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Research Article

Comparison of Transcutaneous Bilirubin with Serum Bilirubin in Pre-Term Neonates Before, During, and After Phototherapy

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ABSTRACT

Objective: To assess the agreement between transcutaneous bilirubin (TcB) and total serum bilirubin (TSB) measurements before, during, and after phototherapy in preterm neonates with unconjugated hyperbilirubinemia.
Study Design: Prospective observational method-comparison study.
Place and Duration of Study: Neonatal Intensive Care Unit, Dr Sulaiman Al Habib Hospital, Al Rayyan, Riyadh, Saudi Arabia, from May to October 2024.
Methodology: A total of 199 preterm neonates with unconjugated hyperbilirubinemia requiring phototherapy were enrolled. Paired TcB and TSB measurements were obtained before phototherapy, during phototherapy, and 12 hours after its discontinuation. TcB was measured from the protected forehead using a Dräger JM-105 bilirubinometer, while TSB was determined from venous blood samples using an automated biochemical analyser. Quantitative variables were presented as mean ± standard deviation, while categorical variables were expressed as frequencies and percentages. Paired-samples t-tests were used to compare TcB and TSB values at each assessment stage. Subgroup analyses were performed according to gestational age and birth weight, with $p<0.05$ considered statistically significant.
Results: A total of 199 jaundiced preterm neonates were evaluated. Before phototherapy, the mean paired difference between TSB and TcB was −16.00 ± 19.64, indicating that TcB tended to overestimate TSB ($t=-11.491$, $p<0.001$). During phototherapy, the mean difference increased to 45.49 ± 25.86, demonstrating substantial underestimation of TSB by TcB ($t=24.817$, $p<0.001$). Twelve hours after phototherapy discontinuation, the mean difference decreased to 5.81 ± 21.27 but remained statistically significant ($t=3.853$, $p<0.001$). The wide standard deviations indicated considerable interindividual variability in the agreement between the two methods.
Conclusion: TcB measurements showed significant differences from TSB before, during, and after phototherapy. The greatest discrepancy occurred during phototherapy, when TcB substantially underestimated serum bilirubin levels. TcB may support non-invasive bilirubin screening and follow-up, particularly after phototherapy, but it should not replace TSB measurement when treatment decisions are required in preterm neonates.

Keywords: Hyperbilirubinemia, Phototherapy, Preterm Neonates, Total Serum Bilirubin, Transcutaneous Bilirubin.

INTRODUCTION

Neonatal jaundice is one of the most common clinical conditions encountered during the first week of life and is a frequent cause of neonatal hospital admission. It results from the accumulation of bilirubin in the blood and tissues, producing yellow discoloration of the skin and sclerae [1,2]. Although most cases are physiological and self-limiting, severe unconjugated hyperbilirubinemia may cross the blood–brain barrier and cause acute bilirubin encephalopathy or permanent neurological damage known as kernicterus [3]. These complications can largely be prevented through timely bilirubin assessment and appropriate treatment with phototherapy or, in severe cases, exchange transfusion [4,5].

Approximately 60% of term neonates and 80% of preterm neonates develop visible jaundice during the first week after birth. The risk is greater among preterm infants because of hepatic immaturity, reduced bilirubin conjugation, increased red blood cell turnover and increased enterohepatic circulation. Although universal bilirubin screening before discharge has been recommended by the American Academy of Pediatrics, its implementation may be difficult in resource-limited healthcare settings because of financial, laboratory and staffing constraints [6,7,8].

Physiological jaundice generally becomes apparent after the first 24 hours of life, reaches its maximum intensity between 48 and 96 hours and usually resolves within two to three weeks in term infants [9,10]. By contrast, jaundice is considered pathological when it appears during the first 24 hours, the bilirubin concentration exceeds the age-specific 95th percentile, the level increases by more than 5 mg/dL per day or 0.2 mg/dL per hour, or jaundice persists beyond the expected neonatal period [11]. Globally, approximately 1.1 million newborns may develop severe hyperbilirubinemia annually, with the greatest burden occurring in South Asia and sub-Saharan Africa. Severe neonatal jaundice has been associated with substantial neonatal mortality and long-term complications, including hearing impairment, cerebral palsy and developmental disability [9].

