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Original Article

Comparison of Invasive Measurement and Two Non-Invasive Measurements in the Diagnosis of Neonatal Hyperbilirubinemia

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ABSTRACT

Aim: Reliable non-invasive methods are required for the diagnosis of indirect hyperbilirubinemia (IHB) in infants. We compared the measured total serum bilirubin (TSB) levels against the transcutaneous and BiliCam methods.

Materials and Methods: This analytical study was performed in a neonatal intensive care unit of a hospital in Turkey. We included 70 infants whose families gave voluntary and written consent, including those infants with a low, medium, and high risk of hyperbilirubinemia, birth weight >1,500 g, and late preterm infants. We measured the TSB and compared it with bilirubin levels obtained via the transcutaneous and BiliCam measurement methods. The relationships between the data were determined using descriptive statistical methods; continuous data showing normal distribution were analyzed using Pearson correlation coefficient, and data that were not normally distributed were analyzed using Spearman correlation analysis.

Results: A statistically significant and positive correlation was observed between the levels of TSB and transcutaneous bilirubin before phototherapy (PT), whereas a moderate relationship was observed in these values after PT ($p<0.01$). A significant positive and moderate relationship was observed between the TSB levels and bilirubin levels measured using BiliCam before PT ($p<0.01$), and a weak relationship was observed between these values after PT ($p<0.05$).

Conclusion: Our results show that considering measurement of TSB as a reference method, the transcutaneous and BiliCam methods can be used as screening methods to detect IHB.

Keywords: Hyperbilirubinemia, transcutaneous, infant, smartphone

Introduction

Clinically, hyperbilirubinemia is observed in at least two-third of infants during their first week of life (1).

Risk factors for hyperbilirubinemia include uridine diphosphate-glucuronyl transferase 1A1 polymorphism in breast-fed infants (2), vitamin D deficiency (3), birth weight <2,500 g, pathological weight loss, exclusive breastfeeding (4), glucose 6 phosphate dehydrogenase (G6PD) deficiency (5), ABO and Rh incompatibility (6), late preterm babies (>40 weeks) (7), sibling history of jaundice, cephalohematoma (8), and some drugs used in pregnancy (9).

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Previous studies have reported the various complications of hyperbilirubinemia. A previous study showed a relationship between neonatal total serum bilirubin (TSB) levels and asthma diagnosis before the age of 7 years (10). Cochlear and auditory functions may be affected in babies with indirect hyperbilirubinemia (IHB) (bilirubin levels >20 mg/dL) for a long time (11). Infants with hyperbilirubinemia at birth are at higher risk of being diagnosed with sensorineural hearing loss (12). Hyperbilirubinemia may be associated with neurodevelopmental disorders (13). Kernicterus continues to be reported to date, particularly in developing countries, and this is a public health problem (14). Cases of kernicterus have also been reported Turkey (15,16). Thus, the diagnosis and treatment of hyperbilirubinemia is necessary.

Measurement of TSB levels is one of the methods used in the diagnosis of IHB. Central laboratories typically consider measurement of TSB as the gold standard for the detection of IHB, and this method is used to evaluate the efficiency of other bilirubin measurement methods. However, determination of TSB levels requires collection of venous blood, which is an invasive method and is painful for the infants and may not always guarantee sufficient blood supply (1). The environment in the neonatal intensive care unit is stressful for babies. Separation from the mother and exposure to recurrent pain are the main factors causing stress in these babies. If the recurrent pain in these is left untreated, these infants may experience permanent neurological and behavioral problems in the future and it may impair their pain perceptions and neuroendocrine stress responses. Pain awareness, approach to pain, and pain control and treatment are extremely important in infants. The most effective approach for controlling pain is to reduce painful interventions (17). In addition, early discharge without appropriate follow-up, lack of knowledge of the mother, cultural practices, and the use of traditional treatments may limit or delay the detection of jaundice and its subsequent treatment (18). Therefore, reliable non-invasive interventions are needed to detect hyperbilirubinemia.

