

Study of Correlation of Cord Blood Bilirubin with Neonatal Hyperbilirubinemia

Shruthi Reddy¹, Rahatull Zeba², Amit Sekerey³, Piush Raj⁴, Monika Singh⁵ and Om Prakash Singh⁶

¹JR III Pediatrics, Narayan Medical College and hospital Jamuhar, Sasaram

²JR III Pediatrics, Narayan Medical College and hospital Jamuhar, Sasaram

³Pediatrics Assistant Professor, Narayan Medical College and hospital Jamuhar, Sasaram

⁴SRPediatrics, Narayan Medical College and hospital Jamuhar, Sasaram

⁵SRPediatricsNarayan Medical College and hospital Jamuhar, Sasaram

⁶Professor Pediatrics, Narayan Medical College and hospital Jamuhar, Sasaram

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ABSTRACT

Background: Neonatal hyperbilirubinemia is a frequently encountered clinical condition during the early neonatal period. Early identification and prompt management are essential to prevent serious complications such as kernicterus. This study aimed to determine the correlation between cord blood bilirubin (CBB) levels and the subsequent development of neonatal hyperbilirubinemia. **Methods:** This was a prospective observational study conducted over 18 months w.e.f. Jun 2023 – Nov 2024 at the department of Pediatrics and Neonatology, Narayan medical college & hospital, Jamuhar, Bihar. A total of 131 neonates were enrolled based on predefined inclusion and exclusion criteria. Cord blood was collected at birth for total, direct, and indirect bilirubin estimation. At 48 hours of life, clinical assessment for jaundice was performed and venous blood samples were taken to measure serum bilirubin. Maternal and neonatal variables were analyzed to explore their associations with hyperbilirubinemia. **Results:** Among 67 neonates with CBB ≥ 2 mg/dL, 52 (77.61%) developed jaundice, while only 4 (6.25%) out of 64 neonates with CBB < 2 mg/dL developed jaundice. Diagnostic evaluation of CBB ≥ 2 mg/dL yielded a sensitivity of 92.86%, specificity of 80.00%, positive predictive value of 77.61%, negative predictive value of 93.75%, and overall accuracy of 85.50%. Significant associations were found between neonatal jaundice and maternal age ($p=0.0134$), gestational age ($p=0.005$), and mode of delivery ($p<0.001$). Vaginal delivery and gestational age of 37–39 weeks were associated with higher risk. Other variables such as parity, maternal comorbidities, Rh incompatibility, and neonatal sex showed no significant association. **Conclusion:** Cord blood bilirubin estimation at birth is a simple, non-invasive, and reliable screening tool for early prediction of neonatal hyperbilirubinemia. A CBB threshold of ≥ 2 mg/dL demonstrates high sensitivity and diagnostic accuracy, making it valuable in identifying neonates at risk, especially those born vaginally and between 37–39 weeks of gestation.

Keywords: Cord blood bilirubin (CBB), Neonatal jaundice, Hyperbilirubinemia, , Gestational age, Mode of delivery



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INTRODUCTION

Jaundice is a common clinical condition in newborns, affecting approximately 60% of term infants and 85% of preterm infants in the first week of life¹ Neonatal hyperbilirubinemia (NHB) results from increased bilirubin synthesis due to the breakdown of fetal hemoglobin and underdeveloped hepatic conjugation and excretion processes in the neonatal liver² Although neonatal jaundice is generally a benign condition, in some cases it can develop into severe hyperbilirubinemia, increasing the risk of bilirubin neurotoxicity and kernicterus, which may lead to irreparable brain damage³.

Risk factors associated with hyperbilirubinemia include maternal age, ethnicity, maternal diabetes, prematurity, altitude, polycythemia, male gender, trisomy 21, cephalhematoma, Rh isoimmunization, ABO incompatibility, oxytocin-induced labor, breastfeeding, dehydration, delayed bowel movements, and a family history of neonatal jaundice⁴⁻⁶. In term infants, neonatal hyperbilirubinemia (NHB) usually results from an increased bilirubin load due to relative polycythemia, reduced erythrocyte lifespan, immature hepatic absorption and conjugation, and increased enterohepatic circulation⁷.

Timely diagnosis of infants predisposed to significant hyperbilirubinemia is critical, as neonatal hyperbilirubinemia is the most common cause of readmission in the first week of life⁸⁻¹¹. Severe hyperbilirubinemia may lead to kernicterus, a preventable but catastrophic condition characterized by motor impairment, auditory neuropathy, and oculomotor abnormalities. All newborns discharged within 48 hours should receive a follow-up examination within 2 to 3 days to detect significant jaundice immediately^{12,13}. However, this suggestion is difficult to implement in some healthcare facilities, particularly in underdeveloped countries, due to logistic constraints.

Numerous studies have investigated prognostic indicators for gestational age (GA) to enable early intervention. Cord blood bilirubin (CBB) measurement has been proposed as a straightforward, inexpensive and non-invasive method for screening newborns at risk of hyperbilirubinemia. Research suggests that elevated bilirubin levels in the umbilical cord correlate with an increased likelihood of requiring phototherapy, suggesting that processes associated with hyperbilirubinemia may occur throughout late fetal development¹⁴. Kardum et al (2021)¹⁵ found that cord blood bilirubin (CBB) assessment is a reliable indicator for early detection of severe anemia, allowing immediate treatment and reducing the risk of severe jaundice¹⁵.

ABO incompatibility is an important factor in hemolytic disease of the newborn (HDN), which occurs in approximately 15–20% of pregnancies and significantly increases the risk of hyperbilirubinemia requiring intervention^{16,17}. Research indicates that infants with ABO incompatibility have an increased incidence of severe jaundice in the first 48 hours of life, which must be detected and treated immediately¹⁸⁻²⁰.

The prevalence of severe anemia is influenced by geographical and ethnic differences, uncertainty in laboratory bilirubin measurements and breastfeeding rates²¹⁻²⁴. Several predictive techniques for neonatal hyperbilirubinemia have been investigated, including transcutaneous bilirubin determination, end-tidal carbon monoxide analysis, pre-discharge bilirubin estimation to the hour, and umbilical cord bilirubin concentration²⁵⁻²⁷. Cord blood bilirubin (CBB) estimation is an accessible, non-invasive screening tool that may be especially beneficial in resource-limited settings for the prediction of neonatal hyperbilirubinemia (NHB) and early discharge decision-making¹⁵.

Given the prevalence of neonatal jaundice and the need to immediately identify infants at risk, our study aims to assess the efficacy of cord bilirubin levels as a prognostic indicator of neonatal hyperbilirubinemia. By correlating cord blood bilirubin (CBB) with the future occurrence of severe hyperbilirubinemia, we aim to improve the early diagnosis and care of at-risk babies, thereby reducing the prevalence of severe neonatal

hyperbilirubinemia (NHB) and its associated consequences

Neonatal hyperbilirubinemia is one of the most common clinical conditions requiring evaluation and treatment in the newborn period. Early identification of neonates at risk can significantly reduce morbidity, hospital stays, and the risk of complications such as kernicterus. Traditional monitoring methods often detect hyperbilirubinemia after clinical signs have emerged, which may delay timely intervention. Establishing bilirubin levels in cord blood offers a non-invasive, easily obtainable, and early biomarker for predicting neonatal jaundice. Studying its correlation with subsequent hyperbilirubinemia can help establish it as a valuable screening tool, especially in resource-limited settings where early follow-up may be challenging.

Despite advancements in neonatal care, delayed recognition of hyperbilirubinemia continues to be a significant cause of neonatal morbidity and, in severe cases, neurodevelopmental impairment. Clinicians currently rely on postnatal bilirubin measurements or visual assessments, which may not reliably identify at-risk neonates before clinical jaundice becomes apparent. There is a critical need to explore reliable predictors available immediately after birth. This study addresses the gap by investigating whether bilirubin levels in cord blood, assessed at the time of delivery, can be used to accurately predict the development of significant neonatal hyperbilirubinemia, thus enabling timely intervention and improved neonatal outcome.

Aims and objectives

- To study the correlation of cord blood bilirubin with neonatal hyperbilirubinemia.
- To determine whether cord bilirubin level could predict risk of development of significant hyperbilirubinemia among neonates.

This study was a hospital-based prospective observational study conducted to evaluate the correlation of cord blood bilirubin with neonatal hyperbilirubinemia.

The study was carried out in the Department of Pediatrics and Neonatology at Narayan Medical College & Hospital (NMCH), Jamuhar, Bihar, a tertiary care rural teaching hospital

The study was conducted over a period of 18 months, from 1st June 2023 to 30th November 2024.

All live-born neonates delivered at NMCH, fulfilling the inclusion and exclusion criteria, were enrolled in this study irrespective of their mode of delivery.

Ethical Consideration

Approval for the study was obtained from the Institutional Ethical Committee (IEC) of Narayan Medical College & Hospital prior to the commencement of the study. Written informed consent was taken from

the parents or legal guardians of all neonates included in the study.

Inclusion Criteria

All neonates delivered at NMCH, irrespective of the mode of delivery, were included in the study.

Exclusion Criteria

- Neonates were excluded from the study if they had any of the following conditions:
- Surgical problems or congenital malformations.
- Chromosomal anomalies or dysmorphic features.
- Perinatal asphyxia.
- History of maternal intake of anti-convulsant drugs.
- Major apparent congenital anomalies.
- Neonates born to mothers with sepsis.
- Neonates with Apgar score less than 7 at 5 minutes.

METHOD OF DATA COLLECTION

After taking detailed antenatal, obstetric, and medical history of the mother, relevant clinical details were recorded in a pre-designed proforma.

Each neonate was clinically examined immediately after birth. The gestational age of each newborn was assessed using the New Ballard Scoring System.

