Doi: 10.32677/IJCH.2017.v04.i04.021

Original Article

Validity of transcutaneous bilirubin in the first few days of life

Pearl Mary Varughese[1], Peter Prasanth Kumar Kommu[1], K Ravichandran[2], Lalitha Krishnan[1]

From [1]Department of Pediatrics, [2]Department of Bio-statistics, Pondicherry Institute of Medical Sciences, Puducherry, India

Correspondence to: Lalitha Krishnan, Department of Pediatrics, Pondicherry Institute of Medical Sciences, Puducherry, India. E-mail: lalitha_krishnan@hotmail.com

Received – 21 April 2017 Initial Review – 16 May 2017 Published Online - 06 November 2017

ABSTRACT

Objective: To study the validity of transcutaneous bilirubin (TCB) at different time points between 24 and 96 h of life. **Materials and Methods:** The study was conducted in a tertiary newborn center from November 2014 to June 2016. We have included all babies above 34 weeks of gestation while babies with established direct hyperbilirubinemia, neonatal septicemia, major congenital/gastrointestinal malformations and those on phototherapy were excluded from the study. After taking into consideration the inclusion and exclusion criteria, 396 babies were recruited. The correlation was analyzed using the ROC curves, and r values and plots of agreement were done using Bland Altman charts. **Results:** A total of 662 paired TCB and serum bilirubin readings were performed in 396 healthy neonates, at designated time points between 24 and 96 h of life. Mean TCB was found to be uniformly higher than total serum bilirubin (TSB) for all variables such as sex, birth weight, gestational age, and growth of the baby with an excellent correlation (r=0.698-0.932). The correlation between TCB and serum bilirubin improved as age increased with maximum correlation at 96 h (r=0.981). Test of agreement showed that at 24 h the TCB overestimated serum bilirubin by 1.5±0.7 mg/dl which decreased to 0.8±0.6 mg/dl by 96 h. **Conclusion:** TCB and TSB correlated significantly between 24 and 96 h of age. However, there is overestimation by TCB initially which decreases at 96 h.

Key words: *Bilirubinometry, Serum Bilirubin, Transcutaneous Bilirubin*

Neonatal hyperbilirubinemia is observed during the 1st week of life in approximately 60% of the healthy infants [1]. Rising bilirubin levels in neonates usually reach the maximum between 72 and 120 h of life, and there is an increased rate of readmission before 6 days of life for hyperbilirubinemia [2]. American Academy of Pediatrics guidelines stress the need for routine pre-discharge measurement of bilirubin levels. With increasing demand for a shorter length of hospital stay for babies after delivery, there is an increased risk of unrecognized or delayed hyperbilirubinemia resulting in an increase in the incidence of babies affected with kernicterus [3]. The conventional method of measuring serum bilirubin requires repeated blood sampling which not only causes undue pain to the babies but also emotional stress to the parents [4]. Over the past three decades, transcutaneous bilirubinometry has emerged as a safe, simple, cost-effective non-invasive modality in the screening and monitoring of jaundiced newborns [5,6].

Several published studies reveal that there is a correlation between transcutaneous bilirubin (TCB) and total serum bilirubin (TSB) over the first few days of life. We planned this study to assess the correlation between TCB and TSB between 24 and 96 h of age and also to see if skin color had any effect on TCB measurements.

MATERIALS AND METHODS

This was a prospective observational study conducted on unborn babies more than 34 weeks gestational age, from November 2014 to June 2016 in a neonatal unit of a medical college hospital in South India. The exclusion criteria were babies with established direct hyperbilirubinemia, neonatal septicemia, and major congenital/gastrointestinal malformations and those started on phototherapy. The study protocol was approved by the Institutional Review Board and Ethics Committee. Written informed consent was obtained from the mothers for using their baby's de-identified data. Confidentiality was maintained throughout the study.

