Original Article

Prognostic Value of Direct Bilirubin in Neonatal Hyperbilirubinemia

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ABSTRACT

Objective. To determine the prognostic value of indirect and direct hyperbilirubinemia in neonates with jaundice.

Methods. A cohort of 92 consecutive neonates reporting with hyperbilirubinemia to a tertiary care center were followed up till well and discharged, or, till death to assess risk and rate of mortality.

Results. The baseline median values of total, direct and indirect bilirubin in the cohort of 21.8, 1.6 and 18.6 mg/dl, respectively, were used as cut-offs for high and low levels. Using survival analyses *i.e.* Kaplan-Meier plots, logrank tests and multivariate Cox proportional hazards regression models to adjust for other strong predictors such as receipt of breastfeeding, being small for gestational age (SGA) and exchange transfusion, high direct bilirubin (≥ median value of 1.6 mg/dl) was independently associated with a higher and faster mortality.

Conclusion. This study showed that direct bilirubin has independent and additive prognostic value and due attention should be given to newborns with raised levels. **[Indian J Pediatr 2007; 74 (9) : 819-822]** *E-mail : manjumamtani@rediffmail.com*

Key words : *Direct bilirubin; Neonatal hyperbilirubinemia; Neonatal jaundice*

For over a decade, it has been strongly argued[1] that direct bilirubin measurements offer little, if any, assistance in evaluating patients with neonatal hyperbilirubinemia. It has been emphasized that direct bilirubin can remain nonspecifically elevated, is prone to measurement errors and is therefore of little clinical value. Consequently, its use has been relinquished from the current recommendations relating to the management protocols for neonatal hyperblirubinemia hinging on the total – rather than direct – serum bilirubin measurements.[2] Arguably, however, the clinical conditions[3] most likely to be associated with an expectation of increase in direct bilirubin are more common in the developing world. It can thus be expected that direct bilirubin measurements can potentially improve the clinical management. Current management protocols for neonatal hyperbilirubinemia do not place much emphasis on the measurements of direct bilirubin. The objective of this study was to evaluate if direct biliubin measurement can contribute to outcome and prognosis of the neonate. To directly address this issue, we conducted a cohort study for the risk and rate of mortality in subjects with neonatal hyperbilirubinemia in central India. Here, we report our findings that demonstrated that increased direct bilirubin levels can independently and additively improve the prognostication of subjects with neonatal hyperbilirubinemia.

METHODS

Study Subjects and Study Protocol

The present cohort study was conducted at the Department of Pediatrics, Indira Gandhi Government Medical College, Nagpur, India – a tertiary care center – during the period of July 1998 to June 2000. Consecutive neonates with hyperbilirubinemia reporting to the study center during the study period were recruited in the study. We submit that the total bilirubin level for clinical management of hyperbilirubinemia is age- and gestational age- dependent but for the purpose of this study, hyperbilirubinemia was defined as total serum bilirubin levels[4] exceeding 15 mg / dl if the age of the

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neonate at the time of the bilirubin measurement was <15 days. If the age at the time of bilirubin measurement was ≥15 days then a total serum bilirubin concentration exceeding 2 mg/dl was used as the criterion to define hyperbilirubinemia. After recruitment into the study the subjects were followed till the subjects either recovered, or were discharged against medical advice or died. As a part of the clinical protocol, all subjects received phototherapy based on the total serum bilirubin levels. Phototherapy was given as blue light with peak output between 425 – 475 nm and irradiance <5 $mW/cm^2/nm$. The decision to give blood exchange transfusion was based on the birth weight as well as total serum bilirubin.

