A study on correlation between estimation of bilirubin by transcutaneous bilirubinometry and laboratory method

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ABSTRACT

Background

Presently, transcutaneous determination of bilirubin has become a valuable method for the early detection of neonatal hyperbilirubinemia. This study aims to determine the correlation of transcutaneous bilirubin with serum bilirubin levels and assess the factors affecting transcutaneous bilirubin measurement in term and late preterm neonates.

Materials and Methods

The study included 100 newborns born at a gestational age of 35 to 41 weeks, with a birth weight of 2100 to 3500 g. Simultaneous measurement of bilirubin by traditional (invasive) and transcutaneous (non-invasive) methods was carried out within 15 minutes of admission in the neonatal intensive care unit. Parallel transcutaneous measurements were taken in the forehead and sternum. Statistical analysis was done to determine the correlation of transcutaneous (forehead and sternal) bilirubin with serum bilirubin levels and assess the factors affecting transcutaneous bilirubin measurement.

Results

In the range of serum bilirubin concentrations till 15 mg/dl, a close correlation was observed between serum bilirubin (TSB) estimated by direct spectrophotometric method and transcutaneous bilirubin (TcB) estimated by "Bilitest 2000". TcB estimation on the sternum was better correlated than that on the forehead (r-value: 0.962 vs. 0.935). TcB had a good correlation with TSB across the range of 12.5 - 20.5 mg/dl. Neonatal hypothermia significantly decreased TcB estimation (p =0.021, F=4.227). Respiratory distress, convulsion, sclerema and shock in neonates did not affect TcB estimation.

Conclusion

TcB has a good correlation with TSB in term and late preterm neonates. Thus TcB readings can be used to screen and manage neonatal hyperbilirubinemia cases.

Keywords

neonatal hyperbilirubinemia; newborn; jaundice; serum bilirubin; transcutaneous bilirubin; laboratory bilirubin determination.

INTRODUCTION

Neonatal jaundice is a common clinical condition encountered in neonates. It is caused due to a mismatch between bilirubin production and excretion [1]. In the first week after birth this condition is reported in approximately 60% of term and 80% of preterm babies.[2] Most cases resolve spontaneously, with only 20% requiring medical interventions [3]. A very high level of bilirubin is toxic to the brain and may lead to an irreversible bilirubin-induced encephalopathy known as kernicterus [4].

Significant hyperbilirubinemia mandating phototherapy is seen in 5-10% of all newborns [5]. As premature babies have higher incidence of neonatal jaundice, they require therapeutic intervention more frequently than term newborns [6]. Continuous clinical monitoring and estimating serum bilirubin at regular intervals is undertaken to prevent bilirubin-induced neurological damage [7].

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Total serum bilirubin (TSB) estimation involves extracting venous blood of the patient. Frequent monitoring requires repeated blood sampling which involves pain in newborns and leads to parental anxiety and distress [8]. Assessment and follow-up of all the newborns is challenging in a resource-constrained developing country. Thus, a pre-discharge stratification of the neonates depending on the risk factors for development of significant jaundice is necessary. Bhutani *et al.* [9] had suggested adopting an hour-specific nomogram for risk stratification of healthy term neonates prior to discharge. But these guidelines cannot be universally applied in an Indian setup due to other risk factors like higher proportion of neonates with a low serum albumin level at birth, higher prevalence of glucose-6 phosphate dehydrogenase deficiency and seasonal variation [10].

In recent years, a new method known as transcutaneous bilirubinometry (TcB) has been developed which is based on reflectance data of multiple wavelengths from the neonate's bilirubin-stained skin. The instrument makes an average of five replicate measurements at a single site and calculates the bilirubin readings in mg/dl (or mmol/l). It is easy to use and minimizes blood loss in an infant. It is used to rapidly screen clinically significant jaundice in a neonate and reduces the necessity of repeat TSB estimation. It provides instant results, which can reduce the necessity of TSB testing and help in the quick discharge of neonates in a resource-constrained setting [11].

