely acknowledged that the approaches used to evaluate the risk of neonatal hyperbilirubinemia, both domestically and internationally, relies on factors such as the newborn's age, measurements of total serum bilirubin and transcutaneous bilirubin, assessment of risk factors (such as hypoxia, acidosis, head hematoma, sepsis, hypoglycemia), and evaluation of neonatal jaundice hour bilirubin nomograms (specifically the Bhutani curve) [7]. A multitude of intricate factors causes neonatal hyperbilirubinemia. Common causes of hyperbilirubinemia include infection, G6PD deficiency, breastfeeding-related jaundice, alloimmunization (such as ABO hemolysis or rhesus monkey incompatibility), and other severe hemolysis. Additionally, several unknown factors contribute to hyperbilirubinemia, which requires further investigation [8]. Vitamin D is soluble in nonpolar solvents, meaning it is fat-soluble. It is a vitamin that acts as a steroid and can enhance bone metabolism. Vitamin D has a role in the metabolism of calcium and phosphorus and supports the proper development of bone marrow cells in fetuses. Vitamin D has a role in many cells' growth, specialization, and programmed cell death. It also regulates the nervous, immunological, endocrine, and other systems.

Additionally, it may decrease the occurrence of cancers, infectious disorders, and allergic reactions [9,10]. Vitamin D is classified as a pro-hormone. Humans get it via dietary supplementation and the natural production of 7-dehydrocholesterol in the skin when exposed to sunshine. Vitamin D3, also known as cholecalciferol, is produced by the skin. Vitamin D enters the bloodstream and is carried to the liver by vitamin D-binding protein (DBP). Vitamin D in dietary supplements may exist as either cholecalciferol or ergocalciferol (also known as vitamin D2).

The lymphatic system takes up both substances as components of chylomicrons. These chylomicrons are broken down into smaller particles, transporting vitamin D to the liver. Liver cell microsomes catalyze the conversion of inactive vitamin D2 and vitamin D3 into 25-hydroxyvitamin D (25-OHD) via 25-hydroxylase [11]. 25-hydroxyvitamin D is the bloodstream's predominant and enduring form of vitamin D. The concentration of 25-hydroxyvitamin D in the bloodstream may serve as an indicator of the body's vitamin D status. 1,25-dihydroxyvitamin D is synthesized from 25-OHD by 1-α hydroxylase in the proximal tubule epithelial cells of the kidney, resulting in a considerable increase in its

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 04, 2024

activity [12]. The liver has a crucial function in turning indirect bilirubin into direct bilirubin, as well as being involved in the synthesis of vitamin D. While the metabolic pathways of the two may vary, they may nonetheless have an impact on each other during the biosynthesis stage in the liver. There is significant interest in the correlation between vitamin D levels and newborn hyperbilirubinemia. Several epidemiological research have examined the association between vitamin D levels and neonatal hyperbilirubinemia. Some findings have shown a negative correlation between blood vitamin D levels and the occurrence of hyperbilirubinemia in newborns [12]. Nevertheless, several research propose no substantial association between blood vitamin D levels and newborn hyperbilirubinemia. There is disagreement or debate around it.

Material and methods

This research was a prospective observational case-control study done in the pediatrics department. The research included 200 babies, with 100 classifieds as cases and 100 as controls. A majority of the caregivers of the infants declined to participate in the study due to their low socioeconomic position, and a smaller proportion of neonates came back for follow-up. The sample size in each group was restricted to just 100 due to these two primary considerations. Subjects were selected as cases or controls based on their serum bilirubin levels. The newborns in the cases group had bilirubin levels within the normal physiological range and did not need any treatment.

