RESEARCH ARTICLE

REVISED Transcutaneous bilirubin level to predict hyperbilirubinemia in preterm neonates [version 3; peer review: 2 approved]

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Abstract

Background: Hyperbilirubinemia commonly occurs in neonates, with a higher prevalence among preterm neonates, which can lead to severe hyperbilirubinemia. Assessment of total serum bilirubin (TSB) and the use of transcutaneous bilirubinometry (TcB) are existing methods which can identify and predict hyperbilirubinemia. This study aimed to determine TcB cut-off values during the first day for preterm neonates, in order to predict hyperbilirubinemia at 48 and 72 hours.
Methods: This cohort study was conducted at Dr. Soetomo General Hospital from September 2018 to January 2019, studying a total of 90 neonates born at ≤35 weeks. They were divided into two groups (Group I: 1000-1500 grams; Group II: 1501-2000 grams). The bilirubin levels were measured at the sternum, using TcB at the ages of 12, 24, and 72 hours. TSB measurements were taken on the third day or if the TcB level reached phototherapy threshold ± 1.24 mg/dL and if TcB showed abnormal results (Group I: 5.76-8.24 mg/dL; Group II: 8.76-11.24 mg/dL). Hyperbilirubinemia was defined as TSB ≥7 mg/dL for Group I and >10 mg/dL for Group II.
Results: In total, 38 Group I neonates and 48 Group II neonates were observed. Approximately onehalf of the neonates in Group I (45%) suffered from hyperbilirubinemia at 48 hours, along with 46% of Group II at 72 hours. The best 24-hour-old TcB cut-off values to predict hyperbilirubinemia at 48 hours were calculated to be 4.5 mg/dL for Group I and 5.8 mg/dL for Group II. The determined 24-hour-old TcB value to predict hyperbilirubinemia at 72 hours was 5.15 mg/dL for Group II.
Conclusion: TcB values on the first day of life can be used as

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hyperbilirubinemia predictors on the following days for preterm neonates. Close monitoring should be managed for those with TcB values higher than the calculated cut-off values.

Keywords
transcutaneous bilirubin, preterm neonates, predict, hyperbilirubinemia

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REVISED **Amendments from Version 2**

We have improved the better English in the manuscript as suggested by the Reviewer.

Any further responses from the reviewers can be found at the end of the article

Introduction

Hyperbilirubinemia is a common condition occurring in the neonatal period[1], with an incidence of approximately 60% in term neonates and 80% in preterm neonates. Preterm neonates have a greater risk of acquiring severe hyperbilirubinemia, which can lead to encephalopathy[2]. This condition is preventable if early detection and prompt treatment can be arranged and correctly managed[1,3,4].

Visual assessment is not reliable especially in the first 24–48 hours, since only 80% of jaundiced babies can be recognized visually if the bilirubin level reaches > 6 mg/dL[5–8]. High bilirubin levels can be dangerous, since preterm neonates have a greater risk of low bilirubin kernicterus[2].

Total serum bilirubin (TSB) measurement remains the gold standard for diagnosing hyperbilirubinemia. However, the drawbacks of this procedure are that it is painful, causes stress to the neonates, has a higher risk of infection, and requires several hours in order to obtain the results[9–11].

Transcutaneous bilirubinometry (TcB) is a non-invasive procedure used to identify hyperbilirubinemia. Several studies have been conducted to validate TcB to assess whether it can be used safely. These studies found that TcB has good correlations with TSB. The use of TcB can also reduce the need for blood sampling by 41–73%[11–13].

Due to the burdens of hyperbilirubinemia, its early detection and prediction are crucial. TSB or TcB is recommended to predict neonatal hyperbilirubinemia for neonates with >35 weeks of gestation[12,14,15]. In a systematic review by Nagar *et al.*[16], most of 22 papers studied the accuracy of TcB to estimate TSB, and TcB could be used in clinical practice to reduce blood sampling. Some studies have used TcB to predict significant hyperbilirubinemia in subsequent days, but all recruited only late preterm and term neonates[5,17]. For preterm neonates, one study was already conducted using TSB measurements at 6–24 hours to predict hyperbilirubinemia in the following hours or days[4]. To the best of our knowledge, there have been no previous studies using TcB to predict subsequent, significant hyperbilirubinemia for older preterm neonates. Therefore, this study aimedto use TcB to predict hyperbilirubinemia in preterm neonates, in order to prevent complications, due to the unreliability of visual assessment.

Methods

Study background and ethical approval

This cohort study was conducted in the neonatal intensive care unit at Dr Soetomo General Hospital for five months (September 2018–January 2019). This study was approved by Dr. Soetomo General Hospital Surabaya Ethics Committee (No. 0586/KEPK/Ix/2018). Parents signed the informed consent form after they understood the information. The study size retrieved in this research used purposive sampling with inclusion and exclusion criteria (a flow diagram is available as *Extended data*)[18,19] during the research period. The sample size was estimated by applying Hulley *et al.*'s[20] formulation, with a confidence interval of 95%, a coefficient correlation of 0.84 and a standard deviation of 1.8[4]. Therefore, the minimum sample size of 20 was applied for each group, classified by infants' body weights. Staying in line with the minimum sample size, the samples were expanded up to 45 infants for each group, with a total sample of 90 infants. However, four datasets were excluded due to missing TSB measurement.

Race and thickness of the skin tissue's melanin layer were taken into account as confounding variables, and as variables able to change the outcomes of others. Therefore, to control for study bias, the subjects addressed for this study were those with similar ethnic backgrounds: Malay Mongoloid.