The results of a study by Akman et al. (19) showed a significant correlation between the non-invasive method for determining transcutaneous bilirubin (TcB) and TSB measurement; a weak correlation was observed in cases of TSB levels >15 mg/dL, but a significant correlation was observed when TSB levels were <15 mg/dL. Furthermore, measurements of TcB levels can be performed reliably, quickly, and easily in infants for the screening of IHB, and babies at a low risk of IHB can also be detected using this method. Additionally, this method prevents unnecessary blood collection from infants (20). Phototherapy (PT) significantly affects the accuracy of transcutaneous bilirubinometry. TSB evaluation is required when considering the treatment of hyperbilirubinemia via TcB measurement (21).

One of the non-invasive methods for the determination of bilirubin levels is the BiliCam-estimated bilirubin (BCB) method. Taylor et al. (22) compared the BiliCam method, which is a new method for detecting IHB using an application downloaded onto a smartphone and a color calibration card placed on the sternum of the baby, with the method for measuring TSB levels. The results of their study showed that BCB had sufficient accuracy and could be used as a screening method to detect IHB. The results of a previous study showed that hyperbilirubinemia could be detected successfully and rapidly using the smartphone application, and the success rate of detection using this method was 85% (23).

To date, no studies have compared the method for determination of TSB with TcB and the BiliCam method. Comparisons of serum bilirubin concentrations measured using the TSB measurement with those measured using the TcB and BCB methods are required to determine which method is more advantageous and in which situation so that the most appropriate method of measurement may be used according to the specific situations. The diagnosis of IHB in infants is crucial; moreover, it is necessary in order to reduce the effects of IHB complications and invasive interventions on the infant. Therefore, in this study, we compared the method for determining TSB levels, as a reference method, with the TcB and BCB methods for the detection of neonatal hyperbilirubinemia.

Materials and Methods

Setting

This analytical study was conducted in a neonatal intensive care unit of a hospital in Turkey.

Participants

The study sample consisted of late preterm and term infants with low, moderate, and high risk of hyperbilirubinemia in a neonatal intensive care unit, diagnosed with IHB, having a birth weight >1,500 g, and requiring PT. Infants whose families volunteered and gave written consent were included in this study. We included healthy infants at a low risk delivered at 38 weeks, infants at intermediate risk delivered at 38 weeks with the presence of risk factors, healthy infants delivered at 35-37 weeks 7 days, and infants at high risk delivered at 35-37 weeks 7 days with the presence of risk factors. Risk factors included iso-immune hemolytic disease, G6PD deficiency, asphyxia, severe lethargy, heat instability, sepsis, acidosis, and/or serum albumin levels <3 g/dL (1).

On the basis of the power analysis made according to the sensitivity and selectivity values obtained from a reference study (22), 70 infants were included in this study to obtain 80% power at 95% confidence level in the power analysis calculated with an 85% sensitivity value and a 65% selectivity value.

Data Collection

The descriptive information of the infants was recorded via the "neonatal descriptive information form," and the results of measurement of bilirubin levels were recorded with the "measurement results registration form". Bilirubin concentrations were measured using the TcB and BCB methods after blood was collected from the infants for the measurement by TSB; the bilirubin concentrations were measured before and after the commencement of PT. Data were collected between January, 2020 and September, 2020.

Measurements Tools

The neonatal descriptive information form was used in this study. This form includes the sociodemographic characteristics of the infants and those features relating to hyperbilirubinemia. It consists of the following questions: Birth weight, current weight, method of delivery, method of feeding, history of jaundice in siblings, blood type of the baby, maternal blood type, and the presence of risk factors for IHB.

The measurement results registration form. This form includes the results of the bilirubin levels measured using the TSB, TcB, and BCB methods before and after PT.

Measurements using a TcB meter. Before the initiation of PT, the bilirubin levels were measured by placing a TcB meter on the sternum of the baby. When measuring with TcB, the area of the sternum was preferred for hygiene reasons and ease of measurement. After the initiation of PT, the measurements were made by placing the bilirubin meter on the covered hip bone, which was not exposed to PT.

Measurements using a smartphone application and color calibration cards (Figure 1). This application, which is freely available online, was downloaded onto an iPhone 5s. This application was used together with color calibration cards measuring 5×5 cm which had hollow squares with a variety of colors. The color calibration card was downloaded onto the phone using the application and a printout was taken.

