Efficacy of transcutaneous bilirubinometry as compared to serum bilirubin in preterm newborn during phototherapy

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Abstract

Transcutaneous measurement of bilirubin is being used for neonatal jaundice. Its utility during phototherapy in preterm babies is not established. The objective of our study was to assess the efficacy of transcutaneous bilirubin (TcB) measurement in comparison to total serum bilirubin in preterm newborns at admission and during phototherapy at the covered skin area (glabella). It was a prospective observational study and conducted at the neonatal intensive care unit of a tertiary care hospital from January 2017 to January 2019. One hundred eligible preterm neonates were enrolled. Babies who were very sick, with poor peripheral circulation, edematous, having conjugated hyperbilirubinemia, with major congenital malformations, already received phototherapy or exchange transfusion were excluded. Paired total serum bilirubin and transcutaneous bilirubin were measured at admission and 6 h and 24 h during phototherapy. TcB was measured from the area (glabella) covered by an eye protector during phototherapy. The sample for TsB was taken within 10 min of TcB measurement. The mean differences between TsB and TcB values at admission and 6 h and 24 h of phototherapy were −0.005 (0.353) mg/dl, −0.350 (0.611) mg/dl, and −0.592 (0.353) mg/dl, respectively. At admission or before starting of phototherapy, the difference (TsB-TcB) was statistically not significant (p = .125), while the difference in these values was statistically significant at 6 h and 24 h of phototherapy.

Conclusion: TcB measurements from the covered skin area in jaundiced preterm infants during phototherapy were not correlated with TsB and cannot be used as an alternate of serum bilirubin testing.

What is known

- HPLC bilirubin measurement is a gold standard test for bilirubin measurement but impractical for day to day use. Serum total bilirubin is used for clinical testing.
- There is evidence for use of transcutaneous bilirubinometry for assessment of bilirubin in term newborn.

What is new

- TcB measurements from a covered skin area in jaundiced preterm newborns under phototherapy were not correlated significantly at 6 h and 24 h of phototherapy, but correlated before phototherapy.
- TcB cannot be used as an alternate of serum bilirubin testing in preterm infants during phototherapy.
Keywords Preterm · Newborns · Transcutaneous · Bilirubin

Introduction

Hyperbilirubinemia is a common occurrence in early neonatal period. About 50% of term and 80% of preterm neonates have serum bilirubin levels greater than 5 mg/dl in the first few days of life. The levels may go up to 15 mg/dl in 6% of term neonates [1]. In preterm populations, the levels are higher and have more serious implications. Timely phototherapy is the mainstay of treatment to avoid exchange transfusion.

HPLC bilirubin measurement is a gold standard test but impractical for day to day use. Serum total bilirubin is used for clinical testing. It, however, requires a needle prick. Transcutaneous bilirubinometry (TcB), on the other hand, is a quick, handy, and non-invasive way of screening for hyperbilirubinemia requiring intervention. It has also been validated to assess jaundice in term neonates at the time of discharge [2]. Once commenced, phototherapy leads to bleaching of the skin, rendering further TcB measurements unreliable. Covering a part of the skin during phototherapy has been shown to give better correlation with TsB values in some studies [3–8]. However, there has been conflicting evidence in studies [9, 10]. These studies were heterogeneous in regard to gestational age and were done in a small number of babies.

Hence, we planned to do an observational study to assess the accuracy of TcB levels on the covered part of the skin, as compared to corresponding TsB levels in jaundiced preterm neonates before the start and during phototherapy.

Trial design and participants

This single-center, prospective observational study was conducted in the neonatal intensive care unit of a tertiary care hospital, in northern India, from January 2017 to January 2019. Preterm neonates requiring phototherapy in the first 2 weeks of life were considered eligible for enrollment into the study. Neonates with poor peripheral perfusion, sepsis, conjugated hyperbilirubinemia, major congenital malformations, and hydrops fetalis were excluded. Those neonates who had already received phototherapy, and the ones who required exchange transfusion, were also excluded. We did not include babies with hemangioma or bruise on the glabella that may have interfered with TcB measurements.

Case enrollment

Preterm neonates with clinical neonatal jaundice were accessed for eligibility. After parental consent, the TcB/TsB levels were measured. As we enrolled Indian Blacks only, hence we did not take skin pigmentation into account.

