

Hepatobiliary Dysfunction in an Egyptian Tertiary Neonatal Intensive Care Unit: Incidence, Risk Factors, and Mortality Outcomes

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Abstract

Background

To determine the incidence and etiologic pattern of hepatobiliary dysfunction (HBD) and to identify independent predictors of HBD and in-hospital mortality in a tertiary Egyptian neonatal intensive care unit (NICU).

Methods

Retrospective cohort study of 236 consecutive NICU admissions at the tertiary referral NICU of Ain Shams University Children's Hospital, Cairo, Egypt (June–December 2024) with multivariable logistic regression models for HBD and all-cause in-hospital mortality.

Results

HBD occurred in 64 of 236 neonates (27.1%): isolated hepatocellular injury in 10.2%, isolated cholestasis in 9.3%, and combined injury in 7.6%. Total parenteral nutrition (TPN) exposure (adjusted odds ratio [aOR] 4.15; 95% confidence interval [CI] 1.68–10.23) and multi-organ failure (aOR 2.95; 95% CI 1.10–7.88) independently predicted HBD (optimism-corrected area under the curve [AUC] 0.75). Mortality was 24.6% (58/236), higher with HBD than without (53.1 vs. 14.0%; $P < 0.001$), rising across phenotypes to 83.3% with combined injury ($P = 0.005$). In the nine-covariate mortality model, respiratory support (aOR 14.36), multi-organ failure (aOR 9.46), TPN exposure (aOR 8.15), cardiac/vasopressor support (aOR 6.47), and lower birth weight (aOR 0.90 per 100 g) were independent predictors (optimism-corrected AUC 0.96). HBD did not independently predict overall mortality (aOR 2.40; 95% CI 0.76–7.57).

Conclusion

HBD affects more than one-quarter of NICU admissions in this Egyptian cohort and is independently associated with TPN exposure and multi-organ failure. HBD marks systemic critical illness rather than independently driving overall mortality.

Key Points

1. HBD occurred in 27.1% of NICU admissions
2. TPN and multi-organ failure predict HBD
3. Mortality rises steeply across HBD phenotypes
4. Death hazard rises > 3-fold after HBD onset

Background

Hepatobiliary dysfunction (HBD) in neonates encompasses a spectrum of liver pathology including isolated cholestasis, isolated hepatocellular injury, and combined injury. Neonatal cholestasis is broadly defined as direct (conjugated) bilirubin exceeding 20% of total bilirubin when total bilirubin exceeds 5 mg/dL, or direct bilirubin greater than or equal to 1 mg/dL when total bilirubin is less than 5 mg/dL. [1] HBD in the NICU extends beyond cholestasis to include hepatocellular injury defined by elevated alanine aminotransferase (ALT), an entity less frequently studied but of potential prognostic significance in critically ill neonates.

The pathogenesis of NICU-acquired HBD is multifactorial. Parenteral nutrition-associated cholestasis (PNAC) is the paradigmatic and most prevalent form, arising from prolonged enteral fasting, suppression of the enterohepatic bile acid circulation, phytosterol-mediated inhibition of the hepatocytic bile salt export pump (BSEP), and the hepatotoxic effects of soybean-based lipid emulsions [2, 3]. Multi-organ failure, sepsis, and hypoxic-ischemic injury further compound hepatic vulnerability by impairing bile acid transport and hepatocellular integrity. [4] Drug-induced hepatobiliary injury—particularly from azole antifungals—represents an additional and increasingly recognized contributor in NICU settings. [5]

Despite this mechanistic understanding, population-level epidemiological data from NICU settings in low- and middle-income countries (LMICs) remain scarce. Published incidence estimates for HBD in NICUs range widely, reflecting heterogeneity in patient populations, definitions used, and institutional practices. [6, 7] In Egypt, high rates of prematurity, prolonged TPN use, culture-proven sepsis, and limited access to fish oil-based lipid emulsions create a distinct risk landscape that has not been rigorously characterized at the population level. Without local epidemiological data, targeted prevention and surveillance protocols cannot be rationally designed or implemented. [8]

This study therefore aimed to: (1) determine the incidence and etiological distribution of HBD in a tertiary Egyptian NICU; (2) identify independent predictors of HBD development and all-cause in-hospital mortality in this cohort; and (3) evaluate whether the individual HBD phenotypes (isolated hepatocellular injury, isolated cholestasis, and combined injury) carry different risks of mortality and prolonged NICU stay.

PATIENTS AND METHODS

Study Design and Setting

This was a retrospective cohort study of consecutively admitted neonates at the NICU of Ain Shams University Children's Hospital, a tertiary academic referral center in Cairo, Egypt, conducted between June 1, 2024 and December 31, 2024. All consecutive NICU admissions during the study period were included, and no diagnostic exclusion criteria were applied; neonates with congenital hepatobiliary disorders (n = 2) and inborn errors of metabolism (n = 6) were retained in the cohort and characterized descriptively. Abdominal ultrasonography was performed in all neonates with cholestasis to exclude

structural hepatobiliary anomalies, including biliary atresia; no case of biliary atresia or surgical gastrointestinal malformation requiring exclusion was identified. The unit is a Level III university NICU with 25 incubator beds that receives exclusively outborn neonates referred from delivery units and hospitals across Greater Cairo and neighboring governorates for surgical, cardiac, and complex medical care. Low-acuity neonates—such as those requiring brief observation for delayed transition, routine glucose monitoring as infants of diabetic mothers, or uncomplicated jaundice treated with phototherapy alone—are managed in the well-baby nursery and are not admitted to the NICU; the study population therefore represents a high-acuity, referral-weighted case mix rather than an unselected newborn population. Primary indications for admission were surgical conditions (45/236; 19.1%), severe neonatal jaundice (43; 18.2%), respiratory distress (31; 13.1%), suspected neonatal sepsis (27; 11.4%), congenital anomalies (28; 11.9%), congenital heart disease (20; 8.5%), poor growth or feeding difficulty (15; 6.4%), hematological disorders (13; 5.5%), neurological disorders (10; 4.2%), metabolic disorders (3; 1.3%), and oliguria (1; 0.4%).

Definitions

Hepatobiliary dysfunction (HBD) was defined as the presence of one or more of the following during NICU admission: (1) Isolated hepatocellular injury: ALT > 50 U/L in the absence of cholestasis. This threshold was applied as a universal pragmatic cutoff and was not adjusted for gestational age, because validated gestational-age-specific ALT reference intervals do not exist for neonates; the cutoff approximates the upper bound of upper-limit-of-normal values used in children's hospitals. [9], (2) Isolated cholestasis: operationally defined as total serum bilirubin > 2 mg/dL with a direct (conjugated) fraction exceeding 20% of total bilirubin, with normal ALT. This operational criterion parallels, but is not identical to, the NASPGHAN/ESPGHAN/FISPGHAN position-paper thresholds, [1] and is addressed in the Limitations. (3) Combined HBD: coexistent hepatocellular injury and cholestasis meeting both criteria above.