When measuring with BCB before PT started, the measurements were made on the sternum of the infant. After the PT had started, the measurements were made over the covered hip bone which was not exposed to PT. Before PT, the sternum area was preferred for hygiene and ease of measurement. Since the sternum was affected by PT, after PT, measurements were made on the covered hip bone. After opening the application on the smartphone, the application is started by placing a color calibration card on the sternum/hip bone of the infant. A red square appears on the smartphone screen. When this red square is matched correctly with the color calibration card and when the lighting is sufficient, it will turn into a green square. The application automatically shows the bilirubin value by capturing both flash and non-flash images using the smartphone camera.

It is recommended that the smartphone be disinfected using 70% isopropanol before and after each measurement to avoid any risk of contamination. Accordingly, we disinfected the smartphone and the TcB meter using 70% isopropanol before and after each measurement. The color calibration card was changed after every measurement.

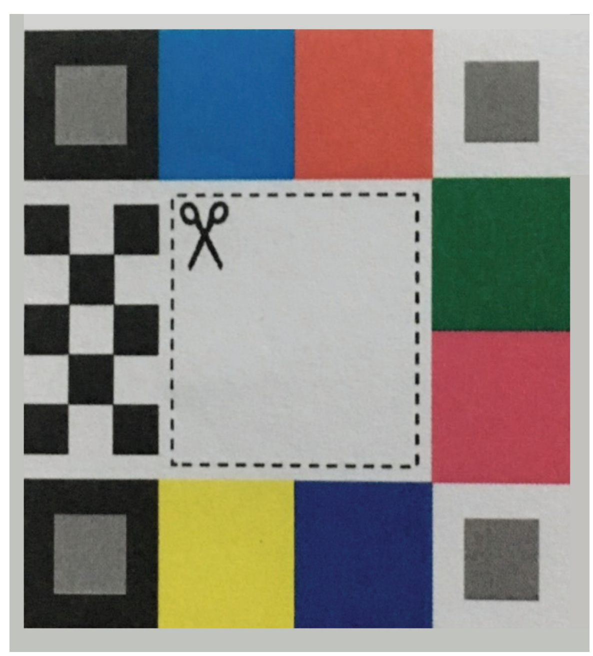

Figure 1. Design of the color calibration card used in our studies

Ethical Considerations

Permission to conduct this study was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee (27.07.2018-E.50623) and the hospital where the research was performed. Parents of the infants included in the sample were informed about this study and their written consent was obtained.

Statistical Analysis

The data obtained from this study were analyzed using the Statistical Package for the Social Sciences (SPSS) 25 package program. Continuous variables were represented as mean ± standard deviation and categorical variables as numbers and percentages. The conformity of the data to normal distribution was examined using the Kolmogorov-Smirnov test. Continuous data showing normal distribution were analyzed using Pearson correlation coefficient, and data that were not normally distributed were analyzed using Spearman's correlation coefficient. Statistical significance was evaluated as $p<0.01$ and $p<0.05$.

Results

The findings regarding the sociodemographic characteristics of the infants included in this study are given in Table I. The average age of the infants was 4.15±1.93 days (range, 1-7 days) and 54.3% of the infants were male.

The birth weight of the infants was 3,161.57±514.14 g and their average weight at the time of this study was 3,007.14±497.56 g. Results regarding the method of delivery showed that 55.7% of the infants were born by normal delivery, and 94.3% of infants had no history of jaundice in their siblings. Determination of the blood groups of the baby showed that 40% of the infants belonged to the A blood group and 90% were Rh positive. Among the mothers, 51.4% belonged to the O blood group, and 12.9% of them were Rh negative. ABO incompatibility was reported in 31.4% of the infants, Rh incompatibility in 14.3% of the infants, and 95.7% of them did not have any risk factors for IHB (Table II).

