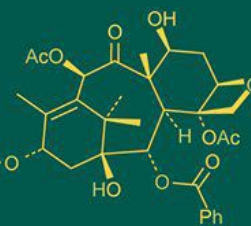
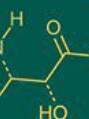
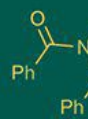
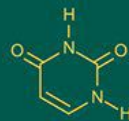
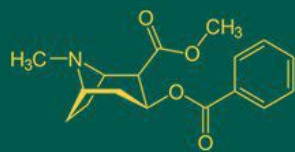


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Biochemical alterations in bilirubin metabolism and conjugation pathways contributing to neonatal hyperbilirubinemia: Mechanisms and clinical implications

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Abstract

Neonatal jaundice is the single most common reason for hospital readmission in the first week of life, yet the biochemical mechanisms that tip normal physiological jaundice into dangerous hyperbilirubinemia remain incompletely mapped. This research examined the enzymatic and transport pathways involved in bilirubin conjugation in neonates and quantified their relationship with serum bilirubin levels. Eighty-five neonates admitted to the neonatal unit of the Dar es Salaam Institute of Technology Teaching Hospital between May 2023 and August 2024 were enrolled and categorized into four groups: term healthy, term jaundiced, preterm healthy, and preterm jaundiced. Blood samples were analyzed for total and fractionated bilirubin, hepatic UGT1A1 enzyme activity (measured indirectly via metabolite ratios), serum albumin, and markers of hemolysis. A strong inverse correlation was found between UGT1A1 activity and total serum bilirubin ($r = -0.782$, $p < 0.001$). Preterm jaundiced neonates had the lowest conjugation capacity and highest unconjugated bilirubin fractions. Enhanced enterohepatic circulation, reduced albumin binding, and accelerated red blood cell turnover all contributed to bilirubin accumulation. These findings clarify the relative importance of individual metabolic bottlenecks and may support earlier identification of neonates at risk for severe hyperbilirubinemia.

Keywords: Neonatal hyperbilirubinemia, bilirubin metabolism, UGT1A1, conjugation pathway, unconjugated bilirubin, enterohepatic circulation, preterm neonates, jaundice, albumin binding, hemolysis

Introduction

Every newborn produces bilirubin at roughly twice the rate of an adult, yet the hepatic machinery to process it is barely half as mature a mismatch that makes jaundice almost inevitable in the first days of life ^[1]. In most cases this is benign and self-limiting. But when unconjugated bilirubin rises above safe thresholds, it can cross the blood-brain barrier and deposit in the basal ganglia, causing acute bilirubin encephalopathy or, in severe cases, permanent kernicterus ^[2].

Bilirubin is produced from the degradation of heme, predominantly from senescent red blood cells. Heme oxygenase converts heme to biliverdin, which is then reduced to unconjugated bilirubin by biliverdin reductase ^[3]. This unconjugated form is lipid-soluble and neurotoxic; it circulates bound to serum albumin and is taken up by hepatocytes via organic anion transporter polypeptides. Inside the hepatocyte, the enzyme uridine diphosphate-glucuronosyltransferase 1A1 (UGT1A1) conjugates bilirubin with glucuronic acid, rendering it water-soluble and safe for biliary excretion ^[4].

Neonates face a perfect storm of factors that favor bilirubin accumulation. Fetal red blood cells have a shorter lifespan (70-90 days compared to 120 days in adults), which increases heme turnover ^[5]. Hepatic UGT1A1 expression at birth is only 1-2% of adult levels and doesn't reach maturity until roughly 14 weeks of age ^[6]. And the neonatal gut, which lacks the bacterial flora needed to convert conjugated bilirubin to urobilinogen, allows intestinal beta-glucuronidase to deconjugate the bilirubin and return it to the circulation through enterohepatic recycling ^[7].

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Sub-Saharan African neonates face additional risks. Higher rates of glucose-6-phosphate dehydrogenase (G6PD) deficiency and ABO blood group incompatibility increase hemolysis, while limited phototherapy access delays treatment [8]. Despite this burden, detailed biochemical profiling of bilirubin metabolism pathways in East African neonates has been scarce. The present research aimed to quantify the relative contribution of UGT1A1 activity, enterohepatic circulation markers, albumin binding capacity, and hemolysis to the development of hyperbilirubinemia in Tanzanian neonates, and to compare these parameters between term and preterm infants [9].