However, studies have reported that the utility of this device may vary between babies of different races, [12] and advocate use of individualized correlation curves for different ethnic groups, which are considered to be are more reliable [13]. Studies have also reported that instrument and neonatal factors affect TcB reading [14, 15]. TcB readings are reported to be unreliable in preterm infants or those who have coexistent illnesses such as anaemia, respiratory distress syndrome, sepsis and acidosis [15]. Further, there is a lack of consensus on whether the reading over the sternum, forehead or an average of both these best correlates with the serum bilirubin [16]. Thus, it is not recommended as an absolute substitute for serum bilirubin estimation. This study aims to determine the correlation of TcB with serum bilirubin levels and assess the factors affecting TcB measurement in term and late preterm neonates in an eastern Indian population.

MATERIAL & METHODS

Study Setting

This prospective observational study was carried out at the Special Newborn Care Unit (SNCU), Department of Paediatrics, Burdwan Medical College Hospital, Purba Bardhaman, India.

Time Period

The study was conducted from March 2015 to February 2016, for one year.

Inclusion Criteria

All babies born after a gestational period of more than 35 weeks were included in our study.

Exclusion Criteria

Babies who were exposed to phototherapy, required exchange transfusion, had congenital malformations, generalized skin rashes (excluding newborn skin rashes), conjugated hyperbilirubinemia and who were more than 28 days old were excluded from our study.

METHOD

Where the clinical team decided to send the neonate's sample for TSB estimation, they simultaneously carried out a TcB measurement using Bilitest 2000. The ultimate decision to start phototherapy was approved by the clinicians depending upon the neonate's TSB result. The TSB threshold for initiating phototherapy depends on the gestational age [threshold = (Gestation ×10) - 100]; e.g. bilirubin threshold for a 36-week gestation infant would be 260 mmol/L (15.2 mg/dl). Neonates not requiring phototherapy were clinically monitored and any further decision for a repeat TSB was made depending on the clinical features. In those requiring phototherapy, TSB estimation was repeated in 6 – 8 h (to check the trend) and then 12 –24 hourly, till it was decided to stop phototherapy. After stopping phototherapy, TSB was repeated after 8 –12 h, to verify that there was no rebound increase in serum bilirubin levels and that the levels were still below the treatment level.

TSB measurement

TSB was measured in the Central Laboratory of the hospital by direct spectrophotometric method based on diazo technique [17] using Erba EM 360 fully automated auto analyser (Erba Diagnostic, Mannheim, Germany).

The diazo technique is based on the principle that

bilirubin reacts with diazotised sulphanilic acid in acidic medium to form pink coloured azobilirubn, the absorbance of which is directly proportional to the concentration of bilirubin. Direct bilirubin being water soluble directly reacts in acidic medium. However indirect bilirubin which is water insoluble is first solubilised using a surfactant, thereafter it reacts like direct bilirubin [17].

TcB measurement

TcB measurement was done using Bilitest 2000 (Technomedica, Russia) within 15 minutes of the collection of a blood sample for TSB. TcB measurement was taken over the mid-sternum area (TcB-S) and the forehead (TcB-F) an average was calculated and tabulated in the proforma accordingly. To determine the precision, Bilitest 2000 TcB measurements were assessed five times in succession in 10 infants.

Transcutaneous bilirubinometers work by directing light of a particular wavelength into the neonate's skin and then measuring the intensity of a specific wavelength that returns. The range of incident wavelengths varies depending on the manufacturer. The bilirubinometer analyzes the spectrum and intensity of optical signal reflected from the neonate's subcutaneous tissues. These signals are then converted into an electrical signal by a photocell. These signals are further analyzed by a microprocessor to generate a serum bilirubin value. Melanin content of the skin, dermal maturity, blood hemoglobin level and serum bilirubin level affect the transcutaneous bilirubinometer measurement [17].

Sample Size

Power calculation with a 95% confidence interval for the limits of agreement with a width of +0.34 standard deviation of the differences between the measurements (TSB and TcB) by the two methods helped us calculate that 100 paired results would be the minimum acceptable sample size. Thus, we included 100 neonates in our study.

