



Serum Bilirubin – Which Method of Estimation is More Accurate?

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Abstract

Objective: Neonatal hyperbilirubinemia is a very common clinical problem encountered by neonates especially in the first week of life. There is an accumulation of bilirubin in the skin and the mucous membrane that causes yellow discoloration of the skin and sclera of the neonates. A Newborn should be examined and monitored for hyperbilirubinemia to prevent brain damage. Wet and dry chemistry methods for evaluating TBiL (total bilirubin) and NBiL (neonatal bilirubin) are available.

Methods: 100 neonates of age two days having physiological jaundice were included in the study. Their serum samples were evaluated for TBiL (total bilirubin) using wet chemistry diazo method and neonatal bilirubin (NBiL) using dry chemistry (reflectance spectrophotometry technology) on Vitros (Ortho Clinical Diagnostics USA) analyzer.

Results: The mean (SD) of NBiL was 8.436 (1.02) mg/dl and that of TBiL was 8.44 (0.56) mg/dl. The mean difference [95% confidence intervals] between the two methods was 0.004[-0.0889 to 0.0969]. The correlation between the two methods was calculated by Pearson's correlation coefficient of 0.971 with an average bias of 0.1 %.

Conclusion: Neonatal bilirubin (NBiL) and total bilirubin (TBiL) can both be used in assessing neonatal jaundice.

Keywords: Serum bilirubin ; Hyper bilirubinemia; Neonatal hyperbilirubinemia

Abbreviations: SBR: Serum Bilirubin; TBil: Total Bilirubin; NBil: Neonatal Bilirubin; G6PD: Glucose 6 Phosphate Dehydrogenase; BIND: Bilirubin Induced Neurological Dysfunction; Bc: Conjugated Bilirubin; Bu: Un Conjugated Bilirubin; TSB: Total Serum Bilirubin; DBil: Direct Bilirubin

Introduction

Bilirubin is the final product of the catabolic breakdown of hemi, a key component of hemoglobin, myoglobin and

cytochromes [1,2]. Due to relative polycythemia and increased red blood cell turnover, increased catabolism of fetal hemoglobin results in increased production of bilirubin. Coupled with the excess production, there is also a deficiency of conjugating enzyme glucuronidase which results in decreased capacity of the liver to conjugate bilirubin and hence this results in elevated levels of unconjugated bilirubin. Hence, in neonatal hyperbilirubinemia, unconjugated bilirubin production is approximately 6 to 8 mg per kg per day, almost twice that of adults. Bilirubin production typically declines to the adult level within 10 to 14 days after birth. However, in

some neonates, this rate of rise for bilirubin levels either continues or accelerates [3].

Neonatal hyperbilirubinemia is a very common clinical problem encountered by neonates especially in the first week of life which manifests as jaundice in approximately 80% of all newborns in the United States. When the total serum bilirubin (TSB) rises above the 95th percentile for age (high-risk zone) during the first week of life, it will be considered as hyperbilirubinemia [4-8]. Several types of bilirubinemia have been reported in neonates including physiological jaundice, pathological jaundice, jaundice due to breastfeeding or breast-milk and hemolytic jaundice including three subtypes due to Rh factor incompatibility, ABO blood group incompatibility and jaundice associated with Glucose-6-phosphate dehydro-genase (G6PD) deficiency [9]. Other causes for pathologic hyperbilirubinemia are Gilbert's syndrome, Crigler-Najjar syndrome, Pyruvate kinase deficiency, Hexokinase deficiency, congenital erythropoietin porphyria, Erythrocyte structural defects among others [10].

In most cases, jaundice is benign and no intervention is needed but approximately 5-10% of them have clinically significant jaundice that require treatment to reduce the serum bilirubin levels in order to prevent bilirubin-induced neurologic dysfunction (BIND), which occurs when bilirubin crosses the blood-brain barrier and binds to brain tissue [11-13]. Hence, the detection of severe hyperbilirubinemia in infants has become one of the most intriguing challenges for neonatologists as the ability of physicians to recognize clinically significant jaundice based on the cephalo caudal progression of jaundice is limited and also to determine when to terminate infant phototherapy [14,1].

Bilirubin can be measured in body fluids by a variety of analytical methods including chromatography, electrophoresis, spectrophotometry, diazo method, peroxidase method, diazo-peroxidase method, direct colorimetric methods etc. Diazo method is the most widely used methods for measurement of different forms of bilirubin. Bilirubin species are classified according to reactivity in "diazo method" as one which reacts with the diazo reagent without the addition of alcohol as direct or conjugated [Bc] and one that reacts in presence of alcohol as un conjugated [Bu] [13,14]. Neonatal Bilirubin (NBiL) can be measured using micro slide technology on Dry chemistry analyzer. NBiL or BuBc slide quantitatively separates unconjugated and conjugated bilirubin, [Bu+Bc=NBiL] not including delta bilirubin, with minimal interference from hemoglobin [13-15]. Direct estimation of Unconjugated bilirubin fraction (Bu) by the dry chemistry method and hence avoiding the traditional calculation (indirect bilirubin = total bilirubin - direct

bilirubin) is a key differentiating factor of the dry chemistry analyzer.

