ORIGINAL RESEARCH PAPER

Neonatology

A PROSPECTIVE COHORT STUDY TO DETERMINE CORRELATION BETWEEN TRANSCUTANEOUS BILIRUBIN AND SERUM BILIRUBIN VALUES IN NEONATES WITH HYPERBILIRUBINEMIA IN A TERTIARY CARE HOSPITAL.

KEY WORDS: Neonate, Bilirubin, Hyperbilirubinemia, Jaundice, Bilirubinometer.

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ABSTRACT

Introduction: Hyperbilirubinemia is a major health condition seen in approximately 60% of term and 80% preterm neonates. Total serum bilirubin (TSB) estimation is the gold standard method and is the most common reason of blood drawing in neonates. Non-invasive method like transcutaneous bilirubin (TCB) estimation is an alternative for bilirubin estimation. Thus, the primary aim of present study is to compare the efficacy of the two methods. **Methods:** This prospective observational study was conducted between 2020-2022 on 836 neonates (>35 weeks, ≥2000gm) at Mazumdar Shaw Medical Centre, Narayana health city, Bangalore aimed to compare TSB and transcutaneous bilirubin (TCB) values using JM-105 bilirubinometer assessed TCB values taken before, during and phototherapy from covered and uncovered skin of different sites and correlated with the serum bilirubin values, through linear regression and Bland Altman analysis. **Results:** Positive correlations were observed between TSB and TCB values at the forehead, sternum, and pubic symphysis, with the strongest correlation at the forehead (r-0.826, p<0.001) pre-phototherapy. Correlation weakened during and after phototherapy, notably at the forehead post-phototherapy (r-0.121, p>0.05). The preferred TCB estimation site was forehead pre- and 12 hours post-phototherapy with mean difference of 2.15±2.1 mg/dl and 0.6±2.13 mg/dl respectively, while sternum was favored site at 24 hours post-phototherapy; with mean difference of 2.01±2.32 mg/dl.TCB cut-off values (13.75mg/dl) demonstrated sensitivity (78.5%) and specificity (64.5%) for discontinuing phototherapy (ROC AUC 0.774, p<0.05). **Conclusion:** TCB proves effective as a non-invasive screening tool for bilirubin estimation, alleviating the need for repeated blood draws. However, caution is advised regarding TCB's reliability during phototherapy monitoring. The study provides valuable insights into TCB's clinical applicability, offering practical recommendations for optimal usage.

INTRODUCTION

Neonatal hyperbilirubinemia is the most common morbidity in the first two weeks of life and is found in approximately 60% of term and 80% of preterm infants.[1] Visible jaundice appears between 24 to 72 hours of life, it usually rises in term infants to a peak level of 12-15mg/dl by 3 days of life and then falls gradually, however in preterm infant peak level occurs on day 3 to 7 of life and serum bilirubin level can rise to over 15 mg/dl.[2,3] Hyperbilirubinemia during neonatal period can be physiological or pathological. It can lead to long term poor neurodevelopmental outcomes depending on the severity of Jaundice. Hence, early recognition and initiation of treatment is the key to the management. Kramer index is a rapid and non-invasive method of visual inspection of the skin, estimation of craniocaudal progression of jaundice by this method gives an indication about increasing hyperbilirubinemia.[4] But this method is observer variant. The gold standard investigation for detecting hyperbilirubinemia is by measuring total serum bilirubin.[5] Laboratory testing of serum total serum bilirubin (TSB) has become one of the most common reasons for drawing blood in the newborn period, which may cause problems like anaemia, osteomyelitis and parental distress.[6,7] So the non-invasive method of measuring jaundice is by using transcutaneous bilirubinometer (TCB), which works on the principle of reflectance densitometer.[8]

The aim of the current study is to find out the correlation between the TCB and TSB using JM-105 bilirubinometer and analyse the impact of maternal and neonatal risk factors on the correlation.

