Vigintiphobia, Unbound Bilirubin, and Auditory Brainstem Responses

Masahisa Funato, MD*; Hiroshi Tamai, MD*; Seiichi Shimada, MD*; and Hajime Nakamura, MD‡

ABSTRACT. *Objective.* **The management of nonhemolytic hyperbilirubinemia in term newborns is controversial. To evaluate the usefulness of serum unbound bilirubin concentrations (UBCs) in the management of hyperbilirubinemia, we compared the concentrations with abnormal auditory brainstem responses (ABRs).**

Methods. **ABRs and serum UBCs in 37 hyperbilirubinemic term newborns (total bilirubin concentrations [TBCs] ≥20 mg/dL and direct bilirubin concentrations <2 mg/dL) were measured before treatment with either phototherapy or exchange transfusions. Eight of these newborns had blood incompatibilities. These hyperbilirubinemic newborns were divided into three groups according to the findings of ABR: group A, normal ABR (n = 18); group B, prolonged latency of wave I only (n = 8); and group C, prolonged interpeak latency of wave I–III/I–V and/or poor amplitude (n = 11).**

Results. **The peak TBC was significantly different between groups A and C (22.8 ± 2.2 mg/dL and 25.4 ± 2.5 mg/dL, respectively; $P < .05$), though there were no differences between groups A and B and between groups B and C. The peak UBCs in groups B (1.27 ± 0.7 μg/dL) and C (1.34 ± 0.37 μg/dL) were significantly higher than in group A (0.78 ± 0.26 μg/dL) ($P < .05$ and $P < .01$, respectively), though there was no significant difference in the peak UBC between groups B and C. Abnormal ABR findings were more clearly associated with the level of UBC at 1.0 μg/dL than that of TBC at 23 mg/dL by multiple logistic regression analysis (odds ratio = 16.6, $P = .0026$, vs 4.2, $P = .1272$).**

Conclusions. **These results suggest that measuring UBC may help in evaluating the possible risk of bilirubin encephalopathy in full-term newborns when there is vigintiphobia (fear of 20).** ***Pediatrics* 1994;93:50–53; *hyperbilirubinemia, vigintiphobia, unbound bilirubin, auditory brainstem responses, newborn.***

ABBREVIATIONS. UBC, unbound bilirubin concentration; TBC, total bilirubin concentration; B/A, bilirubin-albumin molar ratio; ABR, auditory brainstem response.

Since the introduction of blood exchange transfusions for severe neonatal hyperbilirubinemia associated with hemolytic disease of the newborn,[1] bilirubin encephalopathy has been greatly reduced in term newborns. As a preventive measure the administration of anti-Rh globulin to the Rh-negative mother[2] has also contributed enormously to the reduction in hemolytic disease due to Rh isoimmunization.

In spite of such progress in the management of hyperbilirubinemia under several criteria,[3–5] we have not yet been able to achieve a reasonable and confirmatory criterion for the treatment of hyperbilirubinemia. We still continue to have the problem of "vigintiphobia"[6] (fear of bilirubin level: 20 mg/dL) in the management of hyperbilirubinemia in term newborns. Recently, the treatment of nonhemolytic, hyperbilirubinemic newborns with bilirubin concentration ≥20 mg/dL has become controversial.[7,8]

The hypothesis of this study is that unbound bilirubin concentration (UBC) could be a more reliable predictor of bilirubin encephalopathy than total bilirubin concentration (TBC), because unbound or "free" bilirubin is the toxic fraction capable of crossing the intact blood-brain barrier into the brain.[9] So, in dealing with the problem of vigintiphobia, we have tried to evaluate the usefulness of serum unbound bilirubin concentration for the management of neonatal hyperbilirubinemia. Thus we have compared serum TBCs, UBCs, and bilirubin-albumin molar ratios (B/As) with the abnormal auditory brainstem responses (ABRs), which may reflect possible bilirubin encephalopathy in hyperbilirubinemic newborns.[10–13]

SUBJECTS AND METHODS

Between 1987 and 1990, 72 term newborns (≥37 weeks' gestation) with hyperbilirubinemia (TBC ≥ 20 mg/dL) were admitted to our hospital. Newborns with central nervous disorders such as asphyxia, meningitis, intracranial hemorrhage, and brain anomalies were excluded from this study. Direct hyperbilirubinemia (direct bilirubin concentration >2 mg/dL) was also excluded. In 37 of these 72 newborns, results of ABR tests were available just before the newborns received phototherapy or exchange transfusions. Informed consent was obtained from the parents of all of these newborns. Of these, there were 8 with incompatibilities and 29 with nonhemolytic jaundice (4, polycythemia; 3, cephalhematoma; 22, idiopathic jaundice).