In contrast, the newborns in the control group had serum bilirubin levels that fell within the range requiring intervention, such as phototherapy, exchange transfusion, or other treatment types, as the American Academy of Pediatrics recommended. This research included infants who were exclusively breastfed, infants who were born in a hospital, healthy newborns who were born at or after 37 weeks of gestation, and infants born to women who were RH-negative or had O-blood type after confirming their DCT status. The newborn has significant congenital abnormalities, Rh/ABO incompatibility, a history of perinatal asphyxia, meconium aspiration syndrome, pneumonia, sepsis, and conjugated hyperbilirubinemia. Additionally, the newborn has life-threatening abnormalities such as tracheoesophageal fistula (TEF), congenital diaphragmatic hernia (CDH), pulmonary sequestration, or anorectal malformation. Before being released, all moms and caregivers received guidance on breastfeeding, and a certified breastfeeding counselor was accessible to provide counseling and instruction on nursing techniques. As per our hospital's policy, it is recommended that all newborns return for a follow-up appointment on the 5th day to evaluate their newborn examination. During this appointment, regular tests such as thyroid function tests, serum bilirubin, and blood group determination are conducted on every infant. During this period, parents received counseling on this study. On the 5th day after birth, the levels of 25-hydroxy vitamin D were measured in both the mother and the infant, as well as the levels of serum bilirubin, thyroid profile, and blood group. These tests were conducted concurrently. Infants with newborn hyperbilirubinemia, falling within the treatment range as determined by the AAP nomogram, were sent to the infant Intensive Care Unit (NICU) for further medical attention. All 100 newborns in this trial were treated with phototherapy. A neonate required an exchange transfusion; however, the caregivers declined to provide permission for participation in this study. The serum bilirubin concentration was determined using the micro bilirubin technique (Jendrassik and Grof method), whereas the level of 25-hydroxy vitamin D was measured using chemiluminescent immunoassay. The research characterized the state of vitamin D levels as follows. Insufficiency: <20 ng/ml. The range is suboptimal, with a 5-10 ng/ml value. The optimal amount of vitamin D is often between 30 to 50 ng/ml.

Statistical analysis

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 04, 2024

Data analysis was conducted using SPSS version 25. The mean and standard deviation of serum bilirubin and vitamin D levels were studied. Pearson's correlation was used to assess the association among groups. The mean values across groups were evaluated using a Student t-test. A significance level of less than 0.05 was used to determine statistical significance.

Results

This research involved 200 patients, 100 of whom were assigned to the case group and 100 to the control group. The groups were found to have similar characteristics, including the mothers' ages, gestational ages, methods of delivery, birth weights, and genders. The mean values, percentages, and comparative P-values of >.05 indicated that the differences were not statistically significant, as shown in Table 1. No systematic comparison of socioeconomic status was conducted between the cases and controls.

Table 2 displays the average and variability of blood bilirubin and vitamin D levels in both mothers and newborns from both groups. The mean serum bilirubin level in the cases was 18.47 mg/dl, with a standard deviation of 2.45. This was significantly higher than the mean serum bilirubin level in the control group, which was 8.45 mg/dl with a standard deviation of 0.36. The vitamin D levels in the mothers of the cases and controls were 22.67ng/ml and 26.82 ng/ml, respectively. The standard deviations for these levels were 3.89 and 2.95, respectively. The newborns in the cases had a vitamin D level of 12.56 ng/ml with a standard deviation of 2.11, whereas the newborns in the control group had a vitamin D level of 21.35 ng/ml with a standard deviation of 2.17.Upon analyzing the association between groups, it was found that only the vitamin D level of patients exhibited a significant link with their blood bilirubin. The correlation coefficient (r) was -0.34, and the P value was .02 (<0.05), indicating statistical significance. None of the other relationships between groups were statistically significant, as shown by their correlation coefficients in Table 3. There is a negative association between blood bilirubin levels and vitamin D levels in newborns and their mothers, regardless of whether they are cases or controls.Table 2 displays the results of our research, which found that the average vitamin D levels in the mothers of newborns were 22.67 ng/ml for cases and 26.82 ng/ml for controls. The mean difference between the two groups was -6.59 ng/ml. However, the p-value of 0.07 indicates this difference is not statistically significant. This information is also shown in Table 4. Nevertheless, an essential statistical distinction was seen in the vitamin D levels between the cases and controls (P value 0.01). The patients had a mean vitamin D level of 12.56 ng/ml, while the controls had a mean vitamin D level of 21.35 ng/ml, resulting in a mean difference of -8.79.

