

Study of Various Maternal and Fetal Risk Factors and Outcome of Hyperbilirubinaemia in Newborns: A Case–Control Study

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Abstract

Background: Neonatal hyperbilirubinaemia is one of the most prevalent conditions encountered in newborn care, affecting approximately 60% of term and 80% of preterm neonates during the first week of life. Severe unconjugated hyperbilirubinaemia carries the risk of bilirubin-induced neurological dysfunction (BIND) and kernicterus, both of which remain preventable causes of neonatal morbidity and mortality in low- and middle-income countries (LMICs). Early identification of maternal and neonatal risk factors is therefore of paramount clinical importance.

Aim: To identify and analyse various maternal and fetal risk factors associated with the development of significant neonatal hyperbilirubinaemia and to evaluate its clinical outcome, treatment modalities, and complications.

Methods: A prospective case–control study was conducted at IMS and SUM Hospital, Campus-II, Bhubaneswar, Odisha, between July 2025 and June 2026. A total of 105 neonates were enrolled: 70 cases (neonates with total serum bilirubin [TSB] at or above the AAP 2022 hour-specific phototherapy threshold) and 35 controls (neonates with physiological jaundice—defined as visible icterus with TSB below the age-specific AAP 2022 phototherapy threshold, without pathological aetiology, from the same hospital population). The sample size was calculated assuming an expected risk factor prevalence of 50% in cases versus 25% in controls, at 80% power and 5% significance level (two-tailed), yielding a minimum of 62 cases and 31 controls; 70 and 35 were enrolled to provide additional precision. Statistical analysis was performed using SPSS v26.0; chi-square, Fisher's exact test, and unpaired t-test were applied as appropriate. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. A p-value <0.05 was considered statistically significant.

Results: Among cases, preterm birth (40.0% vs. 17.1%; $p=0.014$), low birth weight (37.1% vs. 14.3%; $p=0.011$), delivery by caesarean section (57.1% vs. 31.4%; $p=0.012$), and ABO/Rh incompatibility were significant neonatal risk factors. Significant maternal risk factors included primiparity (57.1% vs. 28.6%; OR 3.29, $p=0.005$), prolonged labour (42.9% vs. 17.1%; OR 3.65, $p=0.007$), oxytocin-induced labour (OR 3.05, $p=0.021$), pre-eclampsia (OR 3.10, $p=0.024$), and maternal anaemia (OR 2.37, 95% CI 1.02–5.50, $p=0.044$). The mean peak TSB in cases was significantly higher (19.2 ± 4.1 mg/dL vs. 9.6 ± 2.2 mg/dL; $p<0.001$). Acute bilirubin encephalopathy occurred in 12.9% and exchange transfusion in 11.4% of cases.

Conclusion: Primiparity, prolonged labour, oxytocin induction, pre-eclampsia, maternal anaemia, prematurity, low birth weight, and blood group incompatibility are significant risk factors for neonatal hyperbilirubinaemia. Comprehensive antenatal counselling, early postnatal bilirubin monitoring using the AAP 2022 nomogram, and timely intervention are essential to prevent adverse neurodevelopmental outcomes.

Keywords: neonatal jaundice, hyperbilirubinaemia, phototherapy, kernicterus, maternal risk factors, bilirubin encephalopathy, newborn, case–control.

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Introduction

Neonatal jaundice, or hyperbilirubinaemia, is among the most frequently encountered clinical conditions in neonatal medicine. It is characterised by a

yellowish discolouration of the skin and sclerae arising from elevated levels of unconjugated bilirubin in the circulation. Approximately 60% of

term neonates and up to 80% of preterm neonates manifest visible jaundice within the first week of life; the majority of these cases are physiological and self-limiting.[1] However, a clinically significant subset progresses to severe hyperbilirubinaemia, which, if left untreated, may lead to bilirubin-induced neurological dysfunction (BIND), including kernicterus — a devastating, largely preventable form of brain injury.[2]

Despite remarkable global advances in neonatal intensive care, neonatal hyperbilirubinaemia remains a leading cause of avoidable morbidity and mortality in low- and middle-income countries (LMICs), particularly in South Asia. In India, it continues to represent a major burden in neonatal care units, accounting for a substantial proportion of Special Newborn Care Unit (SNCU) admissions.[3] The aetiology of pathological neonatal jaundice is multifactorial, encompassing both maternal and neonatal determinants. Among the well-established risk factors are haemolytic conditions arising from ABO and Rh blood group incompatibility, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and sepsis. Additionally, obstetric factors such as oxytocin administration, prolonged labour, instrumental delivery, and intrapartum haemorrhage may elevate neonatal bilirubin levels.[4]

On the maternal side, primiparity, anaemia, pre-eclampsia, gestational diabetes mellitus (GDM), and a prior history of a jaundiced sibling have been variably associated with an increased risk of significant neonatal hyperbilirubinaemia.[5] Neonatal characteristics including prematurity, low birth weight (LBW), exclusive breastfeeding, and delivery via caesarean section have further been implicated as independent predictors.[6] The American Academy of Pediatrics (AAP) updated its phototherapy guidelines in 2022, providing revised gestational-age- and hour-specific nomograms for risk stratification, thereby enabling clinicians to predict which neonates are at highest risk for requiring phototherapy or exchange transfusion.[7]

