Niger J Paediatr 2019; 46 (1):30 – 38

ORIGINAL

Akinbolagbe YO
Ezeaka VC
Akinsulie AO

Evaluation of the Bilicheck® transcutaneous bilirubinometer in jaundiced Nigerian term and preterm neonates

DOI:http://dx.doi.org/10.4314/njp.v46i1.6

Accepted: 19th February 2018

Akinbolagbe YO (✉)
Department of Paediatrics,
Lagos University Teaching Hospital, Idi-araba, PMB 12003.
Lagos, Nigeria.
Email: yesidekush@yahoo.com

Ezeaka VC, Akinsulie AO
Department of Paediatrics,
College of Medicine, University of Lagos/ Lagos University Teaching Hospital, Idi-araba, PMB 12003.
Lagos, Nigeria

Abstract*: Background*: Transcutaneous bilirubinometry has shown some promise as a safe, non-invasive method that correlates well with traditional laboratory methods of estimating serum bilirubin. However there is a paucity of studies done on neonates of African descent.
Aim: The aim of the study was to evaluate the diagnostic performance of the Bilichek® transcutaneous bilirubinometer in jaundiced Nigerian term and preterm neonates.
Methods: This was a cross-sectional study involving 169 jaundiced preterm and term neonates studied over a 4 month period. A total of 200 transcutaneous bilirubin (TcB) readings determined using the Bilichek® device were obtained simultaneously with total serum bilirubin (TSB) readings determined by the diazo method.
Results: The mean (SD) difference between TcB and TSB was 1.5 (2.6) mg/dL (95% CI of 1.2 - 1.9 mg/dL); p= 0.000. There was strong correlation between TcB and TSB with a correlation coefficient (r) of 0.77 (p = 0.000). The 95% limits of agreement were between -3.5mg/dL and 6.6mg/dL. There was poor correlation between TcB and TSB of r= 0.43 at high TSB levels >12mg/dl. The TcB cut-off that most accurately predicted TSB was 11.7mg/dL.
Conclusion: Transcutaneous bilirubinometry is a reliable screening method for assessing severity of hyperbilirubinaemia in black African neonates. However, due to the occurrence of wide disparities, confirmatory serum bilirubin measurements should be done for TcB values above 11.7mg/dL.

Introduction

Neonatal jaundice (NNJ) is one of the most common presenting complaints in the newborn period, occurring in 30-60% of term infants and almost all preterms.[1] In the United States of America, it affects about 60% of term babies and 80% of preterms.[2] In Kenya, Africa, prevalence is about 35%.[3] In Nigeria, mostly from hospital based studies, the prevalence of neonatal jaundice is between 16.9 and 45.6%[4-8] and as high as 71.2% among preterms.[9] Though most cases are self-limiting and do not require treatment, NNJ can lead to serious and life-threatening complications like acute bilirubin encephalopathy (ABE), cerebral palsy and deafness.[10]

The management of neonatal jaundice largely depends on accurate assessment of bilirubin levels. There are various methods of estimating the level of bilirubin in the blood. The methods can be direct from invasive blood sampling or indirect, measured non-invasively via the skin. The gold standard method is High Performance Liquid Chromatography (HPLC), which can assay all forms of bilirubin, including protein-bound bilirubin.[11] Other direct methods include coupling reactions (diazo and vitros), enzymatic methods and spectrophotometric methods.[12] Because it is expensive, cumbersome and highly technical, HPLC is rarely used in clinical practice.[13]

The indirect methods are visual assessment, icterometer, and transcutaneous bilirubinometry which measure bilirubin in the skin as an estimate of total serum bilirubin. Studies have shown high correlation between the intensity of the yellow colour of the skin and serum bilirubin levels.[14-16] Bilirubin estimation from the blood by various laboratory methods is currently the most accurate and widely used method, though fraught with its own deficiencies.[17-19] They require specialized personnel and equipment which are not always readily available in resource poor countries. Repeated blood sampling is also often required with the attendant risk of infection to both patient and healthcare provider. This can also lead to iatrogenic anaemia which is particularly an issue in preterm babies with their smaller blood volumes. These limitations have stimulated a lot of research on transcutaneous bilirubinometry, which started gaining some credence following the development of the first transcutaneous bilirubinometer for clinical use in

Japan during the 1980s.[15] These earlier types such as JM101® and JM102® though useful for screening and research purposes were found to be grossly inaccurate in neonates with low gestational age, low birth weight, dark skin pigmentation and during phototherapy use.[15,20]