Bilirubin measurement protocol

In all study participants, TcB levels were measured simultaneously with TsB. Serum bilirubin levels were measured at three defined time points, just before starting phototherapy and at 6 h and 24 h of continuous phototherapy. During the pilot study, we measured TcB at two different locations: on the covered skin area (glabella) under the eye protector and on the exposed naked skin close to it. We observed higher TcB values under the covered skin than the surrounding uncovered skin area in the pilot study. For TsB measurement, 1–1.5 ml of blood was collected from each neonate. We used venous blood instead of capillary blood as the previous has lesser environmental effect and more accurate values than capillary blood as mentioned in the previous study [11]. At our center, we were using venous blood more frequently than capillary blood for bilirubin measurement.

Peripheral venous blood once collected in plain vials was processed in the laboratory immediately. Our study center was a dedicated pediatric hospital. Our method to measure TsB was calibrated on the range of neonatal hyperbilirubinemia. Bilirubin assay was done by using the diazo method. The principle of bilirubin assay was based on the Jendrassik-Grof method in which diazotized sulfanilic acid reacts with bilirubin to form azo-bilirubin, the latter of which was detected at an optical density of 540 nm. Blood sampling was performed within 10 min of transcutaneous measurement of bilirubin.

Protocol for starting phototherapy

The decision to start phototherapy was based on clinical observation and serum bilirubin level by a pediatrician or a neonatologist. In our unit, we followed NICE (National Institute for Health and Clinical Excellence) guidelines for phototherapy in preterm neonates set by National Collaborating Centre for Women’s and Children’s Health [12]. Preterm neonates with clinical jaundice were tested for TcB and TsB at admission. If the value of TcB/TsB was below the threshold for commencing phototherapy, the neonate was excluded from the study. Phototherapy was started if the value of TcB/TsB was higher than the threshold value. In case of different values between the TcB and TsB, we took the TsB value for the point of action. Exchange transfusion was done if values of serum bilirubin were higher than the reference range or if signs of acute bilirubin encephalopathy were present.

FANEM Bilitron Sky 2006, Super LED Phototherapy was used in our study. The distance between the baby and the
phototherapy unit was 30 cm. Irradiance was kept in the range of 30–50 μW/cm²/nm and measured the same by a handheld radiometer. We used a uniform phototherapy machine for all study participants and the light spectrum was maintained as per standard guidelines.

BiliCare device (BiliCare REF 81000300, Israel, 2015) was used to measure TcB. As per the manufacturer’s recommendation, the device was calibrated each time before use. It gave the average bilirubin value in milligrams per deciliter of three back to back applications. During the treatment with phototherapy, the neonates remained naked, but wearing a diaper and an eye protector. As the babies’ eyes were always covered with an eye protector during phototherapy, we chose the glabella, the area between the eyes, to measure TcB levels to correlate with TsB levels at the same time points. We used a neonatal eye protector of size 10 cm × 3 cm, opaque, non-allergic, and unique design manufactured by Ibis Medical, Kerala, India. It has a stretchable rim that fits the head and minimizes the chances of slipping. In case of any accidental slip of the eye protector, the nursing staff on duty would place it back in the same position.

The phototherapy unit was turned off only during TcB/TsB measurements and feeding. All the doctors involved in the care of these neonates were familiar with the use of BiliCare device as we have been using it for over 2 years in our nursery. We also performed three live demonstrations of its use before the start of the study among our neonatal team.

Sample size

Observations from the study conducted by Lucanova et al. [9] indicated that phototherapy significantly interferes with the accuracy of transcutaneous bilirubinometry. In order to have a correlation coefficient of 0.287, a sample size of 94 neonates was estimated for a study power of 80% and two-tailed alpha of 0.05. This was further rounded off to 100 neonates.

Statistical analysis

Nonparametric tests were used for the outcomes on a continuous scale. The mean of TcB for each measurement time point was compared with the corresponding TsB concentration. Pearson correlation coefficient (r) analysis was performed to assess the correlation coefficient between TcB and TsB. In order to assess the accuracy of non-invasive bilirubin concentration measurement, difference (Δ) between TsB and TcB and their 95% confidence intervals (CI 95%) were evaluated. A p-value of 0.05 was considered statistically significant. The method of Bland and Altman was used to evaluate the agreement between bilirubin levels in blood and skin.

Ethical approval

Written informed consent was taken from the parents or caregiver of the baby before enrollment into the study. The study was approved by the institutional Ethics Committee (letter no: 2823 MC/EC/2016).