Participant eligibility

The inclusion criteria were: 1) birth at ≤35 weeks of gestational age with a birth weight < 2000 g, and 2) parental consent provided on a signed a form. The exclusion criteria were: 1) being diagnosed with hyperbilirubinemia at 12 hoursof age, 2) having any major congenital anomaly, or 3) being discharged from hospital at less than three days old. Neonates who received phototherapy before the observation was complete, missed TSB, or whose parents voluntarily resigned from this study were excluded from the study. The recruited subjects were divided into two groups: neonates with birth weights of 1001-1500 g (Group I) and 1501-2000 g (Group II).

Variables

The bilirubin level of each neonate was measured on the sternum by TcB (Dräger® Jaundice Meter 105) at 12 hours, 24 hours, and 72 hours with ±3 hours tolerance (the TcB measurement could be taken within three hours before/after the exact time). The TSB measurement was taken for each neonate at the age of three days or if the TcB bilirubin level was ≥5.76 (7–1.24) mg/dL for Group I and TcB ≥8.76 (10–1.24) mg/dL for Group II; it had to be taken within six hours before or after the TcB measurement (assumption of TcB standard deviation being ±1.24 mg/dL). The TSB measurement also had to be taken if the TcB measurement showed abnormal results. Hyperbilirubinemia was defined as TSB ≥7 mg/dL for preterm neonates with birth weights of 1000–1500 g and TSB >10 mg/dL for preterm neonates with birth weights of 1501–2000 g as suggested

by the Kaplan *et al.,* in Martin's Neonatal-Perinatal Medicine (2011).[21] TSB was measured in the central laboratory using SIEMENS Dimension® with a modified Doumas[22] reference method, which is a modification of the diazo method described by Jendrassik and Grof in 1938[23]. Internal calibration was completed daily, with a quality control printout. The Indonesian External Quality Assurance Service performed external quality control.

Statistical analysis

The data was analyzed by Microsoft Office Excel, IBM SPSS Statistics Version 21. Receiver operating characteristic (ROC) curve analysis was performed to determined the TcB level cut-off point to predict hyperbilirubinemia at the age of 48 and 72 hoursThe specificity, sensitivity, positive predicted value (PPV), negative predicted value (NPV), and likelihood ratio were calculated.

Results

There were 90 preterm neonates recruited for this study, 40 of whom weighed 1000–1500 grams (Group I) and 50 of whom weighed 1501–2000 grams (Group II). Only 38 neonates in Group I and 48 neonates of Group II were observed until the end of the study. Four neonates were excluded from the study due to missing TSB results.

Maternal and neonatal characteristics are shown in Table 1. For Group I, the mean gestational age of Group I was 32.29 ± 1.84 weeks, with a mean birth weight of 1273.68 ± 177.34 g. Meanwhile, for Group II the mean gestational age was 33.69 ± 1.26 weeks, witha mean birth weight of 1792.70 ± 145.86 g. Based on the risk factors of ABO-incompatibility, one subject of Group I suffered hyperbilirubinemia at the age of 48 hours. Two subjects in Group II suffered hyperbilirubinemia at the age of 48 hours and another two subjects at the age of 72 hours. At the end of the observation, the maximum bilirubin level was 15.2 mg/dl for Group I and 16.33 mg/dL for Group II. Most neonates of Group I (45%) suffered hyperbilirubinemia at the age of 48 hours, while most neonates of group II (46%) at the age of 72 hours (Figure 1). The TSB means in Group I at the ages of 24, 48, and 72 hours were 7.9 mg/dL, 9.16 mg/dL, and 9.3 mg/dL respectively, and 11.01 mg/dL, 10.23 mg/dL, and 11.04 mg/dL respectively, in Group II.

Using TcB bilirubin levels to predict hyperbilirubinemia at the age of 48 hours in Group I preterm neonates

A ROC curve was constructed to determine a hyperbilirubinemia threshold based on the data collected. For Group I, the area under the curve (AUC) of the TcB bilirubin level at the age of 12 hours to predict hyperbilirubinemia at the age of 48 hours was 0.804 (p = 0.002), with a cut-off point of 2.35 mg/dL (sensitivity: 79.20%;specificity: 71.40%). For TcB bilirubin levels at the age of 24 hours to predict hyperbilirubinemia at the age of 48 hours, the AUC was 0.771 (p = 0.06), with a cut-off point of 4.50mg/dL (sensitivity: 87.50%; specificity: 64.26%) (Figure 2a, Figure 2b, Table 2).

Table 1. Maternal and neonatal characteristics of subjects.

Maternal characteristics	Group I (n = 38) n(%)	Group II (n = 48) n(%)
Gestational Age (weeks) (mean ±SD)	32.29 ± 1.84	33.69 ± 1.26
Mode of delivery - spontaneous - c-section - vacuum	 11(29) 26(68) 1(3)	 9(19) 38(80) 1(1)
Maternal Blood Type A B O AB	 7(18.4) 14(36.80) 16(42.10) 1(2.70)	 12(25) 10(20.80) 19(39.60) 7(14.60)
Neonatal characteristics	**Group I (n=38) n (%)**	**Group II (n=48) n (%)**
Birth Weight (g) (mean ±SD)	1273.68 ± 177.34	1792.70 ± 145.86
Hematocrit (%) (mean ±SD)	48.04 ± 10.47	46.99± 8.48
Gender Male Female	 19 (50) 19 (50)	 30(62.50) 18(37.50)
Neonatal blood-type A B O AB	 4(10.50) 11(28.90) 21(55.30) 2(5.30)	 9(18.8) 10(20.8) 24(50) 5(10.4)

*Descriptive analysis was used. Maternal and neonatal rhesus were positive.