The Bilichek® ((Respironics inc., Murrysville, PA, USA) a non-invasive Bilirubin Analyzer,[21] is a multi-wavelength transcutaneous bilirubinometer. It was developed with refinement of the old technology, to counteract the earlier defects or inadequacies. It works by directing light into the skin of the neonate, analyzing the spectrum of optical signals reflected from the neonate's subcutaneous tissues and converting them to an electrical signal by a photocell which is analyzed by a microprocessor to generate a serum bilirubin value. The major skin components which impact the spectral reflectance in neonates are: melanin, dermal maturity, haemoglobin and bilirubin.

The newer multi-wavelength meters such as Bilichek®, subtract the spectral contribution of the known skin components and the bilirubin absorbance is thus quantified, with results displayed directly in either mg/dl or µmol/L.[21] Various studies done worldwide, have reported the device to be quite accurate in estimating serum bilirubin with good correlation, agreement, sensitivity, specificity, and positive predictive value when compared with various laboratory methods.[13,17,22,23] Its accuracy has also been found not to be affected by skin colour, birth weight, gestational age, post-natal age[17,24,25] and phototherapy, if measurement is taken on a patched part of skin.[13,26] Bhutani et al[22] in the US, compared the Bilichek® device with HPLC and found it to be accurate with a good correlation of r = 0.91, mean difference [HPLC-Transcutaneous bilirubin (TcB)] of 0.47mg/dl, sensitivity of 100%, specificity of 88.1%, negative predictive value of 100%, positive predictive value of 32.8% and likelihood ratio of 8.43. However, this study had preponderance of Caucasian neonates who were mostly term and of normal birth weights.

There is a paucity of literature on studies evaluating the utility of the Bilichek® device in Africa, with populations of predominantly black neonates. An earlier study done in 2004 by Slusher et al[24] in Jos, and a recent one by Olusanya et al[27] in Lagos, both in Nigeria found good correlation between the device and serum bilirubin. However they also both obtained wide disparities between both methods, with overestimation by the Bilichek® device, compared with studies done in Caucasian subjects. Rylance and colleagues[28] in Malawi also documented similar findings of good correlation. Some of these African studies excluded patients with certain characteristics like pre-terms, sick neonates, out-born neonates and those receiving phototherapy. This study therefore, aimed to fill some gaps in knowledge about the diagnostic performance of the Bilichek® transcutaneous bilirubinometer, among jaundiced neonates of African descent, in a typical hospital setting.

Materials and Methods

Study site and source population

The study location was at a tertiary health institution, Lagos University Teaching Hospital, in Lagos State. Lagos is a metropolitan megacity in southwest Nigeria. The hospital has about 761 beds, with an average of 170 babies delivered in the labour ward complex monthly. An approximate number of 30 and 33 cases of neonatal jaundice are managed per month in the inborn NICU and Children's Emergency Room/ out born NICU respectively with about 25% of them being preterm neonates. Apparently healthy jaundiced neonates are also seen at the post-natal ward with their mothers or at the outpatient clinic on follow up after discharge from hospital. Averages of 2500 babies are seen annually in these locations at the hospital.
Ethical approval was obtained from the Health research ethics committee of the Hospital. Written informed consent was obtained from the parents of the participants. No extra cost was incurred by the patients on account of the study.

Study design

This was a cross-sectional study among jaundiced neonates involving clinical and laboratory data.

Selection and description of participants

The study was carried out over a four-month period (August 2013 through November 2013). All jaundiced neonates for whom a serum bilirubin measurement was required were eligible for the study. Inclusion criteria were:

1. Postnatal age from birth to 28 days of life,
2. Gestational age of 27 to 41 weeks,
3. Weight range between 1000 and 4995 grams.

These criteria were selected according to the category of patients for which the manufacturers of the Bilichek® device stated that its performance had been clinically proven.[21]
Exclusion criteria[21] were:

1. Exposure to phototherapy before recruitment (those patients used for the study while receiving phototherapy, had an area of forehead patched before commencement),
2. Undergoing an exchange blood transfusion,
3. Skin lesion on measurement site (forehead) such as bruising, nevus, haemangioma, haematomas, birthmarks and excessive hairiness.