Sample size was calculated using the formula: $n=4\ pq/(d)^2$ where p=sensitivity (80%); q=1-p (20%), and d=p x absolute precision. This was done for an anticipated sensitivity of 80% with absolute precision 5% and 0.05 level of significance considering the incidence of hyperbilirubinemia to be 20% and lost to follow-up as 5%. This gave a sample size of 400. After applying inclusion and exclusion criteria, neonates were recruited in the study. The clinical and demographic profile of the mother and the baby was collected using a proforma. At 24 h, the babies were assigned a color code based on Fitzpatrick skin tone scale [7]. The color chart was made on a transparent sheet with each color code separated by half an inch width and the color codes assigned

for each baby were given by the principal investigating officer to avoid the chances of investigator bias.

Transcutaneous bilirubin levels (TCB) were estimated with Drager Jaundice Meter JM-105 by placing the instrument on the baby's sternum. Sternum was taken as the principal site of measurement as several studies have shown excellent correlation with TSB compared to the other sites [8,9]. All measurements were performed with the same device according to the standard described technique. An average of three readings was taken as the TCB value. The probe was cleaned with sterile gauze before using for the next baby.

Approximately 1 ml of venous blood was collected in a Microtainer clot activator tube for assessing TSB level under strict aseptic precautions after the mother was explained about the procedure. Special care was taken to avoid exposure of the collected samples to light. The blood samples were taken to the hospital laboratory within an hour to prevent degradation and processed. Serum bilirubin measurements were measured using the Diazo method (modified Jendrassik--Grof method) in the automated analyzer (Cobas Integra 400 plus from Roche Diagnostics). Although high-performance liquid chromatography (HPLC) estimation is considered the gold standard, "delta bilirubin" or "bilirubin-albumin," is measured by the diazo but not by the HPLC method as it is impractical for routine clinical laboratory use as it is too elaborate, time-consuming and expensive [10]. The maximum interval of time between the transcutaneous measurement and the collection of blood for TSB was 30 min.

All babies were visually examined every 6 h on the 1st day of life by a trained physician and twice a day thereafter. At 24 h, TSB and TCB were done on all babies and later repeated as per attending clinician's discretion. Data were entered in Microsoft Excel and analyzed using the SPSS version 20.0 for Windows software. Pearson's correlation and Bland Altman analysis were used for studying the data.

RESULTS

A total of 396 babies were recruited after applying the eligibility criteria. Demography of the cohort is given in Table 1. The mean TSB of the entire cohort at 24 h was 6.2±1.4 mg/dl and simultaneous mean TCB value was 7.7±1.4 mg/dl. Mean TCB was found to be uniformly higher than TSB for all variables such as sex, birth weight, gestational age, and growth of the baby (Table 1). However, among all variables, there was an excellent correlation between TCB and TSB (r=0.698-0.932).

In our study, most of the babies belonged to the Fitzpatrick skin color codes 3 and 4 with 299 babies (75.5%) in color code 3 and 95 babies (24%) in color code 4 (Fig. 1). TCB correlates better in light skin tone babies (color code 3) than dark skin tone babies (color code 4) with r=0.874 and r=0.856, respectively. A total of 662 paired TCB and serum bilirubin readings were done in the study. Of these, 396 were done at 24 h of age. At 48, 72, and 96 h there were 196, 48, and 22 paired readings, respectively.

Table 1: Demography of the study population

Characteristics	Number (%)	Mean TSB (SD)	Mean TCB (SD)
Sex			
Male	222 (56.1)	5.8 (1.1)	7.4 (1.2)
Female	174 (43.9)	5.9 (1.1s)	7.4 (1.2)
Birth weight			
<2000 g	9 (2.3)	5.5 (1.0)	6.8 (0.9)
2000-2499 g	31 (7.8)	6.1 (0.9)	7.8 (1.0)
2500-2999 g	150 (37.9)	5.8 (1.0)	7.4 (1.2)
3000 g-3499 g	165 (41.7)	5.9 (1.1)	7.4 (1.2)
>3500 g	41 (10.3)	6.1 (1.5)	7.5 (1.3)
Mode of delivery			
Normal vaginal delivery	255 (64.4)	5.9 (1.1)	7.4 (1.2)
Instrumental delivery	19 (4.8)	6.2 (0.9)	7.7 (0.9)
Cesarean delivery	122 (30.8)	5.8 (1.2)	7.3 (1.2)
Gestational age (weeks)			
34-366	28 (7.1)	5.6 (0.9)	7.2 (1.1)
37-396	313 (79)	5.8 (1.1)	7.4 (1.2)
>40	55 (13.9)	6.2 (1.2)	7.8 (1.1)
Growth			
Small for gestational age	46 (11.6)	5.9 (0.9)	7.5 (1.1)
Approximate for gestational age	348 (87.9)	5.9 (1.2)	7.4 (1.2)
Large for gestational age	2 (0.5)	6.2 (1.1)	7.5 (1.3)