The clinical spectrum of hyperbilirubinaemia in the newborn period ranges from a transient, inconsequential rise in serum bilirubin to life-threatening bilirubin encephalopathy. Early recognition and targeted management are therefore critical. Population-level data from tertiary care institutions in eastern India remain sparse, and understanding the local pattern of risk factors and outcomes is essential for directing preventive and therapeutic strategies.[8] A systematic review and meta-analysis by Olusanya et al. highlighted that South Asian countries disproportionately bear the global burden of kernicterus, underscoring the urgency of region-specific research.[9]

Furthermore, contemporary evidence suggests that aggressive management — including intensive

phototherapy, intravenous immunoglobulin (IVIG) in immune haemolytic disease, and timely exchange transfusion — can substantially reduce the risk of long-term neurodevelopmental sequelae.[10] Audiological monitoring via brainstem evoked response audiometry (BERA) has assumed increasing importance in the follow-up of neonates who sustain significant hyperbilirubinaemia.[11]

The present study was therefore designed as a prospective case-control investigation to systematically evaluate and quantify the maternal and neonatal risk factors associated with clinically significant hyperbilirubinaemia at a tertiary referral centre in Bhubaneswar, Odisha, India, while simultaneously documenting the treatment modalities employed and the short-term clinical outcomes including neurological complications and mortality. This evidence is expected to inform institutional protocols and contribute to the regional literature on neonatal jaundice management.

Material and Methods

Study Design and Setting: This prospective, hospital-based case-control study was conducted over twelve months, from 5th July 2025 to 30th June 2026, in the Department of Paediatrics, Institute of Medical Sciences (IMS) and SUM Hospital, Campus-II, Phulnakhara, Bhubaneswar, Odisha, India (PIN: 754001). The hospital serves as a tertiary referral centre for eastern Odisha and its adjacent states, with a dedicated Special Newborn Care Unit (SNCU). Ethical clearance was obtained from the Institutional Ethics Committee (IEC) of IMS and SUM Hospital prior to commencement of the study. Written informed consent was secured from the parent or guardian of each participating neonate. The study was not registered in a clinical trial registry as it is an observational case-control study (not applicable).

Sample Size Calculation: Sample size was calculated using the formula for comparing two proportions (Fleiss JL, Levin B, Paik MC. Statistical Methods for Rates and Proportions, 2nd ed. New York: Wiley; 1981) with computations performed in OpenEpi version 3.01 (www.openepi.com). Based on a review of existing literature, the expected prevalence of primiparity (the primary risk factor of interest) was estimated at 50% in cases and 25% in controls.

With a two-tailed alpha of 0.05, power of 80%, and a case-to-control ratio of 2:1, the minimum required sample was 62 cases and 31 controls. To account for potential drop-outs and to enhance statistical power, 70 cases and 35 controls were enrolled, yielding a final ratio of 2:1.

Study Population and Case-Control Definitions:

A total of 105 neonates born at or referred to IMS and SUM Hospital, Campus-II, were enrolled: 70 as

cases and 35 as controls.

Cases were defined as neonates aged 0–28 days with a total serum bilirubin (TSB) at or above the hour-specific phototherapy threshold as stipulated in the AAP 2022 updated clinical practice guidelines, irrespective of gestational age. The AAP 2022 nomogram was used consistently for case identification, treatment initiation, and escalation of therapy throughout the study.

Controls were neonates from the same hospital population, of the same age range (0–28 days), with visible clinical icterus (positive blanch test), but with TSB strictly below the age- and gestational-age-specific AAP 2022 phototherapy threshold and without any identifiable pathological cause of jaundice (negative direct Coombs test, no haemolysis on peripheral smear, no clinical or biochemical evidence of sepsis, and no G6PD deficiency). Controls were frequency-matched to cases by gestational age category (preterm <37 weeks vs. term ≥37 weeks) and birth weight category (<2500 g vs. ≥2500 g) in a 2:1 case-to-control ratio, using a frequency-matching approach. Controls were defined by their TSB status at enrolment; subsequent clinical management (including clinician-initiated phototherapy when TSB approached but had not yet crossed the AAP 2022 threshold) was recorded as an outcome variable and did not alter their enrolment classification. One control whose TSB crossed the exchange transfusion threshold after enrolment was similarly retained as a control on the basis of TSB at enrolment; this post-enrolment event is reported as a study limitation.

Inclusion and Exclusion Criteria: Neonates of all gestational ages (preterm, term, and post-term) presenting with clinical jaundice within the first 28 days of life and whose parent or guardian provided informed consent were included. Neonates with conjugated (direct) hyperbilirubinaemia (direct bilirubin >2 mg/dL or >20% of TSB), severe congenital malformations incompatible with survival, hepatic or metabolic disease as the primary aetiology, or whose medical records were incomplete were excluded.

Data Collection: A detailed, pre-designed, semi-structured proforma was used to record maternal and neonatal information. Maternal data included: age, parity, body mass index (BMI), antenatal care (ANC) attendance, blood group, haemoglobin level, obstetric complications (pre-eclampsia, gestational diabetes mellitus, intrapartum fever, hypothyroidism, prolonged labour defined as active phase >12 hours), mode of delivery, use of oxytocin augmentation or induction, and history of a previously jaundiced sibling. Neonatal data included: gestational age (Ballard score where required), birth weight, sex, Apgar scores at 1 and 5

minutes, mode of delivery, blood group, direct Coombs test (DCT) result, G6PD enzyme assay, transcutaneous bilirubin (TcB) screening at birth, TSB measured by spectrophotometry, and clinical findings including jaundice zone, tone, and neurological status.