The sample size was determined using the formula for difference of means.[29] From previous study by Leite et al: difference between mean transcutaneous bilirubin (TcB) and total serum bilirubin (TSB) = 0.72 mg/dL and standard deviation of the difference between mean TcB and TSB = 1.57.[23]By substituting in the formula, using a power of 80%, the minimum sample size was calculated to be 154.8; this was rounded off to 155 paired readings. A total of 200 paired readings were done to account for incomplete data, invalid measurements or

lysed blood samples. Paired reading refers to measurement of different variables (TcB and TSB) on the same subject done simultaneously or within a short interval of each other. Patient selection was consecutive until sample size of number of paired readings calculated was reached. Multiple paired readings were done on patients who required repeat bilirubin estimations.

After assessing for the inclusion and exclusion criteria, paired transcutaneous bilirubin measurement (average of five measurements) and blood sample collection for serum bilirubin estimation was done within 30 minutes of each other.[13,23,26]

Blood sample collection and analysis

Blood sample collection for the TSB measurement was by venepuncture as routinely done in the hospital. The specimen bottles were wrapped with aluminum foil paper to protect the specimen from light and avoid photo conversion of bilirubin; then transported to the departmental laboratory and analyzed immediately with a time lag of not more than 15 minutes.[23] Sample analysis was carried out at the Department of Paediatrics Research Laboratory. This laboratory routinely processes about 60 -70% of blood samples from paediatric patients in the hospital for various investigations including serum bilirubin. The total and conjugated bilirubin was quantified by the Malloy and Evelyn (Diazo) Method[30] and the bilirubin values read using the SFRI BSA-3000® Chemistry Auto Analyzer, France according to the manufacturer's instructions. The working reagent used to obtain the diazo derivative was prepared freshly daily, as well as daily calibration and standardization of the spectrophotometer. The sample analysis was done in conjunction with two dedicated laboratory scientists with regular inter-rater comparison of results. Quality control was done 2-4 weekly by analysis of the same sample in two other laboratories in the hospital (Main Chemical Pathology laboratory and Department of Medicine laboratory). Comparison of results was then undertaken with necessary adjustments and recalibration of equipment done if necessary.

Transcutaneous bilirubin measurement

The transcutaneous measurement was done with the non -invasive Bilichek® bilirubin analyzer (Respironics inc., Murrysville, PA, U.S.A.). This is a handheld device that estimates total serum bilirubin via the skin at multi-wavelengths. Measurement was done according to the manufacturer's instructions.[21] For each measurement, a disposable calibration tip (Bilical®) was attached to the fibreoptic probe. If calibration was successful, five consecutive measurements were taken from the forehead, before an average final reading was displayed either in mg/dL or µmol/L according to the setting fixed. For the purpose of this study, the device was set to display results in mg/dL. The process took an average of 30 seconds to 1minute. For patients requiring phototherapy, a phototherapy protective patch (BiliEclipse®) was applied to the forehead before commencement of treatment and subsequent TcB measurements for them were done at the patched area. All transcutaneous measurements were performed by the same researcher (AY).

Data Analysis

Data was initially entered into a Microsoft Excel spreadsheet (2007 version) then analysis was done using the Statistical Package for the Social Sciences (SPSS) for windows software version 20.0. Means and standard deviations were calculated for continuous variables. The difference between means of TcB and TSB were compared using the paired Student's t-test. Linear regression equation of TcB on TSB was generated.
Pearson's correlation co-efficient (r) and coefficient of determination (R^2) were calculated to assess the relationship between the two methods of measurement. Agreement between TcB and TSB was determined using the method of Bland and Altman;[31] the laboratory method was used as the 'gold standard.' For the purpose of the study, the pre-determined acceptable limit of agreement between TcB and TSB was taken as 30 µmol/L or 2 mg/dL.[26] Limits of agreement of the mean differences were given as 95% confidence intervals (CIs).

The performance of the Bilichek® device at high levels of TSB was assessed by segregating subjects into two groups; ≤ 12 mg/dL (low) and >12 mg/dL (high). These levels were based on the institutional protocol of initiating treatment of NNJ at TSB levels of above 12 mg/dL for term babies (depending on postnatal age and presence of risk factors, this level could be lower or higher). For pre-terms the level for treatment is based on the weight (10 × infant weight in kg) or the same level for term babies, whichever is lower. Correlation coefficients and Bland-Altman plots were also generated for these two groups.