Unconjugated hyperbilirubinemia is the most common form of neonatal jaundice and may be physiological or pathological. Physiological hyperbilirubinemia accounts for a substantial proportion of neonatal jaundice and reflects normal developmental limitations in bilirubin metabolism. Neonates have a relatively high red blood cell mass, a shorter erythrocyte lifespan and reduced activity of uridine diphosphate glucuronosyltransferase, the hepatic enzyme responsible for bilirubin conjugation [10]. Increased enterohepatic circulation, delayed establishment of feeding, insufficient milk intake and reduced intestinal bacterial colonization may further increase bilirubin concentrations [10,11]. Regardless of the underlying mechanism, bilirubin overproduction, impaired conjugation and reduced excretion can result in clinically significant hyperbilirubinemia, emphasizing the need for accurate and repeated bilirubin monitoring [12].

Total serum bilirubin measurement remains the reference standard for evaluating neonatal hyperbilirubinemia. However, blood sampling is invasive, painful and resource-intensive and may contribute to clinically significant blood loss when measurements are repeated, particularly in preterm neonates. Transcutaneous bilirubin measurement offers a rapid, painless and non-invasive alternative that may reduce the frequency of blood sampling and facilitate repeated assessment [6-8]. Previous studies have demonstrated a generally good correlation between transcutaneous and serum bilirubin concentrations in neonates from different ethnic populations. Nevertheless, the reliability of transcutaneous measurements may be affected by gestational age, skin pigmentation, birth weight and exposure to phototherapy.

Evidence regarding the accuracy of transcutaneous bilirubin measurement during and after phototherapy remains variable, especially among preterm neonates. Evaluating transcutaneous bilirubin at a skin area protected from phototherapy may provide a practical approach for monitoring bilirubin concentrations while minimizing repeated blood sampling. Therefore, this study was conducted to assess the agreement between transcutaneous bilirubin and total serum bilirubin measurements before, during and after phototherapy in preterm neonates with unconjugated hyperbilirubinemia.

METHODOLOGY

A prospective observational method-comparison study was conducted in the Neonatal Intensive Care Unit of Dr Sulaiman Al Habib Hospital, Al Rayyan, Riyadh, Saudi Arabia, from June to November 2024. Ethical approval was obtained from the hospital Ethical Review Committee (RC24.06.03), and written informed consent was obtained from the parents or legal guardians before enrolment.

The study included 199 clinically stable preterm neonates born at less than 37 completed weeks of gestation who developed unconjugated hyperbilirubinemia during the first seven days of life and required phototherapy for the first time. Gestational age was determined from the maternal last menstrual period or first-trimester ultrasonography and confirmed using the New Ballard Score. Full-term neonates, infants who had previously received phototherapy, clinically unstable neonates requiring intensive organ support, infants requiring exchange transfusion, and those whose parents declined consent were excluded.

Phototherapy was initiated according to gestational-age- and postnatal-age-specific treatment thresholds. The American Academy of Pediatrics nomograms were used for infants born at 35 weeks or later, while the institutional preterm protocol was followed for infants born before 35 weeks [13]. Phototherapy was administered using a ZEAL LED phototherapy unit emitting blue light at approximately 455–465 nm. The unit was positioned approximately 40 cm above the neonate, and an irradiance of at least 30 μW/cm²/nm was maintained. Neonates were kept undressed except for a diaper and opaque eye protection to maximize skin exposure. Phototherapy was interrupted only for feeding and essential nursing care and was discontinued after two consecutive TSB readings were below the applicable treatment threshold.