The ABR was measured with a Nicolet Compact 4 signal averager using monaural 80-dB, 2000 rarefraction click stimuli with a 150- to 1500-Hz band-pass and a 10-millisecond time base. TBC and UBC were measured by the peroxidase-glucose oxidase method[14] using an Arrows UB Analyzer. Serum total protein was measured by the biuret method using a Hitachi 736-40 Automatic Analyzer. Serum albumin was measured by means of electrophoresis on cellulose acetate membrane[15] using a Zyokou CTE-150 Autoanalyzer. Coefficients of variation in measurement of TBC, UBC, and albumin were 1.1 ± 0.3%, 3.1 ± 0.9%, and 1.4 ± 0.5%, respectively. The B/A was also calculated in 32 patients whose serum albumin concentrations were measured at the same time.

The cutoff values of latencies of wave I and interpeak latencies of wave I–III and I–V were defined over the mean +2 SD of the latencies in the 16 nonhyperbilirubinemic normal newborns (TBC < 15 mg/dL) at 3 days of age. Poor amplitude was defined by cutoff values below the mean −2 SD of all the amplitude of waves I, III, V in these normal newborns (Table 1).

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TABLE 1. Auditory Brainstem Response (ABR) Latencies and Amplitudes in Nonhyperbilirubinemic Normal Newborns (N = 16)*

ABR	I	III	V	I–III	I–V
Latency, ms	1.77 ±0.22	4.47 ±0.54	6.72 ±0.63	2.69 ±0.47	4.96 ±0.61
Amplitude, μV	0.204 ±0.053	0.186 ±0.05	0.229 ±0.089		

* Values represent mean ± 2 SD.

Hyperbilirubinemic newborns were divided into three groups according to the findings of ABR: group A had normal ABR (n = 18), group B had prolonged wave I latency only (n = 8), and group C had prolonged wave I–III and/or I–V interpeak latency and/or poor amplitude (n = 11). Statistic analyses were performed by the unpaired Student's *t* test and the Wilcoxon *U* test. Of variables (gender, birth weight, gestation, age, hemolysis, TBC, UBC, and B/A) associated with abnormal ABR findings, TBC and UBC were identified by a multiple regression analysis of Machintosh SPSS Advance Statistics. The cutoff points of TBC and UBC were determined in terms of the maximal values of the χ^2 test with Yates' correction of TBC levels between 22 and 24 mg/dL and of UBC levels between 0.8 and 1.2 μg/dL.

RESULTS

The means ± SD of birth weight, weeks of gestation, and age on admission were 3108 ± 346 g, 38.9 ± 0.9 weeks, and 4.5 ± 2.6 days of life in group A, 3080 ± 335 g, 38.6 ± 0.7 weeks, and 4.5 ± 1.9 days of life in group B, and 3224 ± 507 g, 38.9 ± 1.4 weeks, and 3.5 ± 0.9 days of life in group C, respectively. There were no significant differences in birth weight, gestation, and age on admission among the three groups (unpaired *T*).

The means ± SD of peak TBCs were 22.8 ± 2.2 mg/dL in group A (n = 18), 24.5 ± 3.1 mg/dL in group B (n = 8), and 25.4 ± 2.5 mg/dL in group C (n = 11), respectively. A significant difference of the peak TBC was noticed between groups A and C (Wilcoxon: $P < .05$), though there were no significant differences in the TBCs between groups A and B and between groups B and C (Fig 1). The means ± SD of peak UBCs were 0.78 ± 0.26 μg/dL in group A, 1.27 ± 0.7 μg/dL in group B, and 1.34 ± 0.37 μg/dL in group C, respectively. The peak UBCs in groups B and C were significantly higher than in group A (Wilcoxon: $P < .05$, $P < .01$, respectively), though there was no significant difference in the UBC between groups B and C (Fig 2). The means of peak B/As were 0.7 ± 0.09 in group A (n = 15), 0.78 ± 0.13 in group B (n = 7), and 0.85 ± 0.11 in group C (n = 10). There was a significant difference in the peak B/A between groups A and C (Wilcoxon: $P < .01$), though no significant differences were noticed between groups A and B and between B and C. (Fig 3).

In the logistic regression model, abnormal ABR findings were significantly associated with UBC (Table 2). The adjusted odds of abnormal ABR finding for UBC ≥ 1.0 μg/dL was 16.6 (95% confidence interval, 6.5 to 42.4) times greater than for UBC < 1.0 μg/dL ($P = .0026$). TBC ≥ 23.0 mg/dL was 4.19 (1.6 to 10.7) times more likely to have abnormal ABR findings than TBC < 23.0 mg/dL at the level of $P = .1272$.