Total bilirubin [TBiL] is the commonly used bilirubin fraction for detection of neonatal hyperbilirubinemia. TBiL can be measured using diazo method by Wet technology. TBiL measures both direct and indirect bilirubin along with delta fraction of bilirubin. Delta bilirubin concentrations are negligible in neonates <15-21 days of age and have no clinical utility. Therefore, serum bilirubin measurements at this age can be accomplished by using a slide that does not include the delta fraction. Although it is not common in neonates its existence is not impossible [16]. (Bu + Bc) + Delta bilirubin = TBiL [17]. Thus, the present study was conducted to evaluate if neonatal bilirubin (NBiL) is an acceptable alternative to total bilirubin (TBiL) in the same.

Methods

Ethics

Institutional Ethics Committee permission was obtained prior to the conduct of the study. Written informed consent was obtained from the parent/guardian/legally accepted representative of all the neonates prior to enrolment in the study.

Selection of study participants and study setting

100 neonates of 2-day postnatal age with physiological jaundice in whom bilirubin testing was done as advised by the treating pediatrician during a period of 10 months (from January 2017 to October 2017) at Jayanagar Claudine Hospital, Bangalore were enrolled in the study.

Bilirubin estimation methods

Blood was drawn using vacutainers (Becton-Dickinson USA) containing a clot activator and the serum obtained were used for the estimation of TBiL and Direct bilirubin. Vitros (Ortho Clinical Diagnostics, USA) was used for analyzing bilirubin fractions. Neonatal Bilirubin (NBiL) was measured using micro slide technology by Dry chemistry analyzer. Total bilirubin (TBiL) and Direct bilirubin (DBiL) was measured using Diazo method by Wet technology [18-20].

Statistical analysis

Normality of bilirubin values was assessed using the Kolmogorov-Smirnov test. Quantitative data were expressed as mean and SD. The agreement between the two methods (Dry and Wet chemistry) of measuring NBiL and TBiL were assessed using Bland - Altman plots. The correlation between the NBiL (Dry Chemistry) and TBiL

(Wet Chemistry) was calculated by Pearson's correlation coefficient. All analyses were done at 5% significance using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY and Microsoft Excel 2013.

Results

Demographics

Total 100 neonates having hyperbilirubinemia were analyzed for NBiL values [using dry chemistry] and TBiL values [using wet chemistry].

Bilirubin values by Dry and Wet chemistry

The mean (SD) of NBiL was 8.436 (1.02) mg/dl and that of TBiL was 8.44 (0.56) mg/dl (Table 1).

Mean Bilirubin values	
Neonatal bilirubin	8.436 + 1.02
Total bilirubin	8.44 + 0.56

Table 1: Bilirubin values by Dry and Wet chemistry. All values expressed as Mean + Standard deviation (SD) (mg/dl).

Assessment of agreement between Dry and Wet chemistry methods of measurement of bilirubin using Bland Altman plot (Figure 1): 95% of the values were between 2 SD. The mean difference [95% confidence intervals] between the two methods was 0.004[-0.0889 to 0.0969].

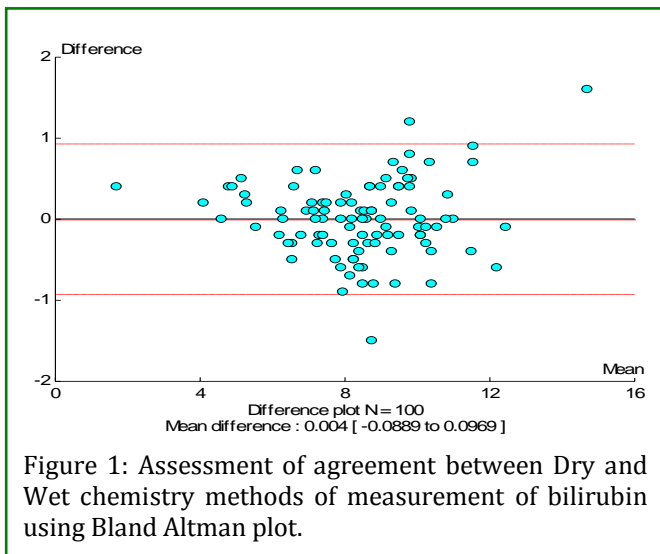


Figure 1: Assessment of agreement between Dry and Wet chemistry methods of measurement of bilirubin using Bland Altman plot.

Correlation between TBiL (Dry) and NBiL (Wet) chemistry methods of measurement of bilirubin (Figure 2): The correlation between the NBiL and TBiL was calculated by Pearson's correlation coefficient of 0.971 with an average bias of 0.1 %.

