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ORIGINAL ARTICLE

BILIRUBIN AS AN INDIRECT MEASURE OF LABORATORY PERFORMANCE OF BILIRUBIN DETERMINATIONS

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ABSTRACT: OBJECTIVE: To correlate Total Serum Bilirubin (TSB) values to the clinical course of hyperbilirubinemia in newborns as this can be an indirect method of quality assurance in the laboratory. **METHODS:** An observational study of bilirubin values from 100 randomly selected case records of newborn jaundice for a period of 6 months. TSB values were determined by diazo reaction on venous blood samples on a semi auto analyzer. MS excel sheet used for statistical analysis. **RESULTS:** Clinical course of hyperbilirubinemia in all subjects correlated well to the reported TSB values in first to last zones corresponding to <6mg/dL and >15mg/dL on 3rd day to 5th day of age. Zones 3, 4 and 5 varied from 7th day of birth, as phototherapy and recovery altered visual assessment of jaundice. One patient was expired with kernicterus had very high TSB value. The median bilirubin values trend downfall which correlated clinically to recovery from jaundice and 33% rapid decline in TSB also indicated the intervention by phototherapy. **CONCLUSIONS:** Bilirubin is one parameter with higher inter laboratory variability since its discovery till today. Hence more quality methods are to be developed to minimize this bias in clinical interpretation of reported bilirubin levels. Our study is an intermediary quality measure useful for both clinicians and lab personnel. This study can be adopted for retrospective quality evaluation and can be adopted for other parameters as well.

KEYWORDS: TSB Total Serum Bilirubin; Hyperbilirubinemia; Kramer's rule; Quality control; VIS variance of Index Scoring {%variance divided by desired coefficient of variation for a parameter, here it is Bilirubin}.

INTRODUCTION: Bilirubin assays are often challenged for their precision and accuracy. Total Serum Bilirubin (TSB) levels in the serum of neonates are a measurement of the excretory function of the liver which is again a function of age, disease status of the organ, enzymatic defects and hemolysis. As a marker for a number of pathological conditions, bilirubin measurement must identify those infants at risk of jaundice and kernicterus. Guidelines for the management of hyperbilirubinemia in a Newborn Infant of 35 or more weeks of gestation by American Academy of Pediatrics rely heavily on the ability of the physician to recognize jaundice and on the measurement of TSB.[1] Hence a laboratory in a pediatric setup must be in a position to determine TSB levels with accuracy and precision to assess the rate of Bilirubin change with time.[2]

Inspite of internal and external quality control in our laboratory, there was a need to innovate a new method of quality control which should also 'involve' clinicians to get an assurance whether these reported Bilirubin values are correlating to the clinical course of the newborn admitted for hyperbilirubinemia. This reassures that these determinations are of a sufficient precision which serves as an indirect measure of laboratory performance.

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MATERIALS & METHODS: This observational study was done in the department of Biochemistry, Niloufer Hospital for women and children, Hyderabad, with the archived case records of Neonatal Intensive Care Unit (NICU). For this kind of study, permission from hospital authority was taken and formal consent from individual subjects is not required. The Case Records for a period of 6 months (i.e., January 2014 to June 2014), a total no. of 100 newborn subjects (Male 55, Female 45) selected at random, were of less than 72 h of age at the time of admission in to NICU for observation of jaundice. All the cases were subjected to phototherapy (PT) and none was given exchange transfusion. The inclusion criteria with regard to sickness level, gestation, birth weight and maternal history were ignored because the objective of this study was to correlate the reported bilirubin (TSB) values with the clinical course and outcome of hospital stay. The exclusion criteria were neonates developing jaundice after a week of birth and who left against medical advice. Babies were examined in broad day light to clinically assess level of jaundice and Kramer's rule was applied.[3] As a routine protocol, Serum obtained from peripheral venous blood samples of the patients were drawn from the newborns without regard to their feed status. The samples were processed to estimate bilirubin values in less than an hour. TSB levels (Both direct and indirect bilirubin) were determined by in vitro LiQuixx BIT kits of Erba Mannheim with the Diazo (Vanden Bergh) reaction End point principle.[4] on Transasia Erba Chem 5 Plus semi auto analyzer. The reports of TSB were verified for the fact that the bilirubin levels were monitored for entire duration of hospital stay, for at least 3 values or 4, with an interval of 12 to 48 h as per the clinician ordering of investigations. But in this study, to maintain uniformity, TSB reports of every 48th hour were taken in to consideration. And various other tests which were done to rule out or confirm the etiologies were also noted, were depicted in Table 1. As per the records, first day post admission (Corresponding to 3rd day after birth), the TSB values are grouped as B1 (B for bilirubin), second TSB report (Age 5th day) as B2, third (Age 7th day) as B3, fourth (9th day) as B4 and fifth (11th day) as B5.MS excel sheet used for statistical analysis and figures.