SELECTION OF SUBJECTS

All the newborn babies who were admitted to the SNCU ward and fulfilled the inclusion and exclusion criteria were included in our study. Details of each neonate were noted in a case report form. This included patient history, antenatal history, clinical examination (including Krammer staging), transcutaneous bilirubin measurement and routine blood investigations. All the neonates were followed up till discharge, transfer or death.

STATISTICAL ANALYSIS

All the categorical data were presented as frequency and percentage. The continuous data was checked for normality using the Kolmogorov-Smirnov test. The parametric data was presented as mean ± standard deviations, while the non-parametric data was presented as median and interquartile range. An independent sample t-test was used to calculate the significance of study parameters within two groups for parametric data. One-way ANOVA test was used to calculate the significance of study parameters between more than two groups for parametric data. A p-value < 0.05 was considered as a level of significance We carried out analyses on a full data set and with smaller subsets of the first observation for each neonate. We assessed the relationship between TcB and TSB using Pearson's correlation coefficient and linear regression. Data was analysed using IBM SPSS version 25.

ETHICAL CLEARANCE

Ethical approval was taken from the Institute Ethical Committee before starting the study (Memo no. BMC/PG/151/1). Parents of all babies admitted to the SNCU who fulfilled the inclusion and exclusion criteria, were provided with written information leaflets and invited to enrol in this study. Thereafter, written consent was obtained from those who were willing to participate in the study.

RESULTS

The demographic, clinical and laboratory characteristics of neonates at the time of admission to the NICU are presented in table 1. Out of 100 newborns in this study, 74 (74%) were term and the remaining 26 (26%) were late preterm. Males were 65 (65%) and females 35 (35%) with a male-to-female ratio of 1.8:1. The mean age of presentation to the hospital was 4.07 ± 2.48 days with a majority of 42 (42%) neonates admitted between the fourth and seventh day followed by 24 (24%) neonates admitted between the first and third day after birth. The majority of the neonates (59 %) developed jaundice between days 4-7. In our study 29 (29 %) neonates had stage III jaundice, 52 (52%) neonates had stage IV jaundice and 19 (19%) neonates had stage V jaundice at the time of admission, based on Kramer's grading. Table 2 presents the different clinical

conditions reported in neonates who presented with jaundice.

Table 1. Demographic, clinical and laboratory characteristics

Parameter	Value
Mean Birth weight	2.6 ± 0.33Kg
Birth weight- range	2.1 - 3.5 Kg
Mean Gestational age	38.02 ± 1.88 weeks
Gestational age-range	35 - 41 weeks
Male to female ratio	1.8:1
Mean postnatal age at admission	4.07 ± 2.48 days
Mean postnatal age at development of jaundice	4.88 ± 2.27 days
Mean hemoglobin levels	14.7 ± 2.24 gm/dl
Babies with hypothermia	17 (17%)
Babies with respiratory distress	13 (13%)
Babies with convulsions	14 (14%)
Babies with sclerema	07 (7%)
Babies with shock	11 (11%)

The blood hemoglobin (Hb) level of neonates at the time of admission was 14.7 ± 2.24 g/dl with 12 (12 %) neonates having Hb less than 12 g/dl, 60 (60%) neonates having Hb between 12-15.9 g/dl, 26 (26 %) neonates having Hb between 16-19.9 g/dl and two (2 %) neonates having Hb 20 g/dl or above.

Table 2. Reported clinical condition along with neonatal jaundice

Diagnosis	Number
ABO incompatibility	12 (12%)
Rh incompatibility	23 (23%)
Hypoxic Ischemic Encephalopathy	34 (34%)
Early onset sepsis (<3 days)	05 (05%)
Late-onset sepsis (>3 days)	19 (19%)
Respiratory distress syndrome	07(07%)

The scatter plot showed a strong direct linear correlation of TcB-F versus TSB (Figure 1a) (Pearson's correlation coefficient, r = 0.935 and R^2 = 0.874 with p ≤ 0.001). Simple linear regression is calculated as TcB-F = 1.736 + 0.839×TSB.