Statistical Analyses

We assessed the association of total, direct and indirect serum bilirubin with the time to and risk of death. We dichotomized each of these continuous variables using the median value as the cutoff. The survival analyses were conducted using Kaplan-Meier plots, logrank tests and multivariate Cox proportional hazards regression models after adjusting for receipt of breastfeeding, being small for gestational age (SGA) and receipt of therapy. SGA was defined as birth weight lower than the 3rd percentile of that expected for the given gestational age. As most of the study subjects had received phototherapy (Table 1) we only adjusted for the effects of blood exchange transfusion. To compare the prognostic value of direct and indirect bilirubin we used two approaches. First, we represented the actual bilirubin measurements as z-values based on the sample mean and standard deviation for bilirubin. We then compared the distribution of the z-values for direct and indirect bilirubin in subjects who survived and died. For these comparisons we used Mann-Whitney tests. Second, we plotted receiver operating characteristic curves for direct and indirect bilirubin and estimated the area under the curves and their standard errors. We compared these estimates of the areas under the curve using a Student's t-test. Finally, to assess whether direct bilirubin measurement can provide additive prognostic information, we used multivariate Poisson regression analysis. All the analyses were conducted using Stata 8.0 (Stata Corp, College Station, Texas) and the significance was assessed at an a-error rate of 0.05.

TABLE 1. Characteristics of the Study Subjects (N = 92)

Gender (N, %)	
Male	57 (62.0)
Female	35 (38.0)
Birth weight (N, %)	
Small for gestational age	35 (38.0)
Normal for gestational age	50 (54.4)
Large for gestational age	7 (7.6)
Preterm neonates (N, %)	17 (18.5)
First stool passed after (mean ± sd) hours	4.6 (3.9)
Breastfeeding given (N, %)	58 (63.0)
Type of delivery (N, %)	
Normal vaginal delivery	77 (83.7)
Caesarean section	14 (15.2)
Forceps delivery	1 (1.1)
Hemoglobin (mean ± sd g/dl)	13.7 (3.4)
Packed cell volume (mean ± sd %)	46.3 (12.2)
Reticulocyte count (mean ± sd %)	3.5 (2.2)
Bilirubin (mean ± sd mg/dl)	
Total	22.0 (5.0)
Direct	2.4 (1.9)
Indirect	19.5 (5.2)
Phototherapy given (N,%)	86 (93.5)
Exchange blood transfusion given (N, %)	42 (45.7)

RESULTS

We recruited a total of 92 cases of hyperbilirubinaemia in the study. The characteristics of these subjects are described in Table 1. Briefly, our study subjects represented predominantly male and average-for-gestational-age neonates born by normal vaginal delivery. Our cohort represented a total of 1039.17 person-days of follow-up. At the end of the follow-up 17 (18.5%) neonates died, 72 (78.3%) recovered while 3 (3.2%) were discharged against medical advice. Following conditions were observed in our study cohort: sepsis (18), polycythemia (8), hypothyroidism (4), glucose-6-phosphate dehydrogenase deficiency (4), idiopathic hepatitis (3), cytomegalovirus infection (2), syphilis (1), duodenal stenosis (1) and duodenal atresia (1). ABO blood group incompatibility was present in the parents of 14 neonates while Rh blood group incompatibility was seen in the parents of 10 neonates.

Receipt of breastfeeding and being SGA were strong predictors of the time to death (Fig. 1, A and B). We thus proceeded to examine the potential influence of total, direct and indirect serum bilirubin on the time to death. The median values for these variables in our study subjects were 21.8, 1.6 and 18.6 mg/dl, respectively. Using these we dichotomized each variable into high (≥median) and low (<median) categories and assessed the influence of the dichotomized variables with the rate of progressing to death. Interestingly, we observed that neither total serum bilirubin nor indirect serum bilirubin predicted the time to death while only high direct bilirubin statistically significantly predicted the time to death after adjusting for the effects of breastfeeding, being SGA and receiving exchange transfusion (Fig. 1, C to E). Contingent upon an alpha error rate of 0.05 and considering the study accrual time the follow-up time and the average survival rates in subjects with direct bilirubin <1.6 or ≥1.6 mg/dL, the power of our study was estimated as 90.82%.