The sensitivity and specificity of the TcB measurement to predict accurately the TSB at a range of values was estimated and plotted on receiver operator characteristic (ROC) curves. The interpretation of the area under the curve is as follows: 0.90 - 1(excellent); 0.80 - 0.90 (good); 0.70 - 0.80 (fair); 0.60 - 0.70 (poor); 0.50-0.60 (fail). The TcB cutoff point with the best sensitivity and specificity to predict TSB was determined. The level of statistical significance was set at $p < 0.05$.

Results

A total of 200 paired readings were taken from 169 babies (multiple readings were taken from some babies). The babies were all of Nigerian origin. The study population consisted of 93 (55.0%) preterm neonates and 76 (45.0%) term neonates. One hundred and twenty and 80 paired TcB and TSB readings were carried out on the two groups respectively. Ninety-one (53.8%) males and 78 (46.2%) females were studied, giving a male: female ratio of 1.2: 1. One hundred and four (61.5%) patients were inborn while 65 (38.5%) were out-born. Table 1

summarizes the baseline characteristics of the subjects.

Table 1: Baseline characteristics of the study population

Variable	n (%) (N=169)	Range	Mean(SD)
Gestational age (weeks)		27-41	35.0 (3.9)
27 - 30	30 (17.8)		
31 - 33	32 (19.0)		
34 - 36	31 (18.3)		
37 - 39	40 (23.6)		
40 - 42	36 (21.3)		
*Birth weight Groups**		1000-4990	2335 (880)
VLBW	40 (23.6)		
LBW	54 (32.0)		
NBW	75 (44.4)		
Sex			
Male	91 (53.8)		
Female	78 (46.2)		
	M:F; 1.2:1		
Postnatal age (hours)		20-456	107 (72)
Site of Birth			
Outborn	65 (38.5%)		
Inborn	104 (61.5%)		
Phototherapy use			
Phototherapy	30 (17.8%)		
No phototherapy	139 (82.2%)		

*VLBW= very low birth weight; LBW= low birth weight; NBW= normal birth weight

Five neonates had TcB readings of 'HIGH', indicating values of above 20 mg/dL. These TcB measurements and their corresponding TSB values were excluded from analysis. This was due to the inability of the transcutaneous bilirubinometer to generate a numeric value for the TcB readings, which made it impossible to assess correlation and differences between the two measurements. Therefore 195 paired readings were eventually analyzed. The number of paired readings per subject ranged between one and three taken between 24-96hr intervals, with 145 (85.8%) of the babies having only one paired reading taken from them. TcB readings were generally higher than TSB levels for most of the paired measurements; 148 (76%). TcB readings were lower than TSB values in 41 (21%) of the paired measurements and were the same as TSB values in 6 (3%) of the paired measurements. Seventy-seven (52%) out of 148 TcB readings, were higher than corresponding TSB values by more than 2 mg/dL, while 17 (41.5%) out of 41 TcB readings, were lower than corresponding TSB values by more than 2 mg/dL. Thirty-three readings were taken from patients receiving phototherapy. Thirty-two of them were preterm while only one was a term neonate. The five excluded paired readings were all from preterm neonates.

The mean (SD) of TcB readings was 11.3 (3.8) mg/dL. This was higher than the mean (SD) TSB level of 9.8 (3.9) mg/dL with a difference of 1.5 mg/dL, SD of 2.6 mg/dL and 95% CI of 1.2-1.9 mg/dL. This difference was statistically significant with a p value of 0.000, t = 8.147.

The overall correlation coefficient (r) between TSB and TcB was 0.77 ($p < 0.001$). Figure 1 shows the scatter diagram of the regression between TcB and TSB.

The correlation coefficient (r) between TSB levels and TcB readings obtained from preterm neonates (gestational age less than 37 weeks) was 0.80 while that for term neonates (gestational age 37 weeks and above) was 0.73. Both correlation coefficients were strongly positive with p values of 0.000. The correlation coefficient in preterm neonates was higher than that for term neonates, however this difference was not statistically significant (p = 0.37, Z - 0.899).