MATERIAL METHODS

A prospective cohort study was conducted at Level III NICU, Mazumdar Shaw Medical Centre, Narayana Health City, Bangalore, India, from August 2020 to May 2022. Ethical approval was obtained from the institute's Ethical and Scientific Research Committee, and parental consent was secured. Neonates with >35 weeks gestation and a minimum weight of 2000gm were included.

Transcutaneous bilirubin (TCB) was measured using the Transcutaneous Bilirubinometer-JM-105(Drager). Measurements from the forehead, sternum, and pubic symphysis were taken. Paired serum samples for total serum bilirubin (TSB) estimation were collected within 30 minutes-1 hour and measured by the VITRIOS 5600 analyzer which works on the principle of Diazo method.

If paired TCB and TSB values indicated initiation of phototherapy at 48±6 hours of life as per modified AAP guidelines[1], the neonates underwent phototherapy covering the areas in forehead (site-1-FH; eye band), sternum (site -2-ST; photo reflective patch) and pubic symphysis (site-3-PS; diaper) as shown in figure-1. Paired TCB and TSB measurements were taken on covered areas (forehead, sternum, and pubic symphysis) and uncovered area of sternum (site-4 -STUC) during (12 hrs of phototherapy) and after 24 hrs of phototherapy.

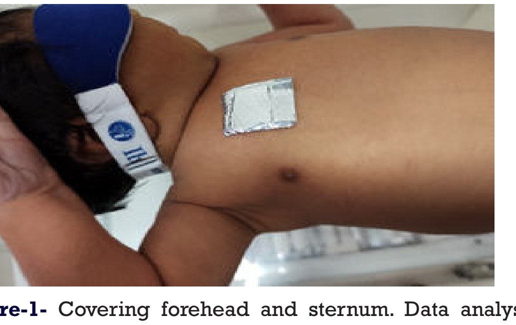


Figure-1- Covering forehead and sternum. Data analysis utilized was SPSS V 27.0. Bland Altman plots assessed agreement between TCB and TSB, while Pearson's correlation and linear regression analyzed the relationship. ROC curve analysis identified TCB cut-off values for discontinuing phototherapy. The study also explored the impact of maternal and neonatal risk factors on TCB and TSB agreement.

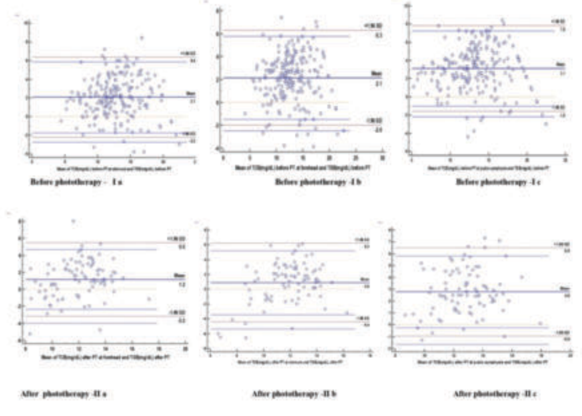
RESULTS

A total of 836 newborns were studied out of which 396 (47.37%) were males while 440 (52.63%) were females. Among which 136 were SGA babies, 648 were AGA babies and 52 were LGA. Gestational age and birth weight wise distribution of neonates as shown in table-1.

Table 1: Distribution of neonates according to gestational age and birth weight.

		Number	Percentage
Gestational Age	Late Preterm (35-37 week)	60	7.17
	Term(37-40weeks)	520	62.20
	Post term (>40weeks)	256	30.63
Birth Weight	< 2 kg	28	3.35
	2 kg – 2.5 kg	120	14.35
	2.5 kg – 3.5 kg	608	72.73
	> 3.5 kg	80	9.57

The initial mean TSB value was 11.82±3.59 mg/dl while mean TCB values at FH, ST and PS were 13.97±3.55mg/dl, 13.9±3.66mg/dl and 14.94±3.64mg/dl respectively. Mean difference (TSB-TCB) Pre-phototherapy at FH, ST and PS were 2.15 ± 2.11, 2.08±2.2 and 3.12±2.41 respectively. Bland Altman plot exhibited statistically significant limits of agreement (p value <0.001) with an interval of -1.982 to 6.279, -2.227 to 6.379 and -1.593 to 7.842 at FH, ST and PS respectively pre-phototherapy than post-phototherapy (graph-1).