TPN exposure was recorded as a binary variable indicating receipt of TPN at any time during NICU admission; TPN duration in days could not be reliably retrieved for all patients, so exposure was not duration-stratified. Parenteral nutrition-associated cholestasis (PNAC) was accordingly designated when cholestasis developed in a TPN-exposed neonate in the absence of another identifiable primary cause. [2]

Multi-organ failure (MOF) was defined as simultaneous dysfunction of ≥ 2 organ systems (respiratory, cardiovascular, renal, hepatic, or hematologic) by standard neonatal criteria. Sepsis was defined as culture-proven bacteremia or fungemia, or clinical sepsis meeting standard neonatal systemic inflammatory response criteria in the presence of a suspected infectious source; in the regression models, sepsis was operationalized as this composite (culture-proven or clinical) binary variable, and blood-culture positivity is additionally reported separately. Hypoxia was operationalized as chart-documented hypoxemia (sustained peripheral oxygen saturation < 90% requiring initiation or escalation

of oxygen or respiratory support) or a documented perinatal hypoxic–ischemic event, abstracted as a binary variable.

Sepsis was defined as culture-proven bloodstream infection or clinical sepsis (clinical signs of infection with supportive laboratory markers prompting a full sepsis evaluation and a therapeutic course of antibiotics); both categories were combined for the binary sepsis variable.

Data Collection

Data were abstracted from NICU case records. Demographic data included gestational age (weeks), birth weight (grams), sex, mode of delivery, and Apgar scores at 1 and 5 minutes. Anthropometric measurements were plotted on the Fenton preterm growth charts. [10] Clinical data included primary diagnosis at NICU admission, TPN exposure, timing of enteral feeding initiation, type and duration of respiratory support, requirement for cardiac/vasopressor support (dobutamine, dopamine, noradrenaline, or adrenaline), presence of umbilical venous catheter (UVC), occurrence of sepsis and MOF, presence of maternal comorbidities, and length of NICU stay. Laboratory parameters recorded included: complete blood count with differential, C-reactive protein (CRP), ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), serum total and direct bilirubin, albumin, prothrombin time (PT), partial thromboplastin time (PTT), international normalized ratio (INR), serum sodium, potassium, blood urea nitrogen (BUN), serum creatinine, and procalcitonin. Blood culture results were recorded.

Statistical Analysis

Categorical variables are presented as frequencies and percentages. Continuous variables are presented as mean $\pm$ standard deviation (SD) for normally distributed data or median with interquartile range (IQR) for skewed distributions. The Kolmogorov-Smirnov test was applied to evaluate normality. Between-group comparisons for categorical variables used the chi-square test or Fisher's exact test as appropriate; for continuous variables, the independent-samples t-test or Mann-Whitney U test was used accordingly.

Two separate multivariable binary logistic regression models were constructed. Model 1 (predictors of HBD development): candidate predictors clinically plausible and associated with HBD at $P < 0.20$ on univariate analysis were considered. No a priori sample-size calculation was performed; all consecutive admissions during the study period were included, and events-per-variable (EPV) ratios are reported for each model. Model 1 entered TPN exposure, multi-organ failure, sepsis (culture-proven or clinical), respiratory support, gestational age, and birth weight. Model 2 (predictors of in-hospital mortality) entered respiratory support, multi-organ failure, TPN exposure, cardiac/vasopressor support, HBD status, hypoxia, and umbilical venous catheter, with gestational age and birth weight. Outcomes (in-hospital mortality and NICU length of stay) were additionally compared across the three HBD phenotypes using the chi-square/Fisher's exact test and the Kruskal-Wallis test.

Multicollinearity was assessed by variance inflation factor (VIF); predictors with $VIF > 5$ were excluded, and VIFs are reported for both models. Variables were entered simultaneously using the enter method. Model calibration was assessed by the Hosmer-Lemeshow goodness-of-fit test; discrimination by the area under the receiver operating characteristic curve (AUC); and explained variance by the Nagelkerke pseudo- R^2 . Results are reported as adjusted odds ratios (aOR) with 95% confidence intervals (CI) and two-tailed P values. Statistical significance was defined as $P < 0.05$. Internal validation of both models used Harrell's bootstrap optimism-correction procedure with 500 resamples: each model was refitted in every bootstrap sample, optimism was estimated as the mean difference between the bootstrap-sample and original-sample AUC, and the corrected AUC was obtained by subtracting this optimism from the apparent AUC. [11] To account for time to death rather than its occurrence alone, Cox proportional hazards regression was additionally fitted with NICU length of stay as the time scale and the same nine covariates; the proportional hazards assumption was examined using scaled Schoenfeld residuals. Because HBD develops during—not before—admission, a second Cox model treated HBD as a time-varying exposure, switching exposure status on the documented day of HBD onset. [12] Given the 22 between-group laboratory and microbiological comparisons, Benjamini–Hochberg false-discovery-rate (FDR) correction was applied; comparisons that did not remain significant at FDR $q < 0.05$ are identified in the corresponding table footnote. [13] Because TPN exposure and HBD accrue over the NICU stay, associations with mortality are susceptible to immortal-time/survivorship bias; [12] a day-14 landmark sensitivity analysis therefore re-estimated Model 2 after excluding neonates who died before day 14 of admission. Given the limited number of outcome events, adjusted estimates with wide confidence intervals were interpreted in light of potential sparse-data bias. [14] Two further sensitivity analyses were performed: (1) re-derivation of the cholestasis component of HBD using the guideline (NASPGHAN/ESPGHAN/FISPGHAN) threshold of direct bilirubin ≥ 1.0 mg/dL, [1] with repetition of the phenotype and mortality analyses; and (2) exclusion of the 43 neonates admitted primarily for unconjugated neonatal jaundice, to assess robustness to case-mix. This study is reported in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement. [15] Complete-case analysis was applied for laboratory variables with missing values; the exact number and percentage of missing values for each laboratory parameter, overall and by HBD status, are reported in Supplementary Table S1, and percentages for laboratory-derived categorical variables use non-missing denominators. All analyses were performed using IBM SPSS Statistics, version 26 (IBM Corp., Armonk, NY, USA), with cross-validation in Python (statsmodels 0.14; survival analyses and bootstrap validation in lifelines and scikit-learn).

Ethical Considerations

This study was conducted in accordance with the ethical standards of the Ain Shams University Faculty of Medicine Institutional Review Board and with the principles of the Declaration of Helsinki (revised 2013). The REC approval number FMASU MS 232/2025. Given the retrospective design based on de-identified data abstracted from existing medical records, the requirement for individual informed consent was waived by the Research Ethics Committee.

