Chronic Auditory Toxicity in Late Preterm and Term Infants With Significant Hyperbilirubinemia

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abstract

BACKGROUND AND OBJECTIVES: Significant hyperbilirubinemia (SHB) may cause chronic auditory toxicity (auditory neuropathy spectrum disorder and/or sensorineural hearing loss); however, total serum bilirubin (TSB) does not discriminate neonates at risk for auditory toxicity. Our objective was to compare TSB, bilirubin albumin molar ratio (BAMR), and unbound bilirubin (UB) for their association with chronic auditory toxicity in neonates with SHB (TSB ≥20 mg/dL or TSB that met criteria for exchange transfusion).

METHODS: Infants ≥34 weeks' gestational age (GA) with SHB during the first 2 postnatal weeks were eligible for a prospective longitudinal study in India. Comprehensive auditory evaluations were performed at 2 to 3 months of age by using auditory brainstem response, tympanometry, and an otoacoustic emission test and at 9 to 12 months of age by using audiometry. The evaluations were performed by an audiologist unaware of the degree of jaundice.

RESULTS: A total of 93 out of 100 infants (mean GA of 37.4 weeks; 55 boys, 38 girls) who were enrolled with SHB were evaluated for auditory toxicity. Of those, 12 infants (13%) had auditory toxicity. On regression analysis controlling for covariates, peak UB (but not peak TSB or peak BAMR), was associated with auditory toxicity (odds ratio 2.41; 95% confidence interval: 1.43–4.07; $P = .001$). There was significant difference in the area under the receiver operating characteristic curves between UB (0.866), TSB (0.775), and BAMR (0.724) for auditory toxicity ($P = .03$) after controlling for covariates.

CONCLUSIONS: Unconjugated hyperbilirubinemia indexed by UB (but not TSB or BAMR) is associated with chronic auditory toxicity in infants ≥34 weeks' GA with SHB.

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Dr Amin conceptualized and designed the study, supervised the study, and drafted the initial manuscript; Drs Saluja and Saili contributed to study design, coordinated and supervised subject enrollment and data collection, and critically reviewed the manuscript; Dr Orlando contributed to study design, supervised auditory data collection, evaluated auditory results, and critically reviewed the manuscript; Dr Wang contributed to study design, conducted the analyses, and reviewed and revised the manuscript; Dr Laroia contributed to study design, validated data collection, and critically reviewed the manuscript; Mrs Agarwal contributed to study design, performed auditory evaluations, collected auditory data, and critically reviewed the manuscript; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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WHAT'S KNOWN ON THIS SUBJECT: Significant hyperbilirubinemia may be associated with auditory toxicity as manifested by sensorineural hearing loss and/or auditory neuropathy spectrum disorder. Total serum bilirubin and bilirubin albumin molar ratio used for the management of significant hyperbilirubinemia are poor predictors of bilirubin-induced neurotoxicity.

WHAT THIS STUDY ADDS: Unbound bilirubin (but not total serum bilirubin or bilirubin albumin molar ratio) is associated with chronic auditory toxicity in late preterm and term infants with significant unconjugated hyperbilirubinemia. Unbound bilirubin is a better predictor of chronic auditory toxicity.

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Significant unconjugated hyperbilirubinemia (SHB) is among the most common readmission diagnoses for neonates throughout the world.[1–4] SHB is considered a sentinel event, and an urgent therapeutic intervention is needed to prevent acute bilirubin encephalopathy that can result in death or kernicterus, including permanent sensorineural hearing loss (SNHL). Although there are specific management guidelines (based on hour-specific total serum bilirubin [TSB], gestational age [GA] of the patient, and presence of clinical risk factors) from the American Academy of Pediatrics (AAP) for the use of phototherapy and exchange transfusion (ET), the guidelines are based on limited evidence.[5] More specifically, TSB correlates poorly with kernicterus.[6,7]

