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Auditory toxicity in late preterm and term neonates with severe jaundice

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Abstract

AIM—Jaundice may cause auditory toxicity (auditory neuropathy and hearing loss). However, total serum bilirubin (TSB) does not discriminate neonates at risk for auditory toxicity. We compared TSB, bilirubin:albumin molar ratio (BAMR), and unbound bilirubin for their association with auditory toxicity in neonates with severe jaundice (TSB ≥342μmol/L, or that met exchange transfusion).

METHOD—Neonates greater or equal to 34 weeks' gestational age with severe jaundice during the first two postnatal weeks were eligible for prospective cohort study, unless they had craniofacial malformations, chromosomal disorders, toxoplasmosis, other infections, rubella, cytomegalovirus, and herpes simplex infections, surgery, or family history of congenital deafness.

RESULTS—Twenty-eight out of 100 neonates (mean gestational age 37.4wks; 59 males, 41 females) had auditory toxicity. Peak unbound bilirubin, but not peak TSB and BAMR, was associated with auditory toxicity (p<0.05) in neonates with severe (TSB <427.5μmol/L) and extreme hyperbilirubinemia (TSB ≥427.5μmol/L). Area under the receiver operating characteristic curve for unbound bilirubin (0.78) was significantly greater (p=0.03) than TSB (0.54) among neonates with severe but not extreme hyperbilirubinemia.

INTERPRETATION—Unbound bilirubin is more strongly associated with auditory toxicity than TSB and/or BAMR in greater or equal to 34 weeks' gestational age neonates with severe jaundice. Unbound bilirubin is a better predictor than TSB in neonates with severe hyperbilirubinemia.

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SUPPORTING INFORMATION

The following additional information may be found online:

The authors have stated that they had no interests which might be perceived as posing a conflict or bias.

Severe jaundice may be associated with kernicterus, and therefore necessitates urgent readmission to the hospital for appropriate evaluations and therapeutic interventions to prevent and/or reduce bilirubin-induced neurotoxicity (BINT).[1] Despite preventive and therapeutic interventions, severe jaundice remains one of the leading causes of neurodevelopmental disabilities, specifically in developing countries with significant impact on societal cost.[2–7] Total serum bilirubin (TSB), the primary biochemical measure used for the management of jaundice, is a poor predictor of BINT, resulting in unwarranted costly interventions to prevent neurotoxicity.[1,2,8] This underscores the importance of identifying a better predictor than TSB for BINT.

The characteristic findings of kernicterus such as choreo-athetoid palsy, supra-nuclear gaze palsy, and language disorder take years to manifest, limiting their use as outcomes to identify predictors of BINT.[1,7] Because of the known predilection of auditory pathway for bilirubin toxicity, auditory evaluation has emerged as a useful, non-invasive, and objective way to evaluate acute BINT in neonates. Although diagnostic evaluation for sensorineural hearing loss (SNHL) using behavioural audiometry is not feasible in neonates, an elevated auditory brainstem-evoked response (ABR) threshold can be used during the neonatal period to evaluate for possible SNHL. In addition, auditory neuropathy spectrum disorder (ANSD), a condition characterized by abnormal or absent ABR and a normal oto-acoustic emission (OAE) test, has been increasingly described with acute BINT in neonates with severe jaundice.[5,9,10] However, TSB has failed to discriminate neonates at risk for bilirubin-induced auditory toxicity.[5,10]

A recent prospective study demonstrated that unbound bilirubin (bilirubin not bound to albumin) is a better predictor of bilirubin-induced ANSD in late preterm and term neonates with severe jaundice than TSB and bilirubin:albumin molar ratio (BAMR).[11] However, this study failed to evaluate for elevated ABR threshold and had few neonates with extreme hyperbilirubinemia (EHB, TSB ≥25mg/dL [multiply by 17.1 to convert to μmol/L] or 427.5μmol/L).[11,12] Therefore, there is a need to validate the usefulness of unbound bilirubin for BINT using a more comprehensive auditory evaluation in a population where EHB is more prevalent. Our objective was to determine whether UB is associated with – and is a better predictor of – bilirubin-induced auditory toxicity than TSB and BAMR in late preterm and term neonates with severe jaundice.