TSB: Total serum bilirubin, SD: Standard deviation

Figure 1: Babies were coded based on Fitzpatrick's skin color codes (a) skin color code 3 (b) skin color code 4

There was a statistically significant positive correlation between TSB and TCBI at 24, 48, 72, and 96 h (p<0.001) (Table 2). As the time progresses, the correlation improves with maximum correlation at 96 h (r=0.981). The Bland Altman plots for the same time intervals were also done which showed that as the time progresses, the TCB overestimates TSB by a lesser value compared to the earlier ones. TCB overestimates TSB by an average of 1.5 mg/dl, 1.3 mg/dl, 0.9 mg/dl, and 0.8 mg/dl at 24, 48, 72, and 96 h, respectively (Table 3, Figs. 2-5).

Table 2: TSB and TCB at designated times and their reliability

Time (n)	24 h (396)	48 h (196)	72 h (48)	96 h (22)
Mean TCB in mg/dl (SD)	7.4 (1.2)	11.6 (1.6)	13 (3.4)	13.2 (2.6)
Mean TSB in mg/dl (SD)	5.9 (1.1)	10.3 (1.6)	12.2 (3.3)	12.4 (2.8)
Mean difference of TCB and TSB	1.5 (0.7)	1.3 (0.6)	0.9 (0.7)	0.8 (0.6)

TSB: Total serum bilirubin, SD: Standard deviation

Table 3: Bland Altman analysis

TSB and TCB difference (hours)	Mean (SD)	Mean+2SD	Mean -2SD
TSB24 and TCB24	−1.54 (0.69)	−0.16	−2.92
TSB48 and TCB48	−1.30 (0.62)	−0.06	−2.54
TSB72 and TCB72	−0.89 (0.65)	0.41	−2.19
TSB96 and TCB96	−0.81 (0.60)	0.39	−2.01

TSB: Total serum bilirubin, SD: Standard deviation

DISCUSSION

The accuracy of transcutaneous bilirubinometry has evolved over the years. The older versions were considered less reliable as they were influenced by skin color, gestational age, birth weight, and other factors [11,12]. The more recent devices are based on micro spectrometry, and studies have shown better correlations with serum bilirubin levels. In our study, there was a good correlation between mean TCB and serum bilirubin values at 24 h with variables such as sex, birth weight, gestational age, and growth of the baby.

There have been studies to show that there is a good correlation in transcutaneous bilirubin levels and capillary and arterial bilirubin levels (r=0.7) [13]. In our study, venous samples were taken and compared with transcutaneous values. The serum bilirubin levels were measured by diazo method in our study. HPLC, though expensive, is the best bilirubin estimate. There are studies showing HPLC measured bilirubin levels showed good correlation at the forehead and sternum though TCB – forehead tend to underestimate and TCB – sternum tends to overestimate [14,15].

