

Hyperbilirubinemia Among African American, Glucose-6-Phosphate Dehydrogenase-Deficient Neonates

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ABSTRACT. *Background.* Although glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is prevalent in African Americans, their risk of associated neonatal hyperbilirubinemia has not been prospectively studied.

Objective. To compare hemolysis and the risk of hyperbilirubinemia among African American, G-6-PD-deficient neonates (study group) and G-6-PD-normal control subjects.

Methods. Consecutive, healthy, term and near-term, male neonates born to African American mothers comprised the patient cohort. G-6-PD testing was performed with umbilical cord blood samples. Routine management included measurement of the end tidal carbon monoxide level corrected for ambient carbon monoxide level (ETCOc) within 4 hours after delivery (assessment of hemolysis), ≥ 1 predischarge bilirubin determination, and additional bilirubin testing as clinically indicated. Indications for phototherapy were identical for study patients and control subjects. Neonates were monitored for the first 1 week of life. ETCOc results, the incidence of hyperbilirubinemia (defined as a transcutaneous or plasma total bilirubin concentration of ≥ 95 th percentile for the hour of life), and the need for phototherapy were compared between the G-6-PD-deficient and G-6-PD-normal groups.

Results. Five hundred male patients were enrolled, of whom 64 (12.8%) were G-6-PD-deficient. ETCOc values (median and interquartile range) were higher among G-6-PD-deficient neonates than among control neonates (2.4 ppm [2.0–2.9 ppm] vs 2.1 ppm [1.7–2.5 ppm]). More G-6-PD-deficient neonates developed hyperbilirubinemia than did control subjects (14 of 64, 21.9%, vs 29 of 436, 6.7%; relative risk: 3.27; 95% confidence interval: 1.83–5.86), whereas 13 (20.3%) met the criteria for phototherapy, compared with 25 control subjects (5.7%) (relative risk: 3.53; 95% confidence interval: 1.91–6.56). No cases of kernicterus were observed.

Conclusions. Within the African American neonatal population, there is a subgroup of G-6-PD-deficient in-

fants with elevated rates of hemolysis, a higher incidence of hyperbilirubinemia, and a greater requirement for phototherapy, compared with G-6-PD-normal control subjects. These newborns should be monitored vigilantly for the development of hyperbilirubinemia. *Pediatrics* 2004;114:e213–e219. URL: <http://www.pediatrics.org/cgi/content/full/114/2/e213>; *glucose-6-phosphate dehydrogenase deficiency, African American, hemolysis, neonatal hyperbilirubinemia, kernicterus, phototherapy, bilirubin, end tidal carbon monoxide.*

ABBREVIATIONS. ETCOc, end tidal carbon monoxide level corrected for ambient carbon monoxide level; G-6-PD, glucose-6-phosphate dehydrogenase; PTB, plasma total bilirubin; TcB, transcutaneous bilirubin; Hb, hemoglobin.

Despite increasing awareness of neonatal hyperbilirubinemia and American Academy of Pediatrics guidelines for the management of this condition, kernicterus continues to be encountered in North America.^{1–8} Because many of the reported neonates were also deficient in glucose-6-phosphate dehydrogenase (G-6-PD), a condition associated with hyperbilirubinemia and kernicterus, an etiologic factor linking these 2 conditions is likely. African Americans, of whom 11% to 13% are known to be G-6-PD-deficient (G-6-PD A⁻), represent the largest subgroup affected in North America.^{9–11} Traditionally, these newborns have not been regarded as being at high risk for G-6-PD deficiency-associated hyperbilirubinemia; Maisels and Newman^{12,13} stated that many American pediatricians do not take G-6-PD deficiency into consideration when evaluating likely causes of severe neonatal hyperbilirubinemia. This view was supported by the finding of Brown et al¹⁴ that few neonates readmitted for treatment of hyperbilirubinemia in the greater New York City area had been tested for G-6-PD deficiency.