METHOD

Study design

This was a prospective large cohort study involving neonates admitted with severe jaundice from 2011 to 2014 at two academic centres in Delhi, India: Sir Ganga Ram Hospital and Kalawati Saran Children's Hospital. The study was approved by the institutional ethics committee. Parental consent for research and publication was obtained for each participant enrolled.

Participants

Neonates more than or equal to 34 weeks' gestational age at birth who were admitted to the Neonatal Intensive Care Unit (NICU) with severe unconjugated hyperbilirubinemia (defined as TSB ≥20mg/dL [342μmol/L] or TSB that met the exchange transfusion criteria according to the American Academy of Pediatrics [AAP] guidelines) during the first two postnatal weeks were eligible for the study.[1,5,11,12] Neonates with the following conditions were excluded: craniofacial malformations; chromosomal disorders; family history of congenital deafness; toxoplasmosis, other infections, rubella, cytomegalovirus, and herpes simplex infections; and surgical interventions. In addition, neonates with a failed hearing screening test before severe jaundice or whose parents lived outside Delhi were also excluded. For $34^{0/7}$ to $34^{6/7}$ weeks' gestational age neonates, TSB concentration that met ET criteria was considered the same as that for $35^{0/7}$ to $37^{6/7}$ gestational age neonates.[1] Gestational age was assessed by obstetric history including first trimester ultrasound, or if obstetric history was unreliable, by Ballard examination.

Evaluation for jaundice

Participants received appropriate evaluation and treatment as outlined in AAP guidelines.[1] The laboratory evaluations including blood group determination of the mother and neonate, direct antiglobulin test, reticulocyte count, peripheral smear, and hematocrit were routinely done. Phototherapy and exchange transfusion were used as per AAP guidelines.[1] Intravenous gamma globulin was used as per the discretion of the attending physician for jaundice secondary to ABO or Rhesus incompatibility.

Bilirubin–albumin binding variables – exposure variables

Blood samples for the measurement of TSB for individual participants were drawn as clinically indicated at the discretion of the attending physician in amber-coloured serum separator tubes to protect from light. TSB was measured immediately (<2hrs) by the institutional clinical laboratory using the colorimetric method. The serum albumin was measured (g/dL [multiply by 151 to convert to μmol/L]) on each participant at the time of admission, with subsequent TSB if jaundice increased despite phototherapy, and before exchange transfusion, using the bromo-cresol green method. The same instrumentation (Beckman Synchron LX – 20 Pro, Beckman Coulter, Inc., Indianapolis, USA) was used for the measurement of TSB and albumin at participating centres. The peak BAMR was calculated for each participant using the peak TSB and the concurrent serum albumin.

Unbound bilirubin was measured using the same aliquot of blood used to measure TSB. For samples collected for TSB measurement before obtaining the consent, residual serum (minimum 50μl) was obtained immediately from the clinical laboratory, which routinely stores extra serum in a −20°C freezer. Blood samples collected after the consent were processed immediately to separate the serum. All serum samples were stored immediately in a −80°C freezer. Frozen samples were shipped to Rochester, USA on dry ice over 48 hours for unbound bilirubin measurement (μg/dL [multiply by 17.1 to convert to nmol/L]). In addition, an aliquot of 10 serum samples with known TSB were sent with each shipment as controls. These controls and study serum samples (n=5) with varying duration of storage (1–6mo) in −80°C were measured for TSB on arrival in the clinical laboratory. Unbound

bilirubin was measured on arrival by the modified peroxidase method at two enzyme concentrations (1:25 and 1:12.5 dilutions) of pre-calibrated peroxidase (Arrows Company, Osaka Japan) using an FDA-approved Arrows UB analyzer UA-1 (Arrows Company).