Table 1: Demographic profile

	Study group (n = 100)		**Control group (n = 100)**		**P value**
	Number / Mean	**Percentage**	**Number / Mean**	**Percentage**	
Mothers' age, in years	25.65±2.54		25.72±2.36		0.12
Gestational age, in weeks	38.47±2.78		38.06±2.23		0.07
Delivery type					
Vaginal delivery	60	60	63	63	0.21
Cesarean section	40	40	37	37	0.34
Gender					
Male	58	58	56	56	
Female	42	42	44	44	0.12
Birth weight, in kg	2.77±0.35		2.89±0.12		0.07
Postnatal age, days	4.87±0.45		4.96±0.59		0.36

Table 2: Maternal and baby Vit D and serum bilirubin.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 04, 2024

	Mean	Sd
Case number = 100		
MaternalvitDng/ml	22.67	3.89
BabyvitDng/ml	12.56	2.11
Sr.bilirubinng/dl	18.47	2.45
Control number = 100		
MaternalvitDng/dl	26.82	2.95
BabyvitDng/ml	21.35	2.17

Table 3: Pearson correlation analysis among various groups

Correlation	r	CIforr	Pvalue
Case			
Maternal vit D vs. baby vit D	0.01	—0.27to0.28	0.13
Maternal vit D vs. sr.bilirubin	—0.04	—0.25to0.33	0.21
Baby vit D vs. sr.bilirubin	—0.34	—0.48to0.07	0.02
Control			
Maternal vit D vs.baby vit D	0.24	—0.07to0.48	0.24
Maternal vit D vs. sr.bilirubin	—0.08	—0.38to0.18	0.23
Baby vit D vs. sr.bilirubin	—0.07	—0.35to0.24	0.16

Table 4: Student t-test difference among various groups

	Mean	Sd	Std. error of the mean	95% Confidence interval ofthe difference		t	df	P
Maternal vit D case vs. maternal vit D control	—1.99	0.33	0.56	—4.11	0.24	—1.45	47	.07
Baby vit D case vs. baby vit D control	—8.58	0.15	0.86	—9.96	—6.47	—9.99	47	0.01

Discussion

The average 25-hydroxyvitamin D level in the mothers of both cases and controls in this research was within the unsatisfactory range, between 20 and 30 ng/ml. A study conducted by Garg R. et al. [13] showed that 98.75% of women in the Indian population had vitamin D levels below 30 ng/ml. The vitamin D levels below the lower end of the normal range in both tests may be attributed to the specific clothing code prevalent in our nation. The average vitamin D level of newborns was within the normal range in the control group but showed a considerable reduction in the cases group. The difference between the two groups was statistically significant. In their research, Mutlu M et al. [14] showed that 83% of newborns who experienced jaundice and had blood bilirubin levels within the normal range had vitamin D levels ranging from 5-14.9 ng/ml. Aletayeb SMH et al. [15] demonstrated that the average vitamin D level of the subjects (referring to jaundiced babies, which aligns with our case definition) was 84.38 nmmo/l (18.75 ng/ml), which falls below the normal range as shown in our research.Our investigation indicates a statistically negligible negative connection between vitamin D levels and serum bilirubin, except in circumstances where the correlation was statistically significant. Mutlu M et al. [14] and Aletayeb SMH et al. [15] demonstrated a significant association between the vitamin D levels of newborns who had

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 04, 2024

hyperbilirubinemia outside the normal range and their blood bilirubin levels.Multicentre research conducted in six developing countries revealed that hyperbilirubinemia was the primary reason for hospital admission in 78% of cases during the first six days after birth. Globally, around 10.5% of neonates born alive need phototherapy treatment for jaundice. Glucose-6-phosphate Dehydrogenase (G6PD) deficiency is a prevalent cause of neonatal jaundice worldwide. It is important to Highlighting that the elevated prevalence of G6PD deficiency in this area contributes to the increased occurrence of hyperbilirubinemia in newborns. The occurrence of vitamin D insufficiency has been documented in pregnant women across several nations, ranging from 18% in the UK to 84% in the Netherlands, with a rate of 80% in Iran[17]. Upon evaluating the vitamin D deficiency and insufficiency levels, we discovered that 16.5% and 78.5% of the moms had low vitamin D levels, respectively. An important constraint of our investigation was the limited sample size. This is attributed to the low socioeconomic condition of the individuals.