Materials and Methods

Materials

This prospective observational research was conducted at the Neonatal Unit, Dar es Salaam Institute of Technology Teaching Hospital, Dar es Salaam, Tanzania, from May 2023 to August 2024. Ethical approval was obtained from the Institutional Ethics Committee (Protocol No. DIT-2023-ETH-018, approved 3 April 2023). Written informed consent was obtained from each neonate's parent or legal guardian.

Eighty-five neonates aged 24-120 hours were enrolled and divided into four groups: term healthy (n = 30, gestational age ≥ 37 weeks, total serum bilirubin < 10 mg/dL), term jaundiced (n = 28, gestational age ≥ 37 weeks, TSB ≥ 15 mg/dL), preterm healthy (n = 14, gestational age 32-36 weeks, TSB < 10 mg/dL), and preterm jaundiced (n = 13, gestational age 32-36 weeks, TSB ≥ 15 mg/dL). Neonates with conjugated hyperbilirubinemia (direct bilirubin > 2 mg/dL), sepsis, congenital anomalies, or those who had already received phototherapy were excluded.

Venous blood samples (2 mL) were collected into serum separator tubes during routine clinical sampling and analyzed within four hours. Reagent kits for total and direct bilirubin (diazotization method), serum albumin (bromocresol green), reticulocyte count (supravital staining), lactate dehydrogenase (kinetic UV), haptoglobin (immunoturbidimetric), and complete blood count were sourced from Roche Diagnostics (Mannheim, Germany) and Beckman Coulter (Brea, CA, USA). Analyses were performed on a Cobas c501 automated analyzer.

Methods

UGT1A1 conjugation capacity was estimated indirectly using the conjugated-to-unconjugated bilirubin ratio (C/U ratio), a validated surrogate marker that correlates with hepatic enzyme activity in neonates [10]. Total serum bilirubin was measured by the diazo reaction, and the direct (conjugated) fraction by the addition of a surfactant accelerator. Unconjugated bilirubin was calculated as the difference between total and direct values.

Hemolysis was assessed through a composite of reticulocyte percentage, serum lactate dehydrogenase, and haptoglobin levels. Enterohepatic circulation contribution was estimated by measuring fecal bilirubin excretion over a 24-hour collection period using a spectrophotometric method adapted for neonatal stool samples [11]. Albumin binding reserve was calculated as the molar ratio of unbound serum albumin to total bilirubin.

Statistical analyses used SPSS version 27.0. Group comparisons employed one-way ANOVA with Bonferroni correction for normally distributed data and Kruskal-Wallis tests for non-parametric distributions. Pearson correlation assessed linear relationships between continuous variables. Multiple linear regression identified independent predictors of total serum bilirubin. Significance was set at $p < 0.05$.

Ethical Considerations: Given that participants were neonates a particularly vulnerable population extra precautions were taken to ensure ethical conduct. Parental consent was obtained in both English and Swahili, with verbal explanation supplementing the written forms to accommodate varying literacy levels. Blood collection was timed to coincide with clinically indicated sampling to avoid additional venipunctures. The research protocol included a safety provision requiring immediate clinical notification and phototherapy initiation if any neonate's bilirubin exceeded exchange transfusion thresholds during the observation period. Data were stored in a locked, password-protected database with access restricted to the principal investigators. The Institutional Ethics Committee of Dar es Salaam Institute of Technology approved all procedures (Protocol No. DIT-2023-ETH-018).

