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BILIRUBIN/ALBUMIN RATIO USEFUL FOR MANAGEMENT OF CHRONIC NEUROLOGIC INJURIES OF HYPERBILIRUBINEMIA

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ABSTRACT

Objective: The aim of this study was to identify thresholds and evaluate sensitivity and specificity of bilirubin-albumin ratio (B/A) in comparison with total serum bilirubin (TSB) in predicting chronic neurologic and auditory impairment. **Methods:** Two hundreds term and near term neonates with severe hyperbilirubinemia were evaluated for bilirubin-induced neurologic dysfunction (BIND) by clinical findings at admission in Ghods children's hospital, Qazvin, Iran. During more than one-year follow up, permanent neurologic injuries and auditory impairment were evaluated by performing Auditory Brain stem Response (ABR) and serial clinical examinations. The relationship of B/A ratio and TSB to BIND were assessed by using receiver operating characteristic curves (ROC). **Results:** Chronic BIND developed in 5% neonates, concluding three frank kernicterus and seven-isolated auditory dysfunction. ROC analysis identified a TSB cutoff 22.7 mg/dl [area under the curve (AUC): 0.967] with a sensitivity of 100% and specificity of 88% and B/A ratio cutoff 5.6 [AUC: 0.953] with sensitivity of 100% and specificity of 75%. **Conclusion:** TSB is a stronger predictor of neurotoxicity than B/A ratio, but using combination of B/A ratio and TSB improves the sensitivity of predictions of BIND to 100%. Threshold values detecting all affected patients for B/A ratio (100% sensitivity) are lower in our study than recommended guideline.

KEYWORDS: kernicterus, auditory brainstem response, auditory impairment, otoacoustic emission.

INTRODUCTION

Jaundice is common in newborn infants. Most jaundice is benign, but because of the potential toxicity of unconjugated bilirubin, newborn infants must be monitored to identify those who might develop severe hyperbilirubinemia.[1]

The development of bilirubin encephalopathy is based on the assumption that when the level of serum-unconjugated bilirubin exceeds the bilirubin binding capacity of albumin (BBCA), lipophilic unconjugated unbound bilirubin readily crosses the blood-brain barrier, resulting in neuronal injury. The infant may die of kernicterus. If the infant survives, then abnormal neurologic findings, including delayed development and hearing impairment, may be detected. There is evidence that high serum levels of unconjugated bilirubin in newborns can produce neurologic injuries.[1,2] In the absence of obstructive jaundice, the serum level concentration of unconjugated bilirubin is best estimated by measuring the total serum bilirubin concentration (TSB). Calculating the "indirect fraction" (TSB minus the "direct reacting" bilirubin concentration) can be misleading in most newborns, because high levels of unconjugated bilirubin can produce an elevated direct fraction (~10 percentage of TSB), which does not represent nontoxic conjugated bilirubin.[2] Recently published management guidelines for jaundiced near-term and term infants by the American Academy of Pediatrics (AAP) are based on the premise that total serum bilirubin is the best available predictor of risk for neurologic injuries such as kernicterus.[3] However, clinical evidence indicates that TSB, beyond a threshold value of ~20 mg/dL (342 µmol/L), is a poor discriminator of individual risk for bilirubin toxicity. Neurologic injuries may rarely occur in healthy term infants with a TSB of <25 mg/dL (428 µmol/L).[4-7] The level currently recommended by the AAP for aggressive intervention, and most infants with a TSB of >25 mg/dL escape without recognized permanent sequel.[7-10]

But because only BF can cross the blood brain barrier, plasma free bilirubin (BF) is thought to be a better indicator of neurotoxicity than TSB.[11-14]

In the absence of an available assay for BF, the B/A ratio might provide a better estimate of BF. The relationship between BF and B/A has been studied by titration of serum samples[15] and by measuring BF in serum of infants with varying B/A.[16] That is why in recent studies

B/A ratio are used as a supplementary indicator to predict BIND and new algorithms, which is based on B/A ratio to determine the exchange is introduced. Otherwise, bilirubin- induced]neurotoxicity may depend on the mutual relation between bilirubin and albumin as their levels and the intrinsic albumin- bilirubin-binding constant (Ka).[17]

Usefulness of the B/A ratio may be limited because many factors influence Ka, Ka may be decreased by drugs (e.g. ceftriaxone) or by plasma constituents (e.g. free fatty acids) that interfere with albumin-bilirubin binding.[18,19] Also in several previous studies performed in Iran, the mean albumin ranges were higher in Iranian infants versus other society, which may influence the B/A ratio.[20]

In this study, we evaluate the bilirubin- albumin ratio as a helper indicator for predicting chronic bilirubin induced neurologic dysfunction (BIND). We hypothesize, that any change in the B/A denominator will have an effect on this ratio's result. The variation between Albumin averages in different societies could possibly change the predicted diagnostics for the known algorithms based on B/A.