Fig 1: Linear regression of TcB on TSB for all the readings

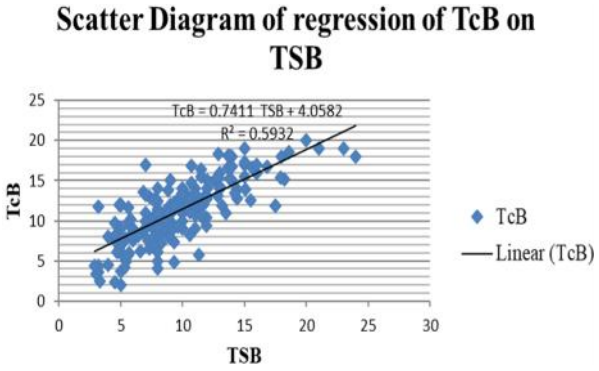
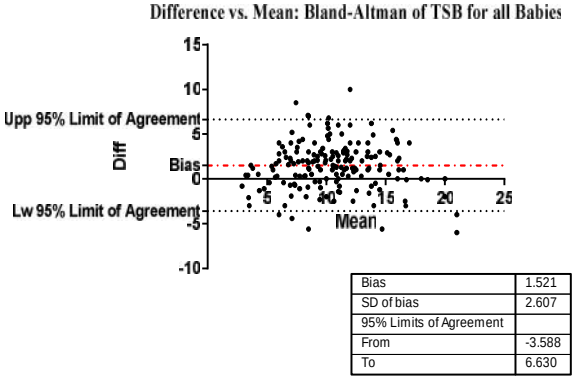


Figure 2 is a Bland-Altman plot of the paired readings showing the agreement between TSB and TcB. It is a plot of the differences between TcB and TSB against the mean or average of the paired values. The red broken line represents the bias (mean TcB-TSB), while the black broken lines show the Upper (UP) and Lower (Lw) limits of agreement (95% Confidence interval of the bias or mean difference). The adjoining table shows the figures for the bias, standard deviation (SD) of bias and the 95% Limits of agreement. It shows a bias of 1.5mg/dL and 95% limit of agreement (imprecision) of between -3.5mg/dL and 6.6mg/dL.

Table 2 shows the correlation between TcB and TSB for the ≤ 12 mg/dL TSB group and the > 12 mg/dL TSB group while Table 3 shows the difference in means of TcB and TSB for the 2 TSB groups. The correlation coefficient (r) between TSB and TcB was 0.64 for the ≤ 12mg/dl TSB group and 0.43 for the ≥ 12mg/dl TSB group.

Fig 2: Bland-Altman plot of differences between TcB and TSB against average or mean of TcB and TSB for all subjects. *Upp (Upper), Lw (Lower), Diff (difference).*



The agreement between TcB and TSB at the two different TSB level groups was also determined by the

method of Bland and Altman. Figures 3 and 4 show the Bland-Altman plots of the difference between TcB and TSB against the mean of TcB and TSB for the ≤ 12 mg/dL and >12 mg/dL TSB level groups respectively. The limits of agreement for the "low" TSB group were between -3.0 and 6.7 mg/dL while that of the "high" TSB group were between -8.0 and 7.8 mg/dL.

Table 2: The correlation between TcB and TSB at TSB levels ≤ 12 mg/dL and > 12 mg/dL

	TSB ≤ 12 mg/dL	TSB > 12 mg/dL
Number of readings	152	43
Correlation coefficient (r)	0.636	0.429
Coefficient of determination (R^2)	0.40	0.18
P value	0.000	0.004
Regression equation	TcB = 3.45 + 0.80TSB	TcB = 10.23 + 0.35TSB

The receiver operating characteristic (ROC) curve plotted to assess the accuracy of TcB to predict TSB is shown in Figure 5. The ROC curve was obtained by plotting the sensitivity and specificity of TcB to predict TSB (gold standard test) at different TcB cut-off levels. In this curve, an area of 1 represents perfect sensitivity and specificity of 100% each. The area under the curve (AUC) for the two diagnostic modalities in this study revealed an area of 0.73 (standard error of 0.05 and 95% CI of 0.63 - 0.83). This showed that the accuracy of TcB as a diagnostic method to predict TSB is "fair." The best TcB cutoff point which maximized sensitivity and specificity to produce this AUC was 11.65 mg/dL with sensitivity of 0.87 and 1- specificity of 0.452.

Table 3: Difference in mean bilirubin readings of subjects at different TSB levels

	TSB ≤ 12 mg/dL	TSB >12 mg/dL
Number of readings	152	43
Mean TcB (SD) mg/dL	10.1 (3.1)	15.7 (2.3)
Mean TSB (SD) mg/dL	8.2 (2.5)	15.4 (2.8)
Mean difference (95% CI) mg/dL	1.9 (1.5 - 2.3)*	0.3 (-0.5 - 1.1) **

Key: Paired t-test comparison between TcB and TSB for TSB values ≤ 12mg/dL and > 12mg/dL; *t statistic 9.349, degree of freedom = 151, p = 0.000, ** t statistic 0.675, degree of freedom = 42, p = 0.503.