Results

We had included neonates of resident Indian Blacks in the population studied. Nine hundred newborns were admitted in the neonatal intensive care unit during the study period. Out of them, 150 preterm newborns were diagnosed for pathological neonatal jaundice and examined for eligibility. Fifty preterm neonates were excluded from the study due to poor peripheral perfusion (n = 5), sepsis (n = 15), already received phototherapy (n = 4), received exchange transfusion (n = 1), conjugated hyperbilirubinemia (n = 6), having major congenital malformations (n = 6), hydrops fetalis (n = 2), refusal for consent (n = 9), and other reasons (n = 2). One hundred newborns were confirmed eligible and participated in the study. All the enrolled cases (n = 100) completed the study and results were analyzed.

The TcB and TsB at admission and 6 h and 24 h during phototherapy were measured for all study participants. Hence, a total of 300 paired samples of TcB and TsB were taken from 100 enrolled newborns. The baseline characteristics are given in Table 1. Mean maternal age, birth weight, and gestational age were 27.89 years, 1488.1 g, and 32.76 weeks, respectively. Only 68% babies received full enteral feeding. Use of antenatal corticosteroids (52%, n = 52), mean Apgar@1 min of 7, mean Apgar@5 min of 9, 78 cases which had CRIB-II score @ Level 1, 22 cases which had CRIB-II score @ Level 2, and no serious comorbidities were observed in the study subjects. The mean TcB (SD) levels at admission and 6 h and 24 h of phototherapy were 14.7 (1.46), 13.58 (1.51), and 11.39 (1.42) mg/dl, while mean TsB (SD) levels at admission, 6 h, and 24 h were 14.08 (1.51), 13.93 (1.29), and 11.98 (1.32) mg/dl.

The mean differences (SD) between TcB and TsB values at admission and 6 h and 24 h of phototherapy were −0.005 (0.353) mg/dl, −0.350 (0.611) mg/dl, and −0.592 (0.353 mg/dl), respectively (Table 2). At admission or before start of phototherapy, the difference (TsB-TcB) was statistically not significant (p = .125), while the difference in these values was statistically significant at 6 h and 24 h of phototherapy.

The sensitivity and specificity for TcB and TsB values at admission and 6 h and 24 h of phototherapy for serum bilirubin levels 11, 13, and 15 mg/dl are given in Table 3. Figures 1, 2, and 3 show the Bland Altman plots for agreement between these two tests at the abovementioned time points.
Discussion

Phototherapy decreases bilirubin through photoisomerization, structural isomerization, and photo-oxidation [13]. Transcutaneous bilirubin estimation has proven efficacy for screening hyperbilirubinemia in full-term neonates. A systematic review concluded that TcB devices reliably estimated bilirubin levels in preterm infants and also could be used to reduce blood sampling in preterm newborns [14]. A meta-analysis by Nagar et al. resulted in TcB devices reliably estimating bilirubin level in preterm infants [15]. The evidence for its use in preterm neonates during phototherapy is lacking. We conducted this observational study in this selected group to assess correlation of TcB with serum bilirubin at start of phototherapy. Our results showed statistically significant difference between TsB and TcB at 6 h and 24 h of phototherapy over the covered skin area, although the difference was not statistically significant at start of phototherapy.

Similar to other studies [5, 9], we used the glabella as a site for transcutaneous measurement of bilirubin. Ozkan H et al. and De Luca D et al. covered the skin with a patch size of 2.5 cm, while we covered the skin with a 10×3 cm eye protector during phototherapy [7, 16]. Lucanova et al. measured transcutaneous bilirubin from the abdomen [9]. Sajjadian, on the other hand, measured transcutaneous bilirubin from both the forehead and sternum and in both healthy and sick newborns [17]. It was a priority for the clinicians to look for unexposed alternative sites for TcB measurement. As the genitals and eyes are covered in all babies receiving phototherapy, these alternative measurement sites may prove convenient for the same. Hence, we used the glabella for measurement of TcB in our study.

Phototherapy is used for treatment of neonatal hyperbilirubinemia to protect the brain from unconjugated bilirubin fraction. Transcutaneous bilirubinometry provides a rapid estimate of total serum bilirubin and can be helpful in monitoring the trends as it can be repeated, non-invasive, reproducible, easier to perform, and cost-effective than TsB.