RESULTS

Epidemiologic Data: (Table 1)

Table 1

Baseline Demographic and Clinical Characteristics of the Study Cohort (N = 236)

Characteristic	Total (N = 236)	Group A: No HBD (n = 172)	Group B: HBD (n = 64)	P-value
Gestational age (weeks), mean ± SD	36.69 ± 2.43	36.74 ± 2.48	36.53 ± 2.32	0.551
Birth weight (g), mean ± SD	2,593.9 ± 703.7	2,618.2 ± 676.6	2,528.8 ± 773.8	0.386
Male sex, n (%)	124 (52.5%)	86 (50.0%)	38 (59.4%)	0.256
Preterm (< 37 weeks), n (%)	78 (33.1%)	56 (32.6%)	22 (34.4%)	0.914
VLBW (< 1,500 g), n (%)	15 (6.4%)	9 (5.2%)	6 (9.4%)	0.39
Apgar score 1 min, mean ± SD	5.91 ± 1.55	6.12 ± 1.49	5.36 ± 1.59	< 0.001
Apgar score 5 min, mean ± SD	8.39 ± 1.15	8.54 ± 1.13	7.98 ± 1.11	< 0.001
NICU length of stay (days), mean ± SD	15.42 ± 13.49	14.23 ± 13.31	18.62 ± 13.56	0.026
Respiratory support required, n (%)	88 (37.3%)	50 (29.1%)	38 (59.4%)	< 0.001
Hypoxia, n (%)	96 (40.7%)	55 (32.0%)	41 (64.1%)	< 0.001
TPN exposure, n (%)	88 (37.3%)	43 (25.0%)	45 (70.3%)	< 0.001
Multi-organ failure, n (%)	26 (11.0%)	9 (5.2%)	17 (26.6%)	< 0.001
Umbilical venous catheter, n (%)	27 (11.4%)	15 (8.7%)	12 (18.8%)	0.055
Cardiac/vasopressor support, n (%)	61 (25.8%)	27 (15.7%)	34 (53.1%)	< 0.001
In-hospital mortality, n (%)	58 (24.6%)	24 (14.0%)	34 (53.1%)	< 0.001
VLBW: very low birth weight; TPN: total parenteral nutrition; NICU: neonatal intensive care unit; SD: standard deviation; HBD: hepatobiliary dysfunction.				

A total of 236 neonates were consecutively enrolled. The cohort comprised 124 males (52.5%) and 112 females (47.5%). Delivery was by cesarean section in 207 neonates (87.7%) and vaginal in 29 (12.3%). The mean gestational age was 36.69 ± 2.43 weeks (range 28–41 weeks), and 78 neonates (33.1%) were preterm (< 37 weeks). Mean birth weight was 2,593.9 ± 703.7 g (range 900–5,000 g); 15 (6.4%) had very low birth weight (< 1,500 g). Mean Apgar scores at 1 and 5 minutes were 5.91 ± 1.55 and 8.39 ± 1.15, respectively. Mean NICU length of stay was 15.42 ± 13.49 days (range 1–101 days). Neonatal sepsis

(culture-proven or clinical) occurred in 117 neonates (49.6%), hypoxia in 96 (40.7%), and TPN exposure in 88 (37.3%). Multi-organ failure was documented in 26 neonates (11.0%), umbilical venous catheters in 27 (11.4%), respiratory support in 88 (37.3%), and cardiac/vasopressor support in 61 (25.8%). Overall, in-hospital mortality was 24.6% (58/236).

Incidence and Etiological Pattern of Hepatobiliary Dysfunction

HBD was identified in 64 of 236 neonates (27.1%). Among the total cohort, isolated hepatocellular injury (ALT criterion only) occurred in 24 neonates (10.2%; 37.5% of HBD cases), isolated cholestasis (bilirubin criterion only) in 22 neonates (9.3%; 34.4% of HBD cases), and combined hepatocellular–cholestatic injury in 18 neonates (7.6%; 28.1% of HBD cases). The median age at HBD onset was 5 days (IQR, 3–14). Baseline gestational age and birth weight did not differ significantly between neonates with HBD (Group B, n = 64) and those without HBD (Group A, n = 172), as summarized in Table 1. Among neonates with a cholestatic component (isolated or combined, n = 40), 28 (70.0%) had received TPN, consistent with parenteral nutrition-associated cholestasis (PNAC) as the predominant cholestatic etiology. Drug-induced HBD was identified in 2 neonates (0.8%), both attributed to azole antifungals, with biochemical resolution after drug withdrawal. Inborn errors of metabolism were documented in 6 neonates (2.5%), and congenital hepatobiliary disorders in 2 (0.8%).

Clinical and Laboratory Characteristics by HBD Status (Table 2)

Table 2
Laboratory and Microbiological Characteristics by HBD Status

Parameter	Group A: No HBD (n = 172)	Group B: HBD (n = 64)	P-value
Hematological			
Thrombocytopenia, n (%)	32 (18.6%)	34 (53.1%)	< 0.001
Anemia, n (%)	36 (20.9%)	32 (50.0%)	< 0.001
Leukocytosis, n (%)	33 (19.2%)	27 (42.2%)	< 0.001
Inflammatory / Microbiological			
CRP, median (IQR) mg/L	3.10 (0.80–23.00)	46.70 (9.40–113.25)	< 0.001
Blood culture positive, n (%)	51 (29.7%)	40 (62.5%)	< 0.001
Klebsiella spp. (any), n (%)	15 (8.7%)	13 (20.3%)	0.026
MDR Klebsiella, n (%)	10 (5.8%)	9 (14.1%)	0.072
Candida spp., n (%)	1 (0.6%)	3 (4.7%)	0.062 ^a
Liver Function			
AST, median (IQR) U/L	44.00 (33.00–59.00)	86.50 (48.50–158.00)	< 0.001
ALT, median (IQR) U/L	16.00 (11.25–24.00)	63.50 (22.75–109.25)	< 0.001
Total bilirubin, median (IQR) mg/dL	6.90 (1.82–10.00)	3.30 (2.27–5.30)	0.002
Direct bilirubin, median (IQR) mg/dL	0.50 (0.40–0.70)	0.80 (0.60–1.55)	< 0.001
Serum albumin, median (IQR) g/dL	3.60 (3.30–3.90)	2.75 (2.30–3.50)	< 0.001
Albumin ≤ 3.5 g/dL, n/N (%) ^b	73/160 (45.6%)	49/64 (76.6%)	< 0.001
PT, median (IQR) seconds	16.50 (14.60–19.68)	20.00 (16.80–25.33)	< 0.001
INR, median (IQR)	1.20 (1.10–1.48)	1.50 (1.23–2.01)	< 0.001

CRP: C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; PT: prothrombin time; INR: international normalized ratio; BUN: blood urea nitrogen; IQR: interquartile range; MDR: multidrug-resistant. ^a Fisher's exact test. ^b Percentages use non-missing denominators (see Supplementary Table S1 for missing-data counts). Benjamini–Hochberg false-discovery-rate correction was applied across the 22 comparisons in this table: all associations with P < 0.01 remained significant at q < 0.05, and *Klebsiella* spp. (any) remained significant (q = 0.032), whereas MDR *Klebsiella* (q = 0.079), *Candida* spp. (q = 0.072), serum sodium (q = 0.112), and hyponatremia (q = 0.562) did not.