MATERIALS AND METHODS

This prospective longitudinal observation study was preformed at Qazvin University Children's Hospital during March 2012 to October 2013. Infants admitted with severe hyperbilirubinemia were examined clinically for acute bilirubin induces neurologic dysfunction (BIND). All the neonates had been assessed previously for auditory function with Otoacoustic Emissions (OAE) in Birthing Hospital. All of 172 neonates were re-evaluated 6 to 20 months after discharge by performing neurological examinations and auditory brainstem response to assess chronic hyperbilirubnemia complications. Twenty-eight cases were questioned for the sign of kernicterus and auditory dysfunction via phone survey. Treatment decisions were made based on total bilirubin (TB) regarding to pediatrics recommended (AAP) guidelines, blinded to B/A ratios.

Two hundred term and near term neonates with age less than 14 days old and bilirubin over 20 were studied. Neonates suspicious, to have metabolic disease chromosome defects, and other diseases that mimic BIND were excluded. Blood samples were collected at admission and immediately sent to a laboratory to measure the TSB and serum albumin and calculate B/A ratio. The TSB was measured by the Doumas *et al.* modified method[21,22] and albumin by the bromocresol green method. Glucose-6-phosphate dehydrogenase (G6PD) assay was performed by fluorescent spot test (qualified test). Other laboratory tests were complete blood test (CBC), mother and infant's blood group and RH, direct coombs.

In this study symptoms and signs of BIND, those that evolve over a prolonged period (chronic BIND) were assessed. Chronic BIND defined as neurological deficits, including hypotonia, active deep tendon reflexes, obligatory tonic neck reflexes, delayed motor skills, movement disorders (choreoathetosis, ballismus and tremor), upward gaze, and sensorineural hearing loss. Auditory impairment was defined as moderate hearing loss (41-60dB), severe hearing loss (61-90dB), profound hearing loss (91and above).

Accurate gestational age was calculated due to pervious sonography and estimated date of conception (EDC). An exact birth weight, which recorded at the birthing hospital, was used in our study. Hemolysis was arbitrarily defined as hematocrit $\leq$ 35% with increased reticulocytes ($\geq$ 6%) in patients with possible isoimmunization. Suspected sepsis was defined as clinical deterioration in the presence of leukocytosis, leukopenia and shift to the left of neutrophils and positive C-reactive protein screen or positive Blood Culture.

Analysis was performed using Excel and MB SPSS Ver.21 statistical software. Independent sample T-test, chi square and receiver operating characteristics (ROC) curve analysis were used. The ethics committee of Qazvin University of Medical Sciences approved the protocol.

RESULTS

Complete examination of pediatricians and assessment of auditory function by Otoacoustic Emissions (OAEs) were performed for all infants in birthing hospitals. In addition, evaluations for jaundice and scheduled follow up were done. Icteric neonates were referred to our hospital for more evaluation and treatment. Birth weight, gestational age and blood type incompatibility were documented in the birthing hospital, as routine. The median age of admission to hospital was 5.8 days. Admission TSB was $\geq$ 20 mg⁄dL. Patient characteristics were summarized in table 1 and 2.

In this cohort, 107 (53.5%) had no risk factors other than severe hyperbilirubinemia. Ten (5%) infants' weighted $\leq$ 2500 gr, 27 (13.5%) neonates had a gestational age from 35 to 37, 6/7 weeks. Totally, 55 (27.5%) neonates had ABO incompatibility, but only eight (4%) neonates had positive direct Coombs. Blood cultures were positive in three of five infants with suspected sepsis. Some had more than one risk factor.

Table 1: Frequency distribution of studied traits (n=200).

Characteristic	No
Boys / girls	102 / 98
ABO incompatibility	55 (27.5%)
RH incompatibility	19 (9.5%)
G6PD deficiency	12 (6%)
Suspected sepsis	5 (2.5%)
Exchange transfusion	18 (9%)
Phototherapy	182 (91%)
Phototherapy + IVIG	1 (0.5%)

Table 2: Means of studied characteristic.