Graph 1: Bland Altman plot for comparing TCB(mg/dL)with TSB(mg/dL) before phototherapy at (Ia) - Forehead, (Ib) - Sternum, (Ic) - Pubic Symphysis and after phototherapy at (IIa) - Forehead, (IIb) - Sternum, (IIc) - Pubic Symphysis

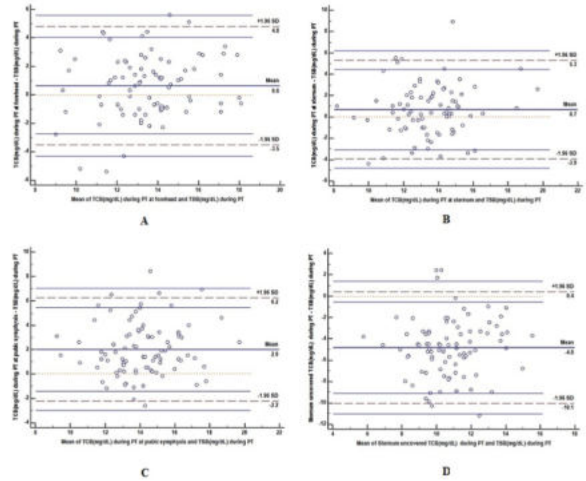
348 out of 836 neonates underwent phototherapy, TCB and TSB values at 12 hr of phototherapy and 24 hr after phototherapy were analyzed. The mean values and mean difference between TCB and TSB at different sites during (12 hrs of phototherapy) and post phototherapy is shown in table-2.

Table 2: Mean and Mean difference of TCB and TSB

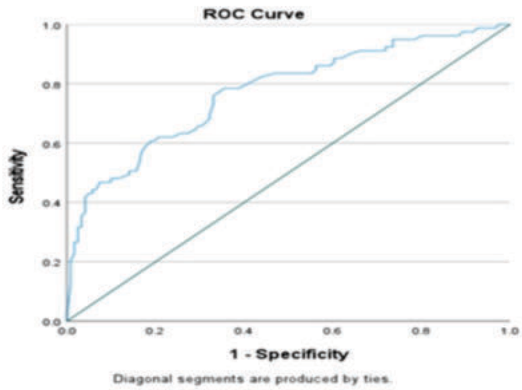
Pair	Paired t test	N	Mean ± SD	Mean difference ± SD	t	p value
1	At First Examination TSB	209	11.82 ± 3.59	2.15 ± 2.11	14.74	<0.001
	At First Examination TCB FH	209	13.97 ± 3.55			
2	At First Examination TSB	209	11.82 ± 3.59	2.08 ± 2.2	13.67	<0.001
	At First Examination TCB ST	209	13.9 ± 3.66			
3	At First Examination TSB	209	11.82 ± 3.59	3.12 ± 2.41	18.77	<0.001
	At First Examination TCB PS	209	14.94 ± 3.64			
4	At 12hr of Phototherapy TSB	87	13.16 ± 2.15	0.64 ± 2.1	-2.82	
	At 12hr of Phototherapy TCB FH	87	13.81 ± 2.44			
5	At 12hr of Phototherapy TSB	87	13.16 ± 2.15	0.7 ± 2.37	-2.78	
	At 12hr of Phototherapy TCB ST	87	13.87 ± 2.36			
6	At 12hr of Phototherapy TSB	87	13.16 ± 2.15	2.01 ± 2.16	-8.68	<0.001
	At 12hr of Phototherapy TCB PS	87	15.17 ± 2.29			
7	At 12hr of Phototherapy TSB	87	13.16 ± 2.15	4.82 ± 2.67	16.82	<0.001
	At 12 hr of phototherapy TCB Sternum uncovered (STUC)	87	8.34 ± 2.34			
8	At 24hrs of Phototherapy TSB	87	11.41 ± 1.88	2.01 ± 2.32	-2.42	
	After 24hrs of Phototherapy TCB FH	87	12.57 ± 2.32			
9	At 24hrs of Phototherapy TSB	87	11.41 ± 1.88	0.92 ± 2.71	-3.17	
	After 24hrs of Phototherapy TCB ST	87	12.33 ± 2.7			
10	At 24hrs of Phototherapy TSB	87	11.41 ± 1.88	2.79 ± 1.91	13.67	<0.001
	After 24hrs of Phototherapy TCB PS	87	14.2 ± 2.13			