Parameter	Group A: No HBD (n = 172)	Group B: HBD (n = 64)	P-value
INR > 1.5, n/N (%) ^b	34/154 (22.1%)	31/63 (49.2%)	< 0.001
Renal Function / Electrolytes			
BUN, median (IQR) mg/dL	10.00 (6.50–16.00)	18.00 (7.75–33.25)	< 0.001
Serum creatinine, median (IQR) mg/dL	0.52 (0.41–0.70)	0.70 (0.40–1.19)	0.004
Creatinine > 1.0 mg/dL, n (%)	13 (7.6%)	21 (32.8%)	< 0.001
Serum sodium, median (IQR) mmol/L	139.00 (137.00–141.25)	140.00 (137.50–144.00)	0.107
Hyponatremia (Na < 135 mmol/L), n/N (%) ^b	23/172 (13.4%)	11/63 (17.5%)	0.562
CRP: C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; PT: prothrombin time; INR: international normalized ratio; BUN: blood urea nitrogen; IQR: interquartile range; MDR: multidrug-resistant. ^a Fisher's exact test. ^b Percentages use non-missing denominators (see Supplementary Table S1 for missing-data counts). Benjamini–Hochberg false-discovery-rate correction was applied across the 22 comparisons in this table: all associations with P < 0.01 remained significant at q < 0.05, and <i>Klebsiella</i> spp. (any) remained significant (q = 0.032), whereas MDR <i>Klebsiella</i> (q = 0.079), <i>Candida</i> spp. (q = 0.072), serum sodium (q = 0.112), and hyponatremia (q = 0.562) did not.			

Compared with neonates without HBD (Group A, n = 172), those with HBD (Group B, n = 64) had significantly lower Apgar scores at 1 minute (5.36 ± 1.59 vs. 6.12 ± 1.49 ; $P < 0.001$) and at 5 minutes (7.98 ± 1.11 vs. 8.54 ± 1.13 ; $P < 0.001$). NICU length of stay was longer in Group B than Group A (18.62 ± 13.56 vs. 14.23 ± 13.31 days; $P = 0.026$). Respiratory support (59.4% vs. 29.1%; $P < 0.001$), hypoxia (64.1% vs. 32.0%; $P < 0.001$), TPN exposure (70.3% vs. 25.0%; $P < 0.001$), multi-organ failure (26.6% vs. 5.2%; $P < 0.001$), and cardiac/vasopressor support (53.1% vs. 15.7%; $P < 0.001$) were all significantly more frequent in the HBD group. In-hospital mortality was also markedly higher among neonates with HBD than those without HBD (53.1% vs. 14.0%; $P < 0.001$).

Median C-reactive protein (CRP) was significantly higher in neonates with HBD than in those without HBD (46.70 [IQR, 9.40–113.25] vs. 3.10 [IQR, 0.80–23.00] mg/L; $P < 0.001$). Culture positivity was also more frequent in the HBD group (62.5% vs. 29.7%; $P < 0.001$). *Klebsiella* species isolation was significantly more frequent among neonates with HBD (20.3% vs. 8.7%; $P = 0.026$), whereas multidrug-resistant *Klebsiella* did not reach statistical significance (14.1% vs. 5.8%; $P = 0.072$). *Candida* species were numerically more frequent in the HBD group (4.7% [3/64] vs. 0.6% [1/172]), but this difference was based on only four isolates overall and did not reach statistical significance (Fisher's exact $P = 0.062$); this observation is exploratory. After Benjamini–Hochberg correction across the 22 laboratory and microbiological comparisons in Table 2, the *Klebsiella* association remained significant (q = 0.032), whereas MDR *Klebsiella* (q = 0.079) and *Candida* (q = 0.072) did not.

Liver-related biochemical abnormalities were markedly more pronounced in neonates with HBD. Median AST was 86.50 U/L (IQR, 48.50–158.00) in Group B compared with 44.00 U/L (IQR, 33.00–59.00) in Group A ($P < 0.001$), and median ALT was 63.50 U/L (IQR, 22.75–109.25) versus 16.00 U/L (IQR, 11.25–24.00), respectively ($P < 0.001$). Median direct bilirubin was 0.80 mg/dL (IQR, 0.60–1.55) in the HBD group compared with 0.50 mg/dL (IQR, 0.40–0.70) in the non-HBD group ($P < 0.001$); median total bilirubin was, in contrast, higher in the non-HBD group (6.90 vs. 3.30 mg/dL; $P = 0.002$), reflecting the high proportion of admissions for unconjugated neonatal jaundice among neonates without HBD. Hypoalbuminemia (albumin ≤ 3.5 g/dL) was more common in Group B (76.6% vs. 45.6%; $P < 0.001$), with a lower median serum albumin (2.75 [IQR, 2.30–3.50] vs. 3.60 [IQR, 3.30–3.90] g/dL; $P < 0.001$). Coagulation abnormalities were more frequent in the HBD group, with elevated INR (> 1.5) in 49.2% versus 22.1% ($P < 0.001$), median INR 1.50 versus 1.20 ($P < 0.001$), and median PT 20.00 versus 16.50 seconds ($P < 0.001$).

Renal dysfunction was also more prominent in neonates with HBD. Median BUN was 18.00 mg/dL (IQR, 7.75–33.25) in Group B compared with 10.00 mg/dL (IQR, 6.50–16.00) in Group A ($P < 0.001$); median serum creatinine was higher (0.70 vs. 0.52 mg/dL; $P = 0.004$), and creatinine > 1.0 mg/dL was present in 32.8% versus 7.6%, respectively ($P < 0.001$). Serum sodium did not differ significantly between groups (median 140.00 [IQR, 137.50–144.00] vs. 139.00 [IQR, 137.00–141.25] mmol/L; $P = 0.107$), and the frequency of hyponatremia (< 135 mmol/L) was comparable (17.5% in Group B vs. 13.4% in Group A; $P = 0.562$).

Missing laboratory data were infrequent (Supplementary Table S1). Missingness was $\leq 1.7\%$ for hepatic parameters (ALT, AST, total and direct bilirubin) in both groups and was highest for coagulation indices (INR missing in 8.1% overall: 10.5% of Group A vs. 1.6% of Group B), reflecting less frequent coagulation testing in clinically stable neonates.