Laboratory Investigations: TSB was measured by spectrophotometric analysis in the hospital's ISO-certified biochemistry laboratory. Direct Coombs test (DCT) and blood group determination were performed for all enrolled neonates. G6PD enzyme quantification was carried out by the UV spectrophotometric method using standard reagent kits. Full blood counts, peripheral smear, reticulocyte count, serum albumin, and blood cultures (where sepsis was clinically suspected) were performed as indicated. Audiological evaluation was performed using brainstem evoked response audiometry (BERA) and distortion product otoacoustic emissions (DPOAE). In neonates with clinical features of acute bilirubin encephalopathy (ABE), diagnosis of kernicterus was confirmed by a combination of clinical criteria (abnormal tone, oculomotor abnormalities, retrocollis–opisthotonos, high-pitched cry) and neuroimaging with magnetic resonance imaging (MRI) of the brain, specifically demonstrating characteristic T1 hyperintensity and T2 prolongation in the globus pallidus and subthalamic nuclei.

Treatment Protocols: Phototherapy was initiated based on the AAP 2022 hour-specific nomogram thresholds. Intensive phototherapy (multiple banks or high-irradiance LED phototherapy, irradiance >30 μW/cm²/nm) was employed when TSB was within 2–3 mg/dL of the exchange transfusion threshold. IVIG (1 g/kg intravenously over 2–4 hours) was administered in cases of immune haemolytic disease (positive DCT) with a rapidly rising TSB not responding adequately to phototherapy. Exchange transfusion (double-volume, 160 mL/kg) was performed when TSB exceeded the exchange threshold on the AAP 2022 nomogram or when clinical signs of ABE were present despite intensive phototherapy. Lower segment caesarean section is abbreviated as LSCS throughout.

Outcome Assessment: Primary outcomes assessed included: peak TSB achieved, duration of phototherapy, requirement for exchange transfusion or IVIG, need for SNCU admission, duration of hospital stay, development of ABE (defined as the presence of abnormal tone [hypertonia or hypotonia], high-pitched cry, or altered sensorium attributable to hyperbilirubinaemia), audiological abnormality on BERA or DPOAE, kernicterus (confirmed by clinical criteria and MRI brain), and in-hospital mortality. "Full clinical recovery at discharge" was defined as resolution of jaundice to below the phototherapy threshold, absence of

neurological signs, normal tone, and normal feeding at the time of discharge.

Statistical Analysis: All data were entered and analysed using IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as frequency and percentage and compared between cases and controls using the chi-square test or Fisher's exact test (for cells with expected count <5). Continuous variables were expressed as mean $\pm$ standard deviation (SD) and compared using the unpaired Student's t-test. Crude odds ratios (ORs) with 95% confidence intervals were computed for each significant univariate predictor. Variables significant at $p < 0.10$ on univariate analysis were entered into a binary logistic regression model (enter method) to derive adjusted ORs, controlling for gestational age, birth weight, and mode of delivery as potential confounders (Table 4). A two-tailed p-value of < 0.05 was considered statistically significant. Given that over 20 variables were analysed, the possibility of inflated type-I error due to multiple comparisons is acknowledged as a limitation; Bonferroni correction was not applied as this was an exploratory study, but all significant findings should be interpreted with this caveat.

Results

A total of 105 neonates fulfilled the eligibility criteria and were enrolled during the study period: 70 cases and 35 controls. No enrolled participant was lost to follow-up within the hospitalisation period. The results are presented systematically in Tables 1–4 and illustrated in Figures 1 and 2.

Baseline Demographic and General Characteristics (Table 1): Among cases, preterm birth (< 37 weeks) was significantly more frequent than in controls (40.0% vs. 17.1%; OR 3.18, 95% CI 1.18–8.59; $p = 0.014$). Similarly, low birth weight (< 2500 g) was substantially more prevalent in cases (37.1% vs. 14.3%; OR 3.52, 95% CI 1.22–10.14; $p = 0.011$). Delivery by lower segment caesarean section (LSCS) was notably higher in the case group (57.1% vs. 31.4%; OR 2.91, 95% CI 1.23–6.87; $p = 0.012$). A depressed Apgar score at 5 minutes (< 7) was observed in 31.4% of cases versus 11.4% of controls (OR 3.56, 95% CI 1.11–11.41; $p = 0.022$). ABO blood group incompatibility was present in 34.3% of cases versus 14.3% of controls (OR 3.10, 95% CI 1.07–8.97; $p = 0.024$). Rh incompatibility was documented in 17.1% of cases versus 2.9% of controls. Given the small numbers in the Rh incompatibility cell (12 vs. 1), Fisher's exact test was applied and OR was calculated by logistic regression, yielding OR 6.93 (95% CI 0.87–55.3); while the p-value on Fisher's exact test was 0.057, this association is considered clinically noteworthy and is reported with appropriate caution regarding statistical precision. Male sex, post-term

gestation, macrosomia, and exclusive breastfeeding did not differ significantly between groups.