Characteristic	Mean	Range
Age (days)	5.8	0.25 – 21
Birth weight (g)	3138	1550 – 4200
Admission TSB, mg/dl	22.4	20 – 38
Admission B/A, mg/g	5.25	3.68 – 9.32
Albumin gr/dL	4.38	2.8 – 5.9

Neonates divided in two groups of term and near term, then using recommended guidelines for term infants with severe hyperbilirubinemia considering risk factor, phototherapy or exchange transfusion performed. All patients received phototherapy. One of the neonates with severe hyperbilirubinemia and continues hemolysis received IVIG besides phototherapy. Exchange transfusions were performed in 18 (6%) patients, median TSB and B/A values in infants receiving exchange transfusions were 27.5 mg/dl and 6.51 mg/g, respectively, compared with TSB 22.9 mg/dl and B/A 5.15 mg/g in those receiving the only phototherapy (Table 3). The exchange was performed due to recommended pediatrics guidelines for term and preterm neonates on the base TSB and blinded to B/A ratio.

Table 3: Means comparison of TSB, Alb and B/A in two group.

Variable	BIND (mean ± SD)	Non BIND (mean ± SD)	P-value
No	10	190	-
Total bilirubin	27.5± 5.2	21.8 ± 2.6	0.001
B/A	6.77 ± 1.29	5.15 ± 0.86	0.001
Albumin	4.29 ± 0.47	4.13 ± 0.63	0.306

At admission, 53 (26.5%) neonates was candidate for exchange and exchange transfusions were performed in 18 infants whose bilirubin were remained over the exchange value after six hours receiving intensive phototherapy or whom suspicious kernicterus. Among 200 neonates attending follow up 188 were healthy, nine had isolated auditory neuropathy, two had frank kernicterus and one infant died with severe BIND. Auditory brainstem response test (ABR) was performed for 91 neonates, nine neonates, had auditory and one of them was completely deaf.

There was a clear relationship between TSB and B/A values with bilirubin induced neuropathy dysfunction (BIND). Mean bilirubin level of BIND neonates was significantly higher than that of neonates without BIND (P<0.001). In addition, B/A ratio was significantly in BIND group. However, there was not a significant difference in mean albumin levels in two groups (Table 3).

ROC analysis identified at the TSB cutoff value of 25 mg/dl, [area under the curve (AUC) 0.967] with a sensitivity of 90% and specificity of 92%. B/A ratio cutoff value for predicting BIND was 8 mg/g (AUC 0.953) with a sensitivity of 40% and specificity of 100% (Fig.1). ROC curves evaluating total serum bilirubin and bilirubin/albumin as predictors of different stages of bilirubin neurotoxicity. TSB, solid line; B/A, dashed line; AUC: area under the curve and standard error (SE) shown.

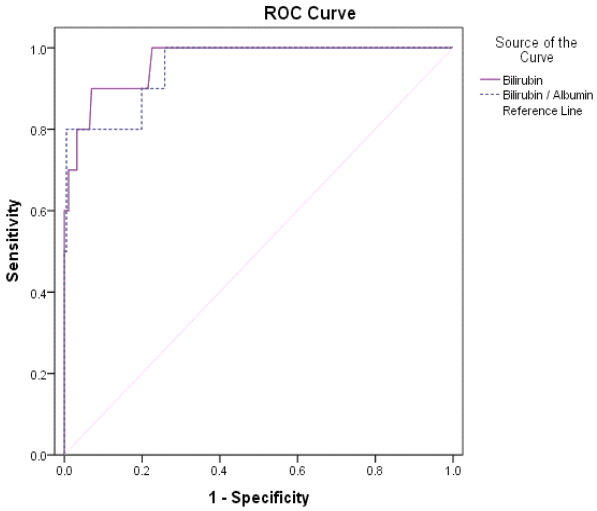

Figure 1: ROC curves of total serum bilirubin and bilirubin/albumin.