Outcomes by HBD Phenotype (Table 3)

Table 3
In-Hospital Mortality and NICU Length of Stay by HBD Phenotype

Group	n	Deaths, n (%)	P-value vs. no HBD ^a	NICU stay, median (IQR), days
No HBD	172	24 (14.0%)	Reference	10 (5–20)
Isolated hepatocellular injury (ALT only)	24	8 (33.3%)	0.033	15 (5–26)
Isolated cholestasis	22	11 (50.0%)	< 0.001	16 (13–20)
Combined hepatocellular–cholestatic injury	18	15 (83.3%)	< 0.001	16 (12–26)
Mortality across the three HBD phenotypes: χ^2 P = 0.005. NICU stay across the three phenotypes: Kruskal–Wallis P = 0.769. Cholestatic component (isolated or combined, n = 40) vs. isolated hepatocellular injury: mortality 65.0% vs. 33.3%, Fisher’s exact P = 0.020; NICU stay P = 0.505. ^a Fisher’s exact test vs. the no-HBD group.				
HBD: hepatobiliary dysfunction; ALT: alanine aminotransferase; IQR: interquartile range; NICU: neonatal intensive care unit.				

In-hospital mortality differed significantly across the three HBD phenotypes: 8 of 24 neonates (33.3%) with isolated hepatocellular injury, 11 of 22 (50.0%) with isolated cholestasis, and 15 of 18 (83.3%) with combined injury died, compared with 24 of 172 (14.0%) neonates without HBD (P = 0.005 across the three phenotypes). Each phenotype carried significantly higher mortality than the non-HBD group (isolated hepatocellular P = 0.033; isolated cholestasis P < 0.001; combined P < 0.001). Neonates with a cholestatic component (isolated or combined, n = 40) had significantly higher mortality than those with isolated hepatocellular injury (65.0% vs. 33.3%; Fisher’s exact P = 0.020). In contrast, NICU length of stay did not differ across phenotypes (median 15, 16, and 16 days, respectively; Kruskal-Wallis P = 0.769), although all exceeded the median stay of neonates without HBD (10 days).

Univariate Predictors of Hepatobiliary Dysfunction:

Univariate analysis identified the following variables as significantly associated (P < 0.05) with HBD development: TPN exposure, positive blood culture, multi-organ failure, Klebsiella species isolation, elevated CRP, respiratory support requirement, hypoxia, lower Apgar scores at 1 and 5 minutes, and longer NICU stay. Candidate predictors entered into Model 1 were: TPN exposure, multi-organ failure, sepsis (culture-proven or clinical), respiratory support requirement, gestational age (retained for clinical plausibility despite P = 0.549 to allow adjustment), and birth weight.

Multivariable Predictors of Hepatobiliary Dysfunction: (Table 4)

Table 4

Multivariable Logistic Regression Analysis: Independent Predictors of Hepatobiliary Dysfunction (Model 1)

Variable	Univariate OR (95% CI)	P-value	Adjusted OR (95% CI) ^a	P-value	VIF
TPN exposure	7.11 (3.76–13.44)	< 0.001	4.15 (1.68–10.23)	0.002	2.08
Multi-organ failure	6.55 (2.74–15.65)	< 0.001	2.95 (1.10–7.88)	0.031	1.23
Sepsis (culture-proven or clinical)	4.03 (2.14–7.58)	< 0.001	1.66 (0.75–3.66)	0.212	1.45
Respiratory support	3.57 (1.96–6.48)	< 0.001	1.20 (0.54–2.69)	0.655	1.60
Gestational age (per week)	0.97 (0.86–1.08)	0.549	1.14 (0.95–1.35)	0.151	1.73
Birth weight (per 100 g)	0.98 (0.94–1.02)	0.385	0.98 (0.92–1.04)	0.492	1.65
^a Adjusted odds ratio from simultaneous entry multivariable logistic regression (enter method). Model fit: Hosmer–Lemeshow $\chi^2 = 10.37$, $P = 0.240$; apparent AUC 0.78; bootstrap optimism-corrected AUC 0.75 (500 resamples); Nagelkerke $R^2 = 0.27$; EPV = 10.7; maximum VIF = 2.08.					
TPN, total parenteral nutrition; NICU, neonatal intensive care unit; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; EPV, events per variable; VIF, variance inflation factor; AUC, area under the receiver operating characteristic curve.					

Six candidate variables—TPN exposure, multi-organ failure, sepsis (culture-proven or clinical), respiratory support, gestational age, and birth weight—were entered into multivariable logistic regression Model 1. On multivariable analysis, TPN exposure and multi-organ failure remained independent predictors of HBD. TPN exposure was associated with a fourfold increase in the odds of HBD (aOR 4.15; 95% CI 1.68–10.23; $P = 0.002$), and multi-organ failure with a threefold increase (aOR 2.95; 95% CI 1.10–7.88; $P = 0.031$). Sepsis, respiratory support, gestational age, and birth weight did not retain independent significance after mutual adjustment (all $P > 0.05$). Model calibration was acceptable (Hosmer–Lemeshow $\chi^2 = 10.37$, $P = 0.240$), discrimination was good (apparent AUC 0.78; bootstrap optimism-corrected AUC 0.75), and explained variance was moderate (Nagelkerke $R^2 = 0.27$). The events-per-variable ratio was 10.7, and no significant multicollinearity was detected (maximum VIF 2.08).

All-Cause In-Hospital Mortality and Its Predictors; (Table 5)

Table 5

Multivariable Logistic Regression Analysis: Independent Predictors of In-Hospital Mortality (Model 2)

Variable	Univariate OR (95% CI)	P-value	Adjusted OR (95% CI) ^a	P-value	VIF
Respiratory support requirement	43.31 (16.11–116.39)	< 0.001	14.36 (1.56–132.41)	0.019	4.71
Multi-organ failure	38.33 (10.91–134.69)	< 0.001	9.46 (1.47–60.88)	0.018	1.37
TPN exposure	57.18 (19.37–168.74)	< 0.001	8.15 (2.00–33.20)	0.003	2.18
Cardiac/vasopressor support	50.05 (21.31–117.54)	< 0.001	6.47 (1.93–21.68)	0.002	2.29
HBD status	6.99 (3.64–13.43)	< 0.001	2.40 (0.76–7.57)	0.137	1.27
Hypoxia	33.28 (12.50–88.59)	< 0.001	1.00 (0.09–11.70)	0.999	4.56
Umbilical venous catheter	4.04 (1.77–9.21)	0.001	0.98 (0.23–4.15)	0.973	1.15
Gestational age (per week)	0.81 (0.72–0.91)	< 0.001	1.11 (0.79–1.55)	0.553	1.75
Birth weight (per 100 g)	0.93 (0.89–0.97)	0.001	0.90 (0.81–0.997)	0.043	1.65
^a Adjusted odds ratio from simultaneous entry multivariable logistic regression (enter method). Gestational age and birth weight were included in the model a priori as core neonatal prognostic covariates. Model fit: Hosmer–Lemeshow $\chi^2 = 6.05$, $P = 0.641$; apparent AUC 0.97; bootstrap optimism-corrected AUC 0.96 (500 resamples); Nagelkerke $R^2 = 0.78$; EPV = 6.4 (below the conventional threshold of 10; estimates should be interpreted with caution); maximum VIF = 4.71 (all VIF < 5). The wide CI for respiratory support reflects sparse data (only 5 of 58 deaths occurred without respiratory support).					
HBD: hepatobiliary dysfunction; TPN: total parenteral nutrition; OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval; EPV: events per variable; VIF: variance inflation factor; AUC: area under the receiver operating characteristic curve.					