Clinical approach to hyperbilirubinemia in newborn jaundice	No.
Term	90
Preterm	10
Hemolysis (Rh, ABO, Thalassemias)	2
Sickness(sepsis, asphyxia)	1
Cholestasis	2
Kernicterus	1
Breast feeding	5
G6PD deficiency	0
Physiological	89

Table 1: Cases of hyperbilirubinemia and their etiologies

RESULTS: As the values did not have normal distribution, median values of B1, B2, B3, and B4 were taken, extrapolated in Table 2 & Figure 1. Out of all one hundred cases, a single death occurred of kernicterus, which correlating to rapid rise of B2.For each subject, the difference (or change) in TSB value (B1-B2; B2-B3 so on), either increase or decrease is calculated for each interval and averages were depicted in Table 2 & Figure 2 as percentage of change.

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Day After Birth	Group	Total No. of Cases	Median TSB (mg/dL)
3rd	B1	100	16.3
5th	B2	100	15.4
7th	B3	98	12.5
9th	B4	69	11.3
11th	B5	24	10

Table 2: TSB values as per the hospital stay

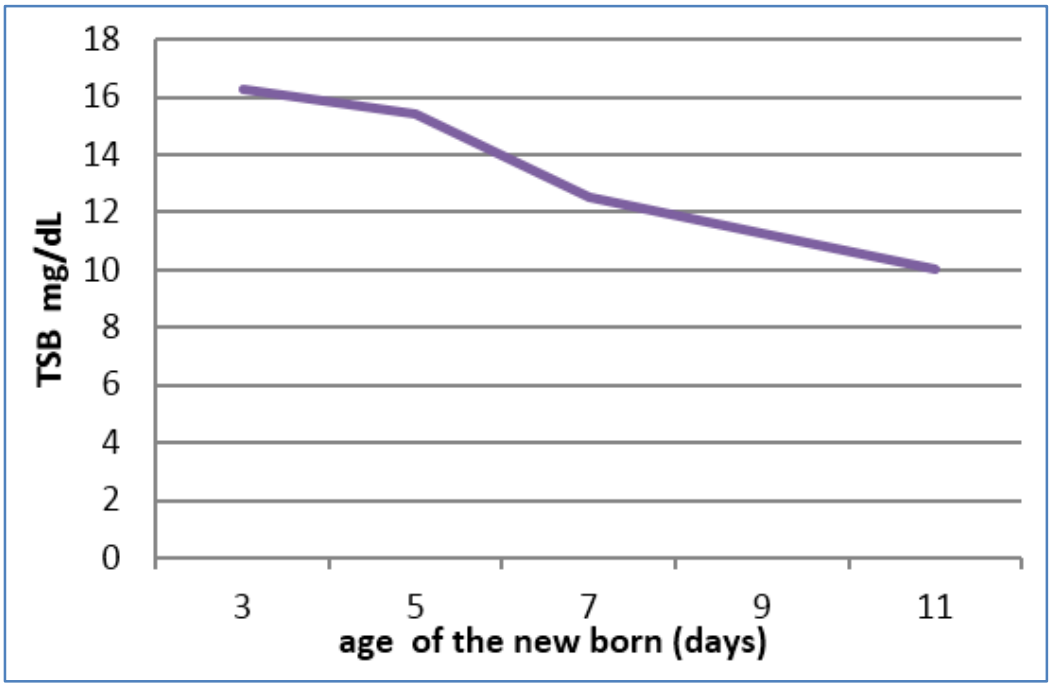

Fig. 1: Trend line showing downfall of TSB

Change of averages	mg/dL	%
B1/B2 (3-5)	1.62	21
B2/B3 (5-7)	2.5	33
B3/B4 (7-9)	0.67	9
B4/B5 (9-11)	2.8	37

Table 3. Decline of TSB levels with time

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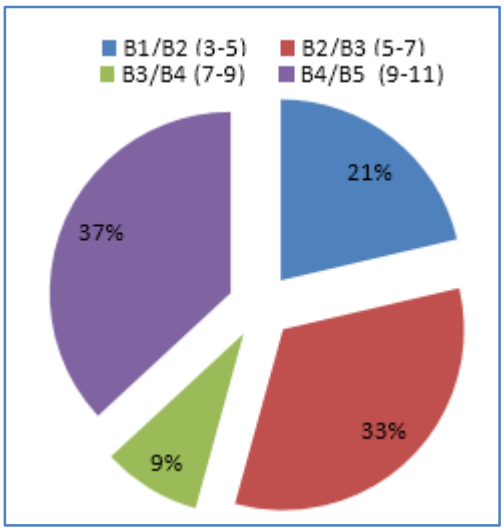

Fig. 2: Rate of Decline OF TSB levels with time

Bilirubin values were also grouped as per the Kramer's rule and correlated to the dermal zone staining. Percent of variation from the rule was shown for each zone in Table 4.

Table 4: Day wise Bilirubin values correlation to icterus (Dermal staining as per Kramer's rule) shown as percentages %. Total no. of reports was shown in the brackets ( ).