The effect of postnatal age on transcutaneous bilirubinometry values has not been fully explored. In 1993, Knudsen et al. conducted a study in all the neonatal intensive care unit babies and found that in multiple linear regression analysis, gestational age, and postnatal age showed a negative but significant correlation. Most of the effects were explained with the increased maturity and thickness of skin and changes in the amount of albumin-bilirubin binding. The difference was most obvious in preterm babies [16]. In 2000, a study of 95 newborn babies found no significant effect of postnatal age on Bilicheck accuracy when it was compared with HPLC bilirubin values (r 0.16; *t*-test for non-zero slope, t 1.53; p 0.05) [17]. Ebbesen et al. studied 261 jaundiced infants with gestational age of 25-43 weeks and found that as the postnatal age increased, the TCB values were lower than serum bilirubin values [18]. In our study, there was a significant correlation as the postnatal age advances, with maximum correlation at 96 h (r=0.981), attributing it to the skin maturity and changes in albumin-bilirubin binding. Bland Altman plots also show that at 96 h, the agreement between TCB and TSB is excellent when compared to the values at earlier time intervals. Thus, there is a good correlation and excellent agreement of TSB and TCB at 96 h. This could also mean that the TCB can replace TSB as the time progresses. Once the babies are assigned a risk category according to the Bhutani's TSB monogram [19], they could be followed up from72 h onward with a TCB value rather than a TSB value; thus, reducing the number of blood samples and unnecessary pricks for the newborn. The study contained only 7.1% of late preterm babies, so the effect of TCB on postnatal age in preterm babies <34 weeks could not be assessed.

The usual peak of TSB occurs at age 72-120h, usually resolves by age 7-10 days, and the outcome is benign. Only approximately 2-5% require readmission to a hospital for treatment [19]. In a country like ours, with less number of mothers delivering in a fully equipped hospital, and even lesser amount of these babies remaining in the hospital for more than 48 h, a follow-up criteria have to be established. In our hospital, the babies were advised discharge after 72 h of life. An hour-specific correlation can predict the risk of hyperbilirubinemia in babies; thus, preventing the future development of kernicterus.

The correlation of skin color with TCB has yet to be defined irrespective of race and place of origin. In USA, with the increasing number of migrants, there is a whole diversity of people with different skin colors and ethnic backgrounds. However, in India, where the population is fairly distributed into major skin color groups, only a small minority having the extremes of color (too light skinned or too dark skinned). Despite JM 103 and Bilicheck accounting for skin pigmentation in their design, the relationship between TCB and TSB is different in black infants. Color-coded scales were rarely used for the comparison, and more studies showed the imprecision of the bilirubinometry mostly based on race and ethnic backgrounds. The Fitzpatrick scales were used for this study; color code 3 has a higher correlation when compared to color code 4. This was similar to the studies done that showed that darker skin tones have a poorer correlation [13,20]. Maisels et al. showed that JM 103 can overestimate TSB in black infants and the imprecision of Bilicheck increases as a degree of skin pigment increases [21].

The present study is the first of its kind to be conducted in the South Indian population; however, it has some limitations too. First, it is not a population-based study, and it represents the data of a single tertiary care hospital in South India. Second, most of the babies in the study belonged to Fitzpatrick skin color codes 3 and 4 with very few babies falling into the other categories. Third, the timing of assessment at 24 h is too early for physiological jaundice peak. Another limitation is that the samples for all the designated time intervals are not the same; hence, it could have affected the validity of the data. The Bland Altman plots though showed great