Approximately 2 ml of cord blood was collected immediately after delivery under aseptic conditions from the placental end of the umbilical cord in a red-top vacutainer tube. This procedure was done irrespective of the mode of delivery (normal vaginal delivery or cesarean section).

The collected cord blood samples were sent immediately to the clinical laboratory for estimation of total, direct, and indirect bilirubin levels using standard biochemical methods.

Further, venous blood samples of the same neonates were collected at 48 hours of life to measure total, direct, and indirect serum bilirubin levels. These samples were also sent immediately to the laboratory for analysis.

Statistical Analysis:

The statistical analysis was performed with SPSS version 21.0. The data were presented in the form of mean (standard deviation) and percentage (%). The chi-square test was used to compare categorical variables, while the independent t-test was used to assess discrete variables between groups. An analysis of variance (ANOVA) was used to compare more than two groups. A ROC (Receiver Operating Characteristic) curve was used to evaluate the performance of a binary classifier by plotting the true positive rate (sensitivity) against the false positive rate (1-specificity) at various threshold settings. A p-value of 0.05 was considered statistically significant.

RESULTS:

Table 1: Distribution of neonates according to their jaundice status

N=131		n	%
Jaundice Status	Present	52	39.69
	Absent	79	60.31

Table 1 and Figure 1 shows the distribution of neonates according to their jaundice status. Out of the total 131 neonates included in the study, 52 (39.69%) developed neonatal jaundice, while 79 (60.31%) did not exhibit significant hyperbilirubinemia. This indicates that approximately 40% of the neonates experienced jaundice during the early neonatal period, emphasizing the importance of early detection and monitoring to prevent complications.

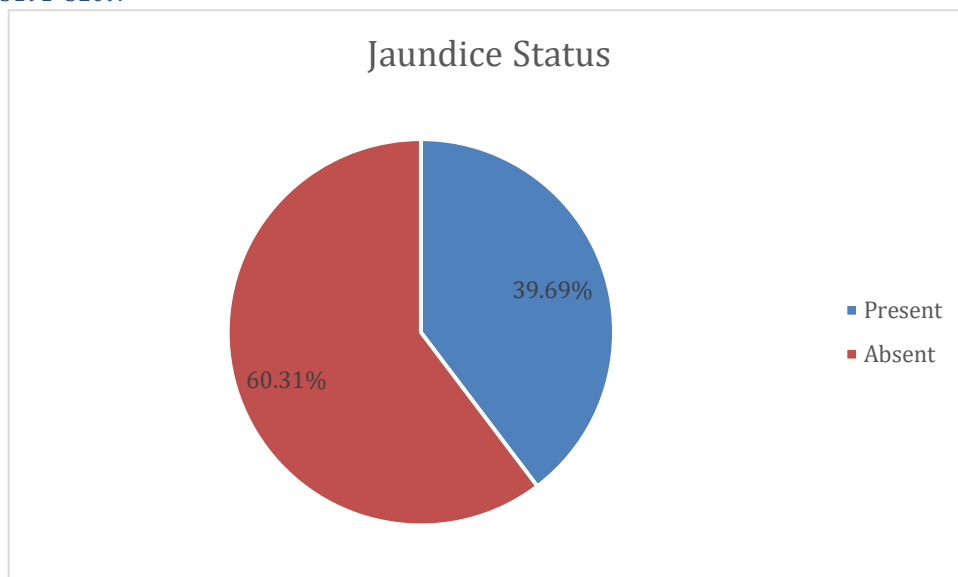


Figure 1: Pie chart show the distribution of neonates according to their jaundice status

Table 2: Association of maternal age between with and without jaundice

		With Jaundice (n=52)		Without Jaundice (n=79)		p-Value
		n	%	n	%	
Maternal age (years)	18-24 years	8	15.38	24	30.38	0.0134
	25-29 years	39	75.00	37	46.84	
	30-34 years	5	9.62	16	20.25	
	≥34 years	0	0.00	2	2.53	

Table 2 and Figure 2 shows the distribution of neonates according to maternal age and its association with neonatal jaundice. Among the total 52 neonates who developed jaundice, the majority (75.00%) were born to mothers aged 25-29 years, while only 15.38% were born to mothers aged 18-24 years, and 9.62% to mothers aged 30-34 years. Notably, no jaundiced neonates were born to mothers aged 34 years and above. In contrast, among the 79 neonates who did not develop jaundice, a higher proportion (30.38%) were born to mothers aged 18-24 years, while 46.84% were from the 25-29 years group, 20.25% from the 30-34 years group, and 2.53% from mothers aged 34 years and above. The p-value (0.0134) indicates a statistically significant association between maternal age and the occurrence of neonatal jaundice.

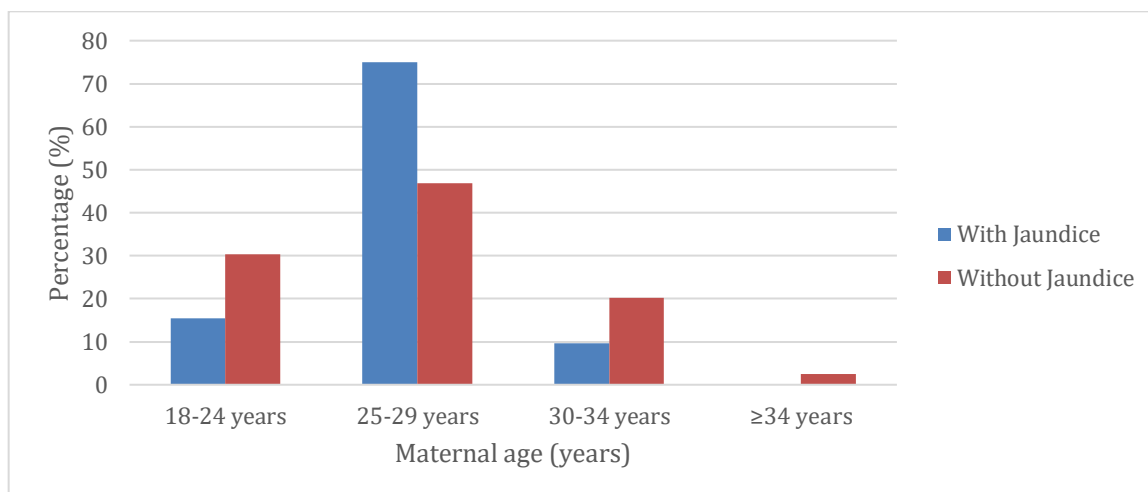


Figure 2: Bar chart show the distribution of patients according to Maternal age (years) in neonates with and without jaundice

Table 3: Association of maternal gravida/parity with neonatal jaundice

		With Jaundice (n=52)		Without Jaundice (n=79)		p-Value
		n	%	n	%	
Gavida/parity	Primigravida	25	48.08	45	56.96	0.130

	Primipara	15	28.85	26	32.91
	Multipara	12	23.08	8	10.13

Table 3 and Figure 3 show the association of maternal gravida/parity with neonatal jaundice.

Among the 52 neonates who developed jaundice, 48.08% were born to primigravida mothers, 28.85% to primipara mothers, and 23.08% to multipara mothers. In contrast, among the 79 neonates who did not develop jaundice, 56.96% were born to primigravida mothers, 32.91% to primipara mothers, and 10.13% to multipara mothers. The proportion of jaundiced neonates was higher among multiparous mothers compared to non-jaundiced neonates (23.08% vs. 10.13%). However, the p-value (0.130) indicates that the association between maternal gravida/parity and neonatal jaundice is not statistically significant

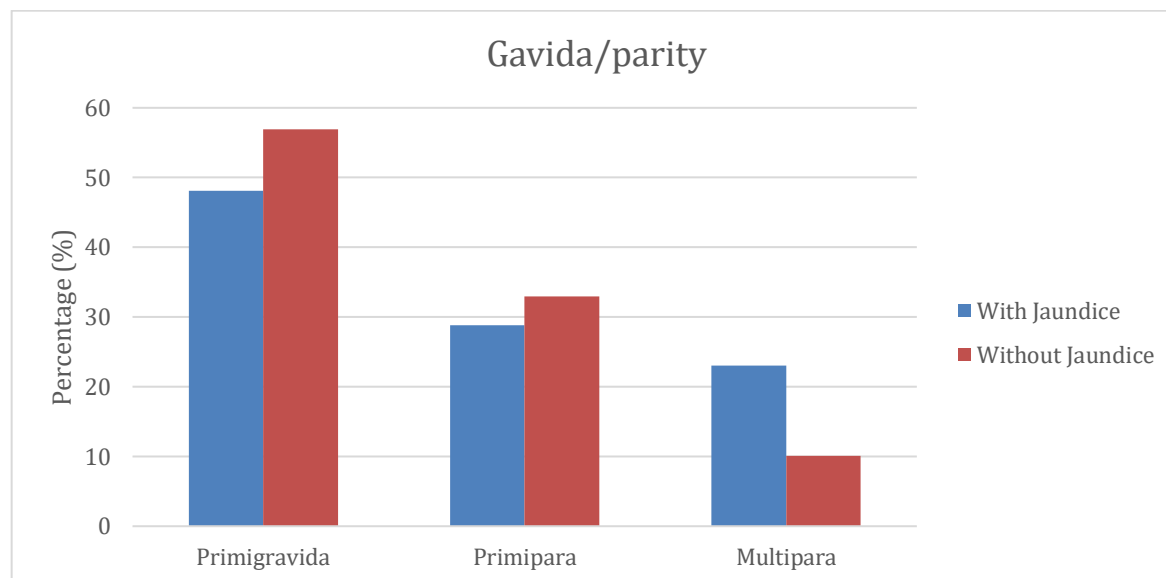


Figure3: Bar chart show the distribution of patients according to maternal gravida/parity in neonates with and without jaundice

Table 4: Association of maternal and neonatal comorbidities between neonates with and without jaundice

Comorbidities	With Jaundice (n=52)		Without Jaundice (n=79)		p-Value
	n	%	n	%	
ABO incompatibility	2	3.85	0	0.00	0.156
Celiac disease	2	3.85	0	0.00	0.156
PROM	4	7.69	2	2.53	0.214
Preeclampsia	2	3.85	0	0.00	0.156
Severe CHNC Hypothyroidism	2	3.85	3	3.80	1.00
Controlled Hypothyroidism	0	0.00	3	3.80	0.276
Well Controlled GDM	0	0.00	0	0.00	-
Leaking PV History	0	0.00	2	2.53	0.518

Table 4 and Figure 4 show the association of maternal and neonatal comorbidities with the occurrence of neonatal jaundice.