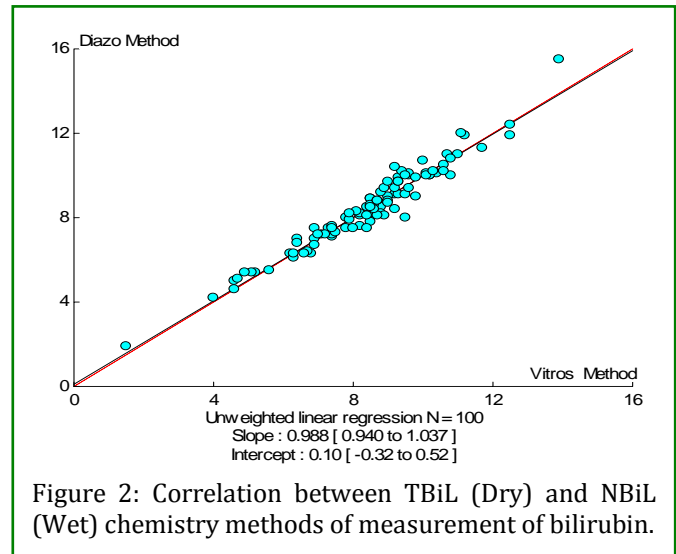


Figure 2: Correlation between TBiL (Dry) and NBiL (Wet) chemistry methods of measurement of bilirubin.

Discussion

The current standard recommendations for evaluating and treating physiological jaundice in neonates' state that all babies should be closely observed for jaundice. Several laboratory tests should be done to diagnose the cause of jaundice in those with early jaundice or bilirubin levels more than 12 to 13 mg/dL (205 to 222 umol/L). If jaundice remains unresolved and not promptly treated, high concentrations of unconjugated bilirubin can ultimately bind to and interact with brain tissue, causing kernicterus, a serious brain injury that can alter and deteriorate the development of the child [12]. In such cases phototherapy should be started to try to keep bilirubin levels below 20 mg/dL (342umol/L). Exchange transfusion is the last resort required if phototherapy fails, regardless of the cause of the jaundice. These recommendations are likely to lead to unnecessary testing and treatment of many jaundiced term infants. Because most jaundiced infants have no underlying illness, and the generally recommended laboratory tests lack sensitivity and specificity, they are seldom useful.

Total serum bilirubin has been the clinical standard for determining risk for kernicterus since almost two decades [21]. However, TBiL takes into account delta bilirubin fraction which is almost negligible in most infants and has no clinical utility. Neonatal bilirubin is estimated as the sum of Bu and Bc. This value is clinically equivalent to total bilirubin without the delta bilirubin fraction and is intended for use in neonates [19]. The present study was conducted to assess if neonatal bilirubin can be used as an acceptable alternative to total bilirubin in neonates with physiological jaundice. In the present study, NBiL and TBiL was measured using dry and wet chemistry

respectively in total 100 neonates of two days of age. There was excellent agreement between the two methods with only 0.4% mean difference in bilirubin values measured by the two methods.

This minor variability between the methods was not significant to influence the clinical decisions

There was an almost perfect (97%) correlation between the dry and wet chemistry methods. In another similar study [18]. It was seen that though NBiL and TBiL difference was statistically significant at certain bilirubin concentrations, it was small and clinically insignificant. TBiL and NBiL methods provide the same results in blood specimens from healthy neonates was seen in another study which concluded that laboratories can use the method they prefer for screening neonates irrespective of their age [19].

Some studies however showed a poor correlation between the two methods

NBiL (Bu Bc) values obtained by the Vitros BuBc slide exceeded the values by the TBiL slide for every specimen. At low bilirubin concentration the difference [(Bu+Bc)-TBiL] was small and at high bilirubin concentration it was large [14,20]. Currently, majority of doctors rely on TBiL in assessing physiological jaundice. The inclusion of NBiL parameter as an acceptable alternative would help overcome misleading interpretations of bilirubin values. Laboratories should aim to be efficient and proficient in measuring bilirubin levels in neonates to enable clinicians to make proper diagnosis and appropriate treatment to the newborns on the basis of accurate measurements of various bilirubin fractions. Our study is limited by the limited sample size recruited from only one site. A similar multi centric study of a larger magnitude would help provide greater evidence to answer our research question.

Conclusion

TBiL and NBiL are both appropriate screening methods for evaluating hyperbilirubinemia in newborns and the confusion that is caused by separating specimens by age is not warranted, based on the similarity of TBiL and NBiL values that this study obtained.

Contributors' Statement

- i. Dr. R. Kishore Kumar conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript.
- ii. Dr. Hari Das collected data, carried out the initial analyses, and drafted the initial manuscript.

- iii. Dr. Arshiya Anjum designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript.
- iv. Dr. S.V. Girish conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.
- v. Dr. Arvind ShenoI critically reviewed the manuscript for important intellectual content.

All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

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