Conjugated Fraction in Neonatal Hyperbilirubinemia- Factors Associated and Influence on Outcome?

Ngangbam Sonamani[1], Ghanshyam Das[2], Waseem Iqbal[3], Shobha B[4], Sunil Bhat[5,*]

[1,3]Fellow, [4]Consultant, [5]Consultant and Head, Dept. of Pediatric Hematology, Oncology and Blood & Marrow Transplantation, Mazumdar Shaw Medical Center, Bangalore
[2]Associate Professor, Dept. of Pediatrics, Kamala Raja Hospital, Gwalior

***Corresponding Author**
E-mail: sunilbhat_9@hotmail.com

Abstract

Objective: To evaluate the factors associated with conjugated hyperbilirubinemia and its contribution to outcome and prognosis of neonates with jaundice.

Design: A prospective and observational study.

Setting: Kamla Raja Hospital, G. R. Medical College, Gwalior (M.P.) for a period of 1 year.

Participants: Consecutive term and late preterm LBW neonates reporting with hyperbilirubinemia to the center were recruited in the study.

Method: Relevant history, clinical findings and appropriate laboratory investigations of the eligible cohort were obtained and the cohort was followed-up till discharge or final outcome to assess the risk, the time to and the rate of mortality(n=126).

Results: The median values of total, conjugated and unconjugated bilirubin in the cohort were 18.6 (IQR 7.2), 2.8 (IQR 2.1) and 15.3 (IQR 6.425) mg/dl respectively. These values were used to dichotomize the variables into high and low. A strong association was observed between perinatal hypoxic ischemia and conjugated hyperbilirubinemia ($p=0.026$, $\chi^2=4.214$, $\kappa=0.144$, odds ratio=3.886). Conjugated hyperbilirubinemia was found to be strongly associated with sepsis indicating the probable role of sepsis in conjugated hyperbilirubinemia ($p<0.001$, $\kappa=0.313$). Unconjugated hyperbilirubinemia contributed more in terms of number of patients (54.77% vs 45.23%), while maximum proportion of death occurred in neonates with conjugated hyperbilirubinemia (71.42% vs 28.58%). The z values for conjugated bilirubin were significantly higher in those neonates who died when compared to those who survived ($p=0.0005$), while the z values for unconjugated bilirubin were similar in neonates who died and who survived (Mann Whitney box plots). Further, comparison of ROC curves (AUC 0.824 vs 0.526; SE 0.0688 vs 0.104; $p=0.0437$) indicated a superior prognostic value of conjugated fraction in predicting death.

Conclusion: The study showed that conjugated bilirubin is a dependable clinical parameter for prognostication of neonates with hyperbilirubinemia. Conjugated bilirubin measurement may be put to greater use in our population where factors associated with conjugated hyperbilirubinemia as sepsis and perinatal hypoxia are quite prevalent**.**

Key words: Conjugated bilirubin, Hyperbilirubinemia, Neonatal Jaundice.

Introduction

Over the years, conjugated fraction of bilirubin has been strongly assumed to be of little, if any, assistance in evaluation and management of neonatal hyperbilirubinemia.[1] It has been emphasized that conjugated bilirubin is prone to inter laboratory and intra laboratory measurement errors and can remain non-specifically elevated due to a variety of transient clinical conditions and leads to diagnosis only on rare occasions. Because of low yield and low specificity, its use has been gradually relinquished from current recommendations relating to management protocols for neonatal hyperbilirubinemia which subsequently rely on the total rather than conjugated serum bilirubin measurements.[2] However, it can be argued that clinical conditions which can be expected to be most likely associated with increase in conjugated bilirubin are more common in developing world. These include gram negative sepsis followed by perinatal hypoxic ischemia and hepatic infections.[3] Impaired bile acid transport at the sinusoidal and canalicular membrane domains due to decreased levels of the putative bile acid transporters (NTCP and canalicular recto-adenosine triphosphatase) mediated by the inflammatory response may account for sepsis associated conjugated hyperbilirubinemia.[4] The cellular mechanisms involved in bile secretion require energy. During hepatic hypoxic ischemia, the decreased level of adenosine triphosphate may compromise the bile secretion processes which are underdeveloped in neonates. Besides, damage to bile canalicular and alteration of bile ducts are postulated to be other mechanisms for conjugated hyperbilirubinemia in perinatal hypoxia.[5] It can thus be expected that conjugated bilirubin measurements can potentially improve evaluation and clinical management in these neonates. Despite its inclusion in routine work-up of neonatal jaundice, conjugated serum bilirubin measurement continues to enjoy less enthusiastic support as a dependable clinical parameter. Henceforth, current management protocols do not give much emphasis on conjugated bilirubin measurement. The current prospective and observational study was conducted to determine if conjugated bilirubin measurement can contribute to prognostication of neonates reporting with jaundice.