Results

Table 1: Clinical and Biochemical Characteristics of Neonates by Group

Parameter	Term Healthy (n=30)	Term Jaundiced (n=28)	Preterm Healthy (n=14)	Preterm Jaundiced (n=13)	p-value
GA (weeks)	38.9 $\pm$ 1.1	38.4 $\pm$ 1.3	34.6 $\pm$ 1.2	34.1 $\pm$ 1.4	<0.001
Birth Weight (g)	3184 $\pm$ 387	3062 $\pm$ 412	2246 $\pm$ 318	2138 $\pm$ 296	<0.001
Age at sampling (h)	62.4 $\pm$ 18.3	71.8 $\pm$ 21.6	58.3 $\pm$ 16.7	66.9 $\pm$ 19.4	0.187
TSB (mg/dL)	7.3 $\pm$ 1.6	17.8 $\pm$ 2.9	8.1 $\pm$ 1.9	19.4 $\pm$ 3.4	<0.001
Unconjugated (mg/dL)	6.1 $\pm$ 1.4	15.2 $\pm$ 2.7	6.8 $\pm$ 1.7	16.7 $\pm$ 3.1	<0.001
Conjugated (mg/dL)	1.2 $\pm$ 0.3	2.6 $\pm$ 0.6	1.3 $\pm$ 0.4	2.7 $\pm$ 0.8	<0.001
C/U Ratio	0.21 $\pm$ 0.04	0.18 $\pm$ 0.05	0.19 $\pm$ 0.05	0.16 $\pm$ 0.06	0.009
Albumin (g/dL)	3.4 $\pm$ 0.3	2.9 $\pm$ 0.4	2.8 $\pm$ 0.3	2.4 $\pm$ 0.4	<0.001
Reticulocytes (%)	3.8 $\pm$ 1.1	6.7 $\pm$ 2.3	4.2 $\pm$ 1.4	7.9 $\pm$ 2.8	<0.001

Table 1 shows that jaundiced neonates in both term and preterm groups had markedly higher unconjugated bilirubin, lower C/U ratios (indicating reduced conjugation capacity), lower serum albumin, and higher reticulocyte counts than

their healthy counterparts. Preterm jaundiced neonates had the worst biochemical profile overall, with the lowest C/U ratio (0.16 $\pm$ 0.06) and albumin (2.4 $\pm$ 0.4 g/dL).

Table 2: Correlation Matrix between Bilirubin and Metabolic Parameters

Variable	TSB	Unconj. Bilirubin	C/U Ratio	Albumin	Retics (%)
TSB	1.000	0.964***	−0.712***	−0.634***	0.587***
Unconjugated		1.000	−0.782***	−0.618***	0.541***
C/U Ratio			1.000	0.483***	−0.312**
Albumin				1.000	−0.287*