The auditory system is highly sensitive to overt bilirubin-induced neurotoxicity; however, few studies have rigorously examined the relationship between SHB and auditory neuropathy spectrum disorder (ANSD) or chronic auditory toxicity (SNHL), which can be assessed much earlier in life than other sequelae of acute bilirubin encephalopathy.[8–12] We recently demonstrated that SHB was associated with a high incidence of acute auditory toxicity as manifested by abnormal auditory threshold and/or acute ANSD in infants ≥34 weeks' GA.[13] However, the natural course of acute auditory toxicity in infants ≥34 weeks' GA with SHB has not been prospectively studied. It is possible that acute auditory toxicity may be reversible and resolve over time or progress as chronic auditory toxicity during infancy. We also demonstrated that unbound bilirubin (UB), but not TSB or bilirubin albumin molar ratio (BAMR), was associated with acute auditory toxicity in infants ≥34 weeks' GA with SHB.[13,14] However, the association of bilirubin biochemical measures (UB, BAMR, and TSB) with chronic auditory toxicity has not been studied. Therefore, our objectives were to evaluate the incidence of chronic auditory toxicity as manifested by ANSD and/or SNHL during infancy and compare UB, TSB, and BAMR for their association with chronic auditory toxicity in infants ≥34 weeks' GA with SHB.

METHODS

Study Design

This was a prospective longitudinal study involving infants ≥34 weeks' GA admitted with SHB from 2011 to 2014 at 2 academic centers in Delhi, India (Sir Ganga Ram Hospital and Kalawati Saran Children's Hospital), who were evaluated earlier for acute auditory toxicity during the neonatal period.[13] The study was approved by the institutional ethics committee. Parental consent was obtained for each subject enrolled.

Subject Population

Infants ≥34 weeks' GA who were admitted to the NICU with SHB (defined as TSB ≥20 mg/dL [342 μmol/L] or TSB that met the ET criteria according to the AAP guidelines) during the first 2 weeks of life were eligible for the study.[1,5,11,12] For infants $34^{0/7}$ to $34^{6/7}$ weeks' GA, we used a TSB concentration that met ET criteria for infants with a GA of $35^{0/7}$ to $37^{6/7}$ weeks.[1] Infants who met the following conditions were excluded because these conditions are often associated with hearing disorders[12]: (1) craniofacial malformations; (2) chromosomal disorders; (3) family history of congenital deafness; (4) toxoplasmosis, other infections, rubella, cytomegalovirus infection, and herpes simplex infections; and (5) surgical interventions at the time of SHB. Furthermore, infants with a failed newborn hearing screening evaluation before SHB or whose parents lived outside Delhi were excluded. GA was evaluated by obstetrical dating criteria, including first trimester ultrasound, or when obstetric history was inadequate, by Ballard examination. Infants received appropriate evaluation and therapy for hyperbilirubinemia as outlined in AAP guidelines and described in an earlier report.[5,13]

Bilirubin-Albumin Binding Variables (Exposure Variables)

The detailed methodology for the collection, shipping, and measurements of bilirubin-albumin binding variables has been previously described.[13] The blood samples for the measurement of TSB for individual participants were drawn as clinically indicated at the discretion of the attending neonatologist and measured immediately (in <2 hours) at the institutional clinical chemistry laboratory by using the colorimetric method. For each participant, the serum albumin was measured (grams per deciliter [multiply by 151 to convert to micromoles per liter]) at the time of admission, with subsequent TSB measurement if jaundice increased despite phototherapy, and before ET by using the bromocresol green method. The peak TSB and the concurrent serum albumin were used to calculate the peak BAMR for each participant.

The same aliquot of blood used to measure TSB was used to measure UB for each participant. UB was measured (micrograms per deciliter [multiply by 17.1 to convert to nanomoles per liter]) by the modified peroxidase method at 2 enzyme concentrations (1:25 and 1:12.5 dilutions) of precalibrated peroxidase (Arrows Company, Ltd, Osaka, Japan) by using an FDA-approved UB analyzer UA-1 (Arrows Company).

Chronic Auditory Toxicity (Outcome Variables)

Each participant had a comprehensive auditory evaluation

(auditory brainstem response [ABR], otoacoustic emission test, and tympanometry) performed in both ears at 2 to 3 months of age by a single audiologist unaware of the degree of hyperbilirubinemia. The methods to evaluate ANSD were identical to those performed to evaluate acute ANSD during the neonatal period and are described in earlier reports.[13,14] Infants with abnormal ABR morphology (absent wave I and III) or absent ABR waveform (absent wave I, III, and V) at 80 dB, but with the presence of cochlear microphonics or normal results from an otoacoustic emission test, were diagnosed with ANSD.