Comprehensive auditory evaluation – outcome variables

Each participant had comprehensive auditory evaluation (ABR, OAE, and tympanometry) performed in both ears after resolution of jaundice and within 96 hours of peak TSB by a single audiologist unaware of the degree of jaundice. Auditory brainstem-evoked responses were recorded with a Biological Navigator Evoked Response System (Bio-logic, Mundelein, IL) using 35 and 80 decibel (dB) broadband stimulus in a quiet room with the participants lying supine in the crib and a skin temperature greater than 35.5°C. The clicks were presented at a repetition rate of 29.9/sec, and three runs of 2000 repetitions were recorded for each ear. The response was amplified (×200 000), band-pass filtered (100–3000 Hz), and averaged over a 20ms time epoch. The two most reproducible runs for each ear were averaged and then used for analysis. Auditory brainstem-evoked responses were also performed using reverse polarity (condensation and rarefaction) to evaluate for cochlear microphonics. The OAE test was administered using DP Echoport ILO 292 (Otodynamics, London, England) on all participants with an 80dB click stimulus. A normal OAE was defined as a replicable response (3dB signal to noise ratio) in the three highest frequency bands (2000, 3000, and 4000Hz). Tympanometry was performed with a Middle Ear Analyzer (Grason Stadler, Milford, NH) using a 256Hz probe tone to exclude middle ear disease.

The audiology tests were evaluated by an experienced audiologist in the USA unaware of the degree of jaundice. Neonates with abnormal ABR morphology (absent wave I and III) or absent ABR waveform (absent wave I, III, and V) at 80dB, but with normal OAE or presence of cochlear microphonics, were diagnosed to have ANSD. Neonates with abnormal ABR morphology or absent ABR waveform at 35 dB, but normal ABR waveform (present wave I, III, and V) at 80dB, were diagnosed to have elevated ABR threshold. Neonates with either ANSD or elevated ABR threshold were deemed to have auditory toxicity.

Risk factors – covariates

Clinical factors such as perinatal asphyxia (Apgar score <3 at 5mins and/or cord pH <7.0), sepsis (culture proven or clinical sepsis requiring at least 7d of intravenous antibiotics), hypoxia ($Pa0_2$ <45mm of Hg), acidosis (pH <7.25), hypoalbuminemia (albumin <3g/dL), and hemolytic disorders (Rhesus incompatibility, ABO incompatibility, glucose-6-phosphate dehydrogenase [G6PD] deficiency, etc.) were prospectively collected.

Sample size calculation

We assumed the area under the receiver operating characteristic (ROC) curve for TSB to be ~0.5 and the correlation of TSB with unbound bilirubin to be ~0.4, based on preliminary findings.[11] A sample size of 100 participants with ~25% prevalence of bilirubin-induced auditory toxicity will achieve 80% power to detect a difference of 0.21 in the area under the curve (AUC) between UB and TSB using a two-sided z-test at a significance level of 5%.[5,10]

Statistical analyses

All analyses were conducted using SAS 9.4 (SAS Institute Inc. Cary, NC). Because the prevalence of neurotoxicity is related to the degree of jaundice, subgroup analyses for neonates with severe hyperbilirubinemia (TSB <25mg/dL [427.5μmol/L]) and EHB were also performed.[12] The Fisher's exact or the chi-square test was used for categorical variables while 2-sample *t*-tests or the Mann–Whitney *U* test were used for continuous variables. Logistic regression analyses were used to evaluate the independent association between each of the bilirubin variables (TSB, UB, and BAMR) and auditory toxicity. Multicollinearity was checked using multiple correlations (variance inflation factors). Variables with significant association ($p \leq 0.15$) to outcomes were included in the regression model. Backward model selection was used to decide the final regression models. ROC curves adjusted for covariates were plotted for each of the bilirubin variables predicting auditory toxicity, and AUCs were compared using the non-parametric test. All analyses were two-sided at the 0.05 level of significance.