Fig 3: Bland-Altman plots of difference between mean TcB and TSB and mean of TSB and TcB for the ≤ 12 mg/dL TSB group. *Upp (Upper), Lw (Lower), Diff (Difference).*

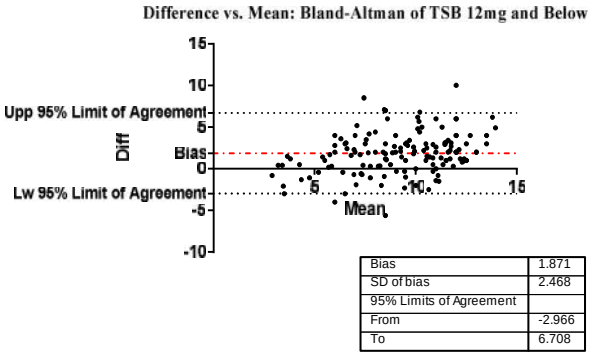


Fig 4: The Bland-Altman plot of the difference between mean TcB and TSB against the mean of TcB and TSB for the > 12 mg/dL TSB group. *Upp (Upper), Lw (Lower), (Diff) Difference.*

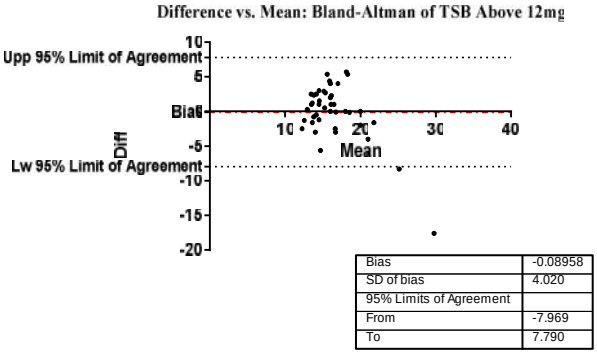
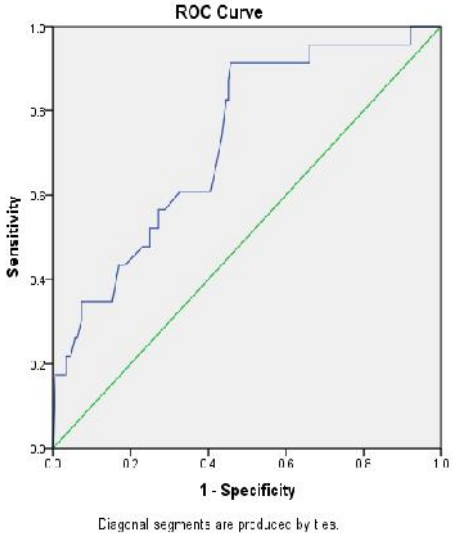


Fig 5: Receiver Operating Characteristic (ROC) curve of plots of sensitivity and 1-specificity of TcB levels in predicting TSB as gold standard test. The blue line represents the curve of the plots.



Discussion

This study revealed that the transcutaneous bilirubin (TcB) readings generally overestimated the corresponding total serum bilirubin (TSB) levels. Also the TcB readings obtained with the Bilichek® device, had strong positive linear correlation with TSB levels. This finding was consistent with previous studies[13,17,22-24,32,33] carried out both in Nigeria and in other regions worldwide. However, the correlation in the present study (r = 0.77) was found to be slightly lower than that of others which ranged from r of 0.79 to 0.98.[13,17,23,24,27,32-35]

These differences could be due to variations between the studies in relation to type of transcutaneous bilirubinometer, laboratory method used for TSB analysis, and population of neonates studied. For instance in other studies done in Nigeria; Owa et al[32] used a different transcutaneous bilirubinometer, JM 102®; Kayode-Adedeji et al[33] used the JM103; Olusanya et al[27] used both Bilichek® and JM 103® and Slusher et al[24] used the Bilichek® device. Rylance et al[28] in Malawi, used

the JM 103®. Furthermore, Owa et al[32] studied well, term neonates; Kayode-Adedeji and colleagues[33] studied only preterms; Olusanya et al[27] studied well, term, inborn neonates; Rylance et al[28] studied both in and outborn, preterm and term neonates, excluding only extremely premature and very sick neonates; while the present study and that of Slusher et al[24] were carried out on both preterm and term neonates born in and out of the hospital, regardless of their health status. Some of the other studies,[13,17,22] had study populations consisting mainly of term and well Caucasian subjects, this could also account for the disparity in the correlation coefficients observed.