Table 1 Baseline characteristics of the study participants

| Baseline characteristics                  | Number | SD | 95% CI     |
|-------------------------------------------|--------|----|------------|
| Male (%)                                  | 50 (50%)|    |            |
| Number of paired sample                   | 300    |    |            |
| Mean maternal age (years)                 | 27.89  | 5.82| 5.11–6.77  |
| Mean weight at birth (g)                  | 1488.1 | 264.37| 232.11–307.11 |
| Mean gestational age (weeks)              | 32.76  | 1.82| 1.60–2.12  |
| Mean postnatal age at the time of admission (h) | 72.36  | 264.37| 16.46–21.77 |
| Number of neonates received enteral nutrition (%) | 68 (68%)|    |            |
| Number of mothers received antenatal steroids (%) | 52 (52%)|    |            |
| Mean Apgar score @1 min                   | 7      |    |            |
| Mean Apgar score @5 min                   | 9      |    |            |
| Number of neonates with CRIB-II score @ Level 1 (%) | 78 (78%)|    |            |
| Number of neonates with CRIB-II score @ Level 2 (%) | 22 (22%)|    |            |
| Number of neonates with serious comorbidities (%) | 0 (0%)|    |            |
| Number of neonates required mechanical support/vasopressor (%) | 0 (0%)|    |            |
| Mean weight loss in neonates since birth to admission (%) | 4.4%|    |            |

SD standard deviation, CI confidence interval, CRIB clinical risk index for babies

Table 2 TcB and TsB measurements and correlation

| Parameters (n = 100) | Mean TcB (mg/dl) | Mean TsB (mg/dl) | Difference b/w TcB and TsB (mg/dl) (n = 100) |
|---------------------|------------------|------------------|-----------------------------------------------|
|                     | Mean (SD)        | Std. error mean  | 95% CI                                        |
| At admission        | 14.7 (1.46)      | 14.08 (1.51)    | −0.005 (0.3532) 0.0353 −0.075 to −0.065 0.154 0.125 |
| At 6 h of phototherapy | 13.58 (1.51)   | 13.93 (1.29)    | −0.350 (0.6108) 0.0611 −0.471 to −0.229 0.437 0.000 |
| At 24 h of phototherapy | 11.39 (1.42)   | 11.98 (1.32)    | −0.592 (0.3532) 0.0353 −0.662 to −0.522 0.296 0.003 |

TcB transcutaneous bilirubin, TsB total serum bilirubin, SD standard deviation, CI confidence interval
TcB cannot substitute TsB as the correlation between these two values is not well established. However, TcB does reduce the frequency of blood sampling and iatrogenic blood loss leading to anemia. It minimizes the pain and stress associated with pricking. This non-invasive measurement is useful to improve quality of neonatal care. Bhutani et al. concluded that universal screening combined with the clinical risk factors provides identification of infants who are at high risk or low risk for development of severe neonatal hyperbilirubinemia [18]. Use of transcutaneous bilirubinometry is debatable in monitoring of newborns during phototherapy. There may be a difference in TcB and TsB as bilirubin concentration in subcutaneous tissue exposed to phototherapy decreases before TsB concentration [19]. The exact time needed for the establishment of the equilibrium between the skin bilirubin to serum bilirubin is not known.

In our study, the correlation coefficient (r) for difference in TsB and TcB was 0.024 at start of phototherapy, and 0.14 at 6 h and 0.088 at 24 h of phototherapy. These results were not correlated at 6 and 24 h of phototherapy and had a positive

| Serum bilirubin level (mg/dl) cut-off value | At admission | At 6 h of phototherapy | At 24 h of phototherapy |
|--------------------------------------------|-------------|------------------------|------------------------|
| > 11 mg/dl                                 | Sensitivity | 100%                   | 96.9%                  | 85.9%                  |
|                                            | Specificity | -                      | 100%                   | 100%                   |
| >13 mg/dl                                  | Sensitivity | 95%                    | 75.9%                  | 66.7%                  |
|                                            | Specificity | 90%                    | 100%                   | 100%                   |
| > 15 mg/dl                                 | Sensitivity | 88.23                  | 98.63                  | -                      |
|                                            | Specificity | 86.36                  | 100                    | 100%                   |

TcB transcutaneous bilirubin, TsB total serum bilirubin

Table 3 Sensitivity and specificity of TcB to predict TsB at certain time points

Fig. 1 Bland-Altman plot at admission
Fig. 2  Bland-Altman plot at 6 h of phototherapy

Fig. 3  Bland-Altman plot at 24 h of phototherapy
correlation at the start of phototherapy. The correlation coefficient of the present study was in contrast to previous studies that ranged from 0.68 to 0.96 [17–20]. TsB was measured over the sternum [21] and the forehead, sternum, and abdomen [22], while we measured it over the glabella. Recently, Alsaedi et al. [21] showed a significant correlation between TcB and TsB before the start and during phototherapy \( (r = 0.85, p < 0.001) \) and \( r = 0.80, p < 0.001 \) in term neonates. The mean difference between TcB and TsB at the start was 0.36 (1.36) mg/dl, whereas it was 0.16 (1.37) mg/dl during the treatment while we have –0.05 (0.353), –0.350 (0.611), and –0.592 (0.353) mg/dl at admission and 6 h and at 24 h of phototherapy. However, our study population were preterm neonates.