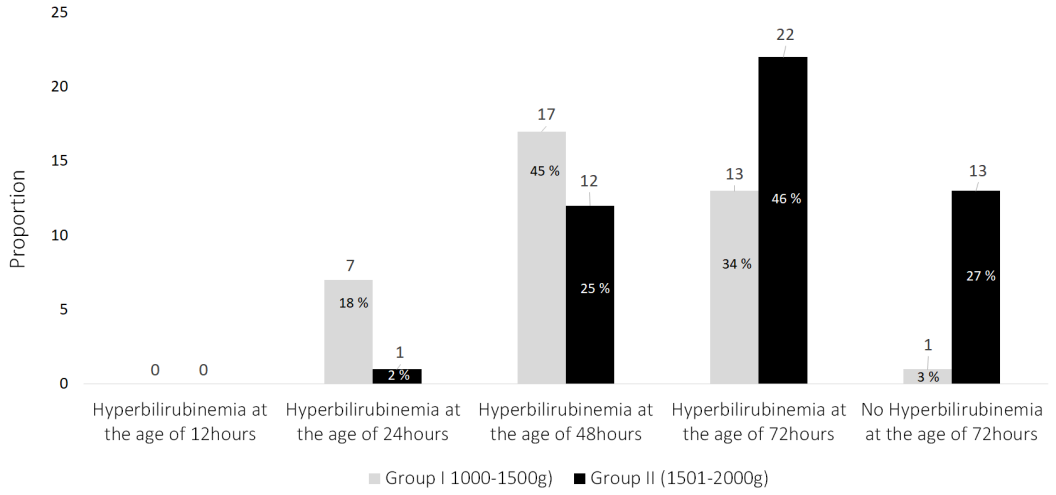

Figure 1. The proportion of hyperbilirubinemia in preterm neonates.

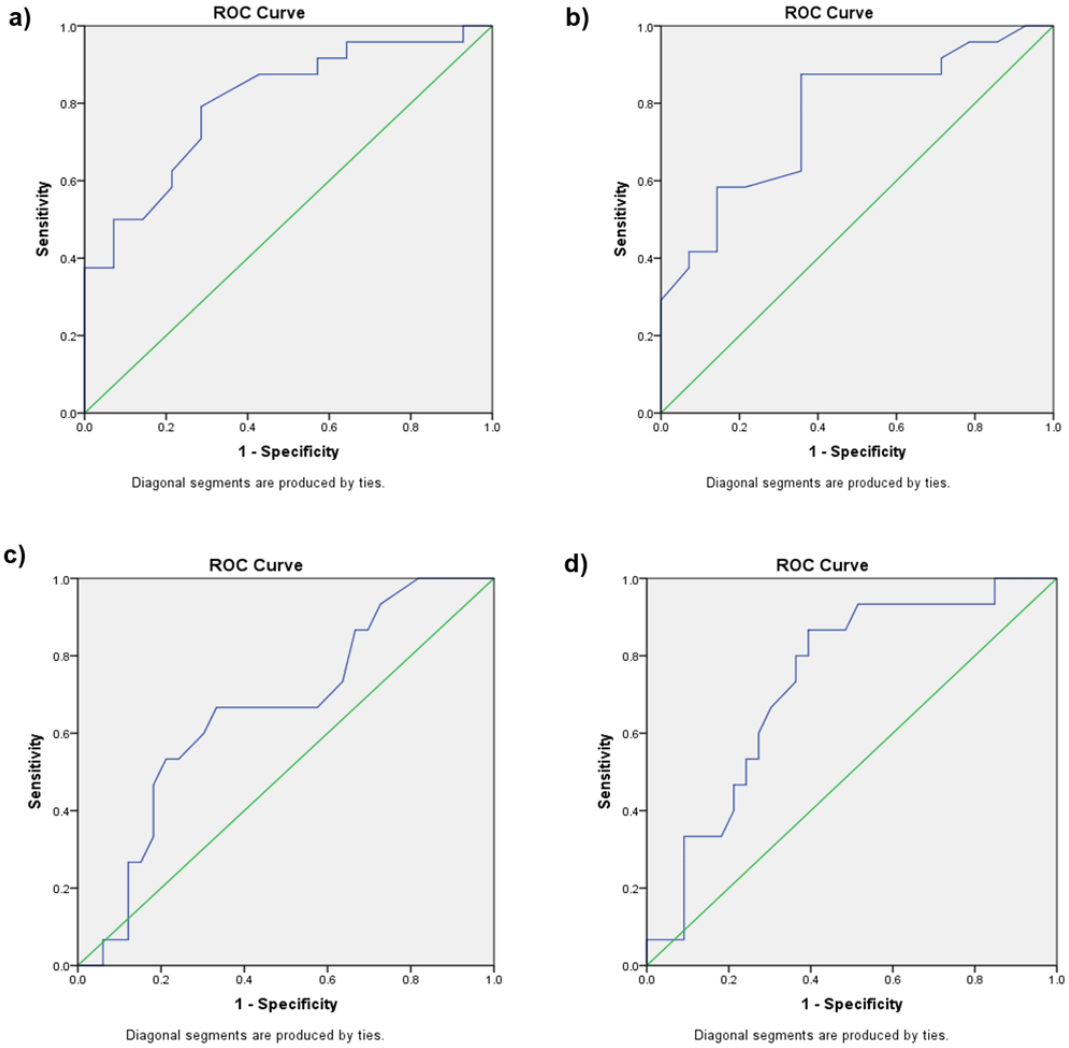

Figure 2. Transcutaneous bilirubinometry (TcB) level to predict hyperbilirubinemia at the age of 48 hours. (**a**) Receiver operating characteristic (ROC) curve for TcB at the age of 12 hours to predict hyperbilirubinemia at the age of 48 hours for Group I. (**b**) ROC curve for TcB at the age of 24 hours to predict hyperbilirubinemia at the age of 48 hours for Group I. (**c**) ROC curve for TcB at the age of 12 hours to predict hyperbilirubinemia at the age of 48 hours for Group II. (**d**) ROC curve for TcB at the age of 24 hours to predict hyperbilirubinemia at the age of 48 hours for Group II.