Among the 52 neonates who developed jaundice, 3.85% had ABO incompatibility, celiac disease, or preeclampsia in the mother, while 7.69% were associated with premature rupture of membranes (PPROM). Additionally, 3.85% of jaundiced neonates had mothers with severe congenital hypothyroidism (CHNC), similar to 3.80% in the non-jaundiced group. Controlled hypothyroidism was observed in 3.80% of mothers in the non-jaundiced group, but none in the jaundiced group.

No cases of gestational diabetes mellitus (GDM) were reported in either group, and a history of leaking per vaginum (PV) was noted in 2.53% of non-jaundiced neonates, with none in the jaundiced group. The p-values for all comorbidities were greater than 0.05, indicating that none of these conditions showed a statistically significant association with the development of neonatal jaundice.

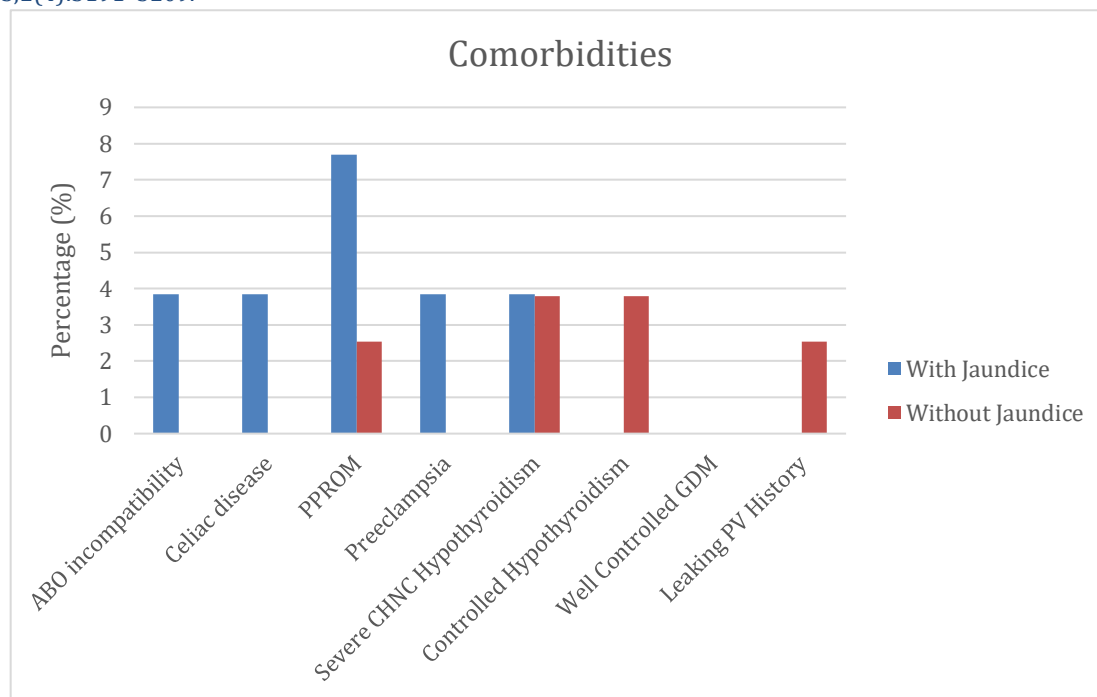


Figure 4: Bar chart show the distribution of patients according to comorbidities in neonates with and without jaundice

Table 5: Association between previous pregnancy complications and the occurrence of neonatal jaundice

		With Jaundice (n=52)		Without Jaundice (n=79)		p-Value
		n	%	n	%	
Previous Pregnancy Complications	Spontaneous Abortion	5	9.62	6	7.59	0.753
	NCIAB	2	3.85	4	5.06	1.000
	D&C	5	9.62	2	2.53	0.113
	UV prolapse	3	5.77	0	0.00	0.065

Table 5 and Figure 5. Association between previous pregnancy complications and the occurrence of neonatal jaundice. Among the 52 neonates who developed jaundice, 9.62% of mothers had a history of spontaneous abortion, compared to 7.59% in the non-jaundiced group, with a p-value of 0.753, indicating no significant association. Similarly, 3.85% of jaundiced neonates' mothers had a history of non-classical incomplete abortion bleeding (NCIAB), compared to 5.06% in the non-jaundiced group, with a p-value of 1.000, suggesting no statistical significance.

A history of dilatation and curettage (D&C) was present in 9.62% of mothers in the jaundiced group, compared to 2.53% in the non-jaundiced group, with a p-value of 0.113, indicating a trend but no statistically significant association. Notably, 5.77% of mothers in the jaundiced group had a history of umbilical vein (UV) prolapse, whereas no cases were reported in the non-jaundiced group (p-value = 0.065), suggesting a possible but non-significant association.

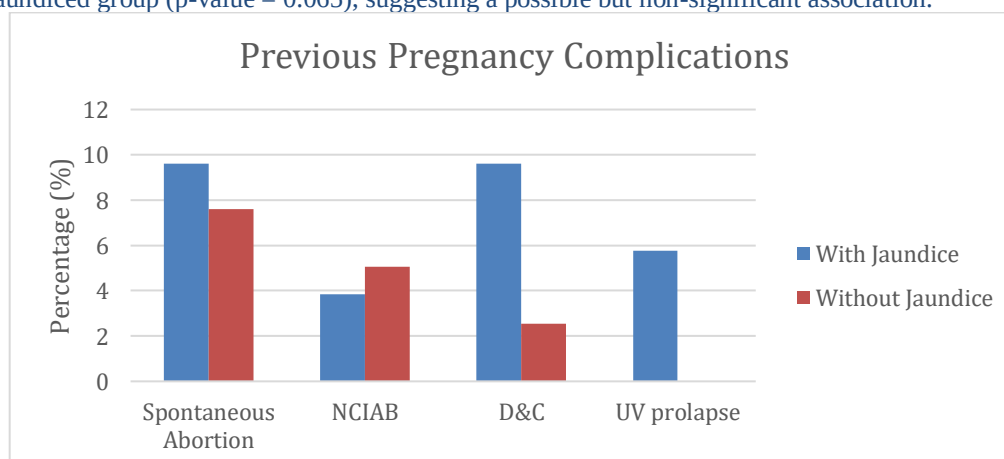


Figure 5: Bar chart show the distribution of patients according to Previous Pregnancy Complications in neonates with and without jaundice

Table 6: Association between maternal blood group and Rh status with neonatal jaundice

		With Jaundice (n=52)		Without Jaundice (n=79)		p-Value
		n	%	n	%	
Maternal Blood Group and Rh Status	A+ve	8	15.38	11	13.92	0.654
	A-ve	2	3.85	2	2.53	
	B +ve	17	32.69	24	30.38	
	B-ve	0	0.00	0	0.00	
	AB+ve	2	3.85	9	11.39	
	AB-ve	0	0.00	0	0.00	
	O+ve	23	44.23	33	41.77	
	O-ve	0	0.00	0	0.00	

Table 6 and Figure 6 show the association between maternal blood group and Rh status with neonatal jaundice.

Among the 52 neonates who developed jaundice, the most common maternal blood group was O+ve (44.23%), followed by B+ve (32.69%), A+ve (15.38%), and AB+ve (3.85%). Similarly, among the 79 neonates who did not develop jaundice, the predominant maternal blood group was O+ve (41.77%), followed by B+ve (30.38%), A+ve (13.92%), and AB+ve (11.39%).

No cases of neonatal jaundice were observed in neonates born to mothers with B-ve, AB-ve, or O-ve blood groups in either group. Additionally, Rh-negative mothers (A-ve and B-ve) accounted for a very small proportion in both jaundiced (3.85%) and non-jaundiced (2.53%) groups.