RESULTS

A total of 124 neonates were admitted to the NICU with severe jaundice, of which eight neonates whose parents lived outside Delhi were excluded. A total of 100 neonates were studied after consent was given. The mean birthweight and gestational age for the neonates were 2703g (standard deviation [SD] 431) and 37.4 weeks (SD 1.3) respectively. The mean peak TSB and unbound bilirubin for neonates were 24.2mg/dL (SD 4.9) and 1.84μg/dL (SD 1.91) respectively. On Bland–Altman analysis, the mean difference between TSB measured in Rochester and India for the control and study samples was 0.04mg/dL (range −2.1 to 2.2). The interclass correlation coefficient between TSB measured in Rochester and India was 0.9968 (95% confidence interval [CI]: 0.991 to 0.998), and confirms stability of samples during storage and shipping.

All neonates received intensive phototherapy with a mean (SD) duration of 40 (22) hours. Eleven neonates received intravenous immunoglobulin. There were 22 neonates with hemolytic jaundice, 13 with hypoalbuminemia, one with hypoxia, one with acidosis, two with polycythemia (hematocrit >65%), four with cephalhematoma, and one with extensive bruising.

Twenty-eight neonates (28%) were found to have auditory toxicity (15 with ANSD and 13 with elevated ABR threshold). None of the neonates had middle ear disease. There was no difference in gestational age, birthweight, sex, asphyxia, sepsis, type of enteral feeding (breast milk or formula), hemolytic disorders, or polycythemia between neonates with and without auditory toxicity (Table I). The mean postnatal age in hours of peak TSB in neonates with and without auditory toxicity were similar (117 [SD 39] vs 114 [SD 49], $p=0.566$). A higher proportion of neonates who developed auditory toxicity were delivered by caesarean section and received exchange transfusion compared to neonates without auditory toxicity (Table I). The peak unbound bilirubin, TSB, and BAMR were significantly higher among neonates with auditory toxicity compared to neonates without auditory toxicity (Table II). On regression analyses using three separate regression models with mode of delivery, sepsis, and exchange transfusion included as covariates, there was a significant association of peak

unbound bilirubin and BAMR, but not peak TSB with auditory toxicity, as shown in Table II. On ROC analyses controlling for covariates (Fig. 1A), unbound bilirubin (0.87, 95% CI: 0.79–0.95) had a larger AUC compared to TSB (0.80, 95% CI: 0.70–0.91) with a trend for significance (p=0.067). There was no difference (p=0.204) in the AUCs between unbound bilirubin and BAMR (0.83, 95% CI: 0.72–0.93) as well as between TSB and BAMR (p=0.315).

A higher proportion of infants with auditory toxicity had BAMR greater than or equal to 1 compared to those without auditory toxicity (28% vs 5% respectively, p=0.003). However, controlling for covariates, there was no significant association between elevated BAMR greater than or equal to 1 and auditory toxicity (OR 3.9, 95% CI: 0.8–19, p=0.082). In neonates with BAMR less than 1 (n=88), the equilibrium binding constant, a measure of bilirubin albumin binding affinity, was similar between neonates with and without auditory toxicity (224 [SD 209] vs 381 [SD 677], p=0.943).

Sub-group analyses

Among 69 neonates with severe hyperbilirubinemia, there were 11 neonates (16%) with auditory toxicity. There was no difference in clinical characteristics between neonates with and without auditory toxicity (Table SI, online supporting information). The peak unbound bilirubin, but not peak TSB and BAMR, was significantly higher among neonates with auditory toxicity compared to neonates without auditory toxicity. There was significant association of peak unbound bilirubin, but not peak TSB and BAMR, with auditory toxicity (Table SII, online supporting information). On ROC analysis (Fig. 1B), unbound bilirubin (0.78, 95% CI: 0.64–0.93) had a significantly (p=0.033) larger AUC than TSB (0.54, 95% CI: 0.36–0.73). Similarly, unbound bilirubin had a larger AUC compared to BAMR (0.61, 95% CI: 0.43–0.80) for auditory toxicity with a trend for significance (p=0.103; Fig. 1B). There was no significant difference in AUCs between BAMR and TSB for auditory toxicity (p=0.500).