All-cause in-hospital mortality occurred in 58 of 236 neonates (24.6%). Mortality was significantly higher among neonates with HBD than among those without HBD (53.1% vs. 14.0%; $P < 0.001$). In multivariable logistic regression Model 2, five variables independently predicted in-hospital mortality: respiratory support requirement (aOR 14.36; 95% CI 1.56–132.41; $P = 0.019$), multi-organ failure (aOR 9.46; 95% CI 1.47–60.88; $P = 0.018$), TPN exposure (aOR 8.15; 95% CI 2.00–33.20; $P = 0.003$), cardiac/vasopressor support (aOR 6.47; 95% CI 1.93–21.68; $P = 0.002$), and lower birth weight (aOR 0.90 per 100 g; 95% CI 0.81–0.997; $P = 0.043$). Gestational age was not independently associated with mortality once birth weight and illness-severity covariates were accounted for (aOR 1.11; 95% CI 0.79–1.55; $P = 0.553$). HBD status itself did not independently predict in-hospital mortality after adjustment for these covariates

(aOR 2.40; 95% CI 0.76–7.57; $P = 0.137$). Hypoxia and umbilical venous catheter use, although strongly associated with mortality in univariate analysis, were not independent predictors in the multivariable model ($P = 0.999$ and $P = 0.973$, respectively). Model calibration was adequate (Hosmer–Lemeshow $\chi^2 = 6.05$, $P = 0.641$), apparent discrimination was high (AUC 0.97), the bootstrap optimism-corrected AUC was 0.96, and the model explained a substantial proportion of variance in mortality (Nagelkerke R^2 0.78; EPV 6.4, below the conventional threshold of 10). No problematic multicollinearity was detected: the maximum VIF was 4.71 for respiratory support (all VIF < 5; Table 5). The wide confidence interval around the respiratory-support estimate reflects sparse data, only 5 of 58 deaths occurred in neonates not requiring respiratory support, and this estimate should be interpreted as directionally informative rather than precisely quantified [14].

Day-14 Landmark Sensitivity Analysis (Supplementary Table S2)

To address potential immortal-time/survivorship bias, Model 2 was re-estimated after excluding the 26 neonates who died before day 14 of admission (median day of death among these, day 6), leaving 210 neonates with 32 late deaths. TPN exposure (aOR 7.50; 95% CI 1.58–35.63; $P = 0.011$) and multi-organ failure (aOR 9.56; 95% CI 1.43–64.02; $P = 0.020$) remained independent predictors of late mortality. Respiratory support (aOR 9.64; 95% CI 0.89–104.89; $P = 0.063$) and cardiac/vasopressor support (aOR 3.05; 95% CI 0.83–11.14; $P = 0.092$) were attenuated. Notably, HBD was independently associated with late mortality in this landmark cohort (aOR 4.03; 95% CI 1.20–13.53; $P = 0.024$). Discrimination remained high (AUC 0.96; 95% CI 0.93–0.99; Hosmer–Lemeshow $P = 0.676$). Because this sensitivity analysis included only 32 events across 7 predictors (EPV 4.6), it should be regarded as exploratory.

Time-to-Event (Cox) Analysis (Supplementary Table S3)

In a Cox proportional hazards model with NICU length of stay as the time scale and the same nine covariates, cardiac/vasopressor support was the only covariate independently associated with the hazard of death (hazard ratio [HR] 3.22; 95% CI 1.43–7.28; $P = 0.005$); respiratory support (HR 7.53; 95% CI 0.97–58.54; $P = 0.054$) and TPN exposure (HR 3.00; 95% CI 0.93–9.70; $P = 0.066$) showed directionally consistent but imprecise associations, and baseline-classified HBD was not associated with the hazard of death (HR 1.19; 95% CI 0.68–2.07; $P = 0.542$). Model concordance was 0.885. The proportional hazards assumption was violated for respiratory support (scaled Schoenfeld residual $P = 0.013$), so its hazard ratio should be interpreted as a time-averaged effect. When HBD was instead modeled as a time-varying exposure switching on the documented day of HBD onset, HBD onset was strongly associated with the subsequent hazard of death (HR 3.43; 95% CI 1.89–6.20; $P < 0.001$), with cardiac/vasopressor support remaining independently associated (HR 2.85; 95% CI 1.31–6.19; $P = 0.008$). This time-varying association is directionally coherent with the day-14 landmark finding and indicates that the onset of HBD marks a period of substantially elevated mortality hazard, although it may partly reflect HBD arising as a manifestation of impending terminal multi-organ deterioration rather than an independent causal effect.

Additional Sensitivity Analyses

When the cholestasis component of HBD was re-derived using the guideline (NASPGHAN/ESPGHAN/FISPGHAN) threshold of direct bilirubin ≥ 1.0 mg/dL,¹ overall HBD incidence was unchanged at 64 of 236 neonates (27.1%), although case membership shifted (48 of 64 cases overlapped with the primary definition, with 16 reclassified in each direction). The mortality gradient across phenotypes persisted (isolated hepatocellular injury 42.9% [12/28], isolated cholestasis 23.8% [5/21], combined injury 80.0% [12/15], versus 16.9% [29/172] without HBD; $P = 0.004$), and crude mortality remained significantly higher with guideline-defined HBD than without (45.3% vs. 16.9%; $P < 0.001$). In the nine-covariate mortality model substituting the guideline-defined HBD variable, results were materially unchanged (respiratory support aOR 14.36, $P = 0.020$; multi-organ failure aOR 10.80, $P = 0.013$; TPN aOR 9.20, $P = 0.002$; cardiac/vasopressor support aOR 7.02, $P = 0.002$; birth weight aOR 0.90 per 100 g, $P = 0.037$; HBD aOR 1.29, $P = 0.671$).