Using TcB bilirubin levels to predict hyperbilirubinemia at the age of 48 hours in Group II preterm neonates

The AUC of TcB bilirubin levels at the age of 12 hours to predict hyperbilirubinemia at the age of 48 hours for Group II was 0.658 (p = 0.083), with a cut-off point of 3.05 mg/dL (sensitivity: 66.7%; specificity: 66.7%). The AUC of TcB bilirubin levels at the age of 24 hours was 0.732 (p = 0.011), with a cut-off point of 5.80 mg/dL (sensitivity: 80%; specificity: 63.6%). (Figure 2c, Figure 2d and Table 3).

Using TcB bilirubin levels to predict hyperbilirubinemia at the age of 72 hours in Group I preterm neonates

The TcB bilirubin levels of Group I at the ages of 12, 24, and 48 hours to predict hyperbilirubinemia at the age of 72 hours showed a very weak AUCs, which were 0.243 (p = 0.386), 0.297 (p = 0.494), 0.500 (p = 1.000), respectively; therefore, no cut-off point could be determined.

Using TcB bilirubin levels to predict hyperbilirubinemia at the age of 72 hours in Group II preterm neonates

Using the TcB bilirubin levels of Group II at the age of 12 hours to predict hyperbilirubinemia at the age of 72 hours showed a weak AUC (0.499, p = 0.991) with a cut-off point of 2.65 mg/dL (sensitivity 60% and specificity 46%). At the age of 24 hours, the TcB AUC was 0.751 (p = 0.008), with a cut-off point of 5.15 mg/dL (sensitivity: 74.3%; specificity: 76.9%). Meanwhile, at the age of 48 hours the TcB AUC was 0.731 (p = 0.015), with a cut-off point of 8.65 mg/dL (sensitivity: 67.6%; specificity: 61%) (Figures 3a–c and Table 4).

Table 2. Transcutaneous bilirubinometry (TcB) bilirubin level cut-off point to predict hyperbilirubinemia at the age of 48 hours for Group I.

TcB level cut-off (mg/dL)		Group I				
		Sn (%)	Sp (%)	PPV (%)	NPV (%)	LR
12 hours old	2.35	79.2	71.4	82.60	66.67	2.78
24 hours old	4.5	87.5	64.3	80.77	64.26	2.45

†Receiver operative characteristic curve analysis was used. Sn: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio.

Table 3. TcB bilirubin level cut-off point to predict hyperbilirubinemia at the age of 48 hours for Group II.

TcB level cut-off (mg/dL)		Group II				
		Sn (%)	Sp (%)	PPV (%)	NPV (%)	LR
12 hours old	3.05	66.7	66.7	47.62	81.48	2.00
24 hours old	5.85	80	63.6	50.00	87.50	2.19

†Receiver operative characteristic curve analysis was used. Sn: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio.

Discussion

This study determined a TcB cut-off value of 4.5 mg/dL at the age of 24 hours for Group I (1000–1500 grams) and 5.8 mg/dL for Group II (1501–2000 grams) as predictive of hyperbilirubinemia at 48 hours. This study could not determine a TcB cut-off value to predict hyperbilirubinemia at the age of 72 hours for Group I as a result of weak correlation. The TcB cut-off value of 5.15 mg/dL at the age of 24 hours was determined as the best predictor for hyperbilirubinemia at the age of 72 hours in Group II. This cut-off level was established with a sensitivity value ranging from 74.3% to 87.5% at 24 hours after birth. Similar studies have already been conducted, but those studies recruited only late preterm neonates. Lavanya *et al.* found that TcB values measured in the first 24–48 hours of life can predict hyperbilirubinemia at more than 48 hours[24]. Bansal *et al.* determined that TcB values > 4.6 mg/dL at 12–24 hours (sensitivity: 83.09%; specificity: 87.37%; PPV: 90.4%; NPV: 78.3%) and > 7.4 mg/dL at the age of 24–48 hours (sensitivity: 93.55%; specificity: 82.11%; PPV: 81.69%; NPV: 95.35%) are predictors for hyperbilirubinemia in the first 48 hours of life[5]. Other studies used TSB values to predict hyperbilirubinemia in the following days. Mayer recruited preterm neonates weighing 1000–1500 grams and determined a capillary TSB value of 3.55 mg/dL at the age of 12 hours as the best predictor of significant hyperbilirubinemia (sensitivity: 94.4%; PPV: 98.1%; NPV: 40%)[4].

Most neonates recruited in this study suffered hyperbilirubinemia prior to 72 hours. This study also showed that smaller babies suffered peak incidence earlier (at 48 hours) than larger babies (at 72 hours). It demonstrates that the higher birth weight babies have the opportunity acquire hyperbilirubinemia later than the lower birthweight babies. The lower birthweight babies have a lower threshold bilirubin, because they have a higher chance of encephalopathy at a lower bilirubin level (high risk infants). The younger gestational age and lower birth weight, lead to a higher prevalence of infants developing hyperbilirubinemia. It is a result of excessive neonatal red cell hepatic and immaturity of the gastrointestinal system. Prematurely delivered infants have a likelihood of slower maturation of hepatic bilirubin uptake and conjugation[25,26]. Hyperbilirubinemia is more prevalent in preterm neonates[4,5,24]) and is usually more severe, with a longer duration compared to that in term neonates[4]. This is caused by increased bilirubin production, decreased bilirubin excretion, increased enterohepatic circulation, lower albumin levels and a weak albumin-bilirubin bond[10,27]. Early detection of hyperbilirubinemia decreases its mortality and morbidity, so the need for reliable methods to predict hyperbilirubinemia is crucial. The use of a non-invasive procedure, like TcB, in the first 6–24 hours of life is recommended as a marker of bilirubin production[28] and it can decrease the need for blood sampling[29].