Among 31 neonates with EHB, there were 17 (55%) neonates with auditory toxicity. There was no significant difference in clinical characteristics between the two groups except for sex and ET (Table SIII, online supporting information). A higher proportion of neonates who developed auditory toxicity were male and received exchange transfusion compared to neonates without auditory toxicity (Table SIII). The peak unbound bilirubin, but not peak TSB and BAMR, was significantly higher among neonates with auditory toxicity compared to neonates without auditory toxicity. On regression analyses controlling for mode of delivery, sex, and exchange transfusion, there was a significant association (p=0.044) of peak unbound bilirubin, but not TSB and BAMR, with auditory toxicity (Table SII). On ROC analysis, although unbound bilirubin had a larger AUC, there was no significant difference (p>0.05) in AUCs between unbound bilirubin (0.89, 95% CI: 0.78–1), TSB (0.84, 95% CI: 0.69–0.98), and/or BAMR (0.86, 95% CI: 0.71–1) for auditory toxicity after controlling for mode of delivery, sex, and exchange transfusion (Fig. 1C).

DISCUSSION

Kernicterus, a preventable neurodisability resulting from severe jaundice, is common in developing countries and also occurs infrequently in developed countries.[3–6,10,13,14] The consensus-based AAP guidelines for the management of severe jaundice in late preterm and term neonates are based on TSB, which has failed to discriminate neonates at risk for kernicterus.[1,8] Identification of a better biochemical measure will not only improve management of severe jaundice, but may also help reduce the morbidity and mortality associated with severe jaundice and related therapeutic interventions.[2,8] Our findings suggest that unbound bilirubin is more strongly associated with auditory toxicity than TSB and BAMR in late preterm and term neonates with severe and EHB. More importantly, unbound bilirubin is a better predictor than TSB and BAMR among neonates with severe hyperbilirubinemia.

The auditory system is the most sensitive neural system to overt BINT.[15] Not surprisingly, we not only found a high incidence of auditory toxicity, but also increased incidence of auditory toxicity with increasing severity of jaundice. Our findings of 16% incidence of ANSD are in agreement with published studies.[5,9–11,16] We also found that elevated ABR threshold is common among neonates with severe jaundice. These findings underscore the importance of comprehensive auditory evaluation in neonates with severe jaundice after its resolution.

The critical issue with TSB as a predictor of BINT is higher sensitivity but lower specificity when TSB is less than 25mg/dL, a condition more common than EHB.[8] The low specificity results in unwarranted aggressive treatment such as exchange transfusion that is not only costly, but may also be associated with adverse effects including death.[2,8] Our findings suggest that unbound bilirubin is not only strongly associated with auditory toxicity but is a better predictor of auditory toxicity than TSB and BAMR among neonates with severe hyperbilirubinemia. Our findings are consistent with the findings of previous studies in similar populations.[11,17] Ahlfors et al. demonstrated in a retrospective study involving 44 neonates, that unbound bilirubin is a better predictor than TSB of a failed hearing screening test as evaluated by automated ABR performed within 4 hours of peak TSB.[17] However, the failed hearing screening test using 35dB automated ABR does not necessarily imply ANSD or SNHL. Similarly, a prospective study involving comprehensive auditory evaluation for ANSD in 44 neonates with severe jaundice demonstrated that unbound bilirubin is a better predictor of bilirubin-induced ANSD than TSB and BAMR.[11] Our study differed from previous studies in participant population characterized by an Asian race and lower birthweight, and inclusion of elevated ABR threshold as an outcome. Clinical studies involving premature neonates also suggest that neurological outcomes of jaundice are more strongly associated with unbound bilirubin than TSB.[18–23]

At extreme TSB levels, the specificity of TSB for auditory toxicity increases as the incidence of auditory toxicity also increases – thus AUCs for TSB and BAMR were greater for neonates with EHB. This partially explains why despite significant association of unbound bilirubin with auditory toxicity, unbound bilirubin does not significantly improve prediction over TSB and BAMR among neonates with EHB. We also found a higher risk for

bilirubin-induced auditory toxicity among male neonates with EHB. Male predisposition to BINT has been previously reported by Seidman et al. who demonstrated that only among male infants, severe jaundice was associated with a significant risk of lower intelligence at 17 years of age.[24]