The p-value (0.654) indicates no statistically significant association between maternal blood group or Rh status and the occurrence of neonatal jaundice. While O+ve and B+ve mothers were more common in both groups,

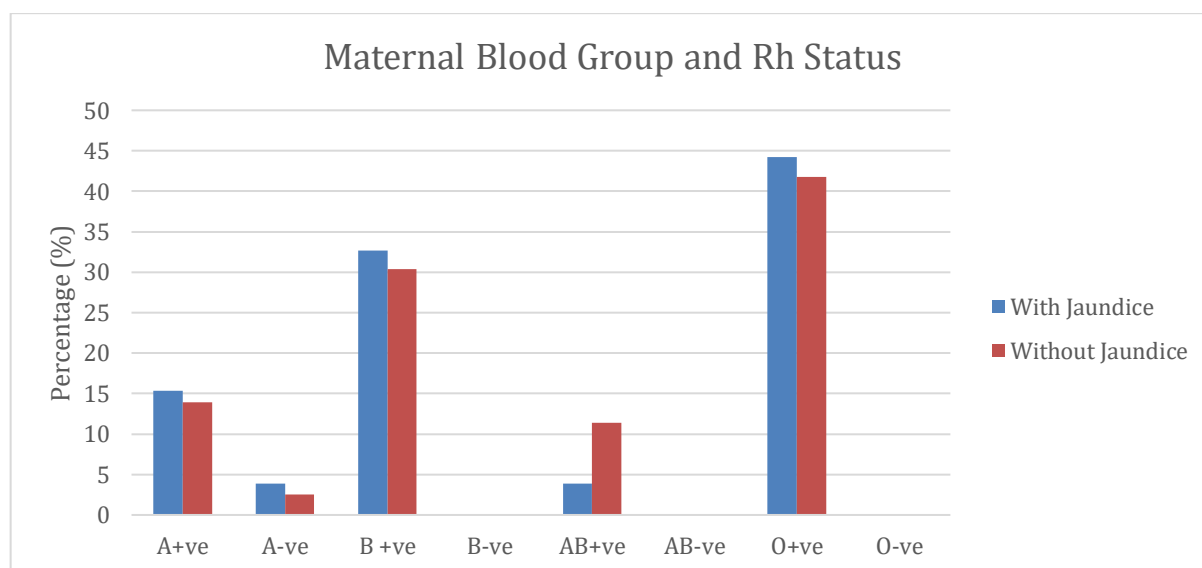


Figure 6: Bar chart show the distribution of patients according to maternal blood group and Rh status in neonates with and without jaundice

Table 7: Association between the period of gestation and the occurrence of neonatal jaundice

		With Jaundice (n=52)		Without Jaundice (n=79)		p-Value
		n	%	n	%	
Period of gestation	<37 weeks	8	15.38	18	22.78	0.005
	37-39 weeks	37	71.15	32	40.51	
	39-41 weeks	7	13.46	28	35.44	
	>41 weeks	0	0.00	1	1.27	

Table 7 and Figure 7 show the association between the period of gestation and the occurrence of neonatal jaundice.

Among the 52 neonates who developed jaundice, the majority (71.15%) were born between 37-39 weeks of gestation, followed by 13.46% born between 39-41 weeks, and 15.38% were preterm (<37 weeks). No cases of neonatal jaundice were observed in neonates born post-term (>41 weeks).

In contrast, among the 79 neonates who did not develop jaundice, 40.51% were born between 37-39 weeks, while a higher proportion (35.44%) were born between 39-41 weeks, and 22.78% were preterm (<37 weeks). Only 1.27% of neonates in this group were born after 41 weeks of gestation.

The p-value (0.005) indicates a statistically significant association between gestational age and neonatal jaundice. This suggests that neonates born between 37-39 weeks are at a higher risk of developing hyperbilirubinemia compared to those born at later gestational ages. Conversely, a greater proportion of non-jaundiced neonates were observed in the 39-41 weeks gestation group, indicating a lower risk of jaundice in this period.

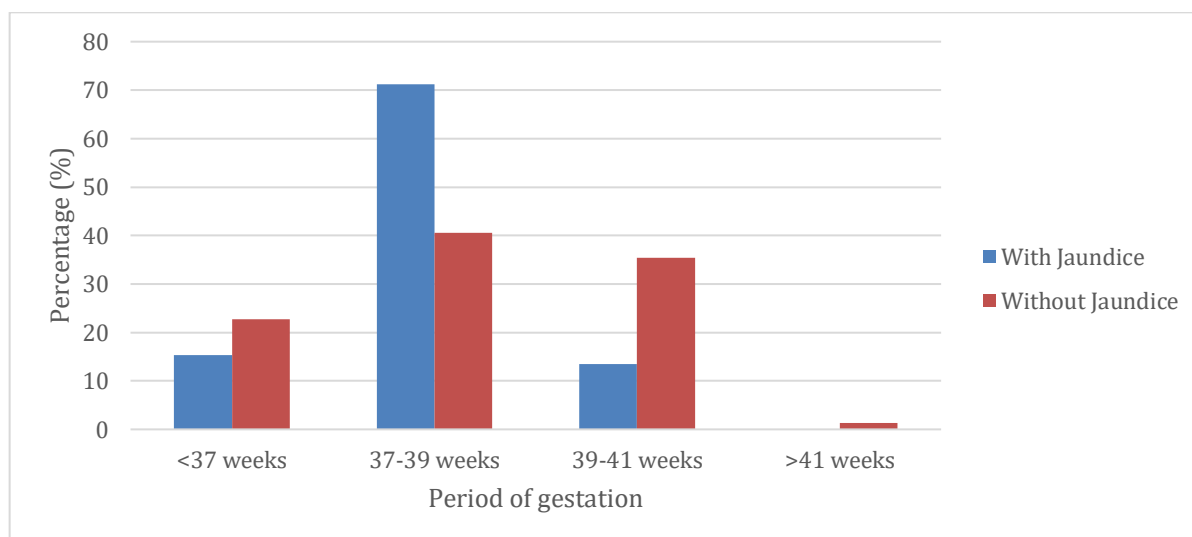


Figure 7: Bar chart show the distribution of patients according to period of gestation in neonates with and without jaundice

Table 8: Association between the mode of delivery and the occurrence of neonatal jaundice

		With Jaundice (n=52)		Without Jaundice (n=79)		p-Value
		n	%	n	%	
Mode of delivery	LSCS	24	46.15	62	78.48	<0.001
	VD	28	53.85	17	21.52	

Table 8 and Figure 8 show the association between the mode of delivery and the occurrence of neonatal jaundice.

Among the 52 neonates who developed jaundice, 46.15% were delivered via Lower Segment Caesarean Section (LSCS), while 53.85% were delivered vaginally (VD). In contrast, among the 79 neonates who did not develop jaundice, a significantly higher proportion (78.48%) were delivered via LSCS, whereas only 21.52% were delivered vaginally.

The p-value (<0.001) indicates a highly significant association between the mode of delivery and neonatal jaundice. These results suggest that neonates born via vaginal delivery (VD) are at a higher risk of developing neonatal jaundice compared to those delivered by LSCS.

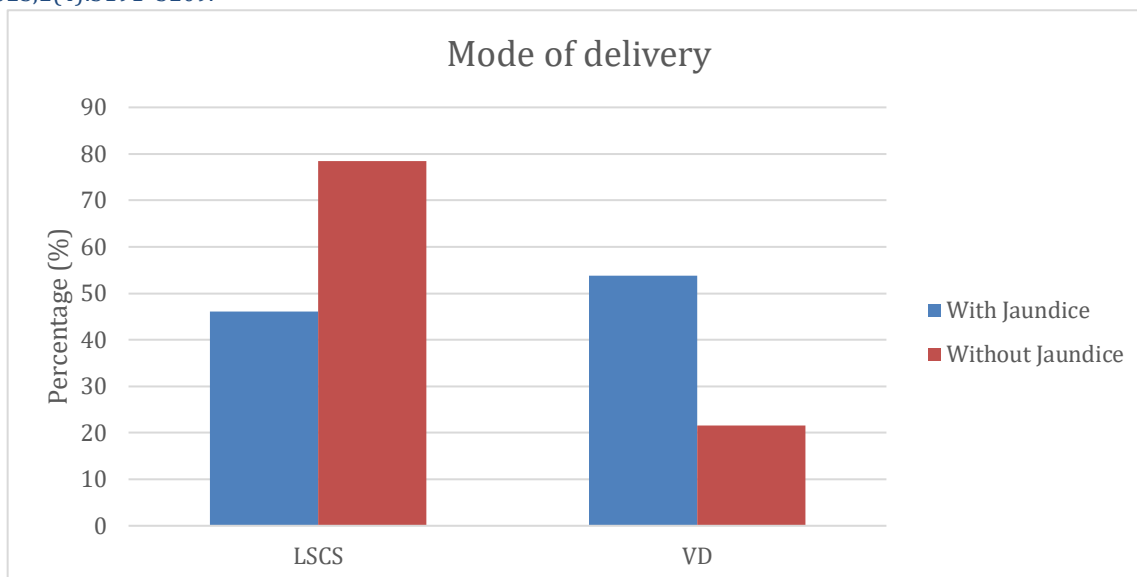


Figure 8: Bar chart show the distribution of patients according to mode of delivery in neonates with and without jaundice.

Table 9: Association between neonatal sex and neonates with and without jaundice

		With Jaundice (n=52)		Without Jaundice (n=79)		p-Value
		n	%	n	%	
Sex	Male	31	59.62	42	53.16	8.13
	Female	21	40.38	37	46.84	

Table 9 and Figure 9 show the association between neonatal sex and the occurrence of jaundice.

Among the 52 neonates who developed jaundice, 59.62% were male, while 40.38% were female. In contrast, among the 79 neonates who did not develop jaundice, 53.16% were male and 46.84% were female. The p-value (8.13) appears to be incorrectly reported and should be checked for accuracy.