In Group I at the ages of 24, 48, and 72 hours, the TSB mean values were 7.9, 9.16, and 9.3 mg/dL, respectively; in Group II, these values were 11.01, 10.23, and 11.04 mg/dL, respectively. This is similar to a previous study that found that TSB mean values in the first five days of preterm neonates were 10–12 mg/dL[21]. However, this study found that some preterm neonates reached a TSB value >15 mg/dL in the first 72 hours. Preterm neonates can have high bilirubin levels in the first days of life,

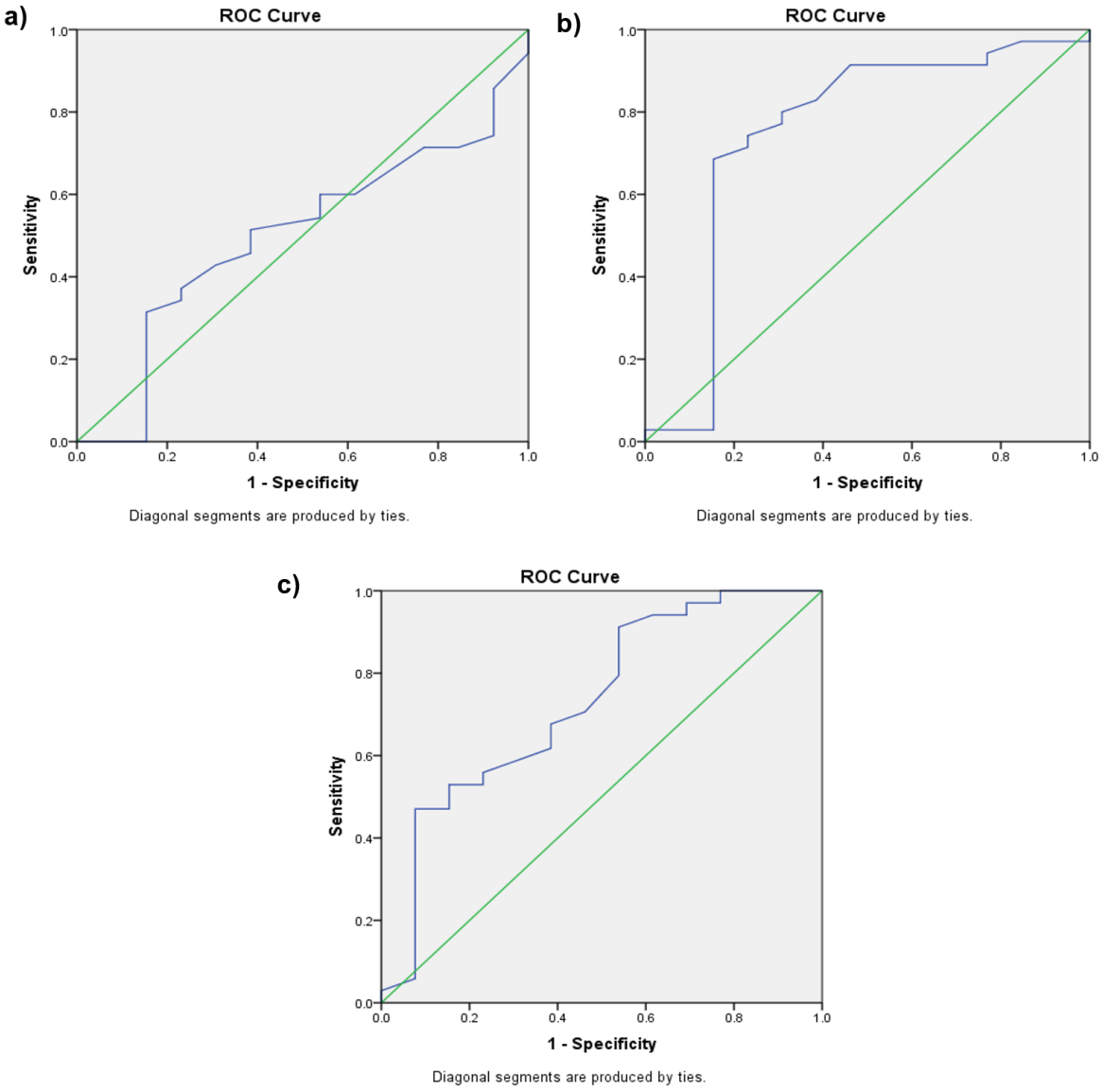

Figure 3. Transcutaneous bilirubinometry (TcB) level to predict hyperbilirubinemia at the age of 72 hours. (**a**) Receiver operating characteristic (ROC) curve for TcB at the age of 12 hours to predict hyperbilirubinemia at the age of 72 hours for Group II. (**b**) ROC curve for TcB at the age of 24 hours to predict hyperbilirubinemia at the age of 72 hours for Group II. (**c**) ROC curve for TcB at the age of 48 hours to predict hyperbilirubinemia at the age of 72 hours for Group II.

Table 4. TcB bilirubin level cut-off point to predict hyperbilirubinemia at the age of 72 hours for Group II.