DISCUSSION

Plasma is but one of the many compartments (including liver, skin, red blood cells, brain, phospholipid membranes, etc.) that compete for binding the miscible (exchangeable) bilirubin load. Plasma is a unique compartment in that it serves as a "mixer" for the miscible pool of bilirubin. The distribution of the bilirubin pool between the various compartments depends on the number of binding sites in each compartment and their affinity for binding bilirubin. The bilirubin-binding affinity is quantified by a binding constant, *K*. Unfortunately, the albumin-bilirubin binding constant, *K*, is not "constant" but varies considerably among newborns. So in a society such as Iran, where the average Albumin is 0.5 dL higher than the established referenced amount, blood exchange should probably be done at a lower TSB/A in order to have a higher sensitivity for this indicator. It is also lower in sick infants and may increase with postnatal age. Furthermore, the effective concentration of albumin, A,

can be reduced by the presence of drugs or compounds that bind to the same locus as bilirubin such as sulfonamides or benzoate, the metabolite of benzyl alcohol.[14-16] The binding constant (ratio of association/dissociation rates) is an order of magnitude lower in undiluted serum and extremely variable between infants.[17-19]

The brain is unique by having a blood-brain barrier that slows the equilibrium between plasma and brain. If the blood-brain barrier is disrupted, bilirubin–albumin moves rapidly into the extracellular space of brain [16] and at sufficiently high B_f bilirubin will produce immediate global neurotoxicity.[19,20]

Comparing TSB with TSB/A ratio to predict chronic BIND, serum total bilirubin was much more sensible and at the point of equal sensitivity, the TSB was more specific. Using TSB as a single predictor of BIND in this study, it was missed to diagnose and treat one of ten neonates (10%) with hyperbilirubinemia injury that lead to permanent profound hearing loss. TSBB/A ratio in this infant was above 8 mg/g and according to a recommended algorithm based on TSB/A ratio,[21-23] exchange transfusion should be done for him. It was found that although TSB is a strong indicator of BIND, but using the B/A ratio as a helper indicator can improve the accuracy of prediction of BIND.

The same as other studies in Iran the average of albumin was higher in our study comparing with those performed in European and American society.[24-26] In addition, the B/A ratio defined in recommended guideline for exchange transfusion had not enough sensitivity in present study and B/A ratio identifying 100% of diseased infants were lower in our neonates. That hypothesis may be B/A ratio should determine considering the mean albumin of the society.

Neurologic injuries is a rare complication of neonatal unconjugated hyperbilirubinemia, and most healthy term infants with a TSB of 25 to 40 mg/dL escape without significant permanent damage. Most of these infants received intensive therapy (phototherapy and/or exchange transfusion).

The 1994 AAP practice parameter for neonatal jaundice was predicated on available evidence[25,26] that neurologic injuries was rare in healthy term infants with elevated TSBs even beyond 30 mg/dL and the assumption that the risk of intervention at TSB levels of <24 to 30 mg/dL may exceed the risk of encephalopathy. Subsequent information supports the contention that neurologic injuries are a rare event but also reveals that TSB is a poor discriminator of patients at risk. The data of a recently study suggest that the concurrent use of B/A ratio and TSB in the management of hyperbilirubinemia may provide a tool for the development of more robust criteria for managing newborn jaundice, and is likely to be better than TSB alone.

CONCLUSION

The need for additional parameters for the use of phototherapy and exchange transfusion in jaundiced newborns is generally agreed on the wide range of currently used ‘‘TSB thresholds’’ are arbitrary. The TSB is a stronger predictor of chronic BIND than TSB/A ratio. When intervention values are adjusted to provide equal sensitivity, the specificity of the TSB is higher. However, using TSB/A ratios in combination with TSB improve the accuracy of prediction of BIND. A lower TSB/A ratio was achieved in our study comparing recommended guideline to identify BIND which may be due, together with mean albumin value of our society. To improve guidelines for managing hyperbilirubinemia and minimize the number of unnecessary and at times dangerous therapeutic interventions, there is need for a national strategy to obtain prevalence and incidence kernicterus data. Therefor it is suggested that the concurrent use of TSB/A ratio and TSB in the management of hyperbilirubinaemia may provide a tool for the development of more robust criteria for managing bilirubin induced neurological dysfunction, and is likely to be better than TSB alone.