Cucuy et al. [22] studied the correlation between TcB and TsB before, during, and after phototherapy in preterm babies. The overall correlation coefficient between TcB and TsB was 0.8. In contrast to our study, they concluded that TcB is a reliable measure of jaundice before 35 weeks gestation. Pendse et al. [23] used aluminum photoprotection system with JM-105 and highlighted positive correlation in infants less than 32 weeks in contrast to our study.

Similar to our findings, Lucanova et al. [9], Murali et al. [10], and Radfar et al. [24] found that TcB was poorly correlated with TsB during phototherapy, irrespective of the site of measurements. Murali et al. and Lucanova et al. also reported a bad correlation between the TsB and TcB after the discontinuation of phototherapy [9, 10]. However, we measured TcB and TsB up to 24 h of phototherapy. All of the above studies concluded that TcB does not appear to be reliable for estimating bilirubin in preterm. Luca D D et al. obtained readings from the forehead and found a good correlation after 4–6 h from the start of phototherapy with a trend of increased difference between TSB and TcB with duration of phototherapy [25]. We used BiliCare device for measurement of TcB similar to other studies [10, 11], while Lucanova et al. [9] used JM-103 similar to other studies [8, 11, 20]. Raimondi et al. compared the three most commonly used devices for TcB measurements, i.e., Bilicheck, JM103, and BiliMed. Pearson coefficients in their study showed good results for Bilicheck \((r = 0.86)\) and JM-103 \((r = 0.85)\) but poor for BiliMed \((r = 0.70)\). Bilicheck and JM-103 had a greater area under the curve than BiliMed when TSB =14 mg/dl was chosen as a threshold value. They concluded that Bilicheck and JM103 (but not BiliMed) are equally reliable [16].

Similar to Taylor JA et al.’s study, we did not find any effect of birth weight, gestational age, and other variables on correlation between TsB and TcB [26]. Although most of the studies included only stable newborns, Jeon J et al. studied the correlation in sick newborns and found a significant correlation between TsB and TcB [27].

The strength of our study includes a prospective study with an adequate sample size. We restricted our study to stable preterm infants. We measured TcB and TsB at different time points during phototherapy. However, our study has certain limitations. All our results were limited to Indian Black neonates and may not be applicable to lighter pigmented groups. We obtained TcB up to 24 h of continuous phototherapy instead of the whole duration. Studies with a longer point of time should address the correlation of TcB and TsB during the entire time of phototherapy. Likewise, we did not address the cost-effectiveness of TcB as well.

The correlation of TsB and TcB before starting phototherapy was significant. But at 6 h and at 24 h of phototherapy, the correlations were not significant. So TcB measurement should not be considered a surrogate for the gold standard TsB measurement during phototherapy. In these cases, TsB should be strongly considered.

**Conclusion**

Our study concluded that TcB measurements by a single TcB device taken from a covered area of the skin in jaundiced, healthy, single-ethnic preterm newborns under phototherapy for a limited period of time did not correlate significantly at 6 h and 24 h of phototherapy, but correlated before phototherapy. Hence, TcB in preterm infants undergoing phototherapy cannot be used as an alternate of serum bilirubin testing.

**Abbreviations** CI, Confidence interval; r, Correlation coefficient; LED, Light-emitting diode; SD, Standard deviation; TcB, Transcutaneous bilirubin; TsB, Total serum bilirubin

**Authors’ Contributions** SG, Study conception and design, Acquisition of data. Analysis and interpretation of data, Drafting of manuscript; Critical revision, Final approval of manuscript; SS, Study conception and design, Acquisition of data. Analysis and interpretation of data, Drafting of manuscript; Critical revision, Final approval of manuscript; KKM, Study conception and design, Acquisition of data. Analysis and interpretation of data, Drafting of manuscript; Critical revision, Final approval of manuscript; MB, Study conception and design, Acquisition of data. Analysis and interpretation of data, Drafting of manuscript; Critical revision, Final approval of manuscript; RC, Study conception and design, Acquisition of data. Analysis and interpretation of data, Drafting of manuscript; Critical revision, Final approval of manuscript; AI, Study conception and design, Analysis and interpretation of data, Drafting of manuscript; Critical revision, Final approval of manuscript.

**Availability of data and material** NA

**Code availability** NA

**Declarations**

**Ethics approval** Approved by Institutional Ethics Committee- 2823 MC/EC/2016.
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Conflict of interest  The authors declare no competing interests.

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