TcB level cut-off (mg/dL)		Group II				
		Sn (%)	Sp (%)	PPV (%)	NPV (%)	LR
12 hours old	2.65	60	46	75.00	30.00	1.11
24 hours old	5.15	74.3	76.9	89.66	52.63	3.22
48 hours old	8.65	67.6	61	82.75	42.10	1.78

[1]Receiver operative characteristic curve analysis was used. Sn: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio.

which can lead to hyperbilirubinemia complications if not recognized and treated properly. The previous study conducted by Bhutani *et al*., also indicated that neonates who suffered from hyperbilirubinemia in the following days had higher percentiles on the first day of life[30]. Therefore, the American Academy of Pediatrics recommends routine checks of TSB or TcB along with risk factor assessments during the first days of life[14].

To the best of our knowledge, this was the first study conducted to predict significant hyperbilirubinemia in preterm neonates weighing 1000–2000 grams using TcB. A limitation of the study was that it could not determine TcB cut-off values to predict hyperbilirubinemia at the age of 72 hours for preterm neonates weighing 1000–1500 grams due to a lack of subjects able to complete the study, since most had already developed significant hyperbilirubinemia by this time. The mothers' rhesus blood groups and the babies' G6PD levels were not obtained. Hopefully, future similar studies will be able to recruit larger populations.

Conclusion

TcB values in the early days of life can be used as a predictor of hyperbilirubinemia in the following days for preterm neonates. Preterm neonates can possess high bilirubin levels in the first few days of their lives. Therefore, daily TcB measurement is important for early identification of hyperbilirubinemia, especially to prevent complications in certain, more vulnerable, preterm neonates. Close monitoring should be arranged for those who have TcB values higher than the cut-off values.

Data availability

Underlying data

Figshare: Datasheet TcB and TSB - Group I. https://doi.org/10.6084/m9.figshare.11948490.v1[31].

This project contains data gathered for neonates in Group I (those 1000–1500 grams).

Figshare: TcB Level and TSB-MTA - Group II. https://doi.org/10.6084/m9.figshare.11948529.v1[32].

This project contains data gathered for neonates in Group II (those 1500–2000 grams).

Extended data

Figshare: Supplemental File - Flow Chart Study of TcB and TSB. https://doi.org/10.6084/m9.figshare.12017586.v1[19].

This project contains a study flow diagram.

Reporting guidelines

Figshare: STROBE checklist for 'Transcutaneous bilirubin level to predict hyperbilirubinemia in preterm neonates'. https://doi.org/10.6084/m9.figshare.11991672.v2[18].

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 17 September 2020

https://doi.org/10.5256/f1000research.27678.r70954

✔ **Tina M. Slusher**
Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA

Language in places is still awkward and could be improved on but message is clearer and concerns have been addressed.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pediatric Global Health, Neonatal Hyperbilirubinemia, Pediatric Critical Care

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 18 Sep 2020

Mahendra Tri Arif Sampurna, Dr. Soetomo Academic Teaching Hospital, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia

Dear Dr. Tina M. Slusher,

Thank you very much for reviewing and helping us to improve this manuscript.
We will do as you suggested, we agree to proofread our manuscript by English proofreading services.

We will be grateful to be working with you in the future.

Thank you again for your kind assistance.

Warm regards,
On behalf of all authors
Mahendra

Competing Interests: No competing interests were disclosed.

Reviewer Report 08 September 2020

https://doi.org/10.5256/f1000research.27678.r70951

Claudio Tiribelli
Italian Liver Foundation, Trieste, Italy

Authors have addressed my concerns.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bilirubin, Jaundice

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 18 Sep 2020

Mahendra Tri Arif Sampurna, Dr. Soetomo Academic Teaching Hospital, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia

Dear Prof. Claudio Tiribelli,

Thank you very much for the review of our manuscript entitled: "Transcutaneous bilirubin level to predict hyperbilirubinemia in preterm neonates."

We sincerely appreciate all the valuable comments and suggestions, which helped us to improve the quality of the article.

Looking forward to working with you in the future

Thank you for your kind attention.

Warm regards,
On behalf of all authors
Mahendra

Competing Interests: No competing interests were disclosed.

Version 1

Reviewer Report 21 May 2020

https://doi.org/10.5256/f1000research.24559.r62845

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Tina M. Slusher
Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA

This study is an interesting study looking at the correlation between transcutaneous bilirubin (TcB) and serum bilirubin and the predictive value of subsequent clinically significant hyperbilirubinemia in neonates. However, there are some problems with the study that need to be addressed.

1. They authors state that TcB has not been used in premature infants with the exception of late preterms. However, in a systematic review by Nager *et al.* most of the 22 articles they mention include very preterm infants. Perhaps the authors need to mean to say in their population but this needs to be clarified.

2. Each country does indeed need to develop their own criteria for diagnosis and treatment levels of hyperbilirubinemia based on risk in their environment, treatments available and other specifics related to their own country. However, authors do need to tell us where there cutoffs for each group came from---are the in country normal, standard cutoffs for their hospital or region or how were they selected.

3. Numbers too small to extrapolate widely to Indonesia or beyond.

4. Rhesus of mothers and infants missing; G6PD status missing if not done state that.

5. Although not a major problem or limitation this article could be improved by having a native English speaker read and make minor edits throughout.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pediatric Global Health, Neonatal Hyperbilirubinemia, Pediatric Critical Care

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 25 Jun 2020

Mahendra Tri Arif Sampurna, Dr. Soetomo Academic Teaching Hospital, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia

1). Reviewer's comments:

This study is an interesting study looking at the correlation between transcutaneous bilirubin (TcB) and serum bilirubin and the predictive value of subsequent clinically significant hyperbilirubinemia in neonates. However, there are some problems with the study that need to be addressed.

