Transcutaneous Bilirubin Measurements and Serum Total Bilirubin Levels in Indigenous African Infants

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ABSTRACT. Objective. The objective of this study was to determine whether transcutaneous bilirubin (TcB) measurements correlate with serum total bilirubin (STB) levels in indigenous, darkly pigmented African newborns with varying degrees of skin pigmentation, some of which had developed kernicterus.

Methods. Jaundiced infants who were ≤2 weeks of age and admitted to Baptist Medical Center-Eku (Eku; n = 29) and Jos University Teaching Hospital (Jos; n = 98) in Nigeria were studied. TcB measurements using the BiliChek were made simultaneously with blood sampling for STB measurements by spectrophotometry before phototherapy.

Results. Using linear regression analysis, we found that measurements of TcB correlated well with those of STB with r values of .90 and .88 for Eku and Jos, respectively. Mean bias and imprecision of TcB measurements as compared with STB measurements for the total population was 0.5 ± 7.6 mg/dL using the method of Bland and Altman. At STB ≥12 mg/dL, correlation (r = .84) and bias and imprecision (−1.2 ± 8.6 mg/dL) of measurements were only slightly poorer. Furthermore, when infants were grouped by degree of skin pigmentation, correlations of TcB and STB measurements remained strong.

Conclusions. From these results, we can conclude that TcB measurements are a useful and reliable index for estimating STB levels in pigmented neonates, including those with hyperbilirubinemia and kernicterus. In the absence of reliable STB measurements, the relatively simple and noninvasive TcB measurements can be an important adjunct in directing phototherapy and exchange transfusions, thereby preventing bilirubin-induced morbidity and mortality in low-technology clinical environments. Pediatrics 2004;113:1636–1641; hyperbilirubinemia, jaundice, kernicterus.

ABBREVIATIONS. STB, serum total bilirubin; TcB, transcutaneous bilirubin; BC, BiliChek; SD, standard deviation; CI, confidence interval; G6PD, glucose-6-phosphate dehydrogenase.

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Virginia University (Morgantown, WV), Eku, and Jos. A total of 131 clinically jaundiced infants who were ≤14 days of age and admitted to Eku and Jos were enrolled between May 2000 and January 2002, regardless of place of birth, gestational age, or health status.

Skin Pigmentation Determinations

Skin pigmentation, as determined through visual observation by the researchers who enrolled patients, was recorded as light, medium, or dark. For each institution, a single dedicated physician performed all skin pigmentation assessments throughout the study.

TcB Determinations

All TcB measurements using the BiliChek (BC, SpectRx, Inc, Norcross, GA) were made on the forehead of each infant and simultaneously with blood sampling for STB measurements and before phototherapy. The BC was selected for use in this study because it has been designed to correct for variabilities in TcB measurements introduced by different degrees of skin pigmentation (melanin levels) as well as other interfering factors, including collagen and hemoglobin.8,11 It is a hand-held device that provides rapid, real-time, noninvasive transcutaneous estimations of STB levels. The instrument requires only an intermittent supply of electrical power to recharge the device. To estimate STB concentrations, this instrument directs a beam of white light at the skin and then performs spectral analysis at >100 individual wavelengths of the reflected light. For each institution, a single dedicated physician performed all TcB measurements throughout the study.

STB Determinations

At Eku, STB and direct bilirubin levels were measured with the Advanced Bilirubinometer Stat-Analyzer, Model BR2 (Advanced Instruments, Inc, Norwood, MA), which quantifies STB directly and direct (conjugated) bilirubin as the diazo derivative in 30-μL blood serum samples, which were obtained via heel-stick. Bilirubin Stat Analyzer Calibrator (Advance Instruments) levels I (5.0 mg/dL) and II (20 mg/dL) were used as daily quality control solutions. At Jos, these parameters were quantified in the diazotized sample (Malloy and Evelyn method) with the Unicam Helios γ spectrophotometer (Unicam, Cambridge, UK), in blood serum obtained by venipuncture. The analyzer was calibrated using the Bilirubin Standard from British Drug House (Canada).

Data Analysis

All data are presented as mean ± standard deviation (SD). Unpaired t tests were performed to compare demographic data between populations at Eku and Jos. Linear regression analysis was performed using the method of least squares. Mean bias ± imprecision (±2 SD of TcB as compared with STB measurements from each hospital and both hospitals combined was calculated using the method of Bland and Altman.13 The mean bias is defined as the mean difference between each paired TcB and STB measurement taken from each patient. Imprecision is defined as ± 2 SD from the mean difference. Limits of agreement of the mean differences are given as 95% confidence intervals (Cls). Differences in skin pigmentation between infants at Eku and Jos were compared by χ² analysis.