Methods

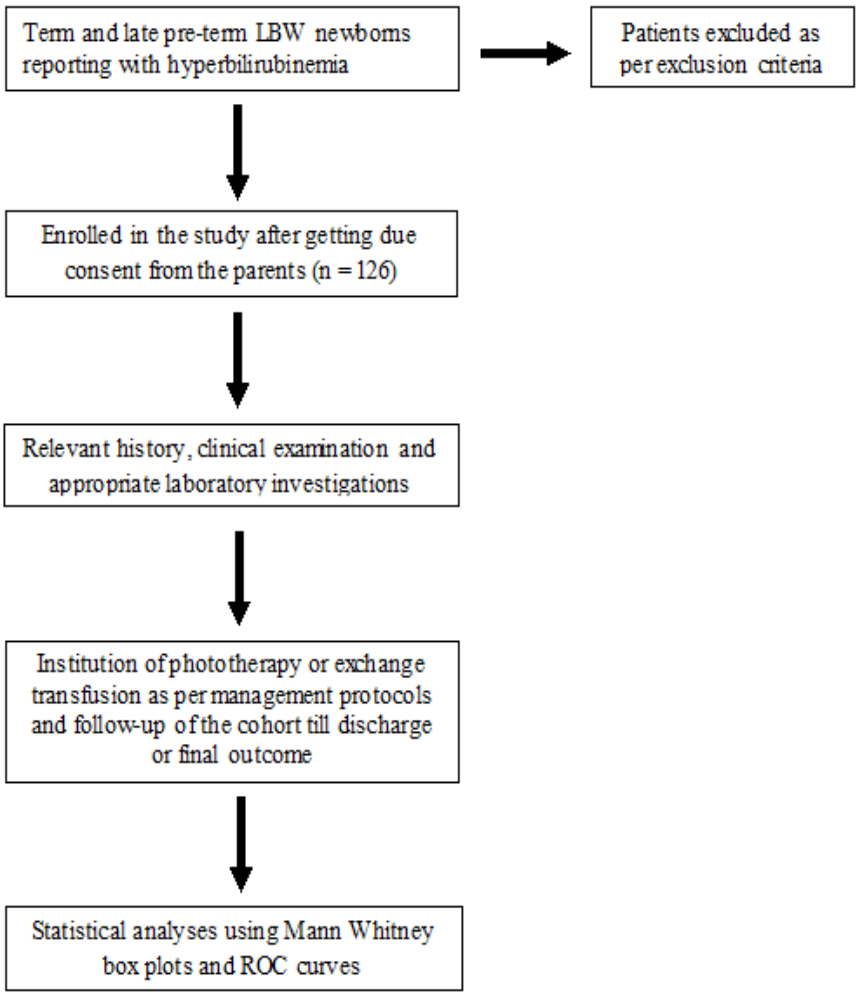
The present study was conducted in the Department of Pediatrics, Kamla Raja Hospital, Gwalior- a tertiary care centre for the period of 1 year. Consecutive term and late pre-term LBW babies (both intra-mural and extramural) with hyperbilirubinemia reporting to the study centre during the study period were included in the study. The following neonates were excluded from the study:

1. Neonates with bilirubin level less than 12 mg/dl if age on admission is less than 15 days.
2. Neonates with bilirubin level less than 2 mg/dl if age on admission is more than 15 days.
3. Preterm babies.
4. Babies with major congenital malformations
5. Large for gestational age babies