Table I. Distribution of the sociodemographic characteristics of the infants

	Mean	Standard deviation
Age (days)		
(Minimum-maximum: 1-7 days)	4.15	1.93
	Frequency	**%**
Gender		
Male	38	54.3
Female	32	45.7

Table II. Distribution of the characteristics of infants with risk of hyperbilirubinemia

	Mean	Standard deviation
Birth weight (g)		
(Minimum-maximum: 2,300-4,450)	3,161.57	514.14
Current weight (g)		
(Minimum-maximum: 2,100-4,000)	3,007.14	497.56
	Frequency	**%**
Method of delivery		
Normal	39	55.7
Caesarean	31	44.3
Method of feeding		
Oral (breast milk and formula)	48	68.6
Oral (breast milk)	10	14.3
Oral and intravenous	7	10.0
Intravenous	5	7.1
History of jaundice in sibling		
No	66	94.3
Yes	4	5.7
Baby blood groups		
A	28	40.0
O	24	34.3
B	11	15.7
AB	7	10.0
Rh positivity of the baby		
Positive	63	90.0
Negative	7	10.0
Mother blood groups		
O	36	51.4
	Frequency	**%**
A	20	28.6
AB	10	14.3
B	4	5.7
Rh positivity of the mother		
Positive	61	87.1
Negative	9	12.9
ABO incompatibility		
No	48	68.6
Yes	22	31.4
Rh incompatibility		
No	60	85.7
Yes	10	14.3
Risk factors		
No	67	95.7
Yes	3	4.3

The distribution of bilirubin levels in the infants measured using the TSB, TcB, and BCB methods before and after PT are shown in Table III.

The mean values of TSB, TcB, and BCB before PT were 17.35±4.86 mg/dL, 15.37±3.75 mg/dL, and 14.14±1.75 mg/dL, respectively, whereas these values after PT were 9.16±3.21 mg/dL, 7.35±2.75 mg/dL, and 8.57±2.53 mg/dL, respectively (Table III).

Before PT, a statistically significant positive and strong relationship was observed between the levels of TSBs and TcB values ($p<0.01$). After PT, a statistically significant positive and moderate relationship was determined between the levels of TSB and TcB ($p<0.01$, Table IV).

Before PT, a statistically significant positive and moderate relationship was observed between the levels of TSB and BCB ($p<0.01$). A statistically significant positive and weak relationship was observed between the levels of TSB and BCB after PT ($p<0.05$, Table V).

Table III. Distribution of bilirubin values before and after phototherapy of infants

	Before PT mean ± SD	Lower-Upper	After PT mean ± SD	Lower-Upper
TSB (mg/dL)	17.35±4.86	5.94-33	9.16±3.21	4.19-22.68
TcB (mg/dL)	15.37±3.75	6.4-22	7.35±2.75	1.7-14.2
BCB (mg/dL)	14.14±1.75	9.6-16.9	8.57±2.53	3.7-14.1

PT: Phototherapy, TSB: Total serum bilirubin, TcB: Transcutaneous bilirubin, BCB: BiliCam-estimated bilirubin, SD: Standard deviation

Table IV. Comparison of the relationship between total serum bilirubin levels and bilirubin levels measured using transcutaneous bilirubin measurement in infants before and after phototherapy

	R	p
Before phototherapy	0.765**	0.000
After phototherapy	0.610**	0.000

**The correlation is significant at the 0.01 level

Table V. Comparison of the relationship between total serum bilirubin levels and bilirubin levels measured using BiliCam in infants before and after phototherapy

	R	p
Before phototherapy	0.572**	0.000
After phototherapy	0.283*	0.017

*The correlation is significant at the 0.05 level
**The correlation is significant at the 0.01 level

Discussion

Our results showed that 51.4% of the women had blood group O, and 12.9% of the mothers were Rh (-) (Table II). ABO incompatibility was reported in 31.4% of the infants and 14.3% of them had Rh incompatibility (Table II). ABO and Rh incompatibility is one of the risk factors for IHB (6). Our findings were similar to those reported in previous studies.

The maximum TSB, TcB, and BCB values before PT were 33 mg/dL, 22 mg/dL, and 16.9 mg/dL, respectively. The maximum TSB, TcB, and BCB values after PT were 22.68 mg/dL, 14.2 mg/dL, and 14.1 mg/dL, respectively (Table III).