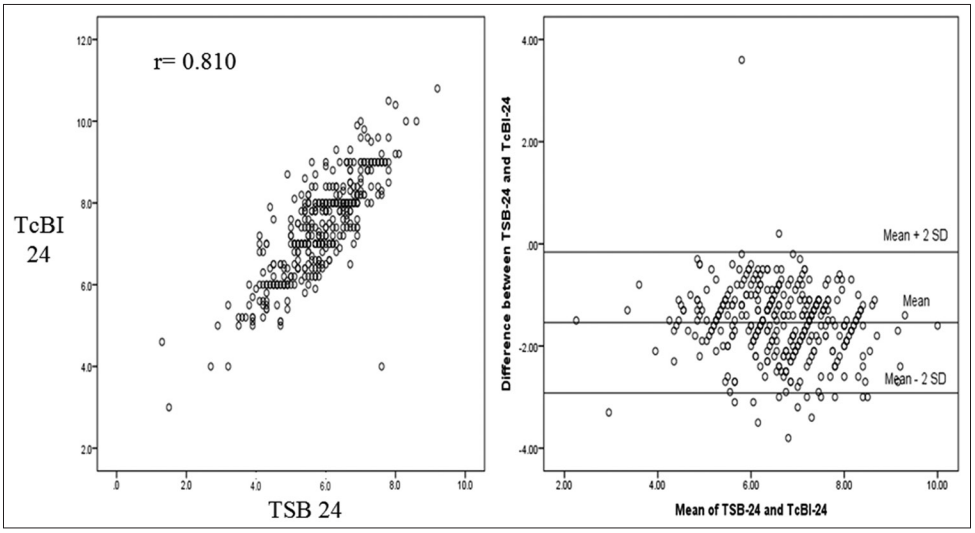

Figure 2: Correlations and Bland Altman plots of agreement of total serum bilirubin and TCBat 24 h

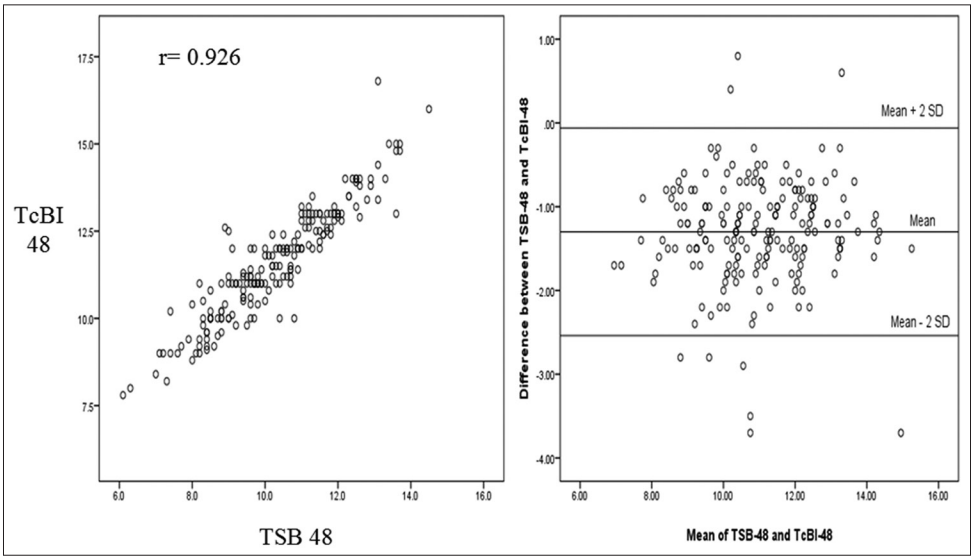

Figure 3: Correlations and Bland Altman plots of agreement of total serum bilirubin and TCB at 48 h