The major strengths of this prospective study are unbound bilirubin measurement using the modified peroxidase method and comprehensive auditory evaluation by an audiologist unaware of bilirubin measures. To minimize underestimation of unbound bilirubin caused by sample dilution with the peroxidase method, a modified peroxidase method was used. The findings of auditory disorders were observed despite immediate and appropriate therapeutic intervention and therefore represent clinically more meaningful and persistent neurotoxicity, compared to earlier, more subtle and transient ABR findings reported by previous studies.[17,23,25] The limitation of the study is that we were unable to evaluate the role of clinical risk factors in bilirubin-induced auditory toxicity.

In summary, unbound bilirubin is more strongly associated with auditory toxicity than TSB and BAMR in neonates of greater than or equal to 34 weeks' gestational age. More importantly, unbound bilirubin is a better predictor than BAMR and TSB of auditory toxicity, specifically when TSB is less than 25mg/dL. Our findings from an Asian population provide additional evidence and lend generalizability for the usefulness of unbound bilirubin in the evaluation of BINT. Future studies are needed to evaluate the natural course of bilirubin-induced auditory toxicity and also the usefulness of unbound bilirubin for predicting jaundice's specific long-term neurological outcomes. Findings of such studies will inform interventional studies to target high-risk neonates using appropriate biochemical measures to prevent or reduce severity of BINT.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

ABR	Auditory brainstem-evoked response
ANSD	Auditory neuropathy spectrum disorder
AUC	Area under the curve
BAMR	Bilirubin:albumin molar ratio
BINT	Bilirubin-induced neurotoxicity
EHB	Extreme hyperbilirubinemia

OAE	Oto-acoustic emission
TSB	Total serum bilirubin

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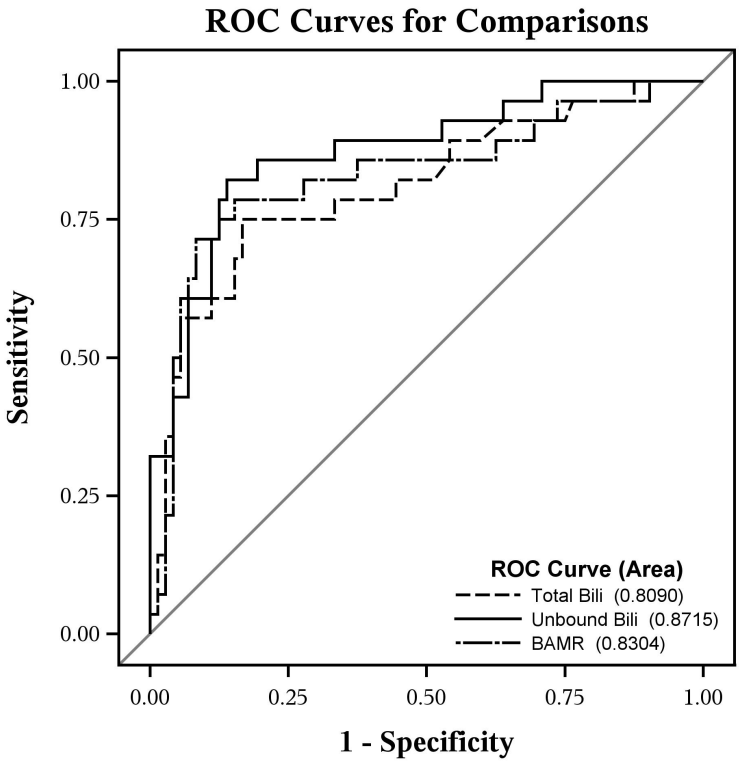
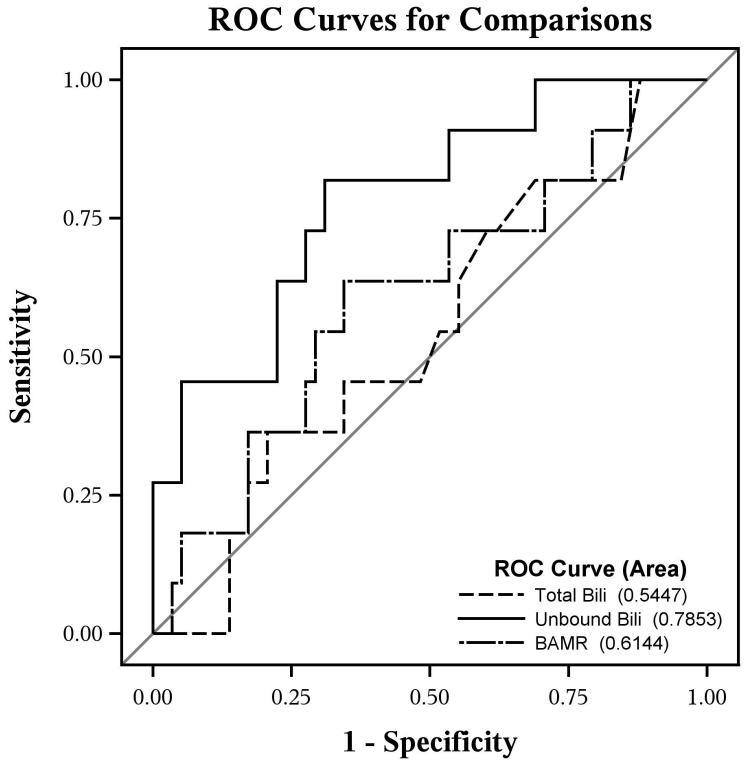
What this paper adds