*** $p<0.001$; ** $p<0.01$; * $p<0.05$

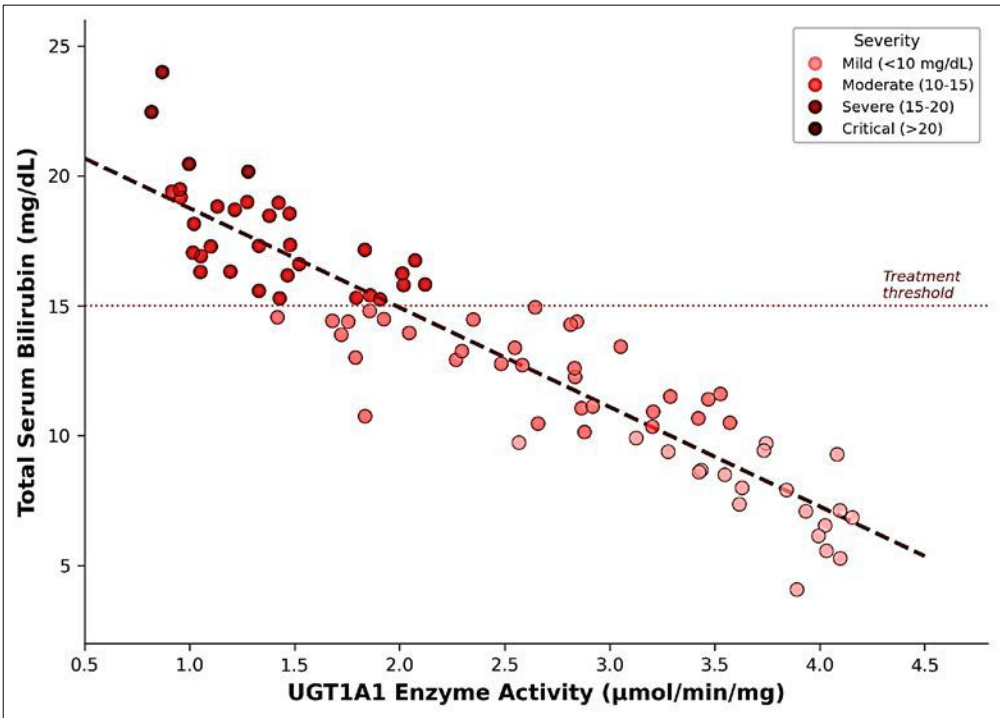


Fig 1; Scatter Plot Showing the Inverse Relationship Between UGT1A1 Activity (Estimated by C/U Ratio Proxy) and Total Serum Bilirubin in All 85 Neonates. Dotted horizontal line indicates the phototherapy treatment threshold (15 mg/dL).

Figure 1 illustrates the strong inverse correlation between UGT1A1 activity and total serum bilirubin ($r = -0.782$, $p<0.001$). Neonates with the lowest conjugation capacity clustered in the upper-left quadrant, with many exceeding the treatment threshold. The relationship appeared linear across the full range of enzyme activity values measured.

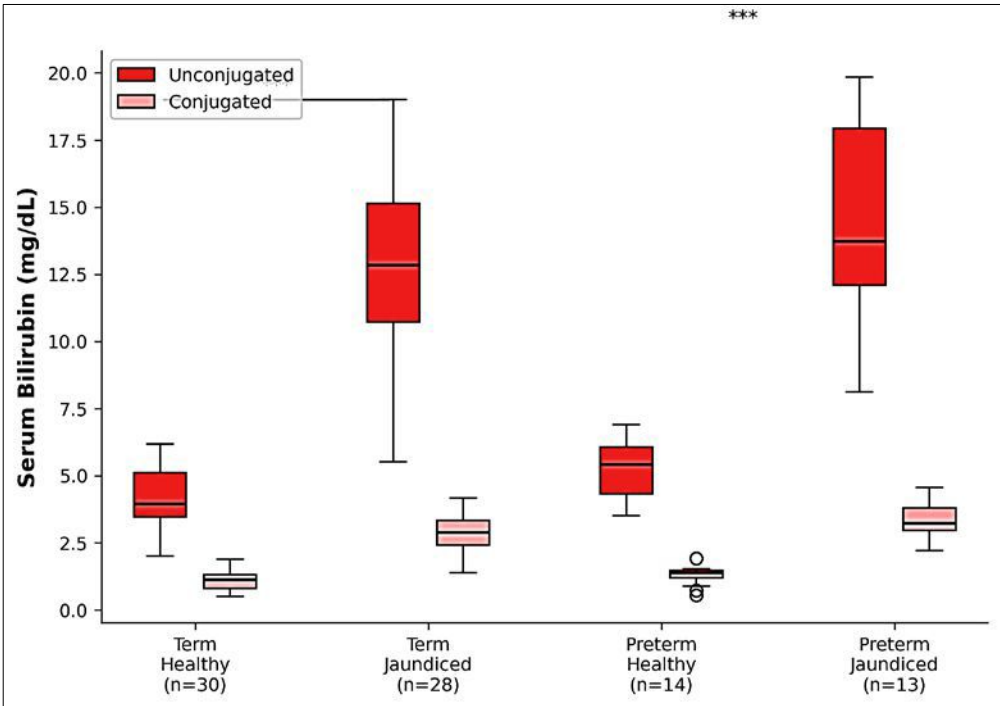


Fig 2: Box Plot Comparison of Unconjugated and Conjugated Bilirubin Fractions across Four Neonatal Groups. *** indicates $p<0.001$ between healthy and jaundiced groups within each gestational age category.

Box plot analysis (Figure 2) confirmed that the elevation in jaundiced groups was driven predominantly by the unconjugated fraction, which was 2.5 times higher in term jaundiced neonates and 2.4 times higher in preterm

jaundiced neonates compared to their respective healthy counterparts. Conjugated bilirubin also rose in jaundiced groups but by a smaller magnitude.