The scatter plot of TcB-S versus TSB (Figure 1b) also reflected a strong direct linear correlation (Pearson's correlation coefficient, r = 0.965 and R^2 = 0.925 with p ≤ 0.001). Simple linear regression is calculated as TCB-S= 1.548 + O.848 ×TSB.

The linear relationship between TcB and TSB measured by using the Pearson correlation coefficient (r) is presented in Table 3. We observed that the correlation of TcB-S with TSB was higher (r=0.962, P<0.01) as compared to the correlation of TcB-F with TSB (r=0.935, P<0.01).
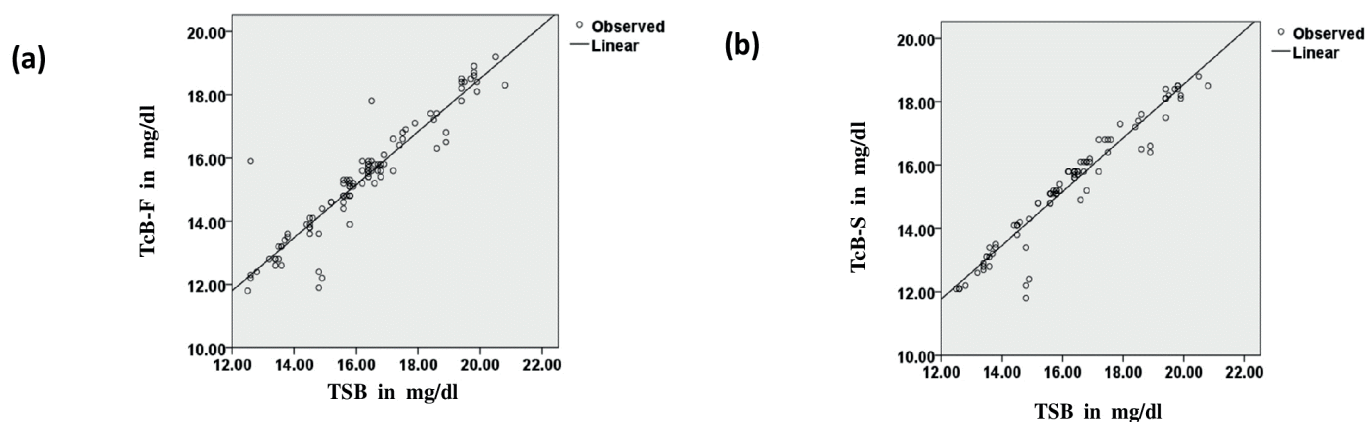
Fig 1. Scatter plots showing strong direct linear correlation of (a) transcutaneous bilirubin measured on forehead with serum bilirubin. (b) transcutaneous bilirubin measured on sternum with serum bilirubin
(TcB-F: Transcutaneous bilirubin on forehead, TcB-S: Transcutaneous bilirubin on sternum, TSB: Total Serum Bilirubin)

Table 3. Pearson correlation coefficient (r) between TcB and TSB depicting better correlation of transcutaneous bilirubin reading over sternum compared to forehead

Variable	TSB	TcB-S	TcB-F
TSB	1.000	0.962*	0.935*
TCB-S		1.000	0.960*
TCB-F			1.000

TSB: total serum bilirubin; TcB-S: transcutaneous bilirubin measurement obtained from mid-sternum area; TcB-F: transcutaneous bilirubin measurement obtained from forehead; *high correlation

The range of TSB in our study was 12.5 - 20.5 mg/dl. We analysed if the different range of TSB affects TcB readings. We observed that TcB readings have a good correlation across the range of TSB (Table 4).