Because 43 neonates (18.2%) were admitted primarily for unconjugated neonatal jaundice—a low-acuity group contributing 4 HBD cases and no deaths—the mortality model was re-estimated after their exclusion ($n = 193$; 58 deaths). Crude mortality was 18.0% among neonates without HBD versus 56.7% among those with HBD ($P < 0.001$), and adjusted estimates were essentially unchanged (respiratory support aOR 13.85, $P = 0.019$; multi-organ failure aOR 9.13, $P = 0.019$; TPN aOR 7.70, $P = 0.004$; cardiac/vasopressor support aOR 6.38, $P = 0.002$; birth weight aOR 0.90 per 100 g, $P = 0.046$; HBD aOR 2.36, $P = 0.142$; apparent AUC 0.96), indicating that the principal findings are not driven by the inclusion of low-acuity jaundice admissions.

DISCUSSION

This retrospective cohort study provides a comprehensive characterization of hepatobiliary dysfunction in a tertiary Egyptian NICU, identifying an overall HBD incidence of 27.1% among 236 consecutive admissions. This rate aligns closely with the 30% reported by Yassein et al. from Benha Children's Hospital, Egypt,⁸ and falls within the range documented across other NICU-based series. [6, 7]

In our cohort of 236 neonates, HBD developed in 64 patients, establishing an overall incidence of 27.1%. Notably, baseline demographic characteristics, including gestational age and birth weight did not differ significantly between neonates who developed HBD and those who did not. The predominance of hepatocellular and mixed injury patterns alongside isolated cholestasis in our cohort contrasts somewhat with high-income country series where PNAC is typically the leading isolated etiology. [6, 16] This difference likely reflects the disproportionate burden of gram-negative bacteremia in our population. Endotoxin released by gram-negative organisms suppresses hepatocytic BSEP expression and impairs canalicular bile flow via TNF- α and IL-1 β -mediated pathways, producing a pattern of mixed hepatocellular-cholestatic injury that mirrors findings from other LMIC NICUs. [4]

Disaggregating the composite HBD outcome revealed a clinically meaningful mortality gradient across phenotypes: 33.3% for isolated hepatocellular injury, 50.0% for isolated cholestasis, and 83.3% for combined injury, and neonates with any cholestatic component had roughly double the mortality of those with isolated ALT elevation (65.0% vs. 33.3%; $P = 0.020$). NICU length of stay, by contrast, did not differ across phenotypes, suggesting that the mortality gradient reflects severity of hepatic and systemic injury rather than cumulative exposure time. These findings indicate that isolated hepatocellular injury and cholestatic dysfunction are not prognostically interchangeable, and support reporting HBD phenotypes separately—rather than solely as a composite—in future neonatal studies.

Candida species were isolated more frequently among neonates with HBD than among those without HBD (4.7% vs. 0.6%), although this difference was not statistically significant (Fisher's exact $P = 0.062$) and was based on only four isolates in the entire cohort. This observation should therefore be regarded as strictly exploratory and hypothesis-generating: it may reflect severity of illness and cumulative invasive care rather than an independent causal pathway. Nonetheless, hepatosplenic candidiasis and disseminated hepatic involvement are recognized complications of invasive neonatal candidemia, [17] and antifungal stewardship remains important in at-risk neonates receiving broad-spectrum antibiotics and TPN.

TPN exposure emerged as the strongest independent predictor of HBD development (aOR 4.15; $P = 0.002$), consistent with a robust body of evidence implicating parenteral nutrition as a dominant modifiable risk factor for PNAC. Moin et al. similarly reported that prolonged parenteral nutrition exposure in very premature and low-birth-weight infants was associated with hepatic dysfunction comparable to that of peers with primary gastrointestinal disease, in a retrospective NICU cohort. [16] A 2025 analysis of very preterm infants demonstrated that a higher parenteral-to-enteral nutrition ratio had strong predictive performance for PNAC, outperforming either PN or enteral nutrition duration alone. [18] A large multicenter observational study corroborates TPN duration, total amino acid dose, and feeding intolerance as independent PNAC risk factors. [19] The pathophysiological mechanisms include phytosterol-mediated inhibition of BSEP, accumulation of hepatotoxic soybean oil-derived lipid peroxidation products, and suppression of the enterohepatic bile acid circulation during enteral fasting. [2, 3] In our unit, as in many Egyptian and other LMIC NICUs, pure soybean-oil-based emulsions remain the only routinely available lipid formulation. Mixed-oil emulsions (containing medium-chain triglycerides, olive oil, and fish oil) and pure fish-oil emulsions—which reduce the phytosterol load, enrich anti-inflammatory omega-3 fatty acids, and have demonstrated reductions in PNAC risk compared with soybean-based emulsions—are not consistently accessible in local settings owing to cost and procurement constraints. [3, 19] Securing formulary access to mixed-oil or fish-oil lipid emulsions, together with protocols prioritizing earlier initiation and advancement of enteral feeding, therefore represents a concrete and addressable systems-level target for HBD prevention in our setting.

The independent association with multi-organ failure (aOR 2.95; $P = 0.031$) reinforces the concept that HBD in the critically ill neonate is frequently a hepatic manifestation of multi-organ dysfunction syndrome rather than a primary hepatic event. Sepsis did not retain independent significance after

adjustment, suggesting its effect on HBD is largely mediated through MOF and TPN exposure as downstream consequences of severe illness. [4]

The multivariable mortality model yielded important clinical insights. The principal independent predictors of in-hospital death—respiratory support (aOR 14.36), multi-organ failure (aOR 9.46), TPN exposure (aOR 8.15), and cardiac/vasopressor support (aOR 6.47)—collectively represent markers of global cardiorespiratory and multi-organ critical illness severity rather than hepatic-specific pathology, and lower birth weight (aOR 0.90 per 100 g) contributed additional independent prognostic information after gestational age and birth weight were forced into the model a priori. The very wide confidence interval around the respiratory-support estimate (95% CI 1.56–132.41) reflects sparse-data conditions, as only five deaths occurred among neonates not requiring respiratory support; the point estimate should accordingly be read as directionally robust but imprecisely quantified. [14] These findings are broadly consistent with Egyptian tertiary NICU mortality data from El-Galaa Teaching Hospital, where hemorrhage, sepsis, and mechanical ventilation were dominant independent predictors of death, [20] and with an Ethiopian cohort in which prematurity, respiratory distress, and sepsis similarly dominated the mortality model. [21]

Crucially, HBD status itself did not independently predict in-hospital mortality after multivariable adjustment (aOR 2.40, 95% CI 0.76–7.57; $P = 0.137$). This finding aligns with a recent analysis of secondary hepatic dysfunction in critically ill children, which found that markers of hepatic injury were not independently predictive of mortality after adjustment for illness severity. [22] HBD thus functions as a prognostic marker, a sentinel of critical illness severity, rather than an independent causal contributor to death. In the day-14 landmark analysis, however, HBD was independently associated with late mortality (aOR 4.03; 95% CI 1.20–13.53), suggesting that whereas early NICU deaths are dominated by cardiorespiratory and multi-organ failure, HBD may carry additional independent prognostic weight among neonates who survive the acute phase. Given the limited number of late deaths ($n = 32$), this observation is hypothesis-generating and requires confirmation in larger prospective cohorts. The time-varying Cox analysis reinforces and refines this interpretation: the hazard of death rose more than threefold after the documented onset of HBD (HR 3.43; 95% CI 1.89–6.20; $P < 0.001$), identifying HBD onset as a clinically actionable sentinel event that should prompt intensified surveillance, review of nutritional strategy, and escalation of supportive care. Because HBD frequently emerges during evolving multi-organ deterioration, part of this association may reflect reverse causation—liver dysfunction appearing as a late manifestation of the dying process rather than a driver of death—and we therefore deliberately avoid causal claims. The convergence of the landmark and time-varying results nonetheless strengthens the conclusion that HBD onset carries prognostic information beyond baseline illness severity.