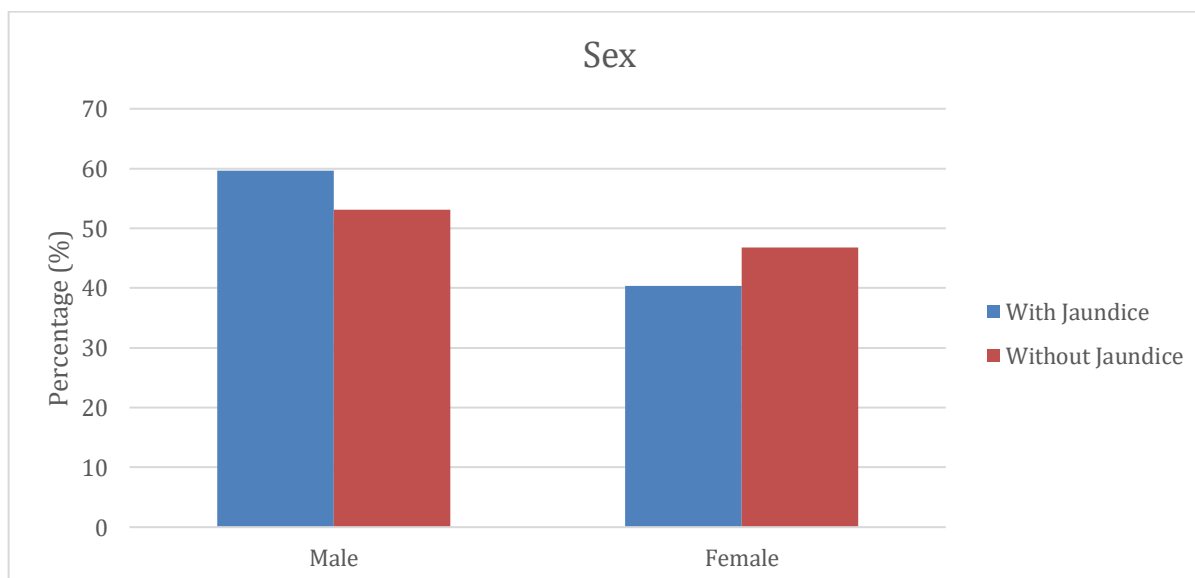


Figure 9: Bar chart show the distribution of patients according to gender in neonates with and without jaundice

Table 10: Association between birth weight and neonates with and without jaundice

		With Jaundice (n=52)		Without Jaundice (n=79)		p-Value
		n	%	n	%	
Births weight	<1.5 kg	5	9.62	0	0.00	0.017
	1.5-1.49 kg	10	19.23	14	17.72	
	≥1.50 kg	37	71.15	65	82.28	
	Mean±SD	2.54±0.61		2.71±0.32		0.034

Table 10 and Figure 10 show the association between birth weight and neonatal jaundice. Among the 52 neonates who developed jaundice, 9.62% had a birth weight of <1.5 kg, whereas none of the 79 non-jaundiced neonates fell into this category. Additionally, 19.23% of jaundiced neonates had a birth weight between 1.5-1.49 kg, compared to 17.72% in the non-jaundiced group. The majority of neonates in both groups had a birth weight of ≥1.50 kg, but the proportion was slightly lower among jaundiced neonates (71.15% vs. 82.28% in the non-jaundiced group). The mean birth weight of neonates with jaundice was 2.54±0.61 kg, whereas it was 2.71±0.32 kg in the non-jaundiced group, with a p-value of 0.034, indicating a statistically significant difference. Furthermore, the p-value (0.017) for birth weight categories suggests that lower birth weight is significantly associated with a higher risk of neonatal jaundice.

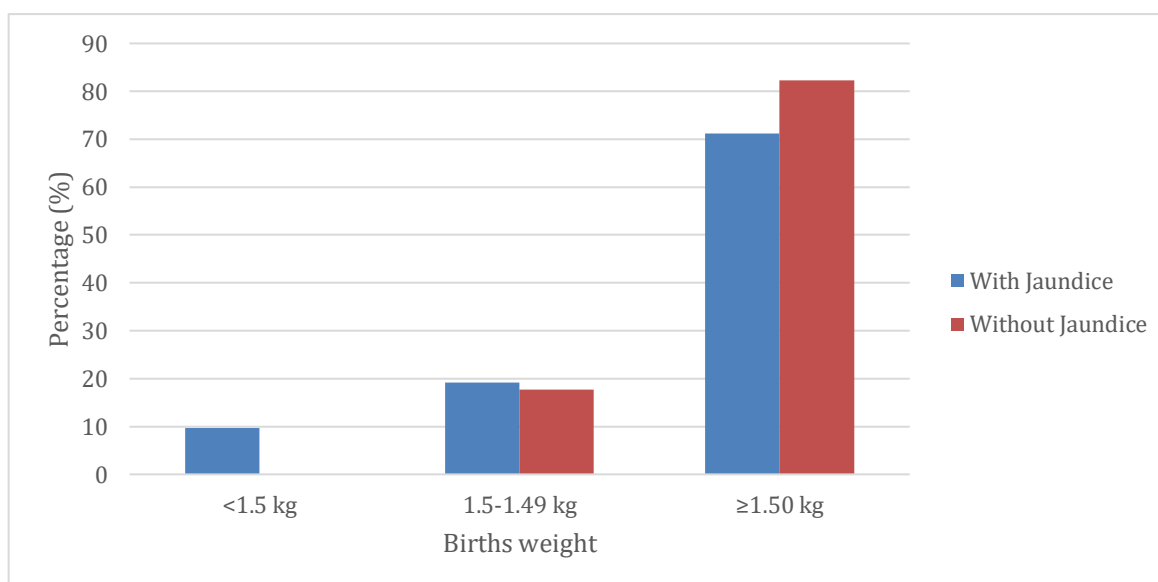


Figure 10: Bar chartshow the mean birth weight in neonates with and without jaundice

Table 11: Association between neonatal anthropometric measurements (length and occipitofrontal circumference) and neonatal jaundice

	With Jaundice (n=52)		Without Jaundice (n=79)		t	p-Value
	Mean	±SD	Mean	±SD		
Length (cm)	46.91	5.87	45.75	8.67	0.84	0.401
Occipitofrontal Circumference (cm)	33.69	4.72	35.72	4.77	-2.38	0.019

Table 11 and Figure 11 show the association between neonatal anthropometric measurements (length and occipitofrontal circumference) and neonatal jaundice. The mean length of neonates who developed jaundice was 46.91 ± 5.87 cm, while it was 45.75 ± 8.67 cm in neonates without jaundice. The t-value (0.84) and p-value (0.401) indicate that there is no statistically significant difference in neonatal length between the two groups. The mean occipitofrontal circumference (OFC) was 33.69 ± 4.72 cm in jaundiced neonates and 35.72 ± 4.77 cm in non-jaundiced neonates. The t-value (-2.38) and p-value (0.019) indicate a statistically significant difference, suggesting that neonates with jaundice tend to have a smaller occipitofrontal circumference compared to non-jaundiced neonates.

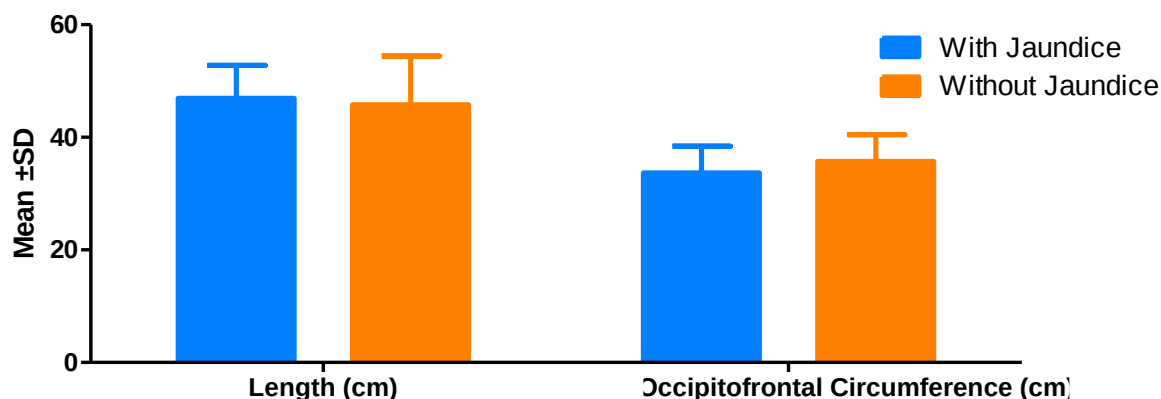


Figure 11: Bar chart show the neonatal anthropometric measurements (length and occipitofrontal circumference) and neonatal jaundice

Table 12: Comparison of cord blood bilirubin levels (total, direct, and indirect) between neonates with and without jaundice

	With Jaundice (n=52)		Without Jaundice (n=79)		t	p-Value
	Mean	±SD	Mean	±SD		
Cord Blood Bilirubin - Total (mg/dL)	2.55	0.74	1.83	0.43	6.92	0.000
Cord Blood Bilirubin - Direct (mg/dL)	0.62	0.42	0.42	0.25	3.32	0.001
Cord Blood Bilirubin - Indirect (mg/dL)	2.18	1.07	1.74	1.11	2.20	0.030

Table 12 and Figure shows the comparison of cord blood bilirubin levels (total, direct, and indirect) between neonates with and without jaundice. The mean cord blood total bilirubin was significantly higher in neonates who developed jaundice (2.55 ± 0.74 mg/dL) compared to those who did not (1.83 ± 0.43 mg/dL), with a t-value of 6.92 and a p-value of 0.000, indicating a highly significant association between elevated total bilirubin at birth and subsequent neonatal jaundice. Similarly, the mean direct bilirubin was significantly higher in the jaundiced group (0.62 ± 0.42 mg/dL) compared to the non-jaundiced group (0.42 ± 0.25 mg/dL), with a t-value of 3.32 and a p-value of 0.001, suggesting a statistically significant difference. The mean indirect bilirubin was also higher in jaundiced neonates (2.18 ± 1.07 mg/dL) than in non-jaundiced neonates (1.74 ± 1.11 mg/dL), with a t-value of 2.20 and a p-value of 0.030, indicating a significant but weaker correlation.