Though current management protocols of hyperbilirubinemia require total bilirubin level to be age- and gestational age dependent, for the purpose of this study hyperbilirubinemia was defined as total serum bilirubin levels more than 12 mg/dl if the age of the neonate at the time of bilirubin measurement was less than 15 days. If the age at the time of bilirubin measurement was more than 15 days, then total serum bilirubin exceeding 2 mg/dl was used as the criteria to define hyperbilirubinemia[6]. The conjugated, unconjugated fractions and the total bilirubin were measured using Ehrlich's reagent. When reacted with Ehrlich's reagent, bilirubin is converted to azobilirubin molecules which give a red purple color in acid, the intensity of which is read colorimetrically. Both conjugated and unconjugated bilirubins give purple azobilirubins with Ehrlich's reagent. Conjugated bilirubin can react in aqueous solution, whereas unconjugated requires an accelerator or solubiliser, such as methanol. Conjugated hyperbilirubinemia is defined as conjugated fraction of more than 20% of the total bilirubin or conjugated fraction of more than 2 mg/dl. After recruitment into the study, the subjects were followed till the subjects either recovered, or were discharged against medical advice or died.

As per recommendations related to management protocols for hyperbilirubinemia, the subjects then either received phototherapy or were treated with exchange transfusion based on the total serum bilirubin levels. Phototherapy was given as blue light with peak output between 425-475 nm and irradiance of at least 30μW/cm^2/nm. The decision to institute exchange transfusion was based on total serum bilirubin levels, age and weight of the neonate.

Factors associated with hyperbilirubinemia were studied and association between these factors with conjugated and unconjugated hyperbilirubinemia independently was studied. The total, conjugated and unconjugated bilirubin values were dichotomized using the median value as the cut off. To evaluate the prognostic value of conjugated and unconjugated bilirubin, two approaches were used. First, distributions of the z values for conjugated and unconjugated bilirubin in neonates who survived and died were compared using Mann Whitney box plots. Second, by plotting ROC curves for conjugated and unconjugated bilirubin with respect to death, the area under curves and their standard errors were compared. All the analyses were conducted using MedCalc version 11.6.1.6(MedCalc bvba software, Ostend, Belgium).



Results

A total of 126 eligible LBW neonates were recruited in the study. At the end of the follow-up, 14(11.11%) neonates died, 107(84.92%) recovered while 5(3.9%) were discharged against medical advice. The following conditions were observed in the cohort: Sepsis 34, ABO incompatibility 17, Polycythemia 11, Neonatal hepatitis 6, Rh incompatibility 6, Hypothyroidism 1, Malrotation of gut 1 and congenital varicella infection 1. The cohort revealed that unconjugated hyperbilirubinemia contributed more in terms of number of patients (45.23% Vs 54.77%), while maximum proportion of death occurred in neonates with conjugated hyperbilirubinemia (71.42% Vs 28.58%). Sepsis (particularly gram negative) was documented in 26.98% of the total study population. Klebsiella species (n,12) was the most predominant isolated organism followed by E. coli (n,4), Pseudomonas aeruginosa (n,3), Staphylococcus species (n,1) and Enterobacter (n,1).The median conjugated bilirubin concentration in infants with sepsis and without sepsis were respectively 3.4 mg/dL (IQR 3.1) and 2.6 mg/dL(IQR 1.555)whereas the median unconjugated bilirubin concentration in corresponding patients were 17.7 mg/dL (IQR 10.075) and 15.12mg/dL (IQR 5.35) respectively. Sepsis was documented in 43.85% of 57 infants with conjugated hyperbilirubinemia and in only 13.04% of 69 infants with unconjugated hyperbilirubinemia. Conversely, conjugated hyperbilirubinemia was found in 71.4% of 34 patients diagnosed with sepsis and in only 53.33% of 92 patients without sepsis indicating the probable role of sepsis in conjugated hyperbilirubinemia ($p<0.001$, $\chi^2=5.743$, $\kappa=0.313$). Perinatal hypoxic ischemia was documented in 17.54% of 57 infants with conjugated hyperbilirubinemia, whereas the same was found in only 7.24% of 69 infants with unconjugated hyperbilirubinemia. Conversely, conjugated hyperbilirubinemia was found in 73.3% out of 15 infants with perinatal hypoxic ischemia and in 41.4% out of 111 infants without perinatal hypoxic ischemia ($p=0.026$, $\chi^2=4.214$, $\kappa=0.144$, odds ratio=3.886), suggesting strong association between perinatal hypoxic ischemia and conjugated hyperbilirubinemia. The median values for total, conjugated and unconjugated bilirubin in the cohort were 18.6, 2.8 and 15.3mg/dl respectively. To evaluate the prognostic value of conjugated and unconjugated bilirubin, two approaches were used.