According to Bland Altman plot the limits of agreement of TCB at 12 hr of phototherapy (-3.533 to 4.822, -3.935 to 5.344 and -2.224 to 6.244) were statistically significant (p-0.0059, 0.0067, <0.001) in covered sites than in uncovered sites shown in graph 2.

The correlation coefficient (r) between TSB and TCB pre-phototherapy (FH 0.826, ST 0.817, PS 0.778) and post-phototherapy (FH 0.121, ST 0.346, PS 0.555) suggested site preferences. Thus, FH was the preferred site for TCB estimation before and during phototherapy. However, PS to be the preferred site for TCB estimation after phototherapy (table 3). ROC analysis indicated 78.5% sensitivity, 64.5% specificity for TCB at 13.75mg/dl in predicting phototherapy discontinuation. (graph 3). Maternal risk factors like Rh factor, ABO blood groups, GDM, PIH, and gestational age showed no statistically significant differences. However, IUGR neonates exhibited strategically significant TCB and TSB differences (p value 0.003). Neonates with asymptomatic hypoglycemia and polycythemia did not show statistically significant differences.



Graph 2: Bland Altman plot for comparing TCB(mg/dL) with TSB(mg/dL) during phototherapy at (A) - Forehead (covered), (B) - Sternum (covered), (C) - Pubic Symphysis (covered), (D) - Sternum (uncovered)



Graph-3: ROC curve

Table 3: Correlation coefficient of TSB and TCB before /during and after phototherapy

Sl. no	Parameters Being Correlated	N	Correlation(R)	P Value
1	At First Examination TSB & TCB FH	836	0.826	<0.001
2	At First Examination TSB & TCB ST	836	0.817	<0.001
3	At First Examination TSB & TCB PS	836	0.778	<0.001
4	At 12hr of Phototherapy TSB & TCB FH	348	0.574	<0.001
5	At 12hr of Phototherapy TSB & TCB ST	348	0.451	<0.001
6	At 12hr of Phototherapy TSB & TCB PS	348	0.528	<0.001
7	At 12 hr of phototherapy TSB & TCB Sternum uncovered (STUC)	348	0.293	0.006
8	After 24hrs of Phototherapy TSB & TCB FH	348	0.121	0.265
9	After 24hrs of Phototherapy TSB & TCB ST	348	0.346	0.001
10	After 24hrs of Phototherapy TSB & TCB PS	348	0.555	<0.001

DISCUSSION

In our study involving 836 neonates (35-41 weeks + 4 days gestational age), with a slight female predominance (M: F- 1:1.1), and a mean birth weight of 2.89 kg, we explored transcutaneous bilirubinometry (TCB) values using Drager JM-105 and compared them with total serum bilirubin (TSB). The mean TCB values at the forehead (FH), sternum (ST), and pubic symphysis (PS) were 13.97±3.55mg/dl, 13.9±3.66mg/dl, and 14.94±3.64mg/dl, respectively.