The authors state that TcB has not been used in premature infants with the exception of late preterms. However, in a systematic review by Nager *et al.* most of the 22 articles they mention include very preterm infants. Perhaps the authors need to mean to say in their population but this needs to be clarified.

Author's response:
Dear Reviewer,
Thank you for your comments.

Yes, indeed you are right, thanks for your suggestion. What we meant here is similar with the study by Mayer (Mayer I, Gursoy T, Hayran M, Ovalı F. Value of twelfth-hour bilirubin level in predicting significant hyperbilirubinemia in preterms Infants. J Clin Med Res. 2014;6(3):190–6) but they used TSB to predict significant hyperbilirubinemia later on. Practice in our hospital so far is using Kramer and few TSB measurement to start phototherapy. So, we give another alternative which is non-invasive, easy to use, and applicable in our setting in Indonesia.

We added in our introduction as :

In a systematic review by Nagar et al.[16], most of 22 articles studied the accuracy of TcB to estimate TSB, and TcB could be used in clinical practice to reduce blood sampling. Some studies have used TcB to predict significant hyperbilirubinemia in subsequent days, but all of them recruited only late preterm and term neonates [5,17] . For preterm neonates, one

study was already conducted using TSB measurements at 6- 24 hours to predict hyperbilirubinemia in the following hours or days [4] . As far as the researchers know, there have been no previous studies using TcB to predict subsequent, significant hyperbilirubinemia for older preterm neonates. Therefore, this study aimed to use TcB to predict hyperbilirubinemia in preterm neonates to prevent complications since visual assessment is unreliable.

2) Reviewer's comment: Each country does indeed need to develop their own criteria for diagnosis and treatment levels of hyperbilirubinemia based on risk in their environment, treatments available and other specifics related to their own country. However, authors do need to tell us where there cutoffs for each group came from---are the in country normal, standard cutoffs for their hospital or region or how were they selected

Author's response:
Thank you for your comments.
We added in our method à variables section as follows:

The bilirubin level of each neonate was measured on the sternum by TcB (Dräger® Jaundice Meter 105) at 12 hours, 24 hours, and 72 hours with ±3 hours tolerance (the TcB measurement could be taken within three hours before/after the exact time). The TSB measurement was taken for each neonate at the age of three days or if the TcB bilirubin level was ≥5.76 (7-1.24) mg/dL for Group I and TcB ≥8.76 (10-1.24) mg/dL for Group II and it had to be taken within six hours before or after the TcB measurement (assumption of TcB standard deviation being ±1.24 mg/dL). The TSB measurement also had to be taken if the TcB measurement showed abnormal results. Hyperbilirubinemia was defined as TSB ≥7 mg/dL for preterm neonates with birth weights of 1000–1500 g and TSB >10 mg/dL for preterm neonates with birth weights of 1501–2000 g as suggested by the Kaplan et al., in Martin's Neonatal-Perinatal Medicine (2011).[21]

3). Reviewer's comment:
Numbers too small to extrapolate widely to Indonesia or beyond.

Author's response:
Dear Reviewer,
This is the study limitation and has been addressed in discussion section.
We added in the discussion section in the last paragraph:

A limitation of the study was that it could not determine TcB cut-off values to predict hyperbilirubinemia at the age of 72 hours for preterm neonates weighing 1000–1500 grams due to a lack of subjects able to complete the study, since most had already developed significant hyperbilirubinemia by this time.

4). Reviewer's comment:
Rhesus of mothers and infants missing; G6PD status missing if not done state that.

Author's response:
Dear Reviewer,
Thank you for your comments.

We even hardly measured Blood Group ABO and Rhesus of the mother and babies since the babies are not yellow. We have not used it as screening program. G6PD measurement is not affordable for our majority of citizen. We need to give consent first when the bilirubin is not yet decrease and rise again after intensive phototherapy, or there is sign of haemolysis

Thank you for your suggestion. Indeed, this is also our limitation that we added in the discussion section on the last paragraph

To the knowledge of the researchers, this was the first study conducted to predict significant hyperbilirubinemia in preterm neonates weighing 1000–2000 grams using TcB. A limitation of the study was that it could not determine TcB cut-off values to predict hyperbilirubinemia at the age of 72 hours for preterm neonates weighing 1000–1500 grams due to a lack of subjects able to complete the study, since most had already developed significant hyperbilirubinemia by this time. The mothers' rhesus blood groups and the babies' G6PD levels were not obtained. Hopefully, future similar studies will be able to recruit larger populations.

5) Reviewer's comment:
Although not a major problem or limitation this article could be improved by having a native English speaker read and make minor edits throughout

Author's response:
Dear Reviewer,
Thank you for your comments

We have already proofread this manuscript by Proofreading Service.

Competing Interests: We declare that no competing interest

Reviewer Report 11 May 2020

https://doi.org/10.5256/f1000research.24559.r62844

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Claudio Tiribelli

Italian Liver Foundation, Trieste, Italy

This is an interesting study investigating in preterm neonates (PTNs) the predictive value of the bilirubin level assessed by transcutaneous technique (TcB) for hyperbilirubinemia (HB). 90 PTNs were and divided into two groups according to the weight (1500 g as diving value). Bilirubin was prospectively measured at different time points (12, 24, 48, and 72 h). Bilirubin level was confirmed by serum bilirubin measurement (TSB) if the TcB was “increased”. ROC curves were used to assess a 24 h TcB value predicting 48 and 72 h HB. Although the data may be of interest, the study suffers several intrinsic weaknesses what must be addressed before being considered further.