- Auditory neuropathy and hearing loss are common among neonates with severe jaundice.
- Unbound bilirubin is associated with auditory toxicity in neonates with severe or extreme hyperbilirubinemia.
- Unbound bilirubin is a better predictor than total serum bilirubin of auditory toxicity in neonates with severe but not extreme hyperbilirubinemia.
- Increased susceptibility of bilirubin-induced auditory toxicity among male neonates with extreme hyperbilirubinemia supports sex-specific differences in bilirubin-induced neurotoxicity.





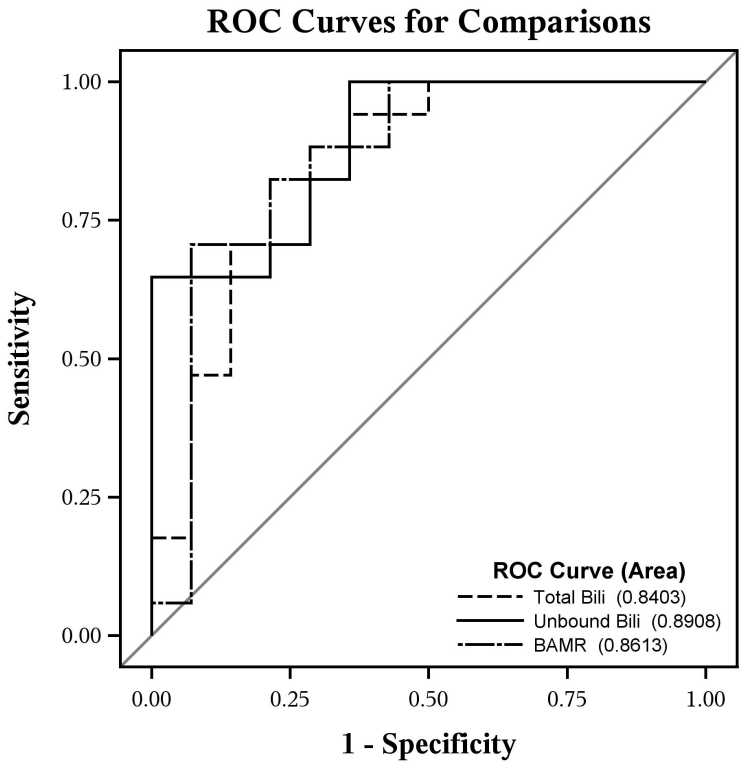


Figure 1.