Hafeez A et al[10] and Costa Posada et al[11] conducted studies with 150 and 217 neonates, including both term and preterm infants, with mean gestational ages of 36.14±1.04 weeks and 27–36 + 6 weeks, respectively. In Hafeez A et al[10] study, the mean TCB and TSB were 13.04±4.07 mg/dl and 12.79±5.49 mg/dl, respectively. The mean difference in bilirubin levels between TSB and TCB was -0.246±2.53 mg/dl. Similarly, in Costa Posada et al[11] study, the mean difference between TCB and TSB before phototherapy at the sternum was 1.07 mg/dl (SD 1.86). Similar results were observed in studies by Chakrabarti R et al[12] and Yang et al[9], using Bilichek[R] as the analyzer. Other studies, conducted by Alsaedi et al, included 151 neonates with gestational ages ranging from 37-41 weeks and a mean birth weight of 3.1 kg. Additionally, a total of 350 term healthy newborns, with a mean gestational age of 38 ± 2 weeks and a mean birth weight of 2.97 ± 0.9 kg, were studied by Bhat JA et al.[13,14]

The Bland Altman plots for TCB and TSB values at different sites revealed varying limits of agreement: FH (-1.982 to 6.279), ST (-2.227 to 6.379), and PS (-1.593 to 7.842). The average differences between TCB-TSB were 2.1483 (FH), 2.0761 (ST), and 3.1249 (PS), indicating TCB overestimation before phototherapy. Correlations were highest at FH (0.826), suggesting it as the preferred site. Garima A et al[15] found differences at forehead, sternum, and interscapular sites ranged from -5 to +6.3 mg/dL, -3.6 to +5.8 mg/dL, and -4.3 to +5.4 mg/dL using JM-103. In Yang et al study, mean differences before phototherapy were 1.4 mg/dL (term) and 2 mg/dL (late-preterm).[9] Castro A et al[16] using Bilicheck reported a 1.4 mg/dL difference with a correlation of 0.8 and limits of agreement (-2.8 to 5.6 mg/dL). Rohsiswatmo R et al[17] (JM-103) noted wider differences with higher bilirubin levels (0.81 mg/dL, limits -2.14 to 3.77 mg/dL). Contrastingly, Murali L et al[18] found poor agreement in Bland–Altman plots (-4.1 to 5.9 mg/dL) at different time points, with a small mean gradient at PT initiation (□0.2 mg/dL), increasing at 12 h (1.4 mg/dL), 24 h (1.5 mg/dL), and after PT cessation (2.0 mg/dL).

In this study, jaundice occurred in 348 out of 836 neonates, necessitating phototherapy. Mean Total Serum Bilirubin (TSB) values at covered areas (FH, ST, PS), uncovered area (ST), and Transcutaneous Bilirubin (TCB) were 13.81 ± 2.44 mg/dl, 13.87 ± 2.36 mg/dl, 15.17 ± 2.29 mg/dl, 8.34 ± 2.34 mg/dl, and 13.16 ± 2.15 mg/dl, respectively. Limits of agreement established intervals of -3.533 to 4.822, -3.935 to 5.344, -2.224 to 6.244, and -10.05 to 0.418 at covered FH, ST, and PS, and uncovered ST after 12 hours of phototherapy. Wider limits at Uncovered ST than covered sites suggest the covered area is preferable for TCB estimation. Correlation coefficient was highest at FH (covered), enhancing its reliability for TCB estimation during phototherapy. A study by Costa Posada et al. at 24 hours showed a mean difference of 7.89 mg/dl (SD 3.11) between uncovered (ST) TCB-TSB, while the covered

(ST) site had a 0.52 mg/dl difference (SD 1.88), supporting lower reliability of uncovered skin during phototherapy.[11] However, Yang et al.'s Bilicheck study indicated mean differences between TCB and TSB during phototherapy of 0.5 mg/dl and 1.7 mg/dl in term and late-preterm infants, respectively.[9] According to Castro A et al., TCB of non-phototherapy exposed (TCB-NE) areas were superior to TCB of photo-exposed areas (TCB-E), with a mean difference of 0.4 mg/dl and a correlation coefficient of 0.74.[16] In contrast, Murali et al.'s study showed wide variation in TCB on exposed skin, similar to TCB of shielded skin, with a small mean gradient between TCB shielded and TSB at initiation (-0.3 mg/dl), but an increase at 12 and 24 hours (-2.2 and -2.5 mg/dl, respectively), and after stopping phototherapy (-1.2 mg/dl).[18] TCB measurements using Bilichek underestimated TSB in 53 (35%) infants, as reported by Alsaedi et al., with a mean difference between TCB and TSB at 6 hours of phototherapy being −0.9897 ± 0.546 and a correlation coefficient of 0.7988.[13]