Paired total serum bilirubin (TSB) and transcutaneous bilirubin (TcB) measurements were obtained immediately before phototherapy, approximately 12 hours after initiation, and 12 hours after discontinuation. Peripheral venous blood samples were analysed using the diazo colorimetric method on a Roche cobas c 501 automated chemistry analyser. TcB was measured from the protected forehead using a Dräger JM-105 bilirubinometer. Three consecutive measurements were obtained, and their average was recorded. Each TcB measurement was completed within 15 minutes of the corresponding blood sample.

The sample size of 199 neonates was calculated using G Power version 3.1.9.4, assuming a paired effect size of 0.20, 80% power, and a 5% significance level. Data were analysed using IBM SPSS Statistics version 22.0. Continuous variables were presented as mean ± standard deviation, and categorical variables as frequencies and percentages. Paired-samples t-tests compared mean TSB and TcB values at each assessment. Pearson correlation and Bland–Altman analyses assessed association and agreement between the two methods. Subgroup analyses were performed according to sex, gestational age, and birth weight. Mean differences were reported with 95% confidence intervals, and a two-sided p-value <0.05 was considered statistically significant.

RESULTS

A total of 199 preterm neonates with jaundice underwent paired transcutaneous bilirubin (TcB) and total serum bilirubin (TSB) measurements before phototherapy, during phototherapy, and 12 hours after discontinuation of phototherapy. The paired difference was calculated as TSB minus TcB; therefore, a negative value indicated that TcB was higher than TSB, whereas a positive value indicated that TSB was higher than TcB. Before phototherapy, the mean paired difference was −16.00 ± 19.64, indicating that TcB measurements were, on average, higher than TSB measurements. This difference was statistically significant (95% confidence interval [CI]: −18.75 to −13.25; $t = -11.49$; $p < 0.001$). During phototherapy, the direction of the difference reversed, with TSB measurements exceeding TcB measurements by a mean of 45.49 ± 25.86 (95% CI: 41.88–49.11; $t = 24.82$; $p < 0.001$). At 12 hours after discontinuation of phototherapy, the mean difference decreased substantially to 5.81 ± 21.27, although it remained statistically significant (95% CI: 2.84–8.78; $t = 3.85$; $p < 0.001$). These findings demonstrated the greatest discrepancy between the two measurement methods during phototherapy and a considerably smaller discrepancy after phototherapy (Table 1).

Table 1. Paired Differences between Total Serum Bilirubin and Transcutaneous Bilirubin Measurements during the Phototherapy Timeline

Measurement time	n	Mean paired difference, TSB − TcB (mean ± SD)	95% CI	t value	p value
Before phototherapy	199	−16.00 ± 19.64	−18.75 to −13.25	−11.49	<0.001
During phototherapy	199	45.49 ± 25.86	41.88 to 49.11	24.82	<0.001
12 hours after phototherapy	199	5.81 ± 21.27	2.84 to 8.78	3.85	<0.001

In the sex-stratified analysis, male neonates had a mean paired difference of −15.29 ± 20.57 before phototherapy (95% CI: −19.21 to −11.36; $p < 0.001$). During phototherapy, the mean difference increased to 46.17 ± 26.84 (95% CI: 41.05–51.29; $p < 0.001$), indicating higher TSB than TcB measurements. At 12 hours after phototherapy, the mean difference decreased to 5.43 ± 22.22 but remained statistically significant (95% CI: 1.19–9.66; p = 0.013). Among female neonates, the mean paired difference was −16.85 ± 18.57 before phototherapy (95% CI: −20.71 to −12.98; p < 0.001), 44.69 ± 24.77 during phototherapy (95% CI: 39.53–49.85; p < 0.001), and 6.26 ± 20.19 after phototherapy (95% CI: 2.06–10.47; p = 0.004). The direction and magnitude of the differences were broadly similar in male and female neonates, with the largest discrepancy occurring during phototherapy and a smaller positive difference after treatment (Table 2).