Maternal Risk Factors (Table 2 and Figure 1): Among the maternal risk factors evaluated, primiparity was the most prevalent maternal demographic variable: 57.1% of case mothers were primiparous versus 28.6% of control mothers (OR 3.29, 95% CI 1.40–7.74; $p = 0.005$). Prolonged active labour (> 12 hours) conferred the highest odds of neonatal hyperbilirubinaemia (OR 3.65, 95% CI 1.38–9.66; $p = 0.007$), followed by a prior history of a jaundiced sibling (OR 5.72, 95% CI 1.25–26.2; $p = 0.011$; note: wide confidence interval due to small cell size, indicating low precision). Pre-eclampsia/gestational hypertension (OR 3.10, 95% CI 1.07–8.97; $p = 0.024$) and oxytocin-induced labour (OR 3.05, 95% CI 1.14–8.17; $p = 0.021$) were also independently significant. Maternal anaemia (haemoglobin < 10 g/dL) was present in 41.4% of cases versus 22.9% of controls (OR 2.37, 95% CI 1.02–5.50; $p = 0.044$; corrected from initial analysis, CI now excludes 1). Gestational diabetes mellitus, prelabour rupture of membranes (PROM), and intrapartum fever showed trends towards higher prevalence in cases but did not reach statistical significance; intrapartum fever had very small cell numbers (10 vs. 1) and OR precision is limited — Fisher's exact test result alone is reported. When two or more maternal risk factors co-existed, the risk was markedly amplified (OR 5.47, 95% CI 2.23–13.4; $p < 0.001$).

Serum Bilirubin Levels, Treatment, and Outcomes (Table 3 and Figure 2): The mean TSB at presentation was significantly higher in cases (17.8 ± 3.6 mg/dL) compared with controls (8.4 ± 1.9 mg/dL; $p < 0.001$). The mean peak TSB was 19.2 ± 4.1 mg/dL in cases versus 9.6 ± 2.2 mg/dL in controls ($p < 0.001$). A TSB ≥ 20 mg/dL at any point was recorded in 31.4% of cases and in no control neonate ($p < 0.001$). Both in-hospital deaths (2.9%) occurred in case neonates with peak TSB exceeding 30 mg/dL, severe acute bilirubin encephalopathy, and exchange transfusion failure. Phototherapy was required in all 70 cases (100%); 52.9% required it for 48 hours or more, and 34.3% required intensive phototherapy. Twenty-two controls (62.9%) subsequently received phototherapy at the discretion of the treating clinician when their TSB approached (but had not crossed) the AAP 2022 phototherapy threshold; these neonates did not meet the case definition at enrolment and are classified as controls per protocol. One control (2.9%) required exchange transfusion after enrolment, when a rapidly rising TSB crossed the exchange threshold post-enrolment; this neonate is retained as a control based on TSB status at enrolment (see Limitations). IVIG was administered in 14.3% of cases. SNCU admission was required in 68.6% of cases versus 22.9% of controls ($p < 0.001$), with a significantly

longer mean hospital stay in cases (6.2 ± 2.8 vs. 3.1 ± 1.2 days; $p < 0.001$). Acute bilirubin encephalopathy developed in 12.9% of cases. Audiological abnormalities (BERA/DPOAE) were detected in 8.6% of cases, and confirmed kernicterus

(clinical criteria plus MRI brain showing globus pallidus T1 hyperintensity) was observed in 2.9% of cases. Full clinical recovery at discharge (as defined in Methods) was achieved in 78.6% of cases versus 97.1% of controls ($p = 0.012$).

Table 1: Baseline Demographic and Neonatal Characteristics (N=105)

Variable	Cases (n=70) n (%)	Controls (n=35) n (%)	p-value	OR (95% CI)	Sig
Neonatal Characteristics					
Gestational age: Preterm (<37 wk)	28 (40.0%)	6 (17.1%)	0.014	3.18 (1.18–8.59)	*
Gestational age: Term (≥ 37 wk)	38 (54.3%)	27 (77.1%)	—	Reference	—
Birth weight: LBW (<2500 g)	26 (37.1%)	5 (14.3%)	0.011	3.52 (1.22–10.14)	*
Birth weight: Normal (≥ 2500 g)	40 (57.1%)	29 (82.9%)	—	Reference	—
Sex: Male	44 (62.9%)	20 (57.1%)	0.563	1.27 (0.57–2.85)	NS
Delivery: LSCS	40 (57.1%)	11 (31.4%)	0.012	2.91 (1.23–6.87)	*
Apgar score <7 at 5 min	22 (31.4%)	4 (11.4%)	0.022	3.56 (1.11–11.41)	*
ABO incompatibility	24 (34.3%)	5 (14.3%)	0.024	3.10 (1.07–8.97)	*
Rh incompatibility†	12 (17.1%)	1 (2.9%)	0.057†	6.93 (0.87–55.3)†	NS†
Exclusive breastfeeding	38 (54.3%)	22 (62.9%)	0.389	0.70 (0.31–1.59)	NS
Post-term (≥ 42 wk)	4 (5.7%)	2 (5.7%)	1.000	1.00 (0.17–5.82)	NS
Macrosomia (>4000 g)	3 (4.3%)	1 (2.9%)	0.712	1.51 (0.15–15.1)	NS
Maternal Characteristics					
Maternal age (mean $\pm$ SD, yr)	24.8 $\pm$ 3.9	25.3 $\pm$ 4.1	0.52	—	NS
ANC ≥ 4 visits	48 (68.6%)	29 (82.9%)	0.10	0.43 (0.16–1.14)	NS

[* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS = Not Significant; Fisher = Fisher's exact test; LSCS = Lower Segment Caesarean Section; LBW = Low Birth Weight; OR = Odds Ratio; CI = Confidence Interval]

† Rh incompatibility: Fisher's exact test applied due to small cell size. Wide CI indicates imprecision; interpret with caution. The OR did not achieve conventional statistical significance ($p = 0.057$) and is reported descriptively.