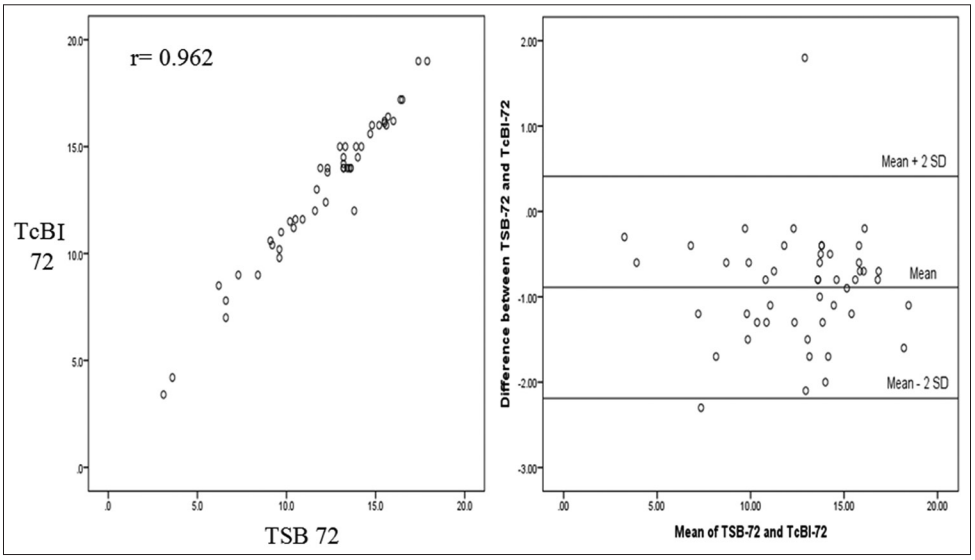

Figure 4: Correlations and Bland Altman plots of agreement of total serum bilirubin and TCB at 72 h

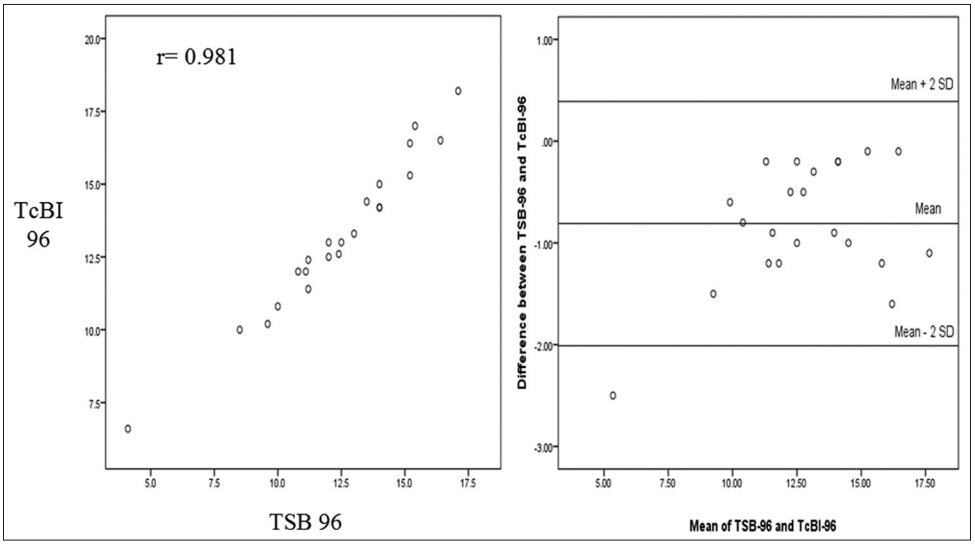

Figure 5: Correlations and Bland Altman plots of agreement of total serum bilirubin and TCB at 96 h

agreement at 72 h and 96 h, they were done for a smaller sample size, and this could have also affected the validity and accuracy. Each institution should be aware of the unique relationship between its laboratory bilirubin values and TCB values. New policies should be made and implemented after understanding this relation. Further studies are needed to be done in a larger population with an equal number of subjects at each designated time spot.

CONCLUSION

This study finds that correlation of TCB and TSB increases as postnatal age advances. Designated TCB indices at different time spots could guide the pediatricians in safe discharge and targeted follow-up in at-risk babies. Transcutaneous bilirubinometry is most efficiently used as a screening test and values near or above age-specific risk cut off points should be confirmed with serum bilirubin values. Lighter skin color in babies showed a better correlation with TCB.