REFERENCES

1. American Academy of Pediatrics. Management of hyperbilirubinemia in healthy term newborn. Pediatrics, 1994; 94: 558-562.
2. Robert M. Kliegman, Bonita M.D. Stanton, Joseph St. Geme, Nina F Schor, Richard E. Behrman. Nelson textbook of pediatrics, 19th edition, 2011; 603.
3. El-Behbishi SN, Shattuk KE, Mohammad AA. Hyperbilirubinemia and transcuteneous bilirubinometry. Clin Chem, 2009; 55(7): 1280-7.
4. Daood M J ,McDonagh AF, Watchko JF. Calculated free bilirubin levels and neurotoxicity Journal of Perinatology, 2009; 29: 14-9.
5. Bhutani VK. Neonatal hyperbilirubinemia and the potential risk of subtle neurological dysfunction. Pediat Res, 2001; 50(6): 679-80.
6. Ostrow JD, Pascolo L, Tiribelli C. Reassessment of the unbound concentrations of unconjugated bilirubin in relation to neurotoxicity in vitro. Pediatr Res, 2003; 54(6): 926-932.
7. American Academy of Pediatrics. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics, 2004; 114(1): 297-316.
8. Watchco JF, Maisel MJ. Jaundice in low birthweight infants: pathobiology and outcome. Arch Dis Child Fetal Neonatal Ed, 2003; 88(6): F455-F458.
9. Shapiro SM. Definition of the clinical spectrum of kernicterus and bilirubin-induced neurologic dysfunction (BIND). J Prinatal, 2005; 25(1): 54-59.
10. Oh W, Tyson JE, Fanaroff AA, Vohr BR, Perritt R, Stoll BJ. Association between Peak Serum Bilirubin and Neurodevelopmental Outcomes in Extremely

Low Birth Weight Infants. Pediatrics, 2003; 112(4): 773-739.
11. Alfors CE, Shapiro SM. Auditory brainstem response and unbound bilirubin in jaundiced (jj) Gunn rat pups. Biol Neonate, 2001; 80(2): 158-162.
12. Calligaris SD, Bellarosa C, Giraudi P, Wennberg RP, Ostrow JD, Tiribelli C. Cytotoxicity is predicted by unbound and not total bilirubin concentration. Pediatr Res, 2007; 62(5): 576-580.
13. Wennberge RP, Alphors CE, Bhutani VK, Johnson LH, Shapiro SM. Toward understanding kernicterus a challenge to improve the management of jaundiced newborns. Pediatrics, 2006; 117(2): 474-485.
14. Alphors CE, Wennberge RP, OstrowJD, Tiribelli C. Unbound (free) bilirubin improving the paradigm for evaluating neonatal jaundice. Clin Chem, 2009; 55(7): 1288-1289.
15. Alphors CE. Criteria for exchange transfusion in jaundiced newborns. Pediatrics, 1994; 93(3): 488-494.
16. Sato Y, Morioka I, Miwa A. Is bilirubin/albumin ratio correlated with unbound bilirubin concentration? Pediatr Int, 2012; 54(1): 81-85.
17. Christian V. Hulzebos, Deirdre E. van Imhoff, Arend F. Bos, Charles E, Alfors. Usefulness of the bilirubin/albumin ratio for predicting bilirubin-induced neurotoxicity in premature infants. Archives of Disease in Childhood. Fetal and Neonatal Edition, 2008; 93(5): 384-388.
18. Odell GB, Cukier JO, Ostrea EM Jr, Maglalang AC, Poland RL. The influence of free fatty acids (FFA's) on the binding of albumin to Bilirubin. J Lab Cclin, 1977; 89(2): 295-307.
19. Robertson A, Karp W, Brodersen R. Bilirubin displacing effect of drugs used in neonatology. Acta Paediatr Scand, 1991; 80(12): 1119-1127.
20. Khosravi N, Samaie H, Mozhdehi, Azar K. Serum Albumin Level in full term neonates in Shahid Akbar Abadi Hospital. J Iran Univ Med Sci, 2001; 23: 15-17.
21. Rutledge JC, Ou CN. Bilirubin and the laboratory. Advances in the 1980s, considerations for the 1990s. Pediatr Clin North Am, 1989; 36(1): 189-198.
22. Doumas BT, Wu TW, Jendrzejczak B. Delta bilirubin absorption spectra, , molar absorptivity, and reactivity in the diazo reaction. Clin Chem, 1987; 33: 769-774.
23. American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics, 2004; 114(1): 297-298.
24. Shahin Ardakani Behjati, Vahid Dana Ghobadi, Vahid Ziaee. Bilirubin/Albumin Ratio for Predicting Acute Bilirubin-induced Neurologic Dysfunction. Iran J Pediatr, 2011; 21(1): 28-32.
25. Iman Iskander, Rasha Gamaleldin, Salma El. Serum Bilirubin and Bilirubin/Albumin Ratio as Predictors of Bilirubin Encephalopathy Pediatrics, 2014; 134: 1330-1339.
26. Ahmadpour KM, Zahedpasha Y, Peydayesh S, Mazloomi A. Assessment of Bilirubin to Albumin ratio as a Criterion of exchange transfusion. Medical journal of Mashhad, 2011; l54: 137-142.