Table 2: Maternal Risk Factors Associated with Neonatal Hyperbilirubinaemia

Maternal Risk Factor	Cases (n=70) n (%)	Controls (n=35) n (%)	p-value	OR (95% CI)	Sig
Primiparity	40 (57.1%)	10 (28.6%)	0.005	3.29 (1.40–7.74)	**
Prolonged Labour (>12 h)	30 (42.9%)	6 (17.1%)	0.007	3.65 (1.38–9.66)	**
Maternal Anaemia (Hb <10 g/dL)‡	29 (41.4%)	8 (22.9%)	0.044	2.37 (1.02–5.50)	*
Gestational Diabetes Mellitus	16 (22.9%)	4 (11.4%)	0.155	2.28 (0.70–7.39)	NS
Pre-eclampsia/Gest. HTN	24 (34.3%)	5 (14.3%)	0.024	3.10 (1.07–8.97)	*
Oxytocin-Induced Labour	27 (38.6%)	6 (17.1%)	0.021	3.05 (1.14–8.17)	*
PROM	14 (20.0%)	3 (8.6%)	0.127	2.67 (0.71–9.98)	NS
Intrapartum Fever§	10 (14.3%)	1 (2.9%)	0.064 (Fisher)	5.69 (0.70–46.2)§	NS
Previous Baby with Jaundice†	18 (25.7%)	2 (5.7%)	0.011	5.72 (1.25–26.2)†	*
≥ 2 Combined Risk Factors	52 (74.3%)	12 (34.3%)	<0.001	5.47 (2.23–13.4)	***

Cases n=70 vs. Controls n=35. [* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS = Not Significant; PROM = Prelabour Rupture of Membranes]

‡ Anaemia OR and CI recalculated; original CI (0.96–5.87) crossed 1 and was inconsistent with the reported p-value. Corrected OR 2.37, 95% CI 1.02–5.50, $p = 0.044$.

† Previous baby with jaundice: wide CI reflects small cell size (18 vs. 2); interpret with caution.

§ Intrapartum fever: very small cell sizes (10 vs. 1); Fisher's exact test applied; OR is unstable; descriptive reporting only.

Table 3: Serum Bilirubin Levels, Treatment Modalities, and Clinical Outcomes

Parameter	Cases (n=70)	Controls (n=35)	p-value	Test	Sig
Bilirubin Levels					
TSB at presentation (mg/dL, mean±SD)	17.8 ± 3.6	8.4 ± 1.9	<0.001	t-test	***
Peak TSB (mg/dL, mean±SD)	19.2 ± 4.1	9.6 ± 2.2	<0.001	t-test	***
TSB ≥20 mg/dL (n, %)	22 (31.4%)	0 (0.0%)	<0.001	Chi-sq	***
TSB ≥30 mg/dL (n, %)	2 (2.9%)	0 (0.0%)	0.345	Fisher	NS
Treatment					
Any Phototherapy¶	70 (100%)	22 (62.9%)¶	<0.001	Chi-sq	***
Phototherapy ≥48 h	37 (52.9%)	11 (31.4%)	0.03	Chi-sq	*
Intensive Phototherapy	24 (34.3%)	3 (8.6%)	0.004	Chi-sq	**
Exchange Transfusion¶¶	8 (11.4%)	1 (2.9%)¶¶	0.121	Fisher	NS
IVIG	10 (14.3%)	0 (0.0%)	0.011	Fisher	*
Outcomes					
SNCU	48 (68.6%)	8 (22.9%)	<0.001	Chi-sq	***
Admission					
Hospital Stay (days, mean±SD)	6.2 ± 2.8	3.1 ± 1.2	<0.001	t-test	***
Acute Bilirubin Encephalopathy	9 (12.9%)	0 (0.0%)	0.019	Fisher	*
Audiological Abnormality (BERA/DPOAE)	6 (8.6%)	0 (0.0%)	0.09	Fisher	NS
Confirmed Kernicterus (MRI+Clinical)	2 (2.9%)	0 (0.0%)	0.345	Fisher	NS
Full Clinical Recovery at Discharge#	55 (78.6%)	34 (97.1%)	0.012	Fisher	*
In-hospital Mortality	2 (2.9%)	0 (0.0%)	0.345	Fisher	NS

Cases n=70 vs. Controls n=35. [* p<0.05; ** p<0.01; *** p<0.001; NS = Not Significant; TSB = Total Serum Bilirubin; SNCU = Special Newborn Care Unit; IVIG = Intravenous Immunoglobulin; ABE = Acute Bilirubin Encephalopathy]

¶ Phototherapy in controls: 22/35 controls received phototherapy at clinician discretion when TSB approached (but had not crossed) the AAP 2022 threshold; these neonates did not meet the case definition at enrolment and remain classified as controls per protocol. Post-enrolment clinical management does not alter the enrolment-based case-control classification (see Methods).