First, we compared the distribution of z values of conjugated and unconjugated bilirubin in those who survived and those who died. Mann Whitney box plots revealed that z values for conjugated bilirubin were significantly higher in those who died when compared to those who survived (p=0.0005), while the z values for unconjugated bilirubin were similar in neonates who died and who survived. Second, comparison of ROC curves of conjugated and unconjugated fractions with respect to mortality (AUC- 0.805 Vs 0.540; SE- 0.0726 Vs 0.0835; p=0.0052) indicated a superior prognostic value of conjugated fraction in predicting death.

Table 1: Other conditions in the cohort

Sepsis	**34**
ABO incompatibility	17
Polycythemia	11
Neonatal hepatitis	06
Rh incompatibility	06
Hypothyroidism	01
Malrotation of gut	01
Congenital varicella infection	01

Table 2: Mann-Whitney test (independent samples)

	Unconjugated fraction	**Conjugated fraction**
Average rank of survived group	63.1920	59.5134
Average rank of died group	65.9643	95.3929
Mann-Whitney U	749.50	337.50
Test statistic Z (corrected for ties)	0.268	3.468
Two-tailed probability	P = 0.7888	P = 0.0005

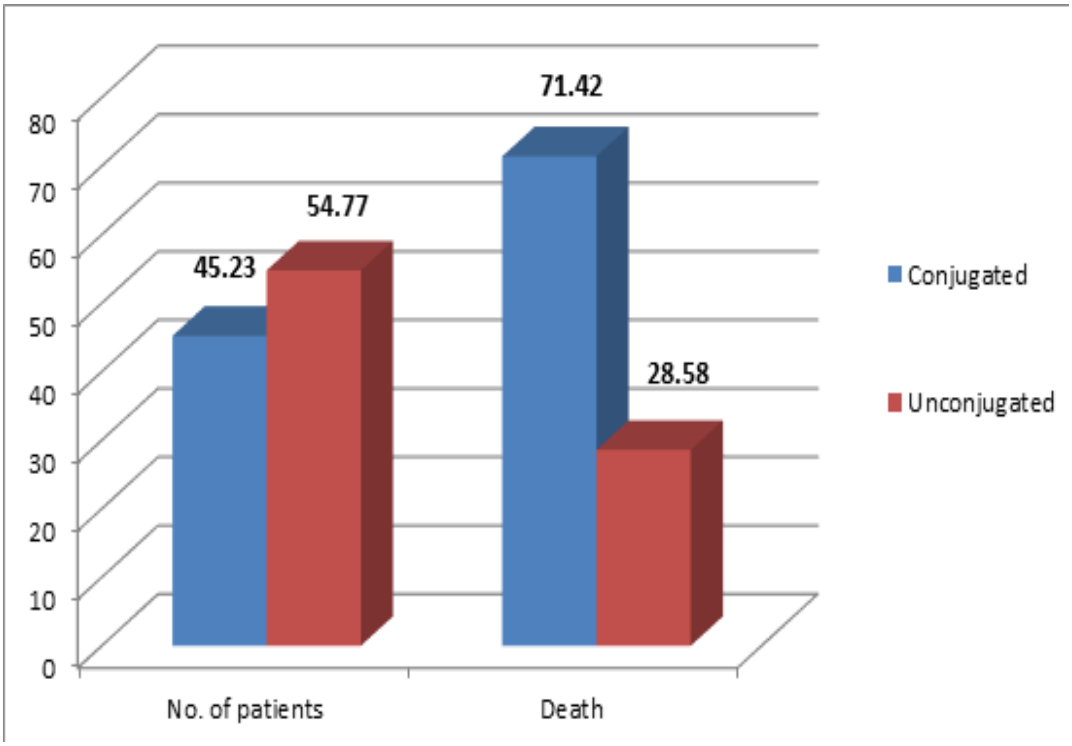


Fig. 1: Distribution of deaths according to type of hyperbilirubinemia

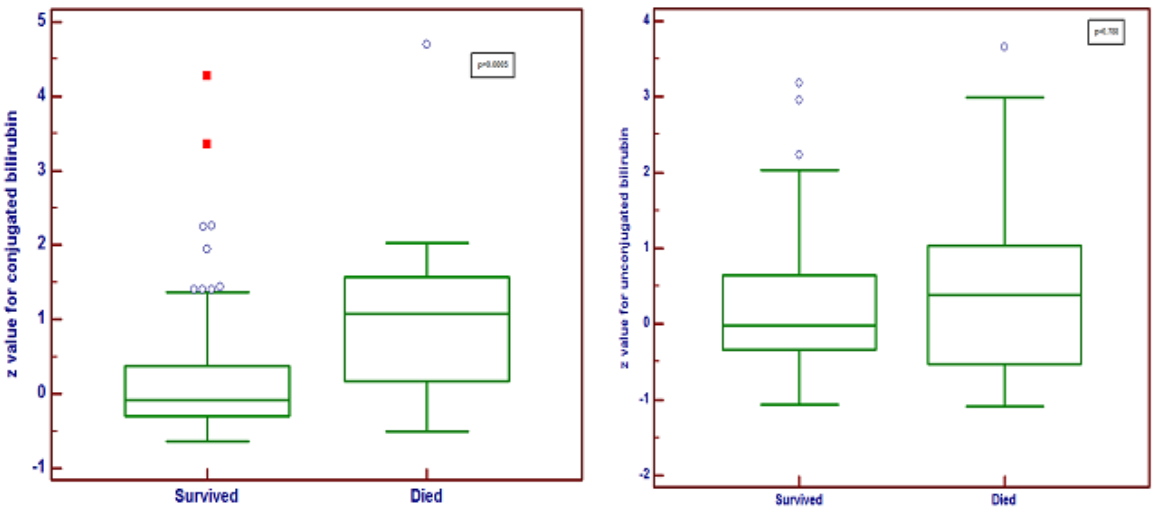


Fig. 2: Mann Whitney box plots showing distribution of z values for conjugated and unconjugated bilirubin in subjects who died or survived.