DISCUSSION

In our study, we observed a good correlation between TcB estimation using Bilitest 2000 and TSB estimation by the diazo method. We further analysed the correlation of TcB-F and TcB-S with TSB. We observed that TcB-S correlates better (r=0.962, p<0.001) with TSB, as compared to TcB-F (r=0.935, p<0.001) (Table 3). Few studies have been done previously to find out if there was any significant difference in TcB measurements on the sternum and forehead. Some of these studies reported that sternal reading had better correlation with serum bilirubin values [17, 18, 19, 20], while others reported that forehead reading had a superior correlation [21, 22]. One study reported that the mean of forehead and sternal readings provided a better correlation with serum bilirubin than either of them individually [23]. Forehead reading was reported to be less reliable owing to the effect of daylight exposure on the forehead skin surface. Other skin sites are reported to be unreliable for assessing jaundice, owing to their variable epidermal thickness [24].

The slope (β) of the regression plot is 0.962 for TcB-S and 0.935 for TcB-F, illustrating a strong correlation, but a slight underestimation of TSB (Table 3). Previous studies suggested that TcB values cannot substitute for TSB estimation particularly for babies with serum bilirubin over 13mg/dl [25]. The range of TSB in our study was 12.5 - 20.5 mg/dl. We observed that TcB readings are reliable across all TSB estimations (Table 4). We cannot confirm if TcB will have a good correlation with TSB beyond this range.

In our study, the mean age of presentation to the hospital was 4.07 ± 2.48 days with a majority 42(42%) of babies brought to the hospital between day 4-7 of postnatal life followed by 24(24%) babies admitted between day 1-3 of postnatal life. The mean age of onset of jaundice was 4.88 ± 2.27 days. The onset of jaundice was also between 4-7 days of age in the majority (59 %) of the cases. This is expected as most term babies develop physiological jaundice after day 3 of life [26]. The mean birth weight of neonates in our study was 2.6 ± 0.33 kg and the mean gestational age at admission was 38.02 ±1.88 weeks (Table 1). The majority (52 %) of the neonates in our study had Kramer zone IV jaundice followed by 29 (29%) babies with stage III jaundice. This correlated with previous studies conducted in India [10, 27].

We observed that neonatal hypothermia (n=17) significantly decreased TcB-S and TcB-F estimation (P value <0.05, F=4.227) (Table 5). Özkan et al.[28]also reported similar findings in their study. During our study, 13 neonates developed respiratory distress. On analysis, we found that respiratory distress did not significantly affect the estimation of TcB (Table 5). A similar finding was reported by Knüpfer M et al. [29]. Fourteen babies had developed clinical convulsions during the study period and TcB estimation was done while injections were being prepared to treat convulsions. Statistical analysis revealed that convulsion did not affect TcB estimation (P=0.454, F=0.805) (Table 5). However, Yamauchi et al. [30] reported that crying and facial expression significantly affected TcB-F estimation and hence suggested that TcB-F readings should be noted when the neonate is calm and quiet.

Amit et al. [24] reported that skin-fold thickness does not affect the TcB readings. In this study, we observed that 8 (8%) babies with clinical sclerema did not have any statistically different TcB and TSB measurements (P=0.148, F=2.013) (Table 5). Özkan et al. [28] studied dermal kinetics concerning shock and reported that clinical shock (prolonged CRT, tachycardia, feeble pulses, oliguria) had negatively affected TcB estimation.

Table 4. Association between different ranges of TSB and TcB values

Serum Bilirubin (in mg/dl)	n	TSB	TcB-S	TcB-F	p-value	F
< 15	29	13.83±0.78	13.09±0.78	13.2±0.88	0.001	7.04
15 – 17.9	52	16.33 ±0.63	15.64±0.59	15.52±0.72	<0.001	23.13
> 18	19	19.43±0.65	17.86±0.73	17.97±0.82	<0.001	26.28

TSB: total serum bilirubin; TcB-S: transcutaneous bilirubin measurement obtained from mid-sternum area; TcB-F: transcutaneous bilirubin measurement obtained from forehead

We further analysed if factors such as hypothermia, respiratory distress, convulsion, sclerema and shock impact the TcB readings (Table 5). We observed that TcB reading is significantly lower than TSB in neonates who had hypothermia (p-value = 0.021).