RESULTS

Of the 131 enrolled infants, data from 127 infants were included for data analysis. Two of the infants were excluded because of missing TcB or STB measurements. Two additional infants (1 from Eku and 1 from Jos) were excluded because of excessive differences between TcB and STB readings (>20 mg/dL, ie, >2 SD from the mean), which were most likely a result of clerical errors. The characteristics of the patients at the 2 study sites are given in Table 1. The gender distribution at Eku was fairly equal, whereas at Jos, there were more boys than girls (60% vs 40%, respectively). There was no difference in mean infant weights between both study sites, STB, TcB, and direct bilirubin levels were higher in infants from Eku than in those from Jos. Fifty-four percent (60 of 111) of infants were observed to be of light pigmentation, with the majority from Jos (95%). Thirty-six percent (40 of 111) of the infants were of medium skin color, with 28 infants from Jos and 12 from Eku. Only 10% (11 of 111) were observed to have dark pigmentation, with the majority from Jos (82%). Skin pigmentation was found to be statistically different between Eku and Jos (P = .003). For n = 16, the degree of pigmentation was not recorded and therefore not included in the data analysis.

The linear regression equations and correlations between TcB and STB measurements at Eku (TcB = 0.70[STB] + 5.0, r = .90, n = 29), Jos (TcB = 0.75[STB] + 4.3, r = .88, n = 98), and the combined populations (TcB = 0.73[STB] + 4.5, r = .92, n = 127) were nearly identical for both hospitals. The mean bias and imprecision of TcB measurements as compared with STB values for Eku, Jos, and the combined populations (Eku + Jos) are shown in Fig. 1. TcB measurements at Eku had a bias of −1.9 mg/dL (95% CI: −3.7 to −0.1) and greater imprecision (±8.9 mg/dL) than those made at Jos, where the bias was only 1.2 mg/dL (95% CI: 0.5–1.9) with an imprecision of ±6.2 mg/dL. Combined data revealed a bias of only 0.5 mg/dL (95% CI: −0.2 to 1.2) and an imprecision of ±7.6 mg/dL.

When infants with STB <12 mg/dL (mean STB: 7.9 ± 2.8 mg/dL; 1.2–11.9; n = 65) and STB ≥12 mg/dL (mean STB: 21.5 ± 8.0 mg/dL; 12.2–42.5; n = 62) were separated, linear regression and correlation were slightly poorer than those for the total range of STB values from all patients studied. Correlation for STB ≥12 mg/dL (r = .84) was slightly stronger than for STB <12 mg/dL (r = .67). When mean bias and

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Eku (n = 29)</th>
<th>Jos (n = 98)</th>
<th>Eku + Jos (n = 127)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17 (59%)</td>
<td>59 (60%)</td>
<td>76 (60%)</td>
</tr>
<tr>
<td>Female</td>
<td>12 (41%)</td>
<td>39 (40%)</td>
<td>51 (40%)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>2.62 ± 0.65</td>
<td>2.75 ± 0.61</td>
<td>2.72 ± 0.62</td>
</tr>
<tr>
<td></td>
<td>(1.40–3.70)</td>
<td>(1.28–4.16)</td>
<td>(1.28–4.16)</td>
</tr>
<tr>
<td>STB, mg/dL</td>
<td>23.5 ± 10.6*</td>
<td>11.9 ± 6.5</td>
<td>14.6 ± 9.0</td>
</tr>
<tr>
<td></td>
<td>(6.7–42.8)</td>
<td>(1.2–31.2)</td>
<td>(1.2–42.8)</td>
</tr>
<tr>
<td>TcB, mg/dL</td>
<td>21.6 ± 8.4*</td>
<td>13.1 ± 5.5</td>
<td>15.1 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>(7.8–37.2)</td>
<td>(4.4–32.9)</td>
<td>(4.4–37.2)</td>
</tr>
<tr>
<td>Direct, mg/dL</td>
<td>4.2 ± 7.8*</td>
<td>0.8 ± 1.3</td>
<td>1.5 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>(0.1–35.6)</td>
<td>(0.1–9.4)</td>
<td>(0.1–35.6)</td>
</tr>
<tr>
<td>Pigmentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>3 (18%)†‡</td>
<td>57 (61%)</td>
<td>60 (54%)</td>
</tr>
<tr>
<td>Medium</td>
<td>12 (70%)†‡</td>
<td>28 (30%)</td>
<td>40 (36%)</td>
</tr>
<tr>
<td>Dark</td>
<td>2 (12%)†‡</td>
<td>9 (9%)</td>
<td>11 (10%)</td>
</tr>
</tbody>
</table>