Major Critiques:

1. The indication when TSB was measured is unclear and confusing. In the abstract it is stated that TSB was assessed before 72 h if TcB showed “abnormal results”. What does this mean? Detailed numerical values must be provided to allow to understand why TSB was performed.

2. On what basis the TSB value of $\geq$ 7 mg/dL in group 1 and > 10 mg/dL HB in group 2 was selected? This needs to be scientifically substantiated. At what time this level was measured?

3. How TSB was measured? Were the lab values confirmed by internal calibration? This must be clarified.

4. Fig 1 shows that the time course of HB is different in the 2 groups being the bilirubin peak reached 24 h later in group 2. This difference accounts for the different TcB cutoff values. This needs to be considered and addressed in the discussion.

5. How was the correlation between TcB and TSB at 72h? This information is important to assess the reliability of the two techniques (see also point #3).

6. The abstract is inaccurate as data reported are different from those indicated in the text (see for example the lack of 48H values).

Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bilirubin, Jaundice

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 25 Jun 2020

Mahendra Tri Arif Sampurna, Dr. Soetomo Academic Teaching Hospital, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia

1). Reviewer's Comment: The indication when TSB was measured is unclear and confusing. In the abstract it is stated that TSB was assessed before 72 h if TcB showed “abnormal results”. What does this mean? Detailed numerical values must be provided to allow to understand why TSB was performed.

Author's response:
Dear Reviewer,
Thank you for your comments.

We added in our method à variables section as follows:
The bilirubin level of each neonate was measured on the sternum by TcB (Dräger® Jaundice Meter 105) at 12 hours, 24 hours, and 72 hours with ±3 hours tolerance (the TcB measurement could be taken within three hours before/after the exact time). The TSB measurement was taken for each neonate at the age of three days or if the TcB bilirubin level was ≥5.76 (7-1.24) mg/dL for Group I and TcB ≥8.76 (10-1.24) mg/dL for Group II and it had to be taken within six hours before or after the TcB measurement (assumption of TcB standard deviation being ±1.24 mg/dL). The TSB measurement also had to be taken if the TcB measurement showed abnormal results. Hyperbilirubinemia was defined as TSB ≥7 mg/dL for preterm neonates with birth weights of 1000–1500 g and TSB >10 mg/dL for preterm neonates with birth weights of 1501–2000 g as suggested by the Kaplan et al., in Martin’s Neonatal-Perinatal Medicine (2011).

2). Reviewer's Comment: On what basis the TSB value of ≥ 7 mg/dL in group 1 and > 10 mg/dL HB in group 2 was selected? This needs to be scientifically substantiated. At what time this level was measured?

Author's response:
Dear Reviewer,
Thank you for your comments.

The cut off 7 and 10 mg/dl are based on the recommendation of Martin Fanaroff (Reference

source: Kaplan M, Wong R, Sibley E, et al.: Neonatal Jaundice and Liver Disease. In: Martin's Neonatal-Perinatal Medicine. 2011; 1443–90.) In this recommendation they use birth weight category regardless postnatal age in hours.

3). Reviewer's comment: How TSB was measured? Were the lab values confirmed by internal calibration? This must be clarified.

Author's response:
Dear Reviewer,

Thank you for your comments.
TSB measurements were performed by retrieving peripheral blood samples of each subject.
Added in methods à variables section :
Total serum bilirubin was measured in the central laboratory using SIEMENS Dimension® with a modified Doumas 22 reference method, which is a modification of the diazo method described by Jendrassik and Grof in 1938 23. Internal calibration was completed daily, with a quality control printout. The Indonesian External Quality Assurance Service performed external quality control.

4). Reviewer's comment: Fig 1 shows that the time course of HB is different in the 2 groups being the bilirubin peak reached 24 h later in group 2. This difference accounts for the different TcB cut-off values. This needs to be considered and addressed in the discussion.

Author's comment:
Dear Reviewer,
Thank you for the comment.

We added in the discussion:

It shows that that higher birth weight has opportunity to be hyperbilirubinemia later than the lower birthweight. The lower birthweight has lower threshold bilirubin, because they have higher chance to become encephalopathy in lower bilirubin level (high risk infants). The younger gestational age and lower birth weight, lead to a higher prevalence of infants developing hyperbilirubinemia. It is a result of excessive neonatal red cell hepatic and immaturity of the gastrointestinal system. Prematurely delivered infants have a likelihood of slower maturation of hepatic bilirubin uptake and conjugation.

5). Reviewer's comment: How was the correlation between TcB and TSB at 72h? This information is important to assess the reliability of the two techniques (see also point #3)

Author's response:
Dear Reviewer,
Thank you for your comments.
We actually did not assess the correlation between TcB and TSB at 72 hours of age. We would like to mention our reasons why we did not perform the assessment as the followings:

1. We were not specifically aiming at performing diagnostic test;
2. We only discovered fewer observed subjects who had reached observation at 72 hours of age thus the sample size at that age was not abundant in number. Most of the infants underwent phototherapy before 72 hours (group 1). Those infants were excluded from the study for further analysis.

6) Reviewer's Comment: The abstract is inaccurate as data reported are different from those indicated in the text (see for example the lack of 48H values).

Author's response:
Dear Reviewer,

Thank you for noticing. We adapted our abstract accordingly

We also found that there was an unsuitable data between those mentioned in the study abstract and the ones in figures. However, we would like to inform that the proportional data provided in the study abstract is correct already meanwhile the ones in figures were attained by integrating the data percentages. Therefore, we are going to amend and adjust the data provided in abstract.

Competing Interests: we declare that no competing interests.