Table 5. Reported clinical condition along with neonatal jaundice

Associated factor	Subset sample size (n)	TSB (in mg/dl)	TcB-S (in mg/dl)	TcB-F (in mg/dl)	p-value	F
Hypothermia	(n=17)	15.75±1.44	14.51 ±1.53	14.41±1.49	0.021	4.227
Respiratory distress	(n=13)	16.45±2.15	15.33±2.02	15.37±2.04	0.306	1.224
Convulsion	(n=14)	16.30±2.35	15.48±1.94	15.38±2.00	0.454	0.805
Sclerema	(n=8)	16.3±1.45	15.22±1.72	15.07±1.74	0.284	1.336
Shock	(n=11)	16.26±1.52	15.13±1.75	15.1±1.79	0.148	2.013

TSB: total serum bilirubin; TcB-S: transcutaneous bilirubin measurement obtained from mid-sternum area; TcB-F: transcutaneous bilirubin measurement obtained from forehead

Shock was seen in 13 (13%) neonates in our study, but we did not find it to affect TcB readings (P=0.148, F=2.013) (Table 5).

Our study was not without limitations. In our study out of 100 newborns, 74(74%) babies were term and the remaining 26 (26%) were late preterm. The ratio of male to female neonates was 1.8:1. This may not be the real situation at the community level. It might be that more male newborns were brought up to the hospital for admission as compared to female newborns as gender preference and discrimination exists in India. The levels of TSB and TcB were correlated in clinically jaundiced neonates before phototherapy, thus it cannot be used to correlate the two parameters during phototherapy or after phototherapy. The subgroup sample size was small when we reported the effect of neonatal hypothermia on TcB estimation. A bigger sample size will help to confirm this finding in the future.

CONCLUSION

In this study, we observed that TcB measured by Bilitest 2000 has a good correlation with TSB estimated by the diazo method in term and near-term infants. The

application of a transcutaneous bilirubinometer can help in reducing the necessity of phlebotomy in neonates. We observed that a transcutaneous bilirubinometer (Bilitest 2000) can reliably be used to screen neonates with serum bilirubin up to 20.5 mg/ dl. Above this value, the correlation of TcB with TSB cannot be confirmed by our study. We also suggest the use of TcB-S instead of TcB-F as this has a better correlation with TSB. In presence of hypothermia, the transcutaneous bilirubinometer falsely underestimated the bilirubin values and hence should be used only when such coexisting conditions have resolved. Respiratory distress, convulsions, sclerema and shock were not found to affect TcB estimation by this device.

ETHICAL CLEARENCE

Ethical approval was taken from the Institute Ethical Committee of Burdwan Medical College before starting the study (Memo no. BMC/PG/151/1).

AUTHORS'S CONTRIBUTION

Data gathering and idea owner of this study: SS, AKD

Study design: SS, AKD, KB

Data gathering: SS, KB

Writing and submitting manuscript: SS, KB, AKD

Editing and approval of final draft: SS, KB, AKD

REFERENCES

1. Hegyi T, Kleinfeld A. Neonatal hyperbilirubinemia and the role of unbound bilirubin. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2021 Dec 24:1-7. https://doi.org/10.1080/14767058.2021.2021177

2. Ghosh S, Ray S, Ghosh TN. Glucose 6 phosphate dehydrogenase deficiency in unexplained neonatal hyperbilirubinemia–A study in neonatal care unit of a tertiary care hospital. *Bangladesh Journal of Medical Science.* 2022;**21**(3):669. https://doi.org/10.3329/bjms.v21i3.59583

3. Hoppenot C, Emmett GA. Neonatal bilirubin triage with transcutaneous meters: when is a blood draw necessary?. Hospital Pediatrics. 2012 Oct 1;2(4):215-20. https://doi.org/10.1542/hpeds.2012-0012