Taylor et al. (22) determined that a cut-off value of 13 mg/dL for BCB. Ercan and Özgün (24) recommend using a TcB cut-off value of 222 µmol/L (12.98 mg/dL). Chokemungmeepisarn et al. (25) recommended using a cut-off value of + 3 mg/dL for TcB. Hulzebos et al. (26) reported the cut-off value of TcB as + 50 µmol/L (2.92 mg/dL). These studies suggest an approximate cut-off value of 13 mg/dL when using these two non-invasive measures. Since our study was conducted without using a cut-off value, high bilirubin values negatively affected our study results.

Thus, our results show a strong relationship between TSB and TcB measurement values before PT, whereas a moderate relationship was observed in these values after PT (Table IV).

Castro et al. (27) showed a significant positive correlation between TSB and TcB in regions not exposed to PT. However, since the reliability of these results was not established, they do not recommended using these results as a guide for clinical decisions regarding the duration of PT (28).

Previous studies examining the reliability of bilirubin measurement methods showed that the measurement of TcB on a skin patch after PT was found to be reliable (28-30).

Hulzebos et al. (26) measured bilirubin levels over the covered hip bone in premature babies with a gestational age of ≤32 weeks. The results of the study by Hulzebos et al. (26) showed that the requirement of a 40% reduction in TSB levels was achieved by using TcB + 50 µmol/L (2.92 mg/dL) as the cut-off level.

We measured bilirubin levels with TcB after PT over the covered hip bone, that is, from under the diaper. The results obtained may not be consistent because of the difficulty in completely protecting the measurement area from PT light because of the constant movement of the baby.

Our results showed a moderate correlation between TSB measurement values before PT and BCB measurement values. After PT, a weak correlation was found (Table V). Taylor et al. (22) reported that a cut-off of 13 mg/dL could be used for BCB before PT. To date, no study has investigated the effect of PT on BCB measurement.

In our study, the bilirubin measurements with BCB were made over the covered hip bone, that is, under the diaper, after PT. This result is thought to be affected by the difficulty of fully protecting the measurement area from PT light due to the baby being mobile, and the difficulty in fixing the color calibration card due to the area where the measurement was made.

Study Limitations

Limitations of this research; those with a gestational age of ≤35 weeks and an age of >7 days and those who had received PT treatment previously were not included in this study. Non-invasive measurements were made from the sternum before PT and over the covered hip bone after PT. No cut-off value was used in TSB values.

Conclusion

Comparison of TSB with TcB and BCB is necessary to determine which method is more advantageous and in which situation to use the most appropriate measurement method for the detection of IHB in order to decrease the effects of IHB complications and limit invasive procedures on the infant. We compared TSB as a reference method with TcB and BCB for the detection of neonatal hyperbilirubinemia. Our results show that TcB and BCB measurement methods can be used for the detection of IHB, considering TSB as a reference method. The TcB and BCB measurement methods can be used to detect bilirubin in the clinic, but if a change in treatment is considered, it should be confirmed by using TSB levels. As our study was conducted without using cut-off values, it may not be reliable for high bilirubin values. In terms of cost and accessibility of the device, the use of a transcutaneous device in neonatal intensive care units may reduce the need for blood sampling for TSB. For TcB and BCB measurements after PT, protecting the measurement area from PT light can increase the reliability of both BCB and TcB measurement values.

Further studies should be performed in order to determine the accessibility and ease of use of the BiliCam method by the parents. Active use of the BCB method can detect IHB.

Ethics

Ethics Committee Approval: Permission to conduct this study was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee (27.07.2018-E.50623) and the hospital where the research was performed.

Informed Consent: Parents of the infants included in the sample were informed about this study and their written consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Z.K.B., B.Ç., Desing: Z.K.B., B.Ç., Data Collection or Processing: Z.K.B., B.Ç., Analysis or Interpretation: Z.K.B., B.Ç., Literature Search: Z.K.B., B.Ç., Writing: Z.K.B., B.Ç.

Conflict of Interest: The authors declared that there were no conflicts of interest.

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