At 9 to 12 months of age, each subject underwent visual reinforcement audiometry testing, a gold standard method for diagnosis of SNHL, by an experienced audiologist using a clinical audiometer (Grason-Stadler Inc, Milford, NH). Normal hearing was defined as having minimum responses to speech and warble tone stimuli (500, 1000, 2000, and 4000 Hz) ≤20 dB HL in the sound field. Infants with either SNHL or ANSD but with a normal tympanometry were deemed to have chronic auditory toxicity.

Risk Factors (Covariates)

Risk factors for kernicterus such as perinatal asphyxia (Apgar score <3 at 5 minutes and/or cord pH <7.0), sepsis (culture proven or clinical sepsis requiring at least 7 days of intravenous antibiotics), hypoxia (PAo_2 <45 mm Hg), acidosis (pH <7.25), hypoalbuminemia (albumin <3 g/dL), and hemolytic disorders (rhesus incompatibility, ABO incompatibility, glucose-6-phosphate dehydrogenase deficiency, etc) were prospectively collected.

Statistical Analyses

All analyses were conducted by using SAS 9.4 (SAS Institute, Inc, Cary, NC). The AAP reference level of 25 mg/dL was used as a subgroup category, and analyses for neonates with TSB <25 mg/dL [427.5 μmol/L]) and TSB ≥25 mg/dL [427.5 μmol/L]) were also performed.[7,13] The 2-sample *t* tests or the Mann–Whitney *U* test were used to analyze continuous variables, and the Fisher's exact test or the χ^2 test was used to analyze categorical variables. The independent association between each of the bilirubin variables (TSB, UB, and BAMR) and chronic auditory toxicity were evaluated by using logistic regression analyses. Multicollinearity was evaluated by using multiple correlations (variance inflation factors) before including continuous variables in regression models. Covariates with significant association ($P \leq .15$) to auditory toxicity were included in the initial regression models. The backward selection method was used to decide the final regression models. Covariate-adjusted receiver operating characteristic (ROC) curves predicting chronic auditory toxicity were plotted for each of the bilirubin variables, and area under the curves (AUCs) were compared by using the nonparametric test. All analyses were 2-tailed, with significance defined as a *P* value <.05.

RESULTS

A total of 100 infants were enrolled in the longitudinal study. Of those, 93 infants (93%) completed auditory evaluations at 2 to 3 months and 9 to 12 months of age. There was no significant difference in GA (mean [SD], 37.4 [1.36] vs 37.2 [0.7]; P = .54), peak TSB (24.3 [4.9] vs 23.1 [3.4] mg/dL; P = .54), or peak UB (1.86 [1.95] vs 2.25 [2.12] μg/dL; P = .53) between the 93 infants who completed auditory evaluations and the 7 infants who did not complete follow-up auditory evaluations, respectively. None of the neonates had an interval history of head trauma, malignancy, or meningitis. None of the neonates had middle ear disease.

Twelve infants (13%) were found to have chronic auditory toxicity (3 with ANSD, 4 with SNHL, and 5 with SNHL and ANSD). Among 9 infants with SNHL, 4 had severe to profound SNHL (>70 dB). Ten of 12 infants (83%) were previously identified to have acute auditory toxicity (ANSD or abnormal auditory threshold), whereas 2 infants had a previously normal auditory evaluation when performed soon after the resolution of SHB.[13] Among 81 infants without chronic auditory toxicity, 16 infants were previously identified to have acute auditory toxicity during the neonatal period.[13] In the original study of 100 infants, out of 28 infants who had acute auditory toxicity, 2 infants did not complete follow-up evaluations, and 10 infants (38%) were identified to have chronic auditory toxicity.[13] Among 72 infants without acute auditory toxicity, 5 infants did not complete follow-up auditory evaluations, and 2 infants (3%) developed chronic auditory toxicity.[13] The positive and negative predictive value of neonatal auditory evaluations for subsequent chronic auditory toxicity during infancy were 0.38 (95% confidence interval [CI]: 0.20–0.59) and 0.97 (95% CI: 0.90–0.99), respectively.