The burden of sepsis and mortality in this cohort warrants explicit contextualization against the unit's case mix. Sepsis was diagnosed in 49.6% of admissions (including culture-negative clinical sepsis), with a positive culture documented in 39.0%, and all-cause in-hospital mortality was 24.6%. These figures are consistent with reports from comparable settings: an earlier surveillance study of two tertiary Cairo

NICUs that included the present unit reported neonatal sepsis in 33.4% of admissions, [23] and in-hospital mortality of 14.2–41.9% has been reported from tertiary NICUs in Egypt and East Africa. [20, 21] Referral status itself is a strong determinant of these rates; in a recent single-center cohort from a comparable lower-middle-income setting, mortality was 21.5% among neonates transferred from other hospitals versus 4.1% among inborn admissions to the same unit. [24] In the present cohort, surgical conditions, congenital anomalies, and congenital heart disease together accounted for 39.4% of admissions in a term-predominant population, and low-acuity neonates are not admitted to the unit. The absolute incidence and mortality estimates reported here therefore characterize a high-acuity tertiary referral population and are most generalizable to similar referral NICUs in low- and middle-income countries.

Limitations

This study has several limitations. First, the retrospective, single-center design limits causal inference and generalizability, and residual confounding by unmeasured illness severity (e.g., validated severity scores such as SNAPPE-II were not available) cannot be excluded. Second, TPN was analyzed as a binary exposure because duration in days could not be reliably retrieved for all patients; PNAC therefore could not be adjudicated against a strict ≥ 2 -week duration criterion, and confounding by indication (sicker neonates receive TPN, and receive it longer) is likely to inflate the TPN–mortality association. Third, because TPN exposure and HBD accrue over the NICU stay, their associations with mortality are susceptible to immortal-time/survivorship bias;¹² the day-14 landmark analysis mitigates but cannot eliminate this, and its own event count (32 deaths; EPV 4.6) limits precision. Fourth, the ALT threshold of > 50 U/L was applied as a universal cutoff because validated gestational-age-specific neonatal ALT norms do not exist; pediatric data suggest hospital upper limits of normal frequently overestimate biological thresholds,⁹ so mild hepatocellular injury may have been misclassified in either direction. Fifth, our operational cholestasis criterion (total bilirubin > 2 mg/dL with direct fraction $> 20\%$) parallels but is not identical to the NASPGHAN/ESPGHAN/FISPGHAN position-paper thresholds;¹ the sensitivity analysis using the guideline threshold of direct bilirubin ≥ 1.0 mg/dL yielded the same overall incidence and materially unchanged model estimates, but 25% of cases were reclassified in each direction, so phenotype-specific estimates should be compared across studies with caution. Sixth, with 58 events and nine covariates (EPV 6.4), the mortality model is below the conventional EPV threshold and susceptible to overfitting and sparse-data bias; bootstrap internal validation suggested minimal optimism (corrected AUC 0.96), but this remains an internal estimate that has not been externally validated, and several adjusted estimates carry wide confidence intervals.¹⁴ Seventh, the study window (June–December) does not span a full calendar year, so seasonal variation in admission case-mix (for example, winter respiratory admissions) could not be captured, which may limit the generalizability of the incidence estimate. Eighth, the proportional hazards assumption was violated for respiratory support in the Cox model ($P = 0.013$), and the time-varying HBD analysis, although mitigating immortal-time bias, may partly capture HBD arising during terminal deterioration; hepatic-specific severity scores and serial biomarker trajectories were not available to disentangle this. Finally, laboratory values represent single

measurements abstracted at clinical decision points rather than serial trajectories, and although missingness was low for hepatic parameters ($\leq 1.7\%$), coagulation indices were missing more often among clinically stable neonates without HBD (Supplementary Table S1), which may modestly bias between-group comparisons of PT and INR.

CONCLUSION

HBD occurs in more than one-quarter of tertiary NICU admissions in this Egyptian cohort and is independently predicted by TPN exposure and multi-organ failure. Mortality rises steeply across HBD phenotypes, and combined hepatocellular–cholestatic injury identifies the highest-risk group. While HBD is strongly associated with in-hospital mortality at the crude level, it does not independently drive overall mortality risk after multivariable adjustment, underscoring its role as a marker of systemic critical illness severity; its independent association with late (post–day-14) mortality and the more-than-threefold rise in the hazard of death after HBD onset in time-varying analysis warrant prospective confirmation.

Declarations

Ethics Approval

This study was conducted in accordance with the ethical standards of the Ain Shams University Faculty of Medicine Institutional Review Board and with the principles of the Declaration of Helsinki (revised 2013). The REC approval number FMASU MS 232/2025. The requirement for individual informed consent was waived by the Research Ethics Committee given the retrospective design based on de-identified medical-record data.

Data Availability

The de-identified individual-level dataset analyzed during the current study is available from the corresponding author on reasonable request.

Competing interests:

Funding: No external funding was received for this study. No personal assistance beyond the contributions of the listed authors was involved in the work being published.

Conflict of Interest

Conflict of Interest: none declared

Declaration of Generative AI Use

During the preparation of this manuscript, the authors used a generative artificial intelligence tool (Perplexity AI) to assist with drafting and language editing of manuscript text, formatting of the manuscript and references to journal style, and verification of numerical consistency between the

abstract, text, and tables. The study design, data collection, statistical analyses, and interpretation of the results were performed by the authors. All AI-assisted content was critically reviewed, verified, and edited by the authors, who take full responsibility for the integrity and accuracy of the entire manuscript.

Authors' Contributions

ME-Conceptualized the outline, literature review, synthesis of data and data analysis, data interpretation, wrote first draft. KR collected data, synthesis of data, data analysis. SA-Conceptualized the outline, synthesis of data and data analysis, data interpretation, important input into editing manuscript. LA-Conceptualized the outline, synthesis of data and data analysis, data interpretation, important input into editing manuscript. IT- Conceptualized the outline, synthesis of data and data analysis, data interpretation, important input into editing manuscript.

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