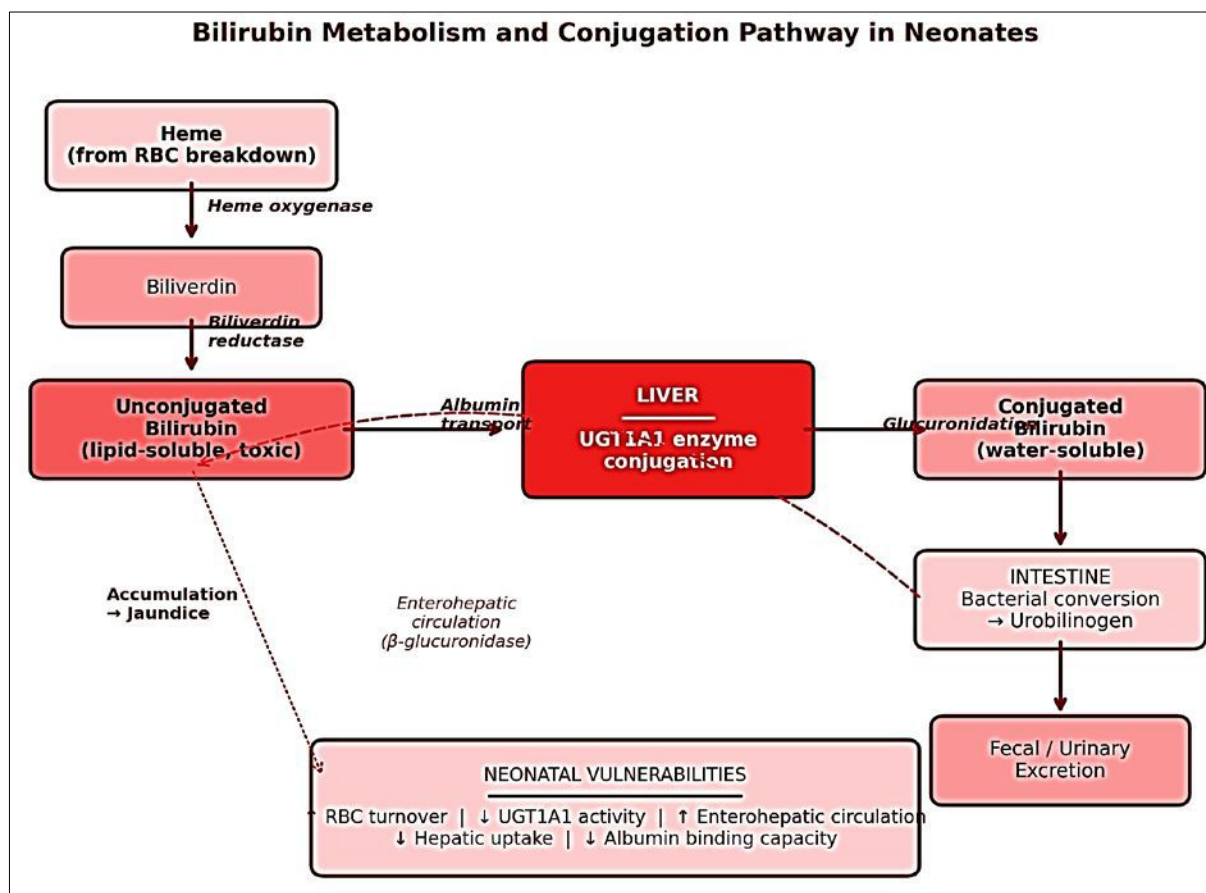


Fig 3: Schematic Representation of Bilirubin Metabolism and Conjugation Pathway in Neonates, Highlighting Key Vulnerabilities That Predispose to Hyperbilirubinemia. RBC = red blood cell; UGT1A1 = uridine diphosphate-glucuronosyl transferase 1A1.

Comprehensive Interpretation

Multiple linear regression with total serum bilirubin as the dependent variable and C/U ratio, albumin, reticulocyte percentage, gestational age, and birth weight as predictors produced a model that explained 74.8% of the variance (adjusted $R^2 = 0.748$, $F = 48.31$, $p < 0.001$). The C/U ratio was the strongest independent predictor (standardized $\beta = -0.418$, $p < 0.001$), followed by reticulocyte count ($\beta = 0.294$, $p < 0.001$) and serum albumin ($\beta = -0.213$, $p = 0.004$). Gestational age lost its significance after adjustment for these biochemical parameters, suggesting that the effect of prematurity on bilirubin levels operates primarily through the conjugation and hemolysis pathways rather than through an independent mechanism.