Conclusion

We found that newborns who experienced jaundice had a low vitamin D level that fell beyond the normal range. Furthermore, there was a strong negative association between their blood bilirubin levels and vitamin D levels. The primary limitation of our research is in the limited size of our study sample, which is confined to a certain location within our nation. In order to establish low vitamin D levels as a risk factor for hyperbilirubinemia, it is necessary to conduct comprehensive research studies in many places around the globe. This will enable us to reduce the future occurrence of hyperbilirubinemia by addressing this risk factor with vitamin D treatment.

References

1. Rathore S., Kumar V.C. and R S., A critical review on neonatal hyperbilirubinemia-an Ayurvedic perspective. J Ayurveda Integr Med, 2020. 11(2): p. 190–196. https://doi.org/10.1016/j.jaim.2018.08.006 PMID: 31628007
2. Ullah S., Rahman K. and Hedayati M., Hyperbilirubinemia in Neonates: Types, Causes, Clinical Examinations, Preventive Measures and Treatments: A Narrative Review Article. Iran J Public Health, 2016. 45(5): p. 558–68. PMID: 27398328
3. Schwartz H.P., Haberman B.E. and Ruddy R.M., Hyperbilirubinemia: current guidelines and emerging therapies. Pediatr Emerg Care, 2011. 27(9): p. 884–9. https://doi.org/10.1097/PEC. 0b013e31822c9b4c PMID: 21926893
4. Karimzadeh P., et al., Bilirubin Induced Encephalopathy. Iran J Child Neurol, 2020. 14(1): p. 7–19. PMID: 32021624
5. Pace E.J., Brown C.M. and DeGeorge K.C., Neonatal hyperbilirubinemia: An evidence-based approach. J Fam Pract, 2019. 68(1): p. E4–E11. PMID: 30724909
6. Maisels M.J., Neonatal jaundice. Pediatr Rev, 2006. 27(12): p. 443–54. https://doi.org/10.1542/pir.27- 12-443 PMID: 17142466
7. Cheng Peng and Xinlin Hou, "2018 Queensland Obstetrics and Neonatal Clinical Guidelines: Neonatal Jaundice", an introduction to the main points. Chinese Journal of Perinatal Medicine, 2020(04): 285- 286–287-288.
8. Mitra S. and Rennie J., Neonatal jaundice: aetiology, diagnosis and treatment. Br J Hosp Med (Lond), 2017. 78(12): p. 699–704. https://doi.org/10.12968/hmed.2017.78.12.699 PMID: 29240507
9. Mingli Yu and Tao Zhang, The effect of vitamin D deficiency during pregnancy on the healthy development of newborns. International Journal of Pediatrics, 2019(09): 683–686.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 04, 2024

10. Dokos C., et al., Inside the "fragile" infant: pathophysiology, molecular background, risk factors and investigation of neonatal osteopenia. Clin Cases Miner Bone Metab, 2013. 10(2): p. 86–90. PMID: 24133523
11. Brannon P.M., et al., Overview of the conference "Vitamin D and Health in the 21st Century: an Update". Am J Clin Nutr, 2008. 88(2): p. 483S–490S.
12. Yang Fan, Vitamin D: Review and Guide Interpretation. Chinese Journal of Maternal and Child Clinical Medicine (Electronic Edition), 2010. 6(04): Page 229–230.
13. Garg R, et al. Prevalence of vitamin D deficiency in Indian women. Int J Reprod Contracept ObstetGynecol 2018;7(6):2222e5.
14. Mutlu M, Çayir A, Çayir Y, O€ zkan B, Aslan Y. Vitamin D and hyperbilirubinaemia in neonates. HK J Paediatr 2013;18(2):77e81 (new series).
15. Aletayeb SM, Dehdashtiyan M, Aminzadeh M, Malekyan A, Jafrasteh S. Com- parison between maternal and neonatal serum vitamin D levels in term jaundiced and nonjaundiced cases. J Chin Med Assoc. 2016;79(11): 614e7.
16. Young Infants Clinical Signs Study Group. Clinical signs that predict severe illness in children under age 2 months: a multicentre study. Lancet. 2008; 12:371(9607):135-42.
17. Bhutani VK. Editorial: building evidence to manage newborn jaundice worldwide. Indian J Pediatr. 2012;79(2):253-