Exchange transfusions were performed as follows: 4 (22.2%) of 18 in group A, 4 (50%) of 8 in group B, and 11 (100%) of 11 in group C.

DISCUSSION

The mechanism of bilirubin encephalopathy has continued to be controversial and has presented very large problem—"a real mess" according to Lucey.[16] An increased unbound bilirubin level,[17,18] impaired blood-brain barrier,[19,20] and intracerebral acidosis,[9,21] are considered to be important pathogenic factors in inducing bilirubin encephalopathy. It has been dem-

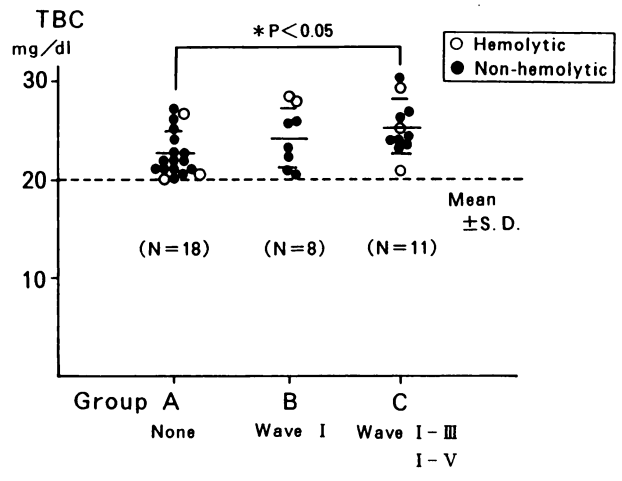

Fig 1. Total bilirubin concentration (TBC) levels and auditory brainstem response (ABR) abnormalities in three groups: group A, normal ABR recording; group B, prolonged peak latency of wave I alone; group C, prolonged interpeak latencies of wave I–III and/or I–V, and/or poor amplitude of wave I, III, and V.

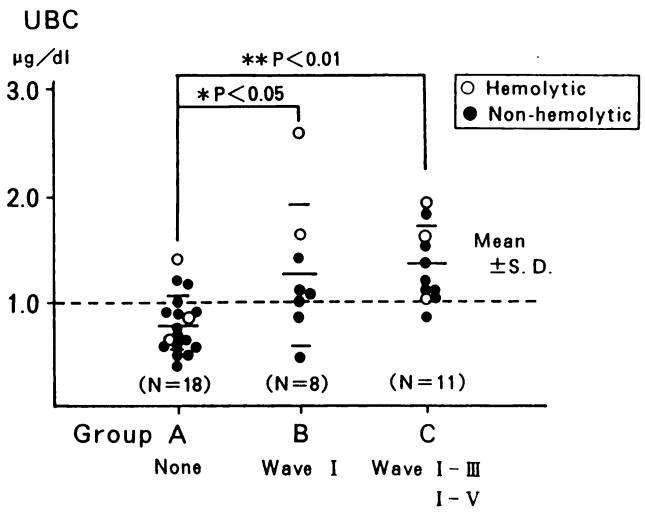

Fig 2. Unbound bilirubin concentration (UBC) levels and auditory brainstem response abnormalities in three groups.

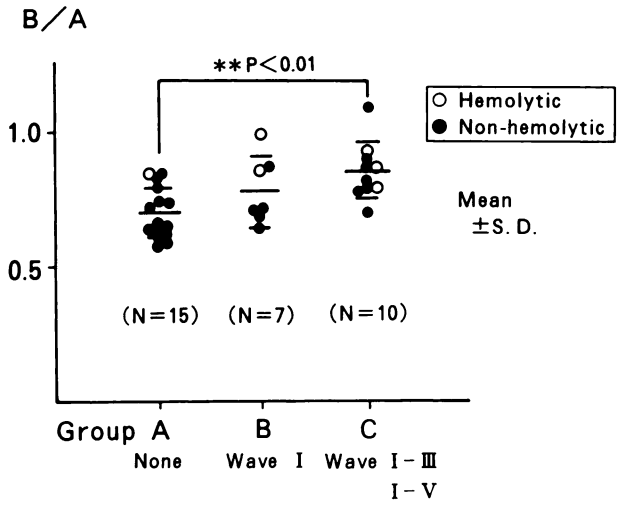

Fig 3. Bilirubin-albumin ratio (B/A) levels and auditory brainstem response abnormalities in three groups.