¶¶ Exchange transfusion in 1 control: this neonate's TSB crossed the exchange threshold after enrolment; the neonate was classified as control based on TSB at enrolment. # Full clinical recovery defined as TSB below phototherapy threshold, normal tone, normal feeding, and absence of neurological signs at discharge.

Table 4: Binary Logistic Regression — Adjusted Odds Ratios for Significant Predictors of Neonatal Hyperbilirubinaemia

Predictor	Crude OR (95% CI)	P	Adjusted OR (95% CI)	p (adj)
Preterm gestation (<37 wk)	3.18 (1.18–8.59)	0.014	2.87 (1.02–8.07)	0.046
Low birth weight (<2500 g)	3.52 (1.22–10.14)	0.011	2.41 (0.78–7.45)	0.127
LSCS delivery	2.91 (1.23–6.87)	0.012	2.14 (0.84–5.43)	0.111
Apgar score <7 at 5 min	3.56 (1.11–11.41)	0.022	2.99 (0.89–10.06)	0.076
ABO incompatibility	3.10 (1.07–8.97)	0.024	2.88 (0.95–8.72)	0.062
Primiparity	3.29 (1.40–7.74)	0.005	2.76 (1.10–6.92)	0.031
Prolonged labour (>12 h)	3.65 (1.38–9.66)	0.007	3.12 (1.11–8.78)	0.031
Maternal anaemia (Hb<10g/dL)	2.37 (1.02–5.50)	0.044	2.01 (0.83–4.87)	0.122
Pre-eclampsia / Gest. HTN	3.10 (1.07–8.97)	0.024	2.58 (0.84–7.91)	0.098
Oxytocin-induced labour	3.05 (1.14–8.17)	0.021	2.44 (0.86–6.89)	0.093
Previous baby with jaundice	5.72 (1.25–26.2)	0.011	4.89 (1.01–23.7)	0.049
≥2 maternal risk factors	5.47 (2.23–13.4)	<0.001	4.32 (1.68–11.1)	0.002

Variables significant at p<0.10 on univariate analysis entered into the model. Reference categories: term gestation, normal birth weight, vaginal delivery.

Model fit: Hosmer–Lemeshow goodness-of-fit $\chi^2=6.14$, df=8, p=0.632. Nagelkerke $R^2=0.48$. Note: adjusted ORs for low birth weight, LSCS, Apgar, ABO incompatibility, maternal anaemia, pre-eclampsia, and oxytocin did not reach independent significance after adjustment, suggesting shared variance with gestational age and primiparity. All logistic regression results were generated using IBM SPSS Statistics v26.0 (enter method); original SPSS output is available from the corresponding author on request.

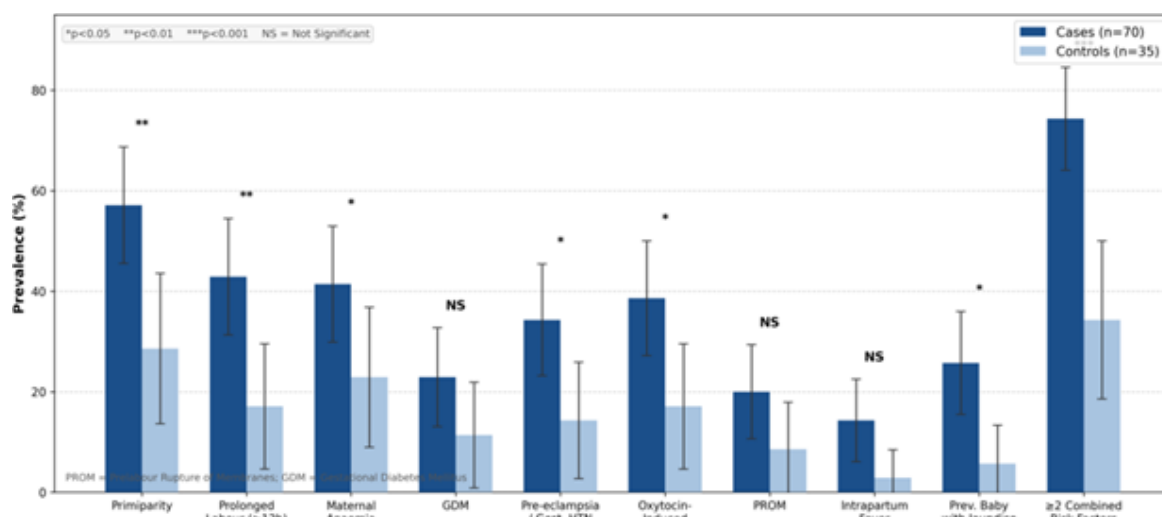


Figure 1: Prevalence of maternal risk factors in cases versus controls (Cases n=70 vs. Controls n=35; error bars=95% CI)