TSB mg/dL	B1(100)	B2(100)	B3(98)	B4(70)	B5(25)
4-5.9 FACE	100	100	75	85	85
6-7.9 CHEST	100	100	75	68	65
8-9.9 ABDOMEN	100	100	65	55	50
10-11.9 THIGHS	100	92	60	60	60
12-14.9 LEGS	95	91	60	25	10
>=15 SOLE	91	91	60	5	3

Table 4

DISCUSSION: TSB level usually rises in full-term infants to a peak of 6 to 8mg/dL by 3 days of age and then falls. A rise to 12mg/dL is in the physiologic range. In premature infants, the peak may be 10 to 12mg/dL on the fifth day of life, possibly rising over 15mg/dL without any specific abnormality of bilirubin metabolism [5]. Physiological jaundice is the main etiology in all these 100 cases studied (89%, Table 1).

It is evident of each patient that the decline in TSB is influenced by the interventions such as double surface or triple surface photo therapy (PT) (or exchange transfusion) which reassures the clinician that the PT machinery is adequately performing. The decrement of TSB levels, (Tables 2, 3 & Fig 1, 2) at 5th day to 7th day (33%) and 9th to 11th day (37%) correlates to the initiation of treatment and liver maturity respectively.[6,7,8] These values correlated well with the clinical course as well as outcome of the subjects, i.e., recovery from jaundice.

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The main focus of the present study is on the precision and accuracy of TSB determinations and how they reflected on the clinical course of jaundice, both preterm and term babies were included as same category and thus their TSB values showed high standard deviations. The recovery time was extended beyond 11th day and this delayed decline might be due to δ bilirubin fraction which appears during recovery phase and also the physiological immaturity of liver in preterm subjects.[5,9] But improved clinical jaundice and recovery of the subjects correlated well, back to their TSB levels.

In this study, Bilirubin levels were also correlated with Kramer scoring based on visual assessment of cephalocaudal progression as shown in table 4. There was a very good correlation of all bilirubin in zones 1 to 5 (TSB <6 to >12mg) from 3rd to 5th day of life (B1 & B2), without intervention, this correlation was above 90%. As the intervention with the Icterus progresses (onset of PT etc.) this correlation falls down and the sensitivity of the Kramer's scale is lost from B3 (9th day) onwards. Even with PT, this correlation was acceptable (65%) only for zones 1, 2, i.e., not beyond the chest. With the management or natural recovery of jaundice, the reversal of dermal staining pattern is not suitable for correlation with the reported TSB values. These observations were consistent with a previous study by Moyer et al and Dhanjal G S et al.,[10,11] Inspite of the decreased sensitivity of the Kramer's rule in B3, B4, and B5 in zones 3, 4 and 5, those TSB well correlated with the clinical course and outcome of the jaundice.

Historically, Bilirubin measurements lack accuracy and reliability thus notorious for their marked interlaboratory variability with coefficient of variation up to 10 to 12 percent for TSB and over 20 percent for conjugated fraction.[5] This was attributed to the lapses in quality control procedures, failure to calibrate instruments properly, and possible matrix effects.[12] Differences in methodologies like transcutaneous Bilicheck (TcB), direct spectrometry (capillary method), and Diazo principle (color reaction) further aggravate the question.[13,14] Quality control (QC) in Biochemistry or any laboratory is an important protocol to ensure lab performance in assay procedures. Both External and Internal quality controls are mandatory for any clinical lab to provide accurate or results of sufficient precision. These two are complementary activities, internal QC being necessary for the daily monitoring of the precision and accuracy of the analytical method, and external QC being important for maintaining long term accuracy of the analytical methods.[15,16,17]

Inspite of these protocol measures, often situations arise, where a trueness of a report is questioned while clinicians have to make critical decisions in potentially life threatening situations like hyperbilirubinemia in newborns especially when bed side TcB value or TSB reports from other laboratory or dermal staining (Kramer's rule) do not match to the reported TSB levels. TcB estimation may be useful screening tool, but it cannot substitute for TSB estimation particularly for babies with serum Bilirubin >13mg/dl.[18]

In our laboratory, with a meticulous care for Bilirubin assay, we maintained External QC by Christian Medical College (CMC), Vellore; of TSB to VIS (variance of Index Scoring) around 100 (<200 desirable) and Internal QC by Transasia Erba Norm and Path; of TSB 0.9-1.9mg/dL for normal controls and 3-6mg/dL for abnormal controls for those 6 months period mentioned. While these values were prospective, indicating good ongoing performance of the laboratory, the results of present study of clinical outcome of recovery or death, well correlated to those reported TSB values from this laboratory. With standard phototherapy systems, a decrease of 22% of the TSB in the initial phase of treatment also in favor of appropriate TSB reported values.[1,7,8] Thus, though retrospectively

shown, it served as an indirect quality measure of the lab performance in TSB determinations. This will surely, reassure clinicians managing hyperbilirubinemia, of reported TSB values.

CONCLUSION: Bilirubin is one parameter with higher inter laboratory variability since its discovery till today. Hence more Quality methods are to be developed apart from internal or external laboratory assessments, to minimize the bias between the lab and clinical assessments. Our study serves as an intermediary quality measure where clinician can also participate in correlating the reports either prospectively or retrospectively, and that can be adopted for retrospective quality evaluation and reassures the clinicians and laboratory personnel as well and can be adopted for other parameters as well, for example, serum Creatinine monitored values in Nephrotic syndrome.

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