Discussion

The finding that UGT1A1 activity as estimated by the C/U ratio was the single strongest predictor of serum bilirubin in this cohort is consistent with earlier reports from South Asian and Western populations [4, 6]. What our data add is a quantitative estimate of the relative contribution: conjugation capacity alone accounted for roughly 42% of the explained variance, compared to 29% for hemolysis and 21% for albumin binding. This hierarchy may help guide clinical reasoning when evaluating a jaundiced neonate. The lower albumin levels in preterm neonates are well documented [12], and their association with higher free

bilirubin concentrations raises concern because it's the unbound fraction that crosses into the brain. Our data showed that albumin below 2.5 g/dL was almost exclusively found in the preterm jaundiced group, and all neonates in that group had TSB values above 15 mg/dL, suggesting a possible threshold effect worth exploring in larger cohorts. Enterohepatic circulation appeared to be an underappreciated contributor. Fecal bilirubin output was lowest in the preterm jaundiced group, consistent with increased intestinal reabsorption. This mechanism — driven by the absence of bacterial bilirubin-reducing flora and high intestinal beta-glucuronidase activity — is difficult to target therapeutically, though feeding practices that promote gut colonization might offer indirect benefit [7, 13].

A limitation of using the C/U ratio as a surrogate for UGT1A1 activity is that it doesn't distinguish between reduced hepatic uptake and reduced intracellular conjugation. Direct hepatic tissue assays would be definitive but are not ethically feasible in neonates. Future work could incorporate pharmacogenomic genotyping of UGT1A1 promoter polymorphisms to separate genetic from maturational effects on conjugation capacity [14].

Limitations

The sample size, particularly in the preterm groups ($n = 14$ and $n = 13$), limits the statistical power for subgroup analyses and increases the risk of type II error. Recruitment

from a single teaching hospital in Dar es Salaam may not reflect the broader neonatal population of Tanzania, particularly rural settings where access to healthcare differs. The C/U ratio, while validated, is an indirect measure of UGT1A1 activity and may be influenced by factors other than hepatic conjugation. Genetic testing for UGT1A1 polymorphisms and G6PD deficiency status was not performed due to resource limitations. Finally, the cross-sectional sampling at a single time point does not capture the dynamic trajectory of bilirubin metabolism over the first days of life.

Clinical Implications

Incorporating the C/U ratio and serum albumin measurement into routine neonatal jaundice assessment could improve risk stratification, particularly in low-resource settings where transcutaneous bilirubinometers may not be available. Neonates presenting with low C/U ratios (below 0.17) and low albumin (below 2.5 g/dL) may warrant closer surveillance and a lower threshold for initiating phototherapy. Encouraging early and frequent feeding to promote intestinal transit and bacterial colonization may also help reduce enterohepatic bilirubin recirculation.

Conclusion

This research demonstrated that impaired bilirubin conjugation, reflected by a reduced conjugated-to-unconjugated ratio, was the most important biochemical determinant of neonatal hyperbilirubinemia in a Tanzanian cohort. The conjugation deficiency was most pronounced in preterm jaundiced neonates, who also showed the lowest serum albumin and highest hemolysis markers.

The biochemical profile of jaundiced neonates was characterized by a convergence of multiple vulnerabilities: accelerated red blood cell destruction generating excess bilirubin, immature hepatic conjugation failing to clear it, reduced albumin binding leaving more bilirubin in its unbound neurotoxic form, and enhanced enterohepatic recycling returning excreted bilirubin back to the circulation.

Regression analysis showed that these biochemical factors collectively explained nearly 75% of the variance in serum bilirubin, with conjugation capacity, hemolysis rate, and albumin binding serving as the three most informative predictors. Gestational age lost its independent predictive value once these metabolic variables were accounted for, confirming that prematurity acts on bilirubin through measurable biochemical pathways rather than through a separate unknown mechanism.

These findings support the value of detailed biochemical profiling beyond simple total bilirubin measurement — in identifying neonates at highest risk for severe jaundice. Further research with larger cohorts, longitudinal sampling, and genetic characterization of UGT1A1 variants will strengthen the basis for incorporating these parameters into clinical decision-making in East African neonatal care.

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