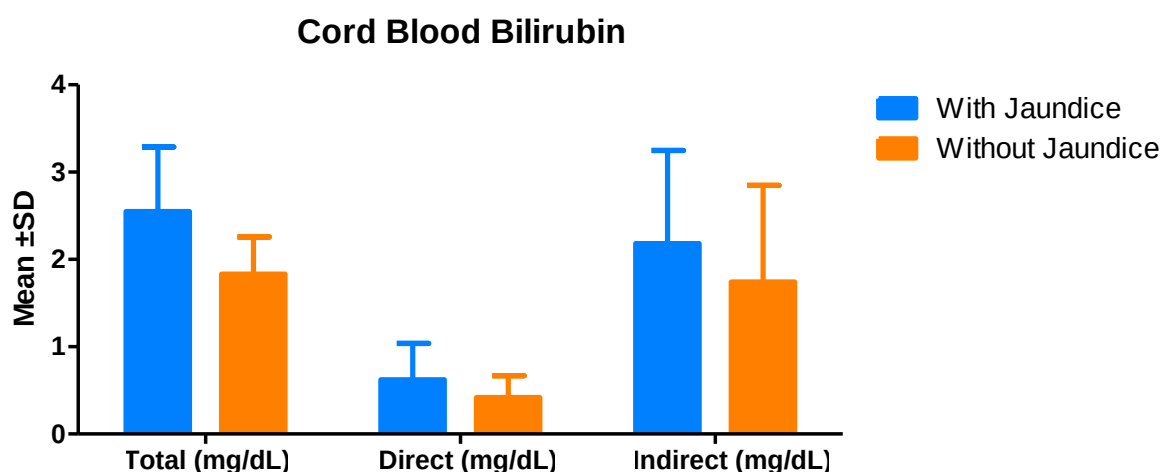


Figure 12: Bar chart show the mean cord blood bilirubin levels (total, direct, and indirect) in neonates with and without jaundice

Table 13: Comparison of serum bilirubin levels after 48 hours between neonates with and without jaundice

	With Jaundice (n=52)		Without Jaundice (n=79)		t	p-Value
	Mean	±SD	Mean	±SD		
Serum Bilirubin After 48 Hours - Total (mg/dL)	15.34	3.59	9.10	2.79	11.08	0.000
Serum Bilirubin After 48 Hours - Direct (mg/dL)	0.50	0.16	0.60	0.69	-1.07	0.286
Serum Bilirubin After 48 Hours - Indirect (mg/dL)	13.63	4.28	8.13	3.17	8.36	0.000

Table 13 and Figure 13 show the comparison of serum bilirubin levels after 48 hours between neonates with and without jaundice. The mean total serum bilirubin in jaundiced neonates was 15.34 ± 3.59 mg/dL, significantly higher than 9.10 ± 2.79 mg/dL in non-jaundiced neonates (p-value = 0.000), indicating a strong association between elevated total bilirubin and neonatal jaundice. Similarly, the mean indirect bilirubin level was 13.63 ± 4.28 mg/dL in jaundiced neonates, significantly higher than 8.13 ± 3.17 mg/dL in non-jaundiced neonates (p-value = 0.000), suggesting that unconjugated hyperbilirubinemia is the predominant form of neonatal jaundice in this study population. However, the direct bilirubin levels did not show a significant difference between the two groups, with 0.50 ± 0.16 mg/dL in jaundiced neonates and 0.60 ± 0.69 mg/dL in non-jaundiced neonates (p-value = 0.286).

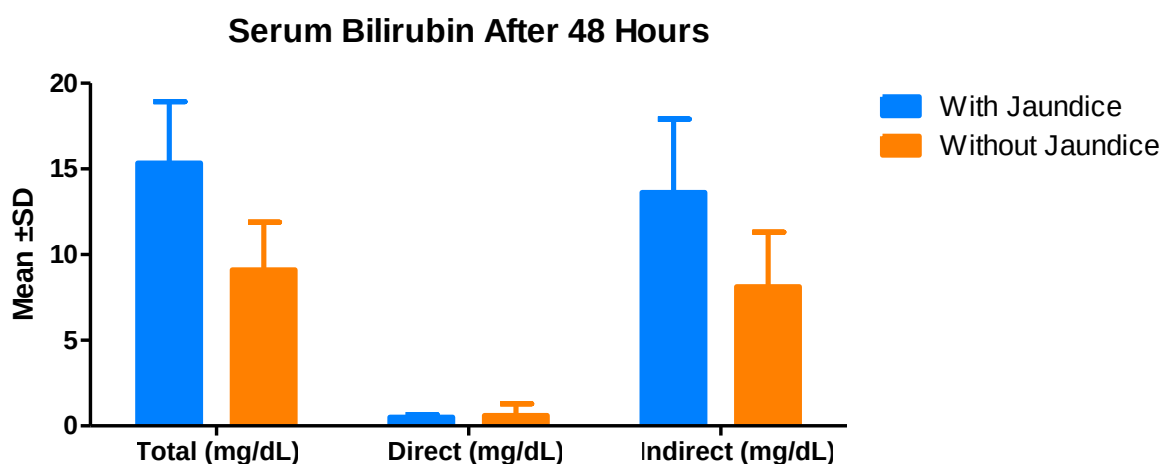


Figure13: Bar chart show the meanserum bilirubin levels after 48 hours in neonates with and without jaundice

Table 14: Correlation between cord blood bilirubin levels and serum bilirubin levels after 48 hours using Pearson correlation analysis

		Serum Bilirubin After 48 Hours - Total (mg/dL)	Serum Bilirubin After 48 Hours - Direct (mg/dL)	Serum Bilirubin After 48 Hours - Indirect (mg/dL)
Cord Blood Bilirubin - Total (mg/dL)	Pearson Correlation	0.411**	-0.108	0.286**
	p-Value	0.000	0.222	0.001
Cord Blood Bilirubin - Direct (mg/dL)	Pearson Correlation	0.155	-0.033	0.170
	p-Value	0.080	0.707	0.055
Cord Blood Bilirubin - Indirect (mg/dL)	Pearson Correlation	0.170	-0.040	0.147
	p-Value	0.055	0.652	0.095

**, Correlation is significant at the 0.01 level (2-tailed).

Table 14 presents the correlation between cord blood bilirubin levels and serum bilirubin levels after 48 hours using Pearson correlation analysis.

A significant positive correlation was observed between cord blood total bilirubin and serum total bilirubin at 48 hours ($r = 0.411$, $p = 0.000$), indicating that higher cord blood bilirubin levels are associated with increased total bilirubin levels

after birth. Additionally, a weaker but still significant correlation was found between cord blood total bilirubin and indirect bilirubin at 48 hours ($r = 0.286$, $p = 0.001$), suggesting that elevated cord blood bilirubin can predict the risk of indirect hyperbilirubinemia in neonates. However, there was no significant correlation between cord blood total bilirubin and direct bilirubin levels at 48 hours ($r = -0.108$, $p = 0.222$), indicating that cord blood bilirubin does not predict conjugated hyperbilirubinemia.

Similarly, cord blood direct bilirubin showed no significant correlation with total bilirubin ($r = 0.155$, $p = 0.080$), indirect bilirubin ($r = 0.170$, $p = 0.055$), or direct bilirubin ($r = -0.033$, $p = 0.707$) at 48 hours, suggesting that direct bilirubin at birth is not a reliable predictor of neonatal jaundice. Likewise, cord blood indirect bilirubin showed only a weak correlation with total bilirubin at 48 hours ($r = 0.170$, $p = 0.055$) and no significant correlation with direct bilirubin ($r = -0.040$, $p = 0.652$) or indirect bilirubin ($r = 0.147$, $p = 0.095$).

Table 15: Diagnostic performance of cord blood total bilirubin in predicting neonatal jaundice at 48 hours

Cord Blood Bilirubin	Cut-off	Sensitivity	Specificity	Area	95% CI		p-Value
					Lower	Upper	
Total (mg/dL)	2.0	92.3	75.3	0.867	0.806	0.927	<0.001

Table 15 and Figure 15 show the diagnostic performance of cord blood total bilirubin in predicting neonatal jaundice at 48 hours. The optimal cut-off value for cord blood total bilirubin was determined to be 2.0 mg/dL, which demonstrated high sensitivity (92.3%) and moderate specificity (75.3%). The area under the curve (AUC) was 0.867, indicating a strong predictive ability of cord blood bilirubin for neonatal jaundice. The 95% confidence interval (CI) ranged from 0.806 to 0.927, suggesting a high level of reliability for this prediction. The p-value (<0.001) confirms that the association is statistically significant.

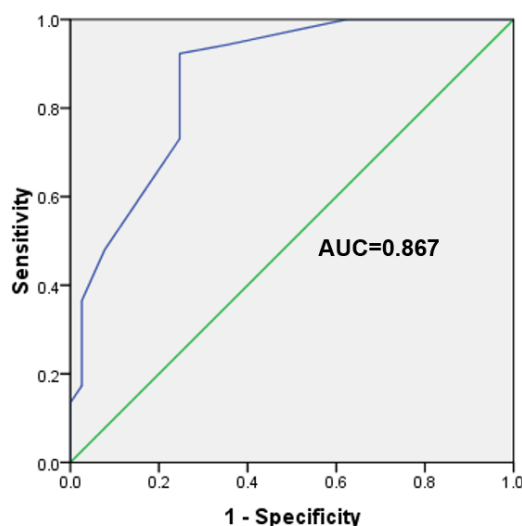


Figure 15: Receiver Operating Characteristic (ROC) curve showing the diagnostic performance of cord blood total bilirubin in predicting neonatal jaundice at 48 hours. The area under the curve (AUC) is 0.867, indicating good discriminatory ability.

Table 16: Cord Blood Bilirubin (D0 Diagnosis of jaundice at 48 hours

Cord Blood Bilirubin	Cut-off	Sensitivity	Specificity	Area	95% CI		p-Value
					Lower	Upper	
Direct (mg/dL)	0.35	86.5	48.0	0.655	0.561	0.750	0.003
Indirect (mg/dL)	1.45	86.5	49.4	0.658	0.563	0.754	0.002

Table 16 and Figure 16 show the diagnostic performance of cord blood direct and indirect bilirubin in predicting neonatal jaundice at 48 hours.