TABLE 2. Multiple Logistic Regression Odds Ratios Associated With Abnormal Auditory Brainstem Response Findings*

Independent Variables	Odds Ratio (95% CI)	*P* Value
Serum UBC		
≥1.0 μg/dL	16.6 (6.5–42.4)	.0026
<1.0 μg/dL	1.0 (referent)	
Serum TBC		
≥23.0 mg/dL	4.2 (1.6–10.7)	.1272
<23.0 mg/dL	1.0 (referent)	

* Abbreviations: CI, confidence interval; UBC, unbound bilirubin concentration; TBC, total bilirubin concentration.

onstrated that increased unbound bilirubin crosses the blood-brain barrier and enhances bilirubin deposits in the brain in vivo.[9,17,22] In vitro, free, unbound bilirubin is more toxic to tissue cells than albumin-bound bilirubin[23] and it inhibits mitochondrial oxidative phosphorylation.[24] So, for many years, increased UBC has been thought to be associated with bilirubin toxicity, though there is no conclusive proof that its measurement is useful in predicting bilirubin encephalopathy.

Since the reports by Hsia et al[25] and Mollison and Cutbush,[26] the "20-mg/dL level" of serum bilirubin has been generally agreed on as a criterion for exchange transfusions in full-term newborns with hemolytic disease. This criterion has been effective in avoiding both death due to kernicterus and brain damage due to bilirubin neurotoxicity, but it is not a perfect standard as Lucey[16] noted. Furthermore, as far as nonhemolytic and noncomplicated jaundice are concerned, we have continued having the problem of "vigintiphobia" (fear of 20). This was presented in an interesting way in a one-act play by Watchko and Oski.[6] In the presentation, there was a quotation from another paper described by Killander et al,[27] who had reported untreated cases with 27.2 mg/dL in peak bilirubin which had not developed kernicterus. This paper had stated: "In full-term infants, it is at present impossible to fix a critical bilirubin level at which exchange transfusions ought to be performed."

Recently, Newman and Maisels[7] have proposed a new criterion for exchange transfusions of ≥25 to 29 mg/dL in bilirubin level for nonhemolytic and well babies. This has induced therewith a large controversy[8] in regard to the criterion associated with "vigintiphobia."

The ABR test is a useful tool for predicting early clinical signs of asymptomatic bilirubin encephalopathy.[10,13] The auditory pathway of the neonate is particularly vulnerable to bilirubin insult, and the damage may result in sensorineural hearing loss,[28] because bilirubin pathologically stains selective subcortical nuclei, including the auditory pathway. ABR abnormalities have been demonstrated in moderately to severely hyperbilirubinemic newborns.[11,12,29,30] Prolongation of latency of wave I (peripheral conduction time), abnormalities in interpeak latencies of wave I–III and/or I–V (central conduction time), and/or decreased or lost amplitude were suggestive of bilirubin encephalopathy.[10] Direct correlations between increasing bilirubin concentrations and abnormal ABR tests have been demonstrated in both animal[31] and infant studies.[11,12,29,30] Nakamura et al[12] reported more reliable correlations between abnormalities of ABR and UBC than of TBC. Wennberg et al[32] reported that kernicterus was not found in jaundiced newborns with less than 20 nmol/L (about 1.16 μg/dL) of unbound bilirubin.

In our study, ABR abnormalities (of latency of wave I, interpeak latencies of wave I–III and I–V, and decreased or lost amplitude) were compared with TBC, UBC, and B/A in hyperbilirubinemic newborns with bilirubin levels greater than 20 mg/dL. As a result, it was demonstrated that the UBC level was the most reliable indication in the prediction of bilirubin encephalopathy in these hyperbilirubinemic newborns. The multiple regression analysis showed that the incidence of ABR abnormalities in newborns with UBC ≥ 1.0 μg/dL was significantly greater than that in newborns with UBC < 1.0 μg/dL (odds = 16.6, P = .0026). This study included a small number of newborns with hemolytic jaundice, but they did not present any significant differences in the multiple regression analysis. As far as these newborns are concerned, further study may be necessary, because they are more vulnerable to bilirubin than are newborns with nonhemolytic, uncomplicated jaundice.[7,25,26]

These results suggest that measurement of UBC may be helpful for evaluating the risk of bilirubin encephalopathy in full-term newborns with hyperbilirubinemia, and the level of UBC may be applicable as a criterion for exchange transfusions in these newborns when vigintiphobia is encountered.

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"Grievances cannot be redressed unless they are known; and they cannot be known but through complaints. . . If these are deemed affronts, and the messengers punished as offenders, who will henceforth send petitions?. . .Where complaining is a crime, hope becomes despair."

—Benjamin Franklin (after a vicious, humiliating public attack on him in 1774)

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