4. Shapiro SM. Bilirubin toxicity in the developing nervous system. *Pediatric neurology*. 2003 Nov **1**;29(5):410-21. https://doi.org/10.1016/j.pediatrneurol.2003.09.011

5. Jaundice in the newborns. *The Indian Journal of Pediatrics*. 2008 Feb;**75**:157-63. 2004:158-219. https://doi.org/10.1007/s12098-008-0024-7

6. Narang A, Kumar P, R. Neonatal jaundice in very low birth weight babies. *Indian J Pediatr* 2001;**68**:307-9. https://doi.org/10.1007/BF02721831

7. Panda SK, Gaurav A, Das P, Swain N, Rath S. A Comparison Between Transcutaneous Bilirubin and Total Serum Bilirubin Levels for the Management of Jaundice in Preterm Neonates by Bland-Altman Plot. *Cureus*. 2021 Oct 2;**13**(10).   https://doi.org/10.7759/cureus.18442

8. Lim Y, Godambe S. Prevention and management of procedural pain in the neonate: an update, American Academy of

Pediatrics, 2016. Archives of Disease in Childhood-Education and Practice. 2017 Oct 1;102(5):254-6. http://dx.doi.org/10.1136/archdischild-2016-311066

9. Bhutani VK, Gourley GR, Adler S, Kreamer B, Dalin C, Johnson LH. Noninvasive measurement of total serum bilirubin in a multiracial predischarge newborn population to assess the risk of severe hyperbilirubinemia. *Pediatrics*. 2000 Aug 1;**106**(2):e17-. https://doi.org/10.1542/peds.106.2.e17

10. Narang A, Gathwala G, Kumar P. Neonatal jaundice: an analysis of 551 cases. *Indian pediatrics*. 1997 May 1;**34**:429-32. https://www.indianpediatrics.net/may1997/429.pdf

11. Stanley Ip, Mei Chung, , John Kulig, , Rebecca O'Brien, Robert Sege *et al.* Technical report, An evidence-based review of important issues concerning neonatal hyperbilirubinemia . *Pediatrics* 2004;**114**:e130-50. https://doi.org/10.1542/peds.114.1.e130

12. De Luca D, Jackson GL, Tridente A, Carnielli VP, Engle WD. Transcutaneous bilirubin nomograms: a systematic review of population differences and analysis of bilirubin kinetics. Arch *Pediatr Adolesc Med*. 2009 Nov;**163**(11):1054-9. https://doi.org/10.1001/archpediatrics.2009.187

13. Varughese, Pearl Mary; Krishnan, Lalitha; Ravichandran, . Does Color Really Matter? Reliability of Transcutaneous Bilirubinometry in Different Skin-Colored Babies. *Indian Journal of Paediatric Dermatology* **19**(4):p 315-320, Oct–Dec 2018. https://doi.org/10.4103/ijpd.IJPD_3_18

14. Raimondi F, Lama S, Landolfo F, Sellitto M, Borrelli AC, Maffucci R, Milite P, Capasso L. Measuring transcutaneous bilirubin: a comparative analysis of three devices on a multiracial population. *BMC pediatrics*. 2012 Dec;**12**:1-5. https://doi.org/10.1186/1471-2431-12-70

15. Carceller-Blanchard A, Cousineau J, Delvin EE. Point of care testing: transcutaneous bilirubinometry in neonates. Clinical biochemistry. 2009 Feb 1;**42**(3):143-9. https://doi.org/10.1016/j.clinbiochem.2008.09.106

16. Agrawal G, Garg K, Sitaraman S, Sarna A. Comparison of diagnostic accuracy of different sites for transcutaneous bilirubin measurement in early preterm infants. *The Indian Journal of Pediatrics*. 2019 Jan **1**;86:32-7. https://doi.org/10.1007/s12098-018-2739-4

17. Puppalwar PV, Goswami K, Dhok A. Review on "evolution of methods of bilirubin estimation". IOSR Journal of Dental and Medical Sciences. 2012;**1**(3):17-28. https://www.iosrjournals.org/iosr-jdms/papers/vol1-issue3/D0131728.pdf