REFERENCES

1. Bhutani VK, Johnson L. A proposal to prevent severe neonatal hyperbilirubinemia and kernicterus. J Perinatol 2009;29 Suppl 1:S61-7.
2. Farhat R, Rajab M. Length of postnatal hospital stay in healthy newborns and re-hospitalization following early discharge. N Am J Med Sci 2011;3:146-51.
3. Romagnoli C, Tiberi E, Barone G, Curtis MD, Regoli D, Paolillo P, *et al.* Development and validation of serum bilirubin nomogram to predict the absence of risk for severe hyperbilirubinaemia before discharge: A prospective, multicenter study. Ital J Pediatr 2012;38:6.
4. Yap SH, Mohammad I, Ryan CA. Avoiding painful blood sampling in neonates by transcutaneous bilirubinometry. Ir J Med Sci 2002;171:188-90.
5. Stillova L, Matasova K, Mikitova T, Stilla J, Kolarovszka H, Zibolen M. Evaluation of transcutaneous bilirubinometry in preterm infants of gestational age 32-34 weeks. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2007;151:267-71.
6. Boo NY, Ishak S. Prediction of severe hyperbilirubinaemia using the Bilicheck transcutaneous bilirubinometer. J Paediatr Child Health 2007;43:297-302.
7. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol 1988;124:869-71.
8. Nagar G, Vandermeer B, Campbell S, Kumar M. Reliability of transcutaneous bilirubin devices in preterm infants: A systematic review. Pediatrics 2013;132:871-81.
9. Kurokawa D, Nakamura H, Yokota T, Iwatani S, Morisawa T, Katayama Y, *et al.* Screening for hyperbilirubinemia in Japanese very low birthweight infants using transcutaneous bilirubinometry. J Pediatr 2016;168:77-81.
10. Jansen PL, Peters WH, Janssens AR. Clinical value of serum bilirubin subfractionation by high-performance liquid chromatography and conventional methods in patients with primary biliary cirrhosis. J Hepatol 1986;2:485-94.
11. Shah B, Gosai DD, Prajapati J. Comparison study between serum and transcutaneous bilirubin measurement with special reference to gestational age. Int J Sci Res 2013;4:2250-3.
12. Samiee-Zafarghandy S, Feberova J, Williams K, Yasseen AS, Perkins SL, Lemyre B. Influence of skin colour on diagnostic accuracy of the jaundice meter JM 103 in newborns. Arch Dis Child Fetal Neonatal Ed 2014;99:F480-4.
13. Amato M, Huppi P, Markus D. Assessment of neonatal jaundice in low birth weight infants comparing transcutaneous, capillary and arterial bilirubin levels. Eur J Pediatr 1990;150:59-61.
14. Chawla D, Jain S, Kaur G, Sinhmar V, Guglani V. Accuracy of transcutaneous bilirubin measurement in preterm low-birth-weight neonates. Eur J Pediatr 2014;173:173-9.
15. Rubaltelli FF, Gourley GR, Loskamp N, Modi N, Roth-Kleiner M, Sender A, *et al.* Transcutaneous bilirubin measurement: A multicenter evaluation of a new device. Pediatrics 2001;107:1264-71.
16. Knudsen A, Ebbesen F. Transcutaneous bilirubinometry in neonatal intensive care units. Arch Dis Child Fetal Neonatal Ed 1996;75:F53-6.
17. Kazmierczak SC, Robertson AF, Briley KP, Kreamer B, Gourley GR. Transcutaneous measurement of bilirubin in newborns: Comparison with an automated Jendrassik-Grof procedure and HPLC. Clin Chem 2004;50:433-5.
18. Ebbesen F, Rasmussen LM, Wimberley PD. A new transcutaneous bilirubinometer, BiliCheck, used in the neonatal intensive care unit and the maternity ward. Acta Paediatr 2002;91:203-11.
19. Bhutani VK, Johnson L, Sivieri EM. Predictive ability of a predischarge hour-specific serum bilirubin for subsequent significant hyperbilirubinemia in healthy term and near-term newborns. Pediatrics 1999;103:6-14.
20. Mahajan G, Kaushal RK, Sankhyan N, Sharma RL, Nakra M. Transcutaneous bilirubinometer in assessment of neonatal jaundice in northern India. Indian Pediatr 2005;42:41-5.
21. Maisels MJ, Ostrea EM Jr, Touch S, Clune SE, Cepeda E, Kring E, *et al.* Evaluation of a new transcutaneous bilirubinometer. Pediatrics 2004;113:1628-35.

Funding: None; Conflict of Interest: None Stated.

How to cite this article: Varughese PM, Kommu PPK, Ravichandran K, Krishnan L. Validity of transcutaneous bilirubin in the first few days of life. Indian J Child Health. 2017; 4(4):549-553.