We therefore compared the distribution of the z-transformed values of direct and indirect bilirubin in those who survived and those who died. The box plots (Fig. 2A) and the results from Mann-Whitney tests

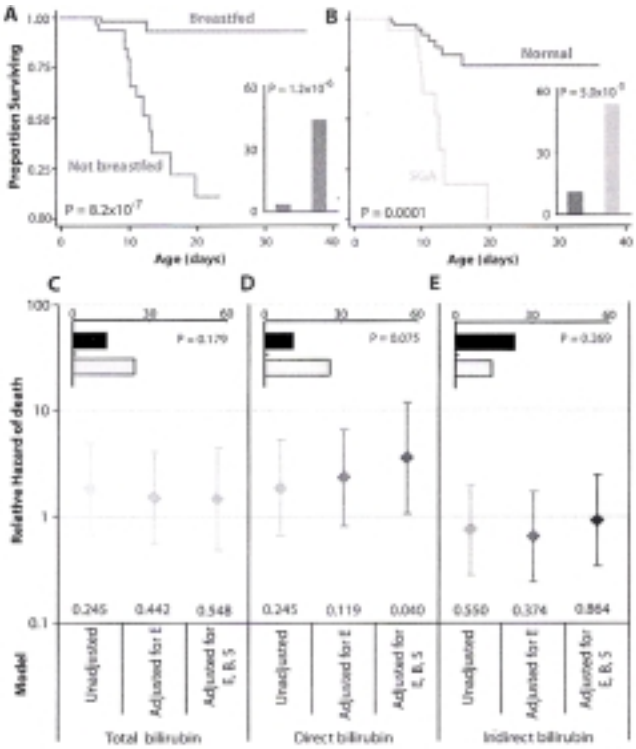

Fig. 1. Influence of the study factors on time to death in the neonates with hyperbilirubinemia. (A-B) Kaplan-Meier plots showing the time to death stratified on the basis of the receipt of breastfeeding (A) and being small for gestational age (B). Insets to A and B show the proportion (%) of subjects who died within each of the color-coded categories. (C-E) Results of Cox proportional hazards regression analyses to assess the influence of total (C), direct (D) and indirect (E) bilirubin on time to death. For each of these predictors, the bilirbin levels were dichotomized as low if less than the median and high if ≥ median value. Three models were run: univariate (unadjusted), adjusted for the receipt of exchange transfusion (adjusted for E) and adjusted for the receipt of exchange transfusion, breastfeeding and being small for gestational age (adjusted for E,B,S). The diamonds and error bars represent the point and 95% confidence interval estimates for the relative hazards while the numbers at the bottom of the plot indicate the significance values for each model. The proportion of subjects who died in the low (gray bars) and high (black bars) bilirubin categories are shown as insets in panels C to E. The significance values for all the insets (in panels A to E) were obtained by the χ^2 test.

indicated that while the z-values for direct bilirubin were significantly increased in those who died, the z-values for indirect bilirubin were similar in subjects who died and subjects who survived. Further, the ROC curve analysis(Fig. 2B) also indicated that the predictive accuracy of direct bilirubin was ~22% better (p = 0.0369) than that of indirect bilirubin. These results reaffirmed those shown in Fig. 1, C to E and underscored the fact that in our study subjects direct bilirubin was a better predictor of time to death than either total or indirect serum bilirubin.