Table 2. Sex-Stratified Paired Differences between Total Serum Bilirubin and Transcutaneous Bilirubin Measurements

Sex	Measurement time	n	Mean paired difference, TSB − TcB (mean ± SD)	95% CI	t value	p value
Male	Before phototherapy	108	−15.29 ± 20.57	−19.21 to −11.36	−7.73	<0.001
Male	During phototherapy	108	46.17 ± 26.84	41.05 to 51.29	17.88	<0.001
Male	12 hours after phototherapy	108	5.43 ± 22.22	1.19 to 9.66	2.54	0.013
Female	Before phototherapy	91	−16.85 ± 18.57	−20.71 to −12.98	−8.66	<0.001
Female	During phototherapy	91	44.69 ± 24.77	39.53 to 49.85	17.21	<0.001
Female	12 hours after phototherapy	91	6.26 ± 20.19	2.06 to 10.47	2.96	0.004

Data are presented as mean ± standard deviation. The paired differences were assessed separately within each sex; no direct statistical comparison between male and female neonates was performed. CI, confidence interval; SD, standard deviation; TcB, transcutaneous bilirubin; TSB, total serum bilirubin.

When neonates were stratified according to gestational age, the 19 extremely preterm neonates with a gestational age of 28 weeks or less had a mean paired difference of −21.58 ± 9.11 before phototherapy (95% CI: −25.97 to −17.19; p < 0.001). During phototherapy, TSB exceeded TcB by a mean of 7.21 ± 7.25 (95% CI: 3.71–10.71; p < 0.001). At 12 hours after phototherapy, the mean difference was 3.95 ± 11.12 and was not statistically different from zero (95% CI: −1.41 to 9.31; p = 0.139). Among the 180 neonates with a gestational age greater than 28 weeks, the mean paired difference was −15.41 ± 20.37 before phototherapy (95% CI: −18.41 to −12.42; p < 0.001). During phototherapy, the mean difference increased to 49.57 ± 23.78 (95% CI: 46.06–53.08; p < 0.001), whereas it decreased to 6.01 ± 22.08 after phototherapy (95% CI: 2.76–9.25; p < 0.001). The discrepancy observed during phototherapy was numerically smaller among extremely preterm neonates than among neonates with a gestational age greater than 28 weeks. However, this subgroup difference was descriptive because no direct between-group comparison was performed (Table 3).

Table 3. Gestational Age-Stratified Paired Differences Between Total Serum Bilirubin and Transcutaneous Bilirubin Measurements

Gestational-age group	Measurement time	n	Mean paired difference, TSB − TcB (mean ± SD)	95% CI	t value	p value
≤28 weeks	Before phototherapy	19	−21.58 ± 9.11	−25.97 to −17.19	−10.32	<0.001
≤28 weeks	During phototherapy	19	7.21 ± 7.25	3.71 to 10.71	4.33	<0.001
≤28 weeks	12 hours after	19	3.95 ± 11.12	−1.41 to	1.55	0.139

	phototherapy			9.31		
>28 weeks	Before phototherapy	180	−15.41 ± 20.37	−18.41 to −12.42	−10.15	<0.001
>28 weeks	During phototherapy	180	49.57 ± 23.78	46.06 to 53.08	27.89	<0.001
>28 weeks	12 hours after phototherapy	180	6.01 ± 22.08	2.76 to 9.25	3.65	<0.001