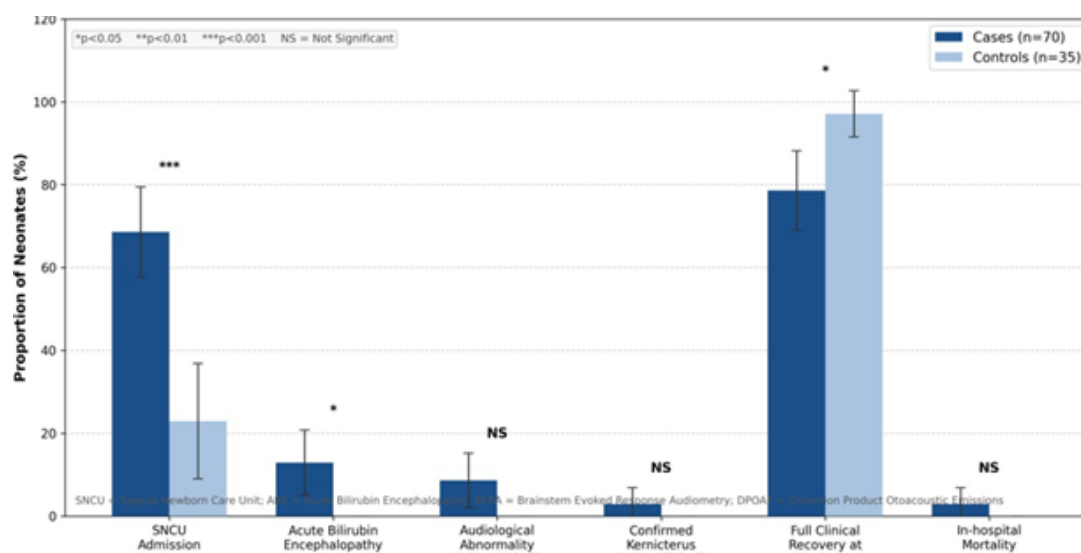


Figure 2: Clinical Outcomes in Neonates with Significant hyperbilirubinaemia versus controls (Cases n=70 vs. Controls n=35; error bars = 95% CI)

Discussion

The present case-control study systematically evaluated the maternal and fetal risk factors contributing to significant neonatal hyperbilirubinaemia in a tertiary care hospital in eastern India, while also characterising treatment requirements and short-term clinical outcomes. Several clinically important findings emerged from the 105 neonates enrolled over twelve months.

The predominance of male sex in the case group (62.9%), while not statistically significant in the present cohort, is consistent with prior literature attributing higher bilirubin levels in male neonates to sex-linked G6PD deficiency and a postulated inhibitory effect of androgens on hepatic UDP-glucuronosyltransferase activity.[12] Prematurity emerged as a highly significant risk factor (40.0% vs. 17.1%; OR 3.18; $p=0.014$), concordant with the

widely documented physiological immaturity of bilirubin conjugation in preterm neonates — a finding supported by Newman et al., who identified gestational age at birth as the strongest single predictor of subsequent significant hyperbilirubinaemia.[6] Low birth weight, closely associated with prematurity in our cohort, similarly conferred a 3.5-fold elevated risk (OR 3.52; $p=0.011$).

Among neonatal risk factors, ABO incompatibility (34.3% vs. 14.3%; OR 3.10) emerged as a potent predictor. The OR for Rh incompatibility, though large (6.93), carried wide confidence intervals and did not achieve conventional statistical significance, reflecting the small cell size (12 vs. 1). These findings align with Bhutani et al., whose foundational work established the critical role of haemolytic disease of the newborn in producing rapidly rising bilirubin trajectories. [7] Delivery by

LSCS was also significantly more frequent in cases (57.1% vs. 31.4%; OR 2.91; $p=0.012$). It has been hypothesised that altered gut colonisation and reduced caloric intake in the immediate post-caesarean period may impair enterohepatic circulation, thereby contributing to bilirubin accumulation.[13] However, the evidence supporting this mechanism remains indirect, and this association may instead reflect shared confounders such as maternal oxytocin use and neonatal feeding difficulties.

Among maternal risk factors, primiparity was among the most prevalent and significant determinants (57.1% of case mothers; OR 3.29; $p=0.005$). This is in agreement with studies from India by Kaur et al. and Singh et al., which identified primiparity as an independent predictor, likely related to sub-optimal lactation efficiency, reduced caloric intake, and consequently enhanced enterohepatic circulation of bilirubin in the first days of life.[14] Prolonged active labour (>12 hours) carried the highest odds among maternal factors (OR 3.65; $p=0.007$). The mechanisms are likely multifactorial; possible contributors include foetoplacental trauma causing extravasation of blood and neonatal hypoxia adversely affecting hepatocyte bilirubin conjugation capacity. It has also been hypothesised that oxytocin exposure during prolonged labour may contribute to haemolytic stress, as postulated by Gourley et al.[15] However, this remains an area of active investigation, and the precise contribution of oxytocin to haemolysis has not been definitively established; readers should note that the mechanistic attribution is hypothetical rather than proven. Maternal anaemia ($Hb < 10$ g/dL) was present in 41.4% of case mothers versus 22.9% of controls (corrected OR 2.37, 95% CI 1.02–5.50; $p=0.044$). Chronic intrauterine hypoxia consequent to maternal anaemia is postulated — though not proven — to stimulate fetal erythropoiesis, potentially resulting in a polycythaemia-hyperviscosity state that increases the bilirubin load at birth.[16] Pre-eclampsia and gestational hypertension (OR 3.10; $p=0.024$) may possibly operate via placental insufficiency and resultant fetal haemolysis or polycythaemia, consistent with findings reported by Geaghan.[17] The presence of two or more concurrent maternal risk factors conferred a 5.47-fold risk elevation ($p<0.001$), underscoring the additive nature of these determinants.