There was no difference in GA, birth weight, sex, mode of delivery, asphyxia, sepsis, type of enteral feeding (breast milk or formula), hemolytic disorders, or polycythemia between infants with and without chronic auditory toxicity (Table 1). There was no difference in serum albumin concentration or bilirubin binding capacity (multiply albumin concentration by 8.8, assuming 1 bilirubin binding site per albumin molecule) between the 2 groups. The mean postnatal age in hours of peak TSB in infants with and without chronic auditory toxicity was similar (117 [SD 39] vs 114 [SD 49], P = .566). A higher proportion of infants

who developed chronic auditory toxicity received an ET compared with infants without chronic auditory toxicity (Table 1).

The peak UB (Fig 1), TSB, and BAMR were higher among infants with chronic auditory toxicity compared with neonates without chronic auditory toxicity (Table 2). A higher proportion of infants with auditory toxicity had a BAMR ≥1 compared with those without auditory toxicity (16% vs 11%, respectively), but the difference was not significant (P = .63). In neonates with a BAMR <1 (n = 82), the calculated bilirubin-albumin equilibrium dissociation constant, a measure of the weakness of bilirubin-albumin binding, was similar between the 2 groups (Table 2).[15,16] In regression analyses in which we used 3 separate regression models with mode of delivery, sepsis, and ET included as covariates, there was a significant association of peak UB (but not peak TSB or peak BAMR) with chronic auditory toxicity (Table 2). For each unit (micrograms per deciliter) increase in UB, the odds of having chronic auditory toxicity increased by a factor of 2.41. In a regression analysis, controlling for covariates, there was no significant association between elevated BAMR ≥1 and chronic auditory toxicity (OR 0.65; 95% CI: 0.10–3.97; P = .64).

In an ROC analysis, controlling for covariates, there was a significant difference in the AUCs between peak TSB, peak UB, and peak BAMR for chronic auditory toxicity (P = .03). UB (0.866) had a larger AUC than TSB (0.775) and BAMR (0.724) for chronic auditory toxicity (Fig 2).

Among 63 infants with TSB <25 mg/dL, there were 5 infants (8%) with chronic auditory toxicity. There was no significant difference in clinical characteristics between infants with and without chronic auditory toxicity (Table 3). The peak UB (but not peak TSB or BAMR) was significantly higher among infants with chronic auditory toxicity compared with infants without chronic auditory toxicity. In a regression analysis controlling for GA and sex, there was a significant association of peak UB (but not peak TSB or BAMR) with chronic auditory toxicity (Table 4). Among 30 infants with TSB ≥25 mg/dL, there were 7 (23%) infants with chronic auditory toxicity. There was no significant difference in clinical characteristics between the

TABLE 1 Clinical Characteristics as a Function of Chronic Auditory Toxicity (N = 93)

	Infants Without Auditory Toxicity (n = 81)	Infants With Auditory Toxicity (n = 12)	P
GA, wks[a]	37.3 (1.4)	37.8 (0.8)	.23[b]
Birth weight, g[a]	2681 (440)	2705 (401)	.87[b]
Sex, n (% male)	49 (60)	6 (50)	.54
Mode of delivery, n (% cesarean delivery)	18 (22)	0 (0)	.11[c]
Serum albumin, g/dL[a]	3.57 (0.60)	3.76 (0.75)	.28[b]
Bilirubin binding capacity, mg/dL[a]	31.4 (5.3)	33.1 (6.6)	.28[b]
Sepsis, n (%)	2 (2)	2 (16)	.08[c]
Asphyxia (Apgar score <3 at 5 min), n (%)	0 (0)	0 (0)	1.00
Hemolytic disorders, n (%)	19 (23)	3 (25)	.99
Polycythemia, n (%)	2 (2)	0 (0)	.99[c]
Breast milk feeding, n (%)	77 (95)	12 (100)	.99[c]
Clinical risk factor, n (%)	22 (27)	5 (41)	.32
ET, n (%)	31 (38)	9 (75)	.02[c]

Clinical risk factors: hemolysis, asphyxia, hypoxia (Pao_2 <45 mm Hg), acidosis (pH < 7.25), or albumin <3 g/dL.
[a] Mean (SD).
[b] Mann–Whitney U test.
[c] Fisher's exact test.