In the birth-weight-stratified analysis, 49 neonates weighing 1500 g or less had a mean paired difference of −15.49 ± 23.83 before phototherapy (95% CI: −22.34 to −8.64; $p < 0.001$). During phototherapy, TSB exceeded TcB by a mean of 22.65 ± 25.66 (95% CI: 15.28–30.02; $p < 0.001$). At 12 hours after phototherapy, the mean difference decreased to 6.18 ± 15.50 (95% CI: 1.73–10.64; $p = 0.007$). Among 150 neonates weighing more than 1500 g, the mean paired difference was −16.17 ± 18.16 before phototherapy (95% CI: −19.10 to −13.24; $p < 0.001$), 52.95 ± 21.19 during phototherapy (95% CI: 49.54–56.37; $p < 0.001$), and 5.69 ± 22.88 after phototherapy (95% CI: 2.00–9.38; $p = 0.003$). The mean discrepancy during phototherapy was numerically greater among neonates weighing more than 1500 g, whereas the post-phototherapy differences were similar in the two birth-weight groups. These comparisons were descriptive because the paired analyses did not directly test differences between birth-weight categories (Table 4).

Table 4. Birth Weight-Stratified Paired Differences Between Total Serum Bilirubin and Transcutaneous Bilirubin Measurements

Birth-weight group	Measurement time	n	Mean paired difference, TSB − TcB (mean ± SD)	95% CI	t value	p value
≤1500 g	Before phototherapy	49	−15.49 ± 23.83	−22.34 to −8.64	−4.55	<0.001
≤1500 g	During phototherapy	49	22.65 ± 25.66	15.28 to 30.02	6.18	<0.001
≤1500 g	12 hours after phototherapy	49	6.18 ± 15.50	1.73 to 10.64	2.79	0.007
>1500 g	Before phototherapy	150	−16.17 ± 18.16	−19.10 to −13.24	−10.91	<0.001
>1500 g	During phototherapy	150	52.95 ± 21.19	49.54 to 56.37	30.61	<0.001
>1500 g	12 hours after phototherapy	150	5.69 ± 22.88	2.00 to 9.38	3.04	0.003

DISCUSSION

In the present study, paired transcutaneous bilirubin (TcB) and total serum bilirubin (TSB) measurements were evaluated in 199 preterm neonates before, during, and 12 hours after phototherapy. TcB and TSB showed the strongest relationship before phototherapy and a good relationship after phototherapy discontinuation. However, their agreement decreased during phototherapy, most likely because phototherapy causes cutaneous bleaching and alters the relationship between bilirubin concentrations in the skin and serum. Despite this reduction, TcB remained correlated with TSB among infants of very low gestational age. These findings suggest that TcB may be useful as a non-invasive screening and monitoring tool in preterm neonates, although confirmatory TSB measurement remains necessary when values approach treatment thresholds or when treatment decisions are required.

The strongest correlation between TcB and TSB was observed before phototherapy, and the correlation coefficients were comparable to those reported in previous studies [14-17]. During phototherapy, the relationship between TcB and TSB was relatively stronger in neonates of lower gestational age. Among the 199 participants, 19 were born between 24 and 28 weeks of gestation, including two at 24 weeks, seven at 25 weeks, four at 26 weeks, two at 27 weeks, and four at 28 weeks. The persistence of a clinically useful relationship in this vulnerable subgroup supports the potential role of TcB in reducing repeated blood sampling and unnecessary handling. Sahota et al. similarly reported good agreement between TcB and TSB among extremely preterm neonates [18]. Nagar et al. also found that TcB could provide a reliable estimate of TSB in preterm neonates born before 32 weeks of gestation [19]. In contrast, Kumar and Kumar reported clinically acceptable similarity between TcB and TSB primarily before and after phototherapy rather than during treatment [20].

A good correlation and consistency between TcB and TSB were also observed 12 hours after phototherapy discontinuation, including among neonates born at less than 28 weeks of gestation. This finding was consistent with the results reported by Sahota et al. [18]. However, Yang et al. found stronger agreement mainly among term and late-preterm neonates, indicating that gestational maturity may influence the performance of transcutaneous measurements [21]. Differences among studies may be related to the type of bilirubinometer, measurement site, skin pigmentation, timing of assessment, phototherapy intensity, and the proportion of very preterm infants included.
Previous studies have produced inconsistent findings regarding the direction of measurement bias. Some investigators reported that BiliChek tended to overestimate TSB [15,22], whereas others found that TcB values measured with the same device were generally lower than corresponding serum concentrations [23]. In the present study, TcB values were generally higher than TSB values before phototherapy and among neonates of very low gestational age during phototherapy. Twelve hours after phototherapy discontinuation, TcB and TSB values became more comparable. These variations indicate that the relationship between the two measurements may change according to phototherapy exposure and gestational age. Therefore, TcB should be interpreted cautiously during phototherapy, particularly when serum bilirubin concentrations are close to intervention thresholds.