Bilirubin-albumin binding variables in infants with severe jaundice.

Receiver operating characteristic (ROC) curves of peak total serum bilirubin (TSB), peak unbound bilirubin (UB), and peak bilirubin:albumin molar ratio (BAMR) as predictors of auditory toxicity, controlling for confounders, for (a) all 100 neonates, for (b) neonates with severe hyperbilirubinemia (TSB <25mg/dL), and (c) for neonates with extreme hyperbilirubinemia (TSB ≥25mg/dL). Figure 1a: 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 AUC for TSB and BAMR but the difference is not significant. Figure 1b: the AUC for UB is significantly greater than the AUC for TSB. The AUC for UB is greater than the AUC for BAMR with a trend for significance. There is no difference in AUCs between TSB and BAMR. Figure 1c: there is no significant difference between the AUCs of TSB, UB, and/or BAMR for auditory toxicity.

Table I

Clinical characteristics as a function of auditory toxicity in neonates with severe jaundice (n = 100)

	Neonates without ANSD and elevated ABR threshold (*n*=72)	**Neonates with auditory toxicity (*n*=28)**	***p***
Gestational age (weeks)[a]	37.3 (1.3)	37.4 (1.3)	0.86
Birthweight (grams)[a]	2687 (416)	2745 (474)	0.54[b]
Gender, *n* (% Male)	41 (57)	18 (64)	0.65
Mode of delivery, *n* (% C-section)	18 (25)	2 (7)	0.05[c]
Serum albumin (g/dL)[a]	3.6 (0.65)	3.5 (0.55)	0.6[b]
Sepsis, *n* (%)	1 (1)	3 (11)	0.06[c]
Asphyxia (Apgar score < 3 at 5min), *n* (%)	0	0	1
Hemolytic disorders, *n* (%)	14 (19)	8 (29)	0.4
Polycythemia, *n* (%)	2 (2)	0 (0)	1.0[c]
Breast milk feeding, *n* (%)	70 (97)	26 (92)	0.31[c]
Clinical risk factor, *n* (%)	22 (30)	13 (46)	0.16
Exchange transfusion, *n* (%)	22 (30)	20 (71)	0.001

Clinical Risk Factor: hemolysis, asphyxia, hypoxia (PaO_2 <45 mm of Hg), acidosis (pH <7.25), albumin <3g/dL.

[a] Mean (standard deviation);

[b] Mann–Whitney *U* test;

[c] Fisher's exact test. ANSD, auditory neuropathy spectrum disorder; ABR, auditory brainstem evoked response.

Table 2

Association between bilirubin–albumin binding variables and auditory toxicity in neonates with severe jaundice (n=100)

	Neonates without ANSD and elevated ABR threshold (*n*=72)	**Neonates with auditory toxicity (*n*=28)**	**Adjusted odds ratio (95% CI)**	***p***
Peak total serum bilirubin (mg/dL[c])[a]	22.9 (3.7) (18.6–47)	27.7 (5.9) (20.2–40)	1.15 (1–1.3)	0.05
Peak bilirubin:albumin molar ratio[a]	0.74 (0.16) (0.44–1.52)	0.91 (0.20) (0.59–1.39)	95(2.9–3167)	0.01
Peak unbound bilirubin (μg/dL[b])[a]	1.24 (0.74) (0.25–3.77)	3.37 (2.92) (0.76–14.12)	2.9 (1.6–5.3)	0.0004

[a] mean (SD) (range), p values were based on logistic regression analyses predicting auditory toxicity;

[b] multiply by 17.1 to convert to nmol/L;

[c] multiply by 17.1 to convert to μmol/L. ANSD, auditory neuropathy spectrum disorder; ABR, auditory brainstem evoked response; CI, confidence interval.