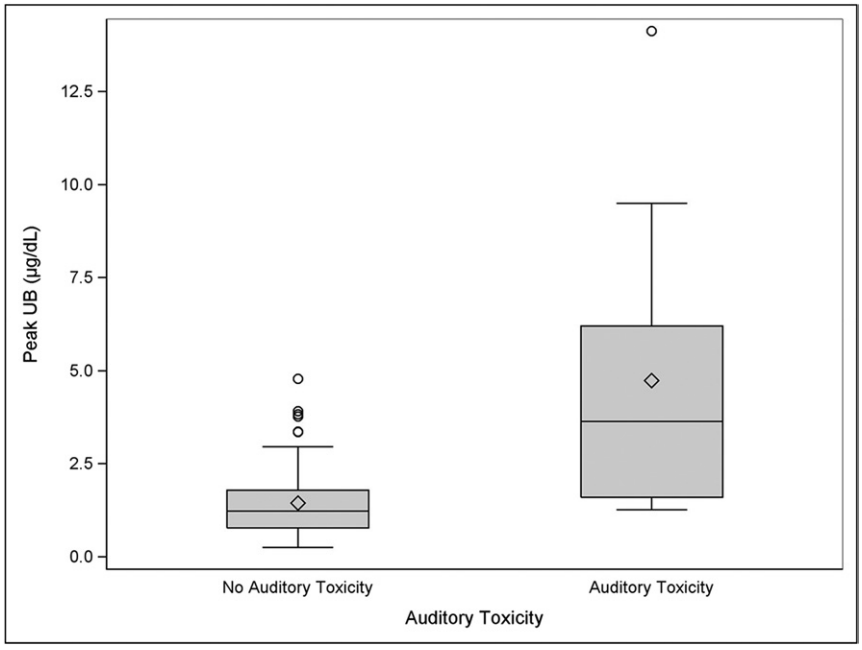

FIGURE 1
UB as a function of chronic auditory toxicity in late preterm and term infants with significant hyperbilirubinemia. In this box plot, the length of the box represents the interquartile range (IQR), or the distance between the 25th and 75th percentiles. The diamond symbol in the box interior represents the group mean. The horizontal line in the box interior represents the group median. The upper fence is defined as the 75th percentile plus 1.5 times IQR. The lower fence is defined as the 25th percentile minus 1.5 times IQR. Observations outside the fences are identified with circles.

TABLE 2 Bilirubin Albumin Binding Variables and Chronic Auditory Toxicity in Infants With Significant Jaundice ($N = 93$)

	Infants Without Auditory Toxicity, Mean (SD) ($n = 81$)	Infants With Auditory Toxicity, Mean (SD) ($n = 12$)	Adjusted Odds Ratio (95% CI)	*P*
Peak TSB (mg/dL[a])	23.6 (4.2)	29 (6.8)	1.10 (0.96–1.25)	.14
Peak BAMR	0.76 (0.17)	0.90 (0.24)	6.65 (0.23–188.5)	.26
Peak UB (μg/dL[b])	1.44 (0.94)	4.74 (3.92)	2.41 (1.43–4.07)	.001

P values were based on logistic regression analyses predicting auditory toxicity.
[a] Denotes multiply by 17.1 to convert to μmol/L.
[b] Denotes multiply by 17.1 to convert to nmol/L.