There was strong linear correlation between TcB and TSB for both preterm (r-0.80) and term neonates (r-0.73) with no statistically significant difference between the correlation coefficients obtained for the two groups. This finding was in line with previous studies done by Deluca et al[25] in Italy and Ahmed and colleagues[36] in the UK who both documented good correlation between TcB and TSB in preterm neonates. Contrary results were reported by Jangaard et al[26] in Halifax, Nova Scotia, of poorer performance of the Bilichek device in preterm neonates, though they stated their small sample size of 63 readings in preterm neonates as a limitation of their study.

The coefficient of determination (R^2) obtained by this study supported the linear relationship between TcB and TSB, but this was not very strong. Also, the slope of 0.74 and intercept of 4.02 obtained from the regression equation generated in the present study, were consistent with values of 0.73 and 4.5 obtained by Slusher and colleagues[24] but contrary to other studies which had higher R^2, low intercepts of approximately 1 mg/dL and slopes close to one.[13,33,34]

The high intercept and low coefficient of determination obtained by this present study indicated the presence of unknown factors or variables contributing to the TcB readings generated which resulted in the TcB readings generally being higher than the corresponding TSB levels. Slusher and colleagues[24] had postulated that the reason for this high intercept could be due to the presence of light absorbing non bilirubin, nutrition-derived yellow pigments (carotenoids) in the skin of the infants which could interfere with TcB measurements since they share similar physical and optical characteristics with bilirubin.

This may also hold true in the population of the present study, since babies here are from the southwest region of Nigeria where their mothers consume diet rich in carotenoids similar to those studied by Adelekan et al[37] which can be transferred to them through breastmilk. However this has not been corroborated.

Mean TcB readings obtained by the Bilichek device® were found to overestimate mean TSB values, with a statistically significant difference. This finding was consistent with other studies done in Nigeria by Slusher et al,[24] and Olusanya et al;[27] in Europe by Ahmed et al[36] and De Luca et al;[25] and by Karon and colleagues in the US.[17] Conversely Bhutani et al[22] in the US, Rubaltelli et al in Europe[13] and Jangaard et al in Canada[26] found an underestimation of TSB by TcB. There was an overestimation of > 2mg/dl in about 52% of the babies compared to the finding by Olusanya et al[27] of 64.5%. Similar findings of wide disparities between TcB and TSB of >2-3mg/dl were also obtained from other studies by Rylance et al[28] in Malawi and Taylor and colleagues[38] in the USA. This US study found this disparity more in black neonates than in Caucasians.

It was noticed that most of the studies above that found an overestimation of TSB had either predominantly black or preterm subjects. This would further support the contribution of melanin and reduced subcutaneous tissue to increased TcB measurements as proposed by Yamauchi[15] and shows that the technology improvements made in the Bilichek device® may not be adequately eliminating the effect of these factors.

Regarding performance of the Bilichek device® at different levels of TSB, the present study found weaker correlation between TcB and TSB at “high” levels of TSB above 12 mg/dL. This finding was also reported by other studies,[23,34,39] but was contrary to the study by Slusher et al[24] who found a stronger correlation at levels above 12 mg/dL. Slusher et al[24] however obtained an underestimation of TSB at “high” levels of TSB which was not corroborated in this study. This may be due to the small sample size of neonates with “high” TSB levels of above 12 mg/dL, compared to the Slusher et al[24] study which consisted of a large population of neonates with severe hyperbilirubinaemia. There was also a poorer agreement between TcB readings and TSB levels, at higher TSB levels similar to the Slusher et al[24] study.

The clinical implication of overestimation of TSB by TcB would be the tendency to increase the number of neonates requiring treatment, but would ensure no case of severe NNJ is missed. In the present study, 152 out of the 195 readings had TSB values 12 mg/dL and below, if only transcutaneous bilirubinometry was applied on them, 78% of blood sampling for TSB measurement would have been avoided. On the other hand, poor agreement obtained, especially at high TSB levels with differences as high as 8mg/dl between TcB and TSB, would necessitate confirmation of the total serum bilirubin level by another method directly from the blood.