For cord blood direct bilirubin, the optimal cut-off value was determined to be 0.35 mg/dL, with a sensitivity of 86.5% and a specificity of 48.0%. The area under the curve (AUC) was 0.655, with a 95% confidence interval (CI) ranging from 0.561 to 0.750. The p-value (0.003) indicates a statistically significant but moderate predictive value.

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For cord blood indirect bilirubin, the cut-off value was 1.45 mg/dL, with a sensitivity of 86.5% and a specificity of 49.4%. The AUC was 0.658, with a 95% CI between 0.563 and 0.754. The p-value (0.002) suggests statistical significance, but the overall predictive value remains modest.

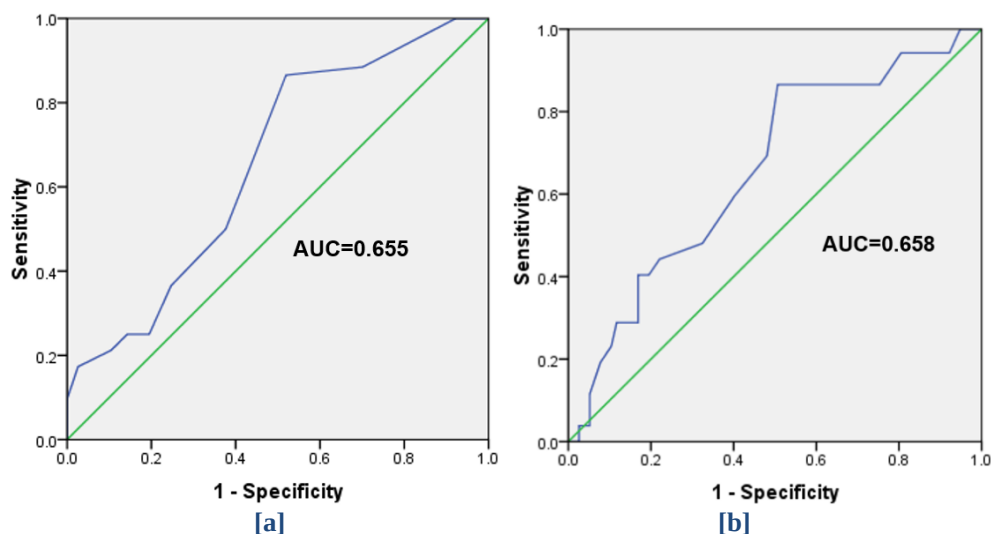


Figure 16: Receiver Operating Characteristic (ROC) curve illustrating the diagnostic ability of cord blood bilirubin [a-Direct, b-Indirect] in predicting the development of neonatal jaundice at 48 hours. The area under the curve (AUC) is 0.655, indicating modest diagnostic accuracy

Table 17a: Correlation between Cord Blood Bilirubin (CBB) levels and the development of neonatal jaundice

Cord Blood Bilirubin (CBB)	Total	Jaundice Present	Jaundice Absent
≥ 2 mg/dL	67	52 (True Positive, TP)	15 (False Positive, FP)
< 2 mg/dL	64	4 (False Negative, FN)	60 (True Negative, TN)
Total	131	56	75

Table 17a presents the relationship between Cord Blood Bilirubin (CBB) levels and the development of neonatal jaundice. Out of 67 neonates with CBB ≥ 2 mg/dL, 52 (77.61%) developed jaundice (classified as true positives, TP), indicating a strong association between elevated cord bilirubin and the risk of hyperbilirubinemia. The remaining 15 neonates (22.39%) with high CBB did not develop jaundice, and are categorized as false positives (FP).

In contrast, among the 64 neonates with CBB < 2 mg/dL, only 4 (6.25%) developed jaundice (false negatives, FN), while the majority, 60 neonates (93.75%), did not develop jaundice (true negatives, TN), suggesting that a low CBB level is generally a good indicator of low risk for jaundice.

Table 18b: Diagnostic Accuracy of Cord Blood Bilirubin (CBB ≥ 2 mg/dL)

Parameter	Formula	Value
Sensitivity	$TP / (TP + FN)$	92.86%
Specificity	$TN / (TN + FP)$	80.00%
Positive Predictive Value	$TP / (TP + FP)$	77.61%
Negative Predictive Value	$TN / (TN + FN)$	93.75%
Accuracy	$(TP + TN) / \text{Total}$	85.50%

Table 18b summarizes the diagnostic performance of using a cord blood bilirubin (CBB) cut-off of ≥ 2 mg/dL for predicting neonatal hyperbilirubinemia. Each metric reflects how well this threshold performs as a screening tool:

Sensitivity (92.86%): This indicates that the test correctly identifies 92.86% of neonates who will develop jaundice. It shows excellent ability to detect true positive cases, making it a reliable early screening parameter for hyperbilirubinemia.
Specificity (80.00%): The test correctly identifies 80% of neonates who will not develop jaundice. This demonstrates a good ability to exclude false positives, reducing unnecessary interventions in healthy newborns.

Positive predictive value (77.61%): Among those neonates with CBB ≥ 2 mg/dL, around 78% actually developed jaundice. This reflects how likely a newborn is to develop jaundice when the test is positive.

Negative predictive value (93.75%): A very high NPV means that when CBB < 2 mg/dL, there is a 94% chance the neonate will not develop jaundice, making it a strong rule-out test.

Accuracy (85.50%): This overall metric shows that 85.5% of all predictions (positive and negative) were correct, confirming the test's clinical reliability.

DISCUSSION

Among 67 neonates with CBB $\geq$ 2 mg/dL, 52 (77.61%) developed jaundice, while only 4 (6.25%) out of 64 neonates with CBB < 2 mg/dL developed jaundice. Diagnostic evaluation of CBB $\geq$ 2 mg/dL yielded a sensitivity of 92.86%, specificity of 80.00%, positive predictive value of 77.61%, negative predictive value of 93.75%, and overall accuracy of 85.50%. Significant associations were found between neonatal jaundice and maternal age ($p=0.0134$), gestational age ($p=0.005$), and mode of delivery ($p<0.001$). Vaginal delivery and gestational age of 37–39 weeks were associated with higher risk. Other variables such as parity, maternal comorbidities, Rh incompatibility, and neonatal sex showed no significant association. In our study, out of 131 neonates, 52 (39.69%) developed neonatal jaundice. The rate of neonatal hyperbilirubinemia we found is higher than the 21.6% reported by Eldho et al. (2022)²⁶ for neonates with ABO incompatibility. Similarly, Bhat et al. (2019)³⁰ reported a lower incidence of pathological jaundice at 11.2%, while Kardum et al. (2021)¹⁵ reported a 14.9% incidence in healthy term neonates. However, our incidence is comparable to the findings of Patil et al. (2024)²⁷, where 50% of neonates developed significant hyperbilirubinemia. The higher incidence in our study may be attributed to the inclusion of a mixed population with varying gestational ages and delivery modes. The findings emphasize that neonatal jaundice remains a significant clinical problem, requiring timely diagnosis and intervention.

In our study, a statistically significant association was found between maternal age and the development of neonatal jaundice ($p=0.0134$). The highest incidence was seen among mothers aged 25–29 years (75%). Sharma et al. (2003)²⁹ stated that pregnancies between 20 and 30 years are most common in the Indian subcontinent. Our findings reinforce the trend of childbearing being most common between 20 and 30 years, although advanced or younger maternal age has not consistently shown an association with neonatal jaundice.

In the present study, the incidence of jaundice was higher among neonates born to multiparous mothers (23.08%), but the association was not statistically significant ($p=0.130$). Eldho et al. (2022)²⁶ found that the rates were similar for first-time mothers (53.3%) and those who had given birth before (46.6%), and this did not significantly affect neonatal hyperbilirubinemia. Bhat et al. (2019)³⁰ also observed no significant association between parity and jaundice. The role of parity as a risk factor appears inconsistent across studies. Our findings indicate that although jaundice was slightly more common among multiparous mothers, gravidity

and parity are not strong predictors of neonatal hyperbilirubinemia.

In the current study, maternal conditions like preeclampsia, hypothyroidism, PPRM, and celiac disease showed no significant association with neonatal jaundice ($p > 0.05$). Eldho et al. (2022)²⁶ also found no significant association between these comorbidities and hyperbilirubinemia in their study population. Similarly, Bhat et al. (2019)³⁰ reported that gestational age, birth weight, and mode of delivery did not significantly impact CBB levels or serum bilirubin. Kardum et al. (2021)¹⁵ also attempted to evaluate the role of infection but found that umbilical cord bilirubin is a poor predictor for neonatal infection (AUC=0.59). These findings support that while maternal comorbidities are essential clinical considerations, they may not independently predict hyperbilirubinemia.

In our study, the gestational age showed a statistically significant association with neonatal jaundice ($p=0.005$). The highest incidence was observed among neonates born between 37 and 39 weeks (71.15%). This aligns with Kardum et al. (2021)¹⁵, who found that neonates with hyperbilirubinemia had significantly lower gestational ages. Bhat et al. (2019)³⁰ reported that 71.18% of their study population were >37 weeks, but gestational age had no significant impact on serum bilirubin levels. Eldho et al. (2022)²⁶ also found no significant difference concerning gestational age. Our findings suggest that gestational age between 37 and 39 weeks may pose a higher risk for jaundice, possibly due to immature hepatic bilirubin metabolism in early-term neonates.

This study showed a strong link between how babies were born and neonatal jaundice ($p<0.001$), with more cases occurring in babies born vaginally (53.85%). This finding is consistent with Ahire et al. (2016)³¹, who reported increased risk of hyperbilirubinemia in neonates born via vaginal delivery due to possible birth trauma or bruising. However, Bhat et al. (2019)³⁰ found no significant difference in serum bilirubin levels based on delivery mode. The male neonates constituted a higher proportion of the jaundiced group (59.62%) compared to females (40.38%). However, the association between gender and jaundice was not statistically significant ($p > 0.05$). This finding agrees with Eldho et al. (2022)²⁶ and Bhat et al. (2019)³⁰.