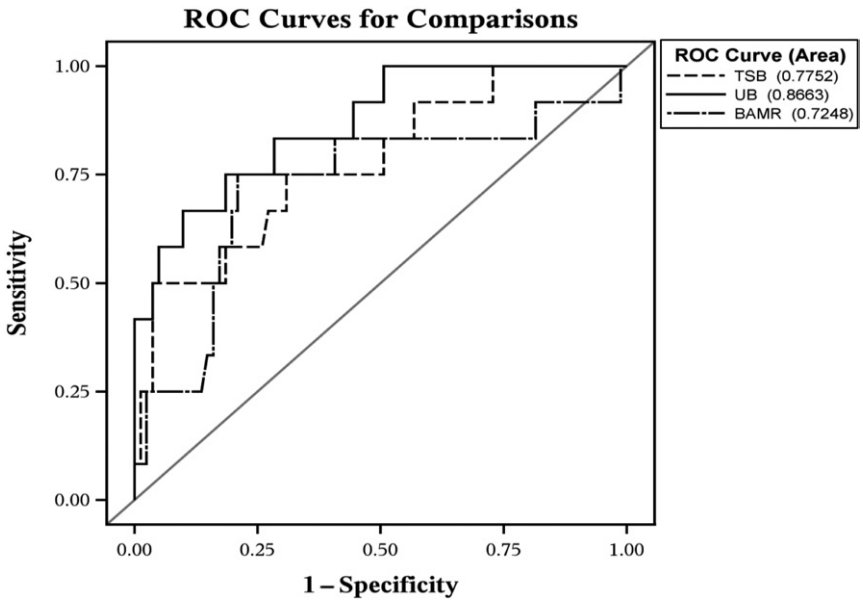

FIGURE 2
Bilirubin-albumin binding variables as predictors of chronic auditory toxicity in late preterm and term infants with significant hyperbilirubinemia. The straight line is the expected curve (unity) if the variable has no predictive value (area under unity curve, 0.5). The area under the curve (AUC) for UB is greater than the AUCs for TSB and BAMR. Controlling for covariates, there is a significant difference in the AUCs between TSB, UB, and BAMR

2 groups (Table 5). The peak TSB and peak UB (but not peak BAMR) were significantly higher among infants with chronic auditory toxicity compared with infants without auditory toxicity. In regression analyses controlling for sepsis, there was a significant association of peak UB (but not TSB or BAMR) with chronic auditory toxicity (Table 4).

DISCUSSION

We had previously reported that acute auditory toxicity, as manifested by an elevated auditory threshold and/or ANSD, is common among infants ≥34 weeks' GA with SHB.[13] Our findings from this longitudinal study suggest that chronic auditory toxicity is also common among infants with SHB. We believe that this is the first report of the natural course of auditory toxicity during infancy among infants ≥34 weeks' GA with SHB. Secondly, our findings suggest that UB (but not TSB or BAMR) is associated with chronic auditory toxicity in infants with SHB.

There is substantial evidence in the literature that SHB may be associated with SNHL and ANSD.[9–12,17–25] The Joint Committee on Infant Hearing also recognizes the TSB level at which an ET is indicated as a significant risk factor for SNHL and ANSD and recommends auditory evaluation.[12] In a previous report, we demonstrated a high incidence (28%) of acute auditory toxicity in infants as manifested by ANSD and/or elevated ABR threshold soon after the resolution of SHB. Our findings from a follow-up of the same cohort suggest a high incidence (13%) of chronic auditory toxicity as manifested by ANSD and/or SNHL. A majority of these infants (83%) with chronic auditory toxicity had acute auditory toxicity during the neonatal period, indicating the usefulness of comprehensive auditory evaluation soon after the resolution of SHB for early identification of infants at risk for chronic auditory toxicity. In addition, our findings also suggest that a normal comprehensive auditory evaluation soon after the resolution of SHB carries a high negative predictive value for subsequent development of chronic auditory toxicity.

Nonetheless, our finding of a small number of infants who developed chronic auditory toxicity despite a normal comprehensive auditory evaluation during the neonatal period indicate that SHB may be associated with a delayed onset of auditory toxicity. This finding underscores the need for a follow-up auditory evaluation of neonates with SHB for early identification and intervention to improve the functional outcome of neonates.[12] We also found that a significant number of infants with acute auditory toxicity had normal auditory evaluations at follow-up, suggesting reversible acute auditory toxicity, which possibly indicates auditory neural plasticity.

SHB is a sentinel event and warrants urgent evaluation and management to prevent kernicterus. TSB, the commonly used bilirubin measure for the management of SHB, has failed to discriminate infants at risk for kernicterus.[7] Our findings suggest that UB (but not TSB or

TABLE 3 Clinical Characteristics as a Function of Chronic Auditory Toxicity in the Subgroup of Neonates With TSB <25 mg/dL ($N = 63$)