* P < .0001, from Jos. † P = .003, from Jos.
imprecision were calculated, TcB measurements overestimated STB levels by 2.1 ± 4.6 mg/dL at STB <12 mg/dL with a 95% CI for the mean bias of 1.5 to 2.7 mg/dL, whereas TcB measurements underestimated STB levels (−1.2 ± 8.6 mg/dL) at STB ≥12 mg/dL with a 95% CI for the mean bias of −2.4 to 0.0 mg/dL (Fig 2).

The characteristics of the infants (n = 20) who were admitted with the diagnosis of kernicterus are given in Table 2. More male infants were admitted than female infants. Mean weight, STB, TcB, and direct bilirubin levels were not statistically different between infant populations at Eku and Jos.

When the correlations between TcB and STB measurements were compared on the basis of light, medium, and dark skin pigmentation, TcB measurements strongly correlated with STB for all degrees of pigmentation (r = .91, .94, and .87, respectively). Mean bias (1.1, 0.8, and −0.2 mg/dL) was similar for light, medium, and dark skin types (Fig 3), respectively, but imprecision increased as the degree of pigmentation increased (±5.4, 7.8, and 11.6 mg/dL, respectively).

**DISCUSSION**

Nigeria is composed of >500 different ethnic/language groups with varying traditional practices of neonatal care and demographics, such as degree of skin pigmentation and incidence of glucose-6-phosphate dehydrogenase (G6PD) deficiency. All of these factors can directly affect the incidence, severity, and consequence of neonatal jaundice reported for various areas of Nigeria. For example, G6PD deficiency and sepsis in combination with exposure to various household chemicals and aflatoxins are believed to be associated with excessive STB levels and responsible for the high mortality and long-term morbidity, such as cerebral palsy in Nigeria. For instance, the reported incidence of G6PD deficiency in jaundiced Nigerian infants throughout the country ranges from a low of 17% to a high of 74%. A likely possibility for the higher TcB and STB levels observed in the Eku newborns might be the high rate of exposure to agents that increase hemolysis in infants with G6PD deficiency. This is supported by observations by Slusher et al in which 65% of mothers of jaundiced infants at Eku...
reported the use of either menthol-containing compounds or naphthalene in the bedding or clothing of their infants as compared with only 23% of the mothers at Jos during the same study period.

The higher mean (23.5 vs 11.9 mg/dL) and the dramatically higher maximum STB values (42.8 vs 31.2 mg/dL), not surprising, are also reflected in a higher number of infants who presented with kernicterus at Eku (41%) than at Jos (8%; Table 2). This could reflect a slower process of seeking medical care. Eku generally serves a more rural population that has transportation difficulties, fewer financial resources, and perhaps a lesser awareness of health issues than the urbanized Jos. These factors may make Eku’s patient population less likely to seek timely health care, which could potentially contribute to the higher levels of STB and the higher rate of kernicterus observed in the Eku infants at the time of admission.

The degree of correlation between TcB and STB measurements at Eku and Jos is similar and satisfactory for clinical decision making. However, in contrast to other studies, the slope of the present relationship is less than unity, and the y intercept is universally and uniformly high (5.0 and 4.3 mg/dL, respectively). The relatively high intercept (mg/dL) for these populations, relative to those of other studies (~1 mg/dL), could reflect the presence of light-absorbing, nonbilirubin, nutrition-derived yellow pigments in the skin of the infants. For instance, the consumption of carotene-containing red palm oil, fruits, and vegetables is relatively high in the study population. Other studies have shown that dietary fat-soluble carotenoids can be transferred through breast milk to these exclusively breastfed infants. Furthermore, skin carotene interference with TcB measurements is likely to occur on the basis of similar physical and optical characteristics of the carotenoids and bilirubin. For instance, both compounds have similar molecular weights (537 vs 585 g/mol), are strongly lipophilic, and have similar light absorption maxima (453 and 490 vs 453 nm) and millimolar absorption coefficients (~134 vs 61). In 1 published case, these similarities in characteristics apparently led to an erroneous diagnosis of jaundice in an adult.