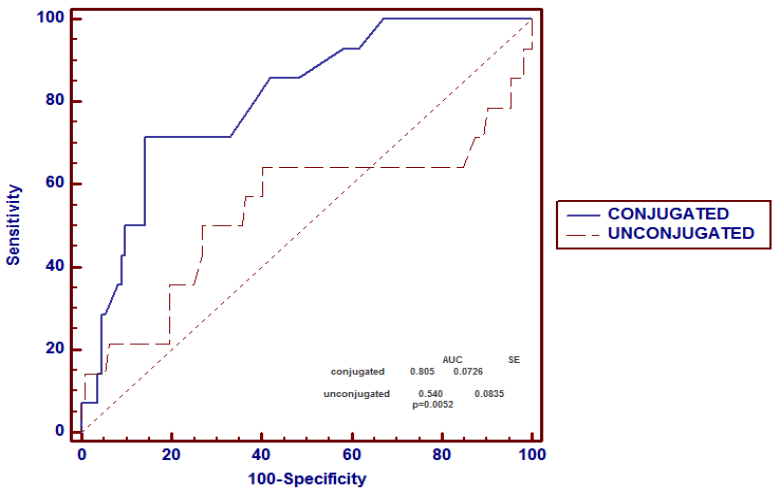


Fig. 3: ROC curve for conjugated and unconjugated bilirubin in predicting death

Discussion

Neonatal cholestatic jaundice may reflect serious underlying pathologies requiring prompt evaluation and initiation of therapy. Common clinical conditions most likely to be associated with an increase in conjugated serum bilirubin concentrations such as neonatal sepsis and perinatal hypoxic ischemia are more prevalent in our population. Therefore conjugated serum bilirubin measurements can be expected to help in evaluating the possible etiologies and direct appropriate therapies in these infants reporting with hyperbilirubinemia and consequently defining its prognostic value in these infants. The present study revealed a strong association between perinatal hypoxic insults and subsequent development of conjugated hyperbilirubinemia. Newborns with perinatal hypoxic insults run a risk of developing conjugated hyperbilirubinemia 3.886 times more as compared with their normal counterparts. Bile secretion processes which are underdeveloped in neonates can become further impaired during hepatic hypoxic insults. The cellular mechanisms involved in bile secretion require energy. During hepatic hypoxic ischemia, the level of adenosine triphosphate decreases. Besides, damage to bile canalicular and alteration of bile ducts are postulated to be other mechanisms for conjugated hyperbilirubinemia .Several other studies by Jacquemen E et al[5] have also identified this as an important causal factors in neonatal in neonatal cholestasis. A study conducted by Filiz Tiker et al[3] to determine the cause and related outcomes of early onset conjugated hyperbilirubinemia found perinatal hypoxic ischemia to be causal factor in 16.7% of 42 neonates with conjugated hyperbilirubinemia with these infants carrying a grave prognosis and concluded that perinatal hypoxic ischemia should be considered as a possible causal factor in neonatal cholestasis. In a series by Linder et al. (1988), out of 93 newborns reporting with unconjugated hyperbilirubinemia only 3 had positive blood culture[9]. Another study of 306 newborns with unconjugated hyperbilirubinemia by Maisels MJ et al.

(1992) identified no cases of sepsis.[10] However, our study document the presence of sepsis in 27% (n=39) of the cohort. A study by Khalil et al[11] demonstrated the presence of conjugated hyperbilirubinemia in 42.5% of newborns with sepsis. Yet another study reported sepsis in 35.7% of 42 neonates reporting with conjugated hyperbilirubinemia[3]. The higher incidence of sepsis in this series and our study could be explained by the fact that the study population in previous studies consisted of infants particularly with unconjugated hyperbilirubinemia, while ours included infants with both conjugated and unconjugated hyperbilirubinemia. This could also explain the possible role of sepsis in conjugated hyperbilirubinemia.