In this study, birth weight was significantly associated with the occurrence of neonatal jaundice ($p=0.017$). The mean birth weight of jaundiced neonates was lower (2.54 ± 0.61 kg) compared to non-jaundiced neonates (2.71 ± 0.32 kg). Kardum et al. (2021)¹⁵ found that neonates with hyperbilirubinemia had significantly

lower birth weights. Patil et al. (2024)²⁷ reported an average birth weight of 2.39 kg among neonates developing jaundice. Similarly, Bhat et al. (2019)³⁰ identified birth weight between 2.5 and 3 kg as the most common among neonates with hyperbilirubinemia. These findings confirm the inverse relationship between birth weight and risk of jaundice.

In the present study, cord blood total bilirubin was significantly higher in neonates developing jaundice (2.55 ± 0.74 mg/dL) compared to non-jaundiced neonates (1.83 ± 0.43 mg/dL; $p=0.000$). Our findings are supported by Eldho et al. (2022)²⁶, who reported a mean CBB of 2.75 mg/dL. Bhat et al. (2019) [30] observed a mean CBB of 2.6 ± 0.8 mg/dL among neonates with pathological jaundice. Patil et al. (2024)²⁷ also found a higher mean CBB (2.66 ± 0.65 mg/dL) in neonates requiring phototherapy. Our study reinforces the clinical relevance of CBB estimation as an early marker for predicting neonatal hyperbilirubinemia.

The Pearson correlation analysis demonstrated a significant positive correlation between cord blood total bilirubin and serum bilirubin levels at 48 hours ($r=0.411$, $p=0.000$). This finding is in agreement with Eldho et al. (2022)²⁶, who reported a positive correlation between CBB and serum bilirubin at 24 hours. Patil et al. (2024)²⁷ also demonstrated a significant correlation ($r=0.087$) between CBB and bilirubin levels on day 3. The findings of Pahuja et al. (2016)²⁸ and Bhat et al. (2019)³⁰ further corroborate this association, confirming that elevated CBB levels at birth predict higher subsequent bilirubin levels.

In this study, the ROC analysis identified an optimal CBB cutoff of 2.0 mg/dL, with a sensitivity of 92.3% and specificity of 75.3% (AUC=0.867). Bhat et al. (2019) [30] found that a cutoff of >3 mg/dL offered a sensitivity of 97.06% and specificity of 99.22%. Eldho et al. (2022)²⁶ suggested a cutoff of ≥ 3.5 mg/dL with a sensitivity of 38.4% and a specificity of 90.4%. Patil et al. (2024)²⁷ identified a cutoff of 2.5 mg/dL with a specificity of 98%. Our findings suggest that lower cutoff values offer higher sensitivity but slightly lower specificity, making them more suitable for early screening purposes in resource-limited settings.

This study, comprising 131 neonates, 52 (39.69%) developed clinically significant neonatal jaundice. Of the 67 neonates with cord blood bilirubin (CBB) ≥ 2 mg/dL, 52 developed jaundice, while among the 64 neonates with CBB <2 mg/dL, only 4 developed jaundice. The diagnostic performance of CBB ≥ 2 mg/dL yielded a sensitivity of 92.86%, specificity of 80.00%, positive predictive value (PPV) of 77.61%, negative predictive value (NPV) of 93.75%, and an overall diagnostic accuracy of 85.50%. These findings suggest that CBB measurement is a valuable, non-invasive, and early screening tool to predict the risk of neonatal hyperbilirubinemia.

The above results are supported by multiple prior studies. Eldho et al. (2022)²⁶ studied 120 healthy term neonates with ABO incompatibility and found a positive correlation between cord bilirubin levels and total serum bilirubin (TSB) at 24 hours. At a CBB cut-off of ≥ 3.5 mg/dL, they reported a sensitivity of 38.4%, specificity of 90.4%, PPV of 52.6%, and NPV of 84.1%. Although their sensitivity was lower compared to our findings, both studies underline the importance of high specificity in ruling out neonates unlikely to develop jaundice.

Similarly, Bhat et al. (2019)³⁰ found a CBB threshold >3 mg/dL to be predictive of severe jaundice with high sensitivity (97.06%) and specificity (99.22%), closely aligning with our study's performance at a slightly lower threshold. They also demonstrated that a CBB level between 2.5–3 mg/dL had a sensitivity of 66.67% and specificity of 92.94%, indicating that risk prediction improves with higher thresholds.

Patil et al. (2024)²⁷ used a CBB cut-off of 2.5 mg/dL and reported specificity of 98% and sensitivity of 56% for predicting phototherapy need. Their study also found a statistically significant correlation between CBB and serum bilirubin at day 3 ($r = 0.087$), echoing our observation of strong correlation between early bilirubin levels and subsequent jaundice development.

Zeitoun et al. (2012)³⁵ also identified CBB thresholds of 2.0–2.15 mg/dL as predictive of significant jaundice, reinforcing the practical utility of CBB ≥ 2 mg/dL. These cut-offs provide reliable early identification of neonates at risk and justify extended monitoring for those falling above the threshold.

Alpay et al. (2000)³² have shown that bilirubin levels measured even within the first 6–24 hours can predict the need for phototherapy, supporting our rationale for using cord bilirubin as an early predictor.

Kardum et al. (2021)¹⁵, in a large cohort of 1360 infants, validated the predictive accuracy of umbilical cord bilirubin with an AUC of 0.80, sensitivity of 76.85%, and specificity of 69.58%. They emphasized CBB's utility particularly in infants with blood group incompatibilities or risk factors such as lower birth weight and infection.

Chary et al. (2017)³³ reported that a CBB level ≥ 2 mg/dL had a sensitivity of 94.12% and specificity of 90.9%, results closely aligning with our current study. Similarly, Kumar et al. (2016)³⁴ used a cut-off of 3 mg/dL in ABO-incompatible neonates and reported specificity of 93% and sensitivity of 97.6%. The consistency across these studies confirms the reliability of cord bilirubin measurement as a cost-effective, non-invasive, and timely method to identify neonates at risk for hyperbilirubinemia.

Limitations:

This study was conducted at a single centre with a relatively small sample size of 131 neonates, which may

limit the generalizability of the findings. Bilirubin levels in cord blood were measured only once, and serial bilirubin monitoring was not performed. Other contributing factors to hyperbilirubinemia, such as genetic predispositions (e.g., G6PD deficiency), were not evaluated. Additionally, external factors like breastfeeding patterns, weight loss, and environmental influences were not considered. The follow-up duration was short, focusing only on early-onset jaundice. We need larger, multicentric studies with extended follow-up and broader assessments to validate and expand upon these findings.

CONCLUSION

This study highlights a significant correlation between higher levels of bilirubin in cord blood and the subsequent development of neonatal hyperbilirubinemia. Among 67 neonates with CBB ≥ 2 mg/dL, 52 (77.61%) developed jaundice, while only 4 (6.25%) out of 64 neonates with CBB < 2 mg/dL developed jaundice. Diagnostic evaluation of CBB ≥ 2 mg/dL yielded a sensitivity of 92.86%, specificity of 80.00%, positive predictive value of 77.61%, negative predictive value of 93.75%, and overall accuracy of 85.50%. Significant associations were found between neonatal jaundice and maternal age ($p=0.0134$), gestational age ($p=0.005$), and mode of delivery ($p<0.001$). Vaginal delivery and gestational age of 37–39 weeks were associated with higher risk. Other variables such as parity, maternal comorbidities, Rh incompatibility, and neonatal sex showed no significant association. Out of 131 neonates, 39.69% developed jaundice. A notable association was found between higher cord blood total, direct, and indirect bilirubin levels and the occurrence of neonatal jaundice. The strong statistical results ($p < 0.001$ for total and indirect bilirubin) indicate that measuring bilirubin in cord blood can be a trustworthy early sign for predicting neonatal hyperbilirubinemia.

Several maternal and neonatal factors were evaluated. Maternal age and gestational age showed statistically significant associations with neonatal jaundice, with the highest risk observed in neonates born to mothers aged 25–29 years and those delivered between 37–39 weeks of gestation. Vaginal delivery was also significantly associated with a higher risk of jaundice compared to LSCS. Birth weight and occipitofrontal circumference (OFC) were important neonatal predictors, with lower birth weight and smaller OFC significantly linked to increased risk. However, maternal parity, blood group, Rh status, and comorbidities such as hypothyroidism, PPRM, or history of abortion did not show significant associations.

Importantly, while the sex of the neonate and mode of delivery exhibited some trends, their direct impact on hyperbilirubinemia was less conclusive without corrected p-values. The high occurrence of indirect (unconjugated) hyperbilirubinemia in affected newborns highlights the importance of early detection and treatment to avoid complications.

In our study cord blood bilirubin (CBB) ≥ 2 mg/dL was a reliable early predictor of neonatal hyperbilirubinemia. With a high sensitivity (92.86%) and negative predictive value (93.75%), this threshold is particularly effective in identifying at-risk neonates soon after birth and in ruling out those unlikely to develop jaundice. The overall diagnostic accuracy of 85.5% supports the clinical utility of CBB estimation as a non-invasive, rapid, and cost-effective screening tool. Incorporating CBB testing into routine neonatal assessment could facilitate timely intervention and help reduce complications related to severe hyperbilirubinemia.

In conclusion, cord blood bilirubin levels, along with gestational age, mode of delivery, and birth weight, serve as valuable predictors for neonatal jaundice. Including cord blood bilirubin testing at birth, particularly for newborns with extra risk factors, can help spot problems early and allow for quick treatment, which can lower the chances of severe hyperbilirubinemia and related health issues.

Conflict of interest none

Funding none

Ethical clearance obtained

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