18. Szabo P, Wolf M, Bucher HU, Haensse D, Fauchère JC, Arlettaz R. Assessment of jaundice in preterm neonates: comparison between clinical assessment, two transcutaneous bilirubinometers and serum bilirubin values. *Acta Paediatr*. 2004 Nov;**93**(11):1491-5. https://doi.org/10.1080/08035250410018328

19. Jeon J, Lim G, Oh KW, Lee NM, Park HW, Chung ML. The forehead is a better site than the sternum to check transcutaneous bilirubin during phototherapy in sick infants. *BMC pediatrics*. 2020 Dec;20:1-5. https://doi.org/10.1186/s12887-020-02450-w

20. Shariati MK, Taleghani NT, Izadi N, Miri A, Tafti RT, Gorji FA. Which Is More Accurate: Transcutaneous Bilirubin Measurement on the Forehead or Sternum? *Archives of Iranian Medicine*. 2022 Aug 1;**25**(8):552-6. Available at: https://doi.org/10.34172/aim.2022.88

21. Thivia Jegathesan, Douglas M. Campbell, Joel G. Ray, Vibhuti Shah, Howard Berger, Robin Z. Hayeems, Michael Sgro, for the NeoHBC; Transcutaneous versus Total Serum Bilirubin Measurements in Preterm Infants. *Neonatology* 19 August 2021; **118** (4): 443–453. https://doi.org/10.1159/000516648

22. Ahn YM, Kim MR, Lee SM, Jun YH. Assessment of neonatal hyperbilirubinemia using a transcutaneous bilirubinometry. *Journal of Korean Academy of Nursing*. 2003 Feb 1;**33**(1):51-9. https://doi.org/10.4040/jkan.2003.33.1.51

23. Maisels MJ, Ostrea EM Jr, Touch S, Clune SE, Cepeda E, Kring E., Gracey K, Jackson C, Talbot D & Hunag R. Evaluatin of a new transctraneous bilirubinometer. *Pediatrics*, 2004; **113** (6), 1628-1635. https://doi.org/10.1542/peds.113.6.1628

24. Amit Y, Jabbour S, Arad ID. Effect of skinfold thickness on transcutaneous bilirubin measurements. *Neonatology*. 1993 Sep 30;**63**(4):209-14. Available at: https://doi.org/10.1159/000243933

25. AKD V. Non-Invasive Estimation of Total Serum Bilirubin by Multi-Wavelength Spectral Reflectance in Neonates Rakesh Lodha Ashok K. Deorari Vishal Jatana Vinod K. Paul. *Indian Pediatrics*. 2000;**37**:771-5. https://www.indianpediatrics.net/july2000/july-771-775.htm

26. Cloherty JP, Eichenwald EC, Stark AR, editors. Manual of neonatal care. Lippincott Williams & Wilkins; 2008.

27. Kumar A, Faridi MM, Singh N, Ahmad SH. Transcutaneous bilirubinometry in the management of bilirubinemia in term neonates. *The Indian Journal of Medical Research*. 1994 May 1;**99**:227-30. https://pubmed.ncbi.nlm.nih.gov/7927581/

28. Özkan HA, Ören H, Duman N, Duman MU. Dermal bilirubin kinetics during phototherapy in term neonates. *Acta paediatrica*. 2003 May;**92**(5):577-81. https://doi.org/10.1111/j.1651-2227.2003.tb02510.x

29. Knüpfer M, Pulzer F, Braun L, Heilmann A, Robel-Tillig E, Vogtmann C. Transcutaneous bilirubinometry in preterm infants. *Acta Paediatrica*. 2001 Aug;**90**(8):899-903. https://doi.org/10.1111/j.1651-2227.2001.tb02454.x

30. Yamauchi Y, Yamanouchi I. Transcutaneous bilirubinometry: variability of TcB measurements on the forehead with crying. *Pediatrics International*. 1991 Oct;**33**(5):655-7. https://doi.org/10.1111/j.1442-200X.1991.tb01881.x