The various mechani-sms postulated in sepsis associated conjugated hyperbilirubinemia include changes in hepatic microcirculation, direct effects from bacterial products and effects caused by endotoxin induced mediators. E. coli endotoxins[4] causing impairment of biliary excretory mechanism has been demonstrated in rat models. Khalil et al[11] in their study of hepato-biliary dysfunction in neonates with septicemia found the prevalence of hepato-biliary dysfunction to be less frequent in babies who died as compared with survivors (43.4% vs 56.7%; $p<0.01$). The presence of conjugated hyperbilirubinemia in neonatal sepsis was found to carry a better prognosis in terms of survival. However, our study demonstrated that higher conjugated bilirubin concentrations were associated with an independently increased risk of death. It also indicated a superior prognostic value of conjugated fraction of bilirubin in predicting death. Similar study by Manju Mamtani et al.[7] (2007) observed a definite and independent prognostic value of conjugated bilirubin in neonatal hyperbilirubinemia.

The limitation of our study is that the study population was too small to extrapolate the results of our findings to the general neonatal population. Our study also did not incorporate derangements in hepatic enzymes while studying the causal association of factors such as perinatal hypoxia and sepsis with conjugated hyperbilirubinemia. Another limitation is that the power of the study was not adequate to establish a non-significant association between unconjugated fraction and risk of death with conviction.

To conclude, the value of conjugated hyperbilirub-inemia needs to be reconsidered in the management of these infants with hyperbilirubinemia from the prognostic and etiologic point of view. In population where neonatal sepsis and perinatal hypoxia contribute significantly to morbidity and mortality, it may be prudent to give more emphasis on conjugated serum bilirubin measurements. There is a need to critically re-examine the current recommendation protocols for management of neonatal hyperbilirub-inemia by giving due consideration to the conjugated fraction.

What Is Already Known?

Conjugated fraction of bilirubin finds little place in current management protocols of neonatal hyperbilirubinemia.

What The Study Adds?

Conjugated bilirubin may be used as a dependable clinical parameter to define outcome in neonatal hyperbilirubinemia in our population where sepsis and perinatal hypoxia contribute significantly to

Implication For Practice

Due consideration should be given to conjugated fraction while managing patients with neonatal

Conflict of Interest: None
Source of Support: Nil

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