<table>
<thead>
<tr>
<th>Total Population</th>
<th>Kernicterus</th>
<th>Gender</th>
<th>Weight (kg)</th>
<th>STB (mg/dL)</th>
<th>TcB (mg/dL)</th>
<th>Direct (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eku (n = 29)</td>
<td>12 (41%)</td>
<td>Male</td>
<td>2.61 ± 0.41 (1.84–3.09)</td>
<td>30.0 ± 9.3 (14.0–42.8)</td>
<td>25.9 ± 7.7 (13.4–37.2)</td>
<td>7.1 ± 10.9 (0.4–35.6)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td></td>
<td>2.33 ± 0.62 (1.31–3.03)</td>
<td>30.0 ± 9.3 (14.0–42.8)</td>
<td>22.0 ± 3.0 (17.4–28.7)</td>
<td>2.4 ± 3.2 (0.3–9.4)</td>
</tr>
<tr>
<td>Jos (n = 98)</td>
<td>8 (8%)</td>
<td>Male</td>
<td>2.61 ± 0.41 (1.84–3.09)</td>
<td>30.0 ± 9.3 (14.0–42.8)</td>
<td>25.9 ± 7.7 (13.4–37.2)</td>
<td>7.1 ± 10.9 (0.4–35.6)</td>
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<tr>
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<td>2.33 ± 0.62 (1.31–3.03)</td>
<td>30.0 ± 9.3 (14.0–42.8)</td>
<td>22.0 ± 3.0 (17.4–28.7)</td>
<td>2.4 ± 3.2 (0.3–9.4)</td>
</tr>
<tr>
<td>Eku + Jos (n = 127)</td>
<td>20 (16%)</td>
<td>Male</td>
<td>2.49 ± 0.51 (1.31–3.09)</td>
<td>28.1 ± 7.7 (14.0–42.8)</td>
<td>24.3 ± 6.6 (13.4–37.2)</td>
<td>5.0 ± 8.5 (0.3–35.6)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
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<td>5.0 ± 8.5 (0.3–35.6)</td>
</tr>
</tbody>
</table>

Fig 3. Mean bias (solid line) ± imprecision (dotted lines) of measurements of TcB and STB for infants of light, medium, and dark pigmentation at Eku and Jos. Kernicteric infants are shown in (Δ).
who had a high carotene intake. This case demonstrates that carotenodermia can also affect the correlation between TcB and STB values. Alternatively, the higher intercept could be related to the relatively high levels of conjugated bilirubin in this population. However, no studies have been published to support this possibility. If this were a possibility, the correlation between these 2 measurements would be expected to be poor in previously reported studies as well. Furthermore, the high mean direct bilirubin value of 4.2 mg/dL observed in Eku was primarily driven by a single patient with a direct bilirubin value of 35.6 mg/dL and the much smaller sample size at Eku (n = 26 vs 97). In fact, median direct bilirubin values were 1.0 vs 0.6 mg/dL for Eku and Jos, respectively.

Even under ideal laboratory conditions, interlaboratory variability of bilirubin measurement has been found to be significant. The laboratory facilities throughout Nigeria vary greatly as they do between the 2 hospitals, which may lead to significant variations in measured STB values. Jos measures STB levels in blood obtained by venipuncture, whereas Eku uses small samples usually obtained by heel-stick. It has been reported that there are significant differences in STB levels from blood drawn by heel-stick method compared with blood obtained by venipuncture. These factors may account for the greater bias and imprecision and wider and higher STB values found for Eku as compared with Jos (Table 1, Fig 1). Another reason for the greater bias and imprecision found in Eku could be the number of infants with hyperbilirubinemia with 50% having a STB >20 mg/dL. However, we found that the correlations between TcB and STB measurements at Eku and Jos were nearly identical (r = .90 and .88, respectively).

The linear regression analysis results do not provide significant evidence that the degree of hyperbilirubinemia affects the accuracy of TcB readings. The cutoff value of 12 mg/dL was selected on the basis of the concentration of STB, for which a physician may consider instituting phototherapy. Both slope and intercept for the low (<12 mg/dL) and high (≥12 mg/dL) STB ranges are similar. However, the correlation coefficient for the low STB range is slightly poorer than that for the high STB subgroup (0.67 vs 0.84, respectively) and similar to that of the combined Eku + Jos populations (0.92; Fig 1).