After 24 hours of phototherapy, the mean TCB values at FH, ST, and PS, as well as TSB, were 12.57 ± 2.32 mg/dl, 12.33 ± 2.7 mg/dl, 14.2 ± 2.13 mg/dl, and 11.41 ± 1.88 mg/dl, respectively. The mean differences were 1.16, 0.91, and 2.78, respectively. Limits of agreement indicated intervals of -3.167 to 5.5, -4.386 to 6.225, and -0.946 to 6.525 at FH, ST, and PS, respectively. However, in contrast to pre- and during-phototherapy stages, the correlation coefficient was highest at PS, making it the preferred site for TCB estimation post-phototherapy. According to a study by Rohsiswatmo R et al, 24 hours after phototherapy, a significant positive correlation between TSB and TCB (r2 = 0.791) was found. TCB tended to overestimate TSB, with a mean difference of 0.43 mg/dL (SD 1.57) and limits of agreement ranging from □2.66 to 3.51 mg/dL.[17] In a study by Castro A et al, the mean difference between TCB and TSB after 24 hours of phototherapy was 0.2 mg/dL.[16] However, it was considered less useful for monitoring rebound. In a similar study by Murali et al, the mean difference between TCB and TSB at uncovered areas (3.14 mg/dl, 2.93 mg/dl at 12 and 24 hrs of phototherapy) was higher than the covered area (1.05 mg/dl, 1.38 mg/dl at 12 and 24 hrs of phototherapy), indicating poor agreement in covered and uncovered skin, rendering it unreliable.[18]

In this study, phototherapy commenced using cut-off values from the TCB normogram by Bhutani et al[1], a modified AAP chart. Determining cut-off values for TCB to initiate phototherapy proved challenging, as TCB tended to overestimate TSB even at bilirubin levels <10mg/dl by 2-3mg/dl. This inconsistency with the normogram for starting phototherapy led to more neonates undergoing phototherapy than expected if TCB was used as the cut-off. The AUC for TCB at the forehead was 0.774, significant with a p-value <0.05. ROC analysis indicated that TCB values at 13.75mg/dl yielded a sensitivity of 78.5% and specificity of 64.5% for predicting the discontinuation of phototherapy in neonates who started treatment.

Contrastingly, Chakrabarti R et al.'s ROC analysis post-phototherapy revealed that transcutaneous measurements at the sternum showed sensitivity of 62%, specificity of 52%, and a likelihood ratio of 1.29 when TCB levels >10.19 mg/dl were considered.[12] The AUC was 0.621, with a p-value of 0.003. Similarly, Chokemungmeepisarn P et al, using Bilicheck, found that TCB values had an accuracy of 92.5%, with sensitivity, specificity, positive predictive value, and negative predictive value of 78.3%, 94.2%, 62.1%, and 97.3%, respectively.[19]

In conclusion, our study utilized the TCB normogram of Bhutani et al[1], a modified AAP chart, to initiate phototherapy, facing challenges in determining cut-off values as TCB tended to overestimate TSB, leading to an increased number of neonates undergoing phototherapy. The ROC analysis highlighted the sensitivity and specificity of TCB values at the forehead and sternum, indicating their potential use in predicting phototherapy discontinuation. Maternal and neonatal risk factors were explored, revealing that certain factors, such as IUGR, impacted the correlation between TCB and TSB. Despite good correlation, the study emphasizes the limitations of TCB in guiding therapeutic decisions, particularly during and after phototherapy. It recommends TCB as a screening tool before phototherapy, with caution in interpreting results for IUGR cases. These findings contribute to the understanding of TCB's applicability and limitations in clinical practice, paving the way for future research in optimizing neonatal jaundice management strategies.

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Conflict Of Interest
None

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