Due to the finding of poor agreement between TcB and TSB at high bilirubin levels, it was expedient to find a cut off level of TcB beyond which its accuracy or reliability was reduced and the assessment of severity of hyperbilirubinaemia by another method may be necessary. This was done by generating ROC curves. A TcB level of 11.7 mg/dL (200 µmol/L) was obtained with an area under the curve (AOC) of 0.73. The TcB cut-off levels obtained by other studies are 14 mg/dL (239 µmol/L) with AOC of 0.98 by Leite et al;[23] 15.2 mg/dL (260µmol/L) by Jangaard et al;[26] bilirubin index of 20 (TSB level of 9.9 mg/dL) by Owa et al;[32] 10.3 mg/dL

with AOC of 0.706 by Olusanya et al;[27] and 14.6 mg/dL (250 µmol/L) by Boo and Ishak.[34] The differences in cut -off levels are likely due to population differences as well as the different methods of obtaining them.

The present study has demonstrated that transcutaneous bilirubin measurements with the Bilichek device correlate with total serum bilirubin levels in neonates of African descent. It has proven to be a reliable screening method to determine the severity of hyperbilirubinaemia in clinically jaundiced babies. However, the finding from this study, of the possibility of wide disparities between TcB and TSB, as well as lower sensitivity and specificity especially at bilirubin levels >11.7 mg/dL, precludes the use of the Bilichek device as a substitute for laboratory measurements. Confirmatory serum bilirubin measurements are recommended at these levels, as well as for any neonate for which the TcB reading suggests need for treatment such as preterm neonates, who may require treatment at bilirubin levels below 12mg/dL.

Due to the ease of use, portability and lack of dependence on electricity, its use transcends the hospital setting and can be used even in primary health care centers in rural communities and homes, for bilirubin monitoring, referral and follow up of patients. Transcutaneous bilirubinometry has been found to be a more accurate screening method for determining severity of hyperbilirubinaemia and need for blood sampling compared to visual assessment[40,41] which is the current method used in most centers in Nigeria. It is currently being used in developed countries like the United Kingdom and United States of America, where it has helped to reduce the number of readmissions for severe NNJ[42] and save costs, as unnecessary blood sampling and laboratory measurements are avoided.[43,44] However its utility in predominantly black neonates as a sole means of bilirubin measurement is somewhat limited, due to the overestimation (with some wide differences) obtained between the instrument and laboratory methods.
Another major drawback of the device as compared to other transcutaneous bilirubinometers such as the JM 103® is the added cost of the disposable calibration pads. This can be circumvented by subsidy of the cost by the manufacturers for low income countries such as ours, or refinement of the technology to make them reusable. More studies should be done to evaluate the effect of melanin on the accuracy of TcB measurements. This would help in the development of transcutaneous bilirubinometers with less disparity of results compared with laboratory methods in neonates of African descent. Thus, the Bilichek® Transcutaneous Bilirubinometer can become an indispensable tool in the management of neonatal jaundice, aiding in early determination of treatment and reduction of blood sampling by health personnel, to achieve Nigeria's goal of reducing the great morbidity and mortality from this easily treatable condition.

Conclusion

Transcutaneous bilirubinometry is a simple, fast and reliable screening method for assessing severity of hyperbilirubinaemia in black term and preterm neonates which can reduce the need for blood sampling. However due to the occurrence of wide discrepancies, it is recommended that confirmatory serum bilirubin measurements are done for neonates with high TcB levels of > 11.7mg/dL or any TcB level suggesting need for treatment.

Acknowledgements

We would like to acknowledge the following individuals who contributed to the success of this work. The Residents and the Laboratory scientists in the Department of Paediatrics of the Lagos University Teaching Hospital for their assistance with blood sample collection and analysis. Dr Remi Ogundimu for help in procurement of the Bilichek® device and Mr Samuel Bodunrin for statistical analysis of data.

Authors' contributions
Akinbolagbe YO: concept and design; data acquisition; analysis and interpretation of data; manuscript preparation, editing and review.
Ezeaka VC: study design; analysis and interpretation of data; manuscript preparation, editing and review.
Akinsulie AO: study design; analysis and interpretation of data; manuscript preparation, editing and review.
Conflict of Interest: None
Funding: None

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