The error plots in Fig 2 indicate that TcB levels in the range <12 mg/dL overestimate (2.1 ± 4.6 mg/dL) STB, whereas those in the higher range underestimate STB (−1.2 ± 8.6 mg/dL). In contrast to other studies, we did not find the BC to be less accurate at measuring bilirubin levels <12 mg/dL. However, with careful review of the data, we found that the lower accuracy at STB ≥12 mg/dL is not clinically important. For example, of the 127 jaundiced infants studied, only 1 (0.8%) infant had a TcB value that would have potentially directed us to a different treatment strategy and, therefore, would have led to a real potential for increased morbidity (ie, not performing an exchange blood transfusion otherwise indicated by STB values). This infant had poor relative values of TcB versus STB of 14.0 and 25.4 mg/dL, respectively. In this case, the jaundice was clinically severe enough that physical observation led us to believe that the TcB value was incorrect and warranted us either to repeat the TcB or to measure an STB level. In the other cases in which the TcB underestimated the STB, treatment plans likely would not have been changed on the basis of our current standards of practice that include a lower threshold for instituting phototherapy or performing exchange blood transfusions than in the United States. This decision is of critical importance if we are to consider using measurements of TcB as a screening tool or as a surrogate measure of STB in hospitals and clinics. For term infants, both hospitals routinely perform exchange transfusion when STB is >20 mg/dL or at 18 mg/dL when the infant has an associated risk factor for jaundice. For premature infants, both institutions perform exchange transfusions on the basis of an infant’s weight. Eku uses a chart, which also factors in the age of the infant. At Jos, transfusions are initiated when STB levels are 10% of the premature infant’s weight, eg, a 1000-g infant would receive a transfusion at 10 mg/dL. In addition, both exchange earlier when there is a rapid rise in STB levels within the first 24 hours of life.

In his article, Rubaltelli et al raised the point that TcB actually measures the bilirubin that has moved from the plasma into the tissue, as opposed to measurements from the laboratory, which measures bilirubin in the serum. The authors suggested that TcB might actually be a better measure of the bilirubin available for moving into the brain. If this were true, then TcB measurements would identify infants who have extreme hyperbilirubinemia and are at risk for kernicterus much earlier than STB measurements. However, this has not been observed in previously published reports. Furthermore, the rationale for this hypothesis is flawed because the movement of bilirubin into the skin is governed by different processes than those that affect bilirubin movement into the brain, not the least of which is the discriminating blood-brain barrier. An indication of the existence of different processes is that bilirubin does not distribute in the same pattern in skin as it does in the brain, where there is focal deposition of bilirubin in kernicteric infants. Furthermore, not all severely jaundiced infants develop kernicterus. In our study, some of the infants were presenting at various stages of kernicterus, and it would be inappropriate to correlate TcB or STB levels at the time of admission with the development of kernicterus.

There exists a slight or moderate effect of pigmentation on the TcB measurements. The slopes for the regression equations decrease gradually from 0.80 to 0.69 (14%), whereas the intercepts remained nearly the same. Furthermore, Fig 3 clearly demonstrates that although the mean bias is nearly the same, the imprecision range significantly increases with the degree of pigmentation. Because a single physician was assigned to assess skin pigmentation at each institution, there was minimal intravariability in assessment; however, intervariability was not measured and may account for the different distribution.
of the skin pigmentation of infants between the study centers.

As one considers the benefits of the TcB, a frequently cited and real advantage is the decreased need for invasive blood sampling, which is both painful and a health risk to the infant and a potential health hazard to the practitioners as well as laboratory personnel, who may be exposed at the bedside possibly to human immunodeficiency virus/acquired immune deficiency syndrome–infected blood. In sub-Saharan Africa, the proper protection of the health care providers is often unavailable altogether or suboptimal.

CONCLUSIONS

We conclude that TcB measurements are a useful and reliable index for estimating STB levels in heavily pigmented neonates before directing phototherapy and exchange blood transfusions in a population in which determining reliable STB levels is often difficult. The relatively simple and noninvasive TcB measurements can be an important adjunct in preventing the high incidence of bilirubin-induced morbidity and mortality in low-technology clinical environments. However, the challenge to make this exciting technology available to those who need it most still remains.

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REFERENCES