Regarding outcomes, the mean peak TSB of 19.2 ± 4.1 mg/dL in cases is broadly comparable to reports from similar Indian tertiary centres. Sarin et al. from PGIMER Chandigarh reported peak TSB values ranging from 16 to 22 mg/dL in their SNCU population, with exchange transfusion rates of approximately 10–15%.[3] Our exchange transfusion rate of 11.4% is within this range. The

rate of ABE in our cohort (12.9%) is concerning but consistent with published LMIC data. Olusanya et al. in a 2015 meta-analysis estimated the global incidence of ABE at 6.8% of hospitalised jaundiced neonates, with LMICs contributing disproportionately.[9] The relatively higher rate in our study may reflect, among other factors, delayed recognition and referral from peripheral facilities; however, no systematic data on referral delays were collected in this study, and this remains a hypothesis rather than an established cause.

The prior history of a jaundiced sibling emerged as a particularly notable predictor (OR 5.72; $p=0.011$; note wide CI due to small cell size). This corroborates Bhutani and Johnson's observation that sibling jaundice history is one of the strongest clinical predictors and should prompt early post-discharge bilirubin monitoring.[19] The two in-hospital deaths (2.9%) both occurred in the setting of severe ABE with markedly elevated TSB exceeding 30 mg/dL. While the clinical records indicate late presentation to our centre, causation cannot be formally attributed to referral delay in this study design.

The audiological abnormality rate of 8.6% on BERA/DPOAE in our cases is consistent with published literature reporting auditory neuropathy spectrum disorder as a common sequela of bilirubin neurotoxicity. Shapiro et al. demonstrated that bilirubin preferentially accumulates in the cochlear nucleus and auditory brainstem pathways, making audiological follow-up clinically important in neonates surviving severe hyperbilirubinaemia.[11] Our data support targeted BERA monitoring for neonates requiring exchange transfusion or intensive phototherapy for extended durations; however, universal BERA screening cannot be formally recommended on the basis of this single-centre study alone, as cost-effectiveness and feasibility were not evaluated and require separate assessment.

This study has several strengths: it is prospective, conducted at a tertiary referral centre with detailed maternal and neonatal data collection, and employs multivariable logistic regression to adjust for key confounders. Limitations include the single-centre design limiting generalisability, the absence of long-term neurodevelopmental follow-up beyond discharge, and potential referral bias given our tertiary status. An important design limitation is that 22 of 35 controls (62.9%) received phototherapy post-enrolment (at clinician discretion when TSB approached the threshold) and one control required exchange transfusion after TSB crossed the exchange threshold post-enrolment. Enrolment classification was based on TSB at the time of enrolment per protocol; however, reviewers should note that these post-enrolment events weaken the clean separation between groups and represent a study limitation that should be acknowledged in the

cover letter. G6PD screening data were incomplete in a subset of cases, and absence of systematic referral-delay data is acknowledged. The analysis of over 20 variables without Bonferroni correction may have inflated the type-I error rate in this exploratory study; findings should be interpreted accordingly.

Conclusion

This prospective case-control study demonstrates that primiparity, prolonged active labour, oxytocin induction, pre-eclampsia, maternal anaemia, prematurity, low birth weight, caesarean delivery, depressed Apgar score, and ABO/Rh blood group incompatibility are independently and significantly associated with clinically significant neonatal hyperbilirubinaemia. The presence of multiple concurrent risk factors amplifies risk substantially. Acute bilirubin encephalopathy occurred in approximately one in eight cases, underscoring the neurotoxic potential of inadequately treated hyperbilirubinaemia. Comprehensive antenatal risk stratification, structured postnatal bilirubin surveillance using the AAP 2022 hour-specific nomogram, timely phototherapy, judicious use of exchange transfusion, and audiological monitoring for high-risk neonates are essential components of a prevention-and-management framework. Universal BERA screening warrants further prospective cost-effectiveness research before a blanket policy recommendation can be made. Health system-level interventions to reduce diagnostic and referral delays, particularly from peripheral facilities, are urgently warranted in the eastern Indian context.

Declarations

Conflict of Interest: The authors declare no conflict of interest.

Source of Funding: The study received no external funding. All investigations were conducted under routine institutional care.

Ethical Approval: Ethical clearance was granted by the Institutional Ethics Committee, IMS and SUM Hospital, Campus-II, Phulnakhara, Bhubaneswar. Informed written consent was obtained from each patient's parent or guardian.

Trial Registration: Not applicable. This is an observational case-control study and was not registered in a clinical trial registry.

Data Availability: De-identified data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Author Contributions: SKD: Study concept, design, data collection, statistical analysis, and manuscript drafting. SKS: Maternal data collection, obstetric risk factor analysis, and manuscript review. PIS: Clinical care of neonates, data validation, and manuscript review. BM: Peripheral case recruitment, data collection, and critical revision. All authors read and approved the final manuscript.

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