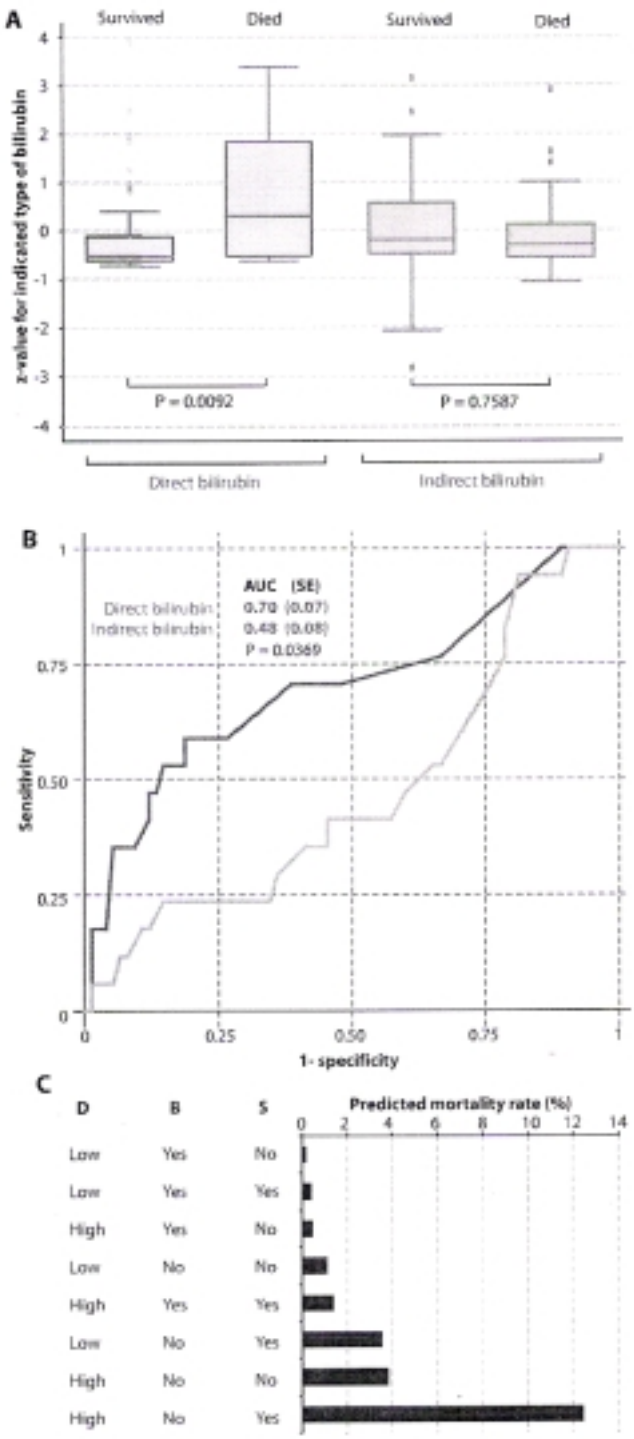

Fig. 2. Comparison of the prognostic performance of direct versus indirect bilirubin. (A) Box plots showing the distribution of the z-values for direct and indirect bilirubin in subjects who survived or died at the end of the follow-up. The significance values for assessing the difference of bilirubin distribution between those who survived and those who died were obtained using the Mann-Whitney test. (B) Receiver operating characteristic curve for the prognostic value of direct and indirect bilirubin in predicting death. The estimates of the area under the curve (AUC) were compared using a two-tailed t-test. SE, standard error. (C) Predicted

probability of death based on direct bilirubin levels (D), receipt of breastfeeding (B) and being small for gestational age (S). The estimates were obtained by Poisson regression analysis after adjusting for the receipt of exchange transfusion.

Lastly, to illustrate the independent and additive effects of direct bilirubin, we conducted multivariate Poisson regression analysis (Fig. 2 C). We observed that after adjusting for the effects of blood exchange transfusion, a combination of information on three predictors – direct bilirubin, receiving breastfeeding and being SGA – displayed a spectrum of variable risk of death and that a child with high direct bilirubin who is SGA and not breastfed has the highest risk of death (12.4%). In contrast, a child with low direct bilirubin, who is not SGA and is breastfed represented the other end of the clinical spectrum with only 0.1% risk of death.

DISCUSSION

In spite of its inclusion in the routine work-up of neonatal jaundice, direct serum bilirubin measurement has enjoyed less enthusiastic support as a dependable clinical parameter.[5] Our study shows that there exists a need to critically consider the value of this parameter from the standpoint of prognosis. While our study was not adequately powered to establish the non-significant association of indirect bilirubin with mortality in neonatal hyperbilirubinemia, we could demonstrate that direct bilirubin ≥1.6 mg/dl was independently associated with a higher and faster mortality. We also observed that while in the SGA neonates an increased value of direct hemoglobin indicated a higher risk of death, in term neonates a lower value of direct serum bilirubin greatly abated the risk of death (Fig. 2C). Combined with simple and effective interventions like breastfeeding[6], the prognostic value of direct bilirubin can be put to clinical use by using a "kinder, gentler approach"[7] to the management of neonatal hyperbilirubinemia. We therefore concluded that serum bilirubin can be an informative clinical conjunct in the evaluation of neonatal hyperbilirubinemia and we, thus, recommend its estimation and use in management of neonatal hyperbilirubinemia.

In a broader context, in populations of the developing world where conditions like sepsis, hepatic infections and other liver diseases are common it may still be prudent to use direct bilirubin measurements. Alternatively, there may be a need to refine the recommendations of management of neonatal hyperbilirubinemia so as to make them more globally relevant by considering the direct bilirubin concentration.

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