	Neonates Without Auditory Toxicity ($n = 58$)	Neonates With Auditory Toxicity ($n = 5$)	*P*
GA, wks[a]	37.2 (1.3)	38.3 (1.5)	.06[b]
Birth weight, g[a]	2648 (424)	2692 (295)	.77[b]
Sex, *n* (% male)	38 (65)	1 (20)	.06[c]
Mode of delivery, *n* (% cesarean delivery)	14 (24)	0 (0)	.57[c]
Serum albumin, g/dL[a]	3.56 (0.5)	3.54 (0.9)	.81[b]
Bilirubin binding capacity, mg/dL[a]	31.3 (4.7)	31.1 (8.2)	.81[b]
Sepsis, *n* (%)	1 (2)	0 (0)	.99[c]
Asphyxia (Apgar score <3 at 5 min), *n* (%)	0 (0)	0 (0)	1.00[c]
Hemolytic disorders, *n* (%)	13 (22)	1 (20)	.99[c]
Polycythemia, *n* (%)	1 (2)	0 (0)	.99[c]
Breast milk feeding, *n* (%)	55 (95)	5 (100)	.99[c]
Clinical risk factor, *n* (%)	15 (26)	1 (20)	.99[c]
ET, *n* (%)	13 (22)	2 (40)	.58[c]

Clinical risk factors: hemolysis, asphyxia, hypoxia (Pa_{O_2} <45 mm Hg), acidosis (pH < 7.25), or albumin <3 g/dL.
[a] Mean (SD).
[b] Mann–Whitney *U* test.
[c] Fisher's exact test.

TABLE 4 Bilirubin Binding Variables and Chronic Auditory Toxicity (Subgroup Analyses)

	Neonates Without Auditory Toxicity	Neonates With Auditory Toxicity	Adjusted Odds Ratio (95% CI)	*P*
Neonates with TSB <25 mg/dL	Mean (SD) ($n = 58$)	Mean (SD) ($n = 5$)		
Peak TSB (mg/dL[a])	21.6 (1.5)	22.8 (1.2)	2.06 (0.92–4.6)	.08
Peak BAMR	0.70 (0.10)	0.77 (0.18)	3714 (0.44–3.10e^{+07})	.07
Peak UB (μg/dL[b])	1.19 (0.70)	1.99 (0.87)	3.31(1.09–10.0)	.03
Neonates with TSB ≥25 mg/dL	Mean (SD) ($n = 23$)	Mean (SD) ($n = 7$)		
Peak TSB (mg/dL[a])	28.6 (4.9)	33.4 (5.4)	1.18 (0.98–1.4)	.07
Peak BAMR	0.92 (0.20)	0.99 (0.24)	8.5 (0.12–596)	.32
Peak UB (μg/dL[b])	2.07 (1.16)	6.69 (4.12)	7.09 (1.15–43)	.03

P values were based on logistic regression analyses predicting auditory toxicity.
[a] Indicates to multiply by 17.1 to convert to μmol/L.
[b] Indicates to multiply by 17.1 to convert to nmol/L.

TABLE 5 Clinical Characteristics as a Function of Auditory Toxicity in the Subgroup of Neonates With TSB ≥25 mg/dL ($N = 30$)

	Neonates Without Auditory Toxicity ($n = 23$)	Neonates With Auditory Toxicity ($n = 7$)	*P*
GA, wks[a]	37.6 (1.5)	37.4 (0.53)	.84[b]
Birth weight, g[a]	2768 (478)	2715 (486)	.79[b]
Sex, *n* (% male)	11 (48)	5 (71)	.39[c]
Mode of delivery, *n* (% cesarean delivery)	4 (17)	0 (0)	.54[c]
Serum albumin (g/dL)[a]	3.6 (0.7)	3.9 (0.6)	.17[b]
Bilirubin binding capacity (mg/dL)[a]	31.8 (6.7)	34.5 (5.5)	.17[b]
Sepsis, *n* (%)	1 (4)	2 (28)	.12[c]
Asphyxia (apgar score <3 at 5 min), *n* (%)	0 (0)	0 (0)	1.00[c]
Hemolytic disorders, *n* (%)	6 (23)	2 (28)	.69[c]
Polycythemia, *n* (%)	1 (4)	0 (0)	.99[c]
Breast milk feeding, *n* (%)	22 (96)	7 (100)	.99[c]
Clinical risk factor, *n* (%)	7 (30)	4 (57)	.37[c]
ET, *n* (%)	18 (78)	7 (100)	.30[c]

Clinical risk factors: hemolysis, asphyxia, hypoxia (Pa_{O_2} <45 mm Hg), acidosis (pH <7.25), or albumin <3 g/dL.
[a] Mean (SD).
[b] Mann–Whitney *U* test.
[c] Fisher's exact test.