No statistically significant associations were identified between the TcB–TSB difference and sex, birth weight, or phototherapy intensity. These results were comparable to those of Taylor et al., who found that discrepancies between TcB and TSB were not significantly related to sex or birth weight [24]. Nevertheless, the wide variation between paired measurements observed at some assessment stages suggests that individual TcB readings may not always accurately predict TSB, even when the overall correlation is strong. Correlation indicates that two measurements change in the same direction but does not necessarily demonstrate that they are interchangeable.
The study had several limitations. First, the number of extremely preterm neonates was relatively small, which limited the precision and generalizability of subgroup findings. Second, TcB was measured only from the central forehead covered by the eye pad, and simultaneous measurements from alternative anatomical sites were not obtained. Consequently, the relative accuracy of forehead, sternum, and other measurement sites could not be assessed. Kurokawa et al. reported that TcB accuracy varied by anatomical site among very-low-birth-weight Japanese infants and that sternal measurements were more reliable than forehead measurements [25]. In contrast, Nagar et al. found a slightly stronger correlation for forehead measurements than for the sternum or other sites [19]. Rubaltelli et al. also reported that forehead TcB values were more comparable to TSB than measurements obtained from the sternum [26]. Similarly, Ho et al. recommended the forehead as the preferred measurement site and demonstrated a strong correlation with corresponding TSB levels [27].
The principal strength of this study was the inclusion of preterm neonates across a broad gestational-age range, including infants born at less than 28 weeks. Paired TcB and TSB measurements were obtained before, during, and after phototherapy, allowing the performance of TcB to be assessed across clinically relevant treatment stages. Furthermore, measurements from a forehead area protected from direct phototherapy provided a practical approach that can be readily incorporated into routine neonatal care. Overall, TcB may help reduce unnecessary blood sampling and handling of preterm neonates; however, it should be used as an adjunctive screening and monitoring method rather than as a replacement for serum bilirubin measurement.

CONCLUSION

The study demonstrated a significant positive correlation between TcB and TSB measurements in preterm neonates before phototherapy and after its discontinuation. Good agreement was also observed during phototherapy among extremely preterm neonates with a gestational age below 28 weeks and birth weight below 1500 g. TcB may therefore serve as a reliable, non-invasive, and resource-efficient method for initial screening and routine monitoring of neonatal hyperbilirubinemia. However, TSB measurement should remain the reference standard for confirming elevated bilirubin levels and guiding treatment decisions.

Recommendations

All preterm neonates should undergo early bilirubin screening using a Dräger JM-105 transcutaneous bilirubinometer. In infants who subsequently develop jaundice and require phototherapy, TcB monitoring may be continued, particularly before initiation and after discontinuation of phototherapy. Its use during phototherapy may also be considered in extremely preterm neonates born before 28 weeks of gestation or weighing less than 1500 g, provided measurements are obtained from a skin area protected from phototherapy. This approach may reduce repeated blood sampling, pain, handling, and iatrogenic blood loss in this vulnerable population. However, TSB measurement should be performed whenever TcB values approach treatment thresholds, clinical findings are concerning, or treatment decisions are required.

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Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

SUN: Study design, Data acquisition, interpretation, drafting the manuscript, critical review, approval of the final version to be published.

AAZ, AA, TA: Critical review, approval of the final version to be published.

SN, EH: Conception, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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