BAMR) is associated with chronic auditory toxicity in infants with SHB. These findings are consistent with our published report of the significant association of UB (but not TSB or BAMR) with acute auditory toxicity in the same cohort of infants with SHB.[13] This consistent finding of the association of UB (but not TSB) with neurologic outcomes confirms the critical role of UB in the pathogenesis of kernicterus. More importantly, our findings also suggest that without the UB data, one might erroneously conclude that SHB is not associated with chronic auditory toxicity.

The ROC curve analyses also strongly suggest that UB is a more sensitive and specific measure of jaundice-associated chronic auditory toxicity in infants with SHB than TSB or BAMR. This is consistent with the findings of ROC curve analyses reported by authors of previous studies for other jaundice-associated neurologic outcomes.[11,13,14,26] These findings are not surprising because UB (but not bilirubin bound to albumin) can cross the intact blood-brain barrier, leading to neurotoxicity. Besides, UB concentration is a better vascular gauge of jaundice severity because it is influenced by and increases with an increase in bilirubin load, a decrease in bilirubin binding capacity, and/or an increase in bilirubin binding dissociation equilibrium constant.[15,16] The slight overlap in UB level between infants with and without chronic auditory toxicity suggests that other unknown clinical factors such as neuronal predisposition to bilirubin toxicity may have a role in pathogenesis. We found no association of clinical risk factors (such as GA, sex, and hemolytic disorders) with chronic auditory toxicity.

The strength of the study is a prospective longitudinal follow-up of a large cohort of infants with SHB

who were previously evaluated for acute auditory toxicity. Secondly, we excluded infants with conditions that may be associated with auditory disorders. Thirdly, evaluations for ANSD and SNHL were performed by using diagnostic methods at an appropriate age and by an audiologist unaware of bilirubin biochemical measures. Fourthly, the accretion rate was excellent and there were no differences in GA and degree of jaundice between infants who completed evaluations and those who failed to complete auditory evaluations. Finally, the UB was measured by the modified peroxidase method to prevent underestimation of UB because of rate-limiting dissociation of bilirubin from albumin. The limitation of the study was that there was not enough power to evaluate the role of hypoxia, acidosis, and asphyxia as risk factors for bilirubin-induced auditory toxicity. Secondly, because our findings are derived from an observational study, an appropriately powered randomized clinical trial is required to establish the causal association of UB with chronic auditory toxicity in infants ≥34 weeks' GA with SHB.

CONCLUSIONS

UB (but not TSB or BAMR) is associated with chronic auditory toxicity as manifested by SNHL and/or ANSD in infants ≥34 weeks' GA with SHB. Secondly, chronic auditory toxicity is common among infants with SHB. With our findings, we provide supporting evidence for the need for close monitoring and comprehensive auditory evaluation for high-risk infants with SHB. More importantly, UB is a better predictor than TSB or BAMR of chronic auditory toxicity in infants ≥34 weeks' GA with SHB. Future studies are required to evaluate the association of SHB with specific adverse long-term neurodevelopmental outcomes as a function of UB because the use of TSB and BAMR in the absence of UB may lead to erroneous conclusions. Such studies are warranted because the findings of these studies will inform interventional studies to target high-risk infants by using appropriate bilirubin biochemical measures and neurodevelopmental outcomes to prevent or reduce the wide spectrum of neurodevelopmental disorders that may be associated with bilirubin-induced neurotoxicity.

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ABBREVIATIONS

AAP: American Academy of Pediatrics
ABR: auditory brainstem response
ANSD: auditory neuropathy spectrum disorder
AUC: area under the curve
BAMR: bilirubin albumin molar ratio
CI: confidence interval
ET: exchange transfusion
GA: gestational age
ROC: receiver operating characteristic
SHB: significant unconjugated hyperbilirubinemia
SNHL: sensorineural hearing loss
TSB: total serum bilirubin
UB: unbound bilirubin

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