The incidence and severity of G-6-PD deficiency-associated neonatal hyperbilirubinemia among African American neonates are largely unknown, because no large prospective study has been performed to date. The primary aim of this study was to determine prospectively the risk of developing hyperbilirubinemia, and the severity thereof, among a cohort of healthy, male, term and near-term, African American, G-6-PD-deficient neonates and to compare the findings with those for G-6-PD-normal counterparts. We also compared the degree of hemolysis, a risk factor for kernicterus, between the 2 groups, using an end tidal carbon monoxide technique.¹⁵

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METHODS

Patients and Treatment

The study was performed in the general care (well-infant) nursery of the University of Chicago Hospitals (Chicago, IL), with the approval of the review board of that institution. This hospital has ~3000 deliveries annually, 87% with African American mothers. Signed informed consent was obtained from at least 1 parent of each neonate enrolled in the study.

Eligible for enrollment were healthy male neonates who were born to African American mothers. Criteria for exclusion included transfer to the neonatal intensive care unit for reasons including major congenital anomalies, sepsis, respiratory distress or oxygen requirement, and hypoglycemia requiring intravenous glucose therapy. The study was limited to male subjects because, inasmuch as G-6-PD deficiency is an X-linked condition, the G-6-PD status can be accurately determined for male subjects, who can be either normal or deficient hemizygotes. Among female subjects, a large number of G-6-PD-deficient heterozygotes, with a form of the condition that may be difficult to diagnose, may be encountered.¹⁶

Because results of the G-6-PD testing usually became available only after discharge, infants in the 2 groups were treated identically, and differentiation into G-6-PD-deficient and -normal groups was performed only retrospectively. For all neonates enrolled in the study, a sample of umbilical cord blood was collected for G-6-PD biochemical analysis. Umbilical cord blood was also used for Rh testing of neonates born to Rh-negative mothers. Care extended to these neonates was routine. Breastfeeding was encouraged, and healthy infants were hospitalized for ~48 hours.

Management of neonatal jaundice was as follows. Nursery protocol included measurement of the end tidal carbon monoxide level corrected for ambient carbon monoxide level (ETCOc) within 4 hours after delivery. At least 1 predischarge transcutaneous bilirubin (TcB) test was performed for each neonate at 48 ± 12 hours of age, except for neonates who were already receiving phototherapy before that age. TcB readings of ≥ 15.0 mg/dL were confirmed with plasma total bilirubin (PTB) determinations. Additional TcB or PTB determinations were performed as deemed necessary according to these tests or the suggestion of hemolytic disease, in combination with clinical evaluations.

Infants with ETCOc readings of < 3.0 ppm, equivalent to the 95th percentile for this population during the first 4 hours of life, were treated routinely. In the event of ETCOc readings of ≥ 3.0 ppm, an immediate bilirubin test was performed. Any infant with a TcB reading or PTB concentration of ≥ 4.0 mg/dL within the first 4 hours of life was evaluated with determination of the blood group and Rh type, direct Coombs' test, complete blood count, and smear for red blood cell morphologic evaluation. Subsequent bilirubin determinations were obtained in accordance with these results and clinical assessments.

The TcB or PTB values were plotted on a nomogram of bilirubin percentiles for the hour of life at which the test was performed.¹⁷ In the event of a discrepancy resulting from the use of the different techniques, the higher value was used. Patients with percentile values of ≥ 75 remained in the hospital for additional observation and bilirubin determinations. All infants who met the criteria for phototherapy underwent ≥ 1 PTB determination before the initiation of phototherapy. During phototherapy, only PTB determinations were performed.

Indications for phototherapy were based on those of the American Academy of Pediatrics Practice Parameter,¹⁸ as follows: PTB concentrations of ≥ 171 $\mu\text{mol/L}$ (10.0 mg/dL) within the first 24 hours, ≥ 206 $\mu\text{mol/L}$ (12.0 mg/dL) between 25 and 48 hours, ≥ 257 $\mu\text{mol/L}$ (15.0 mg/dL) between 49 and 72 hours, and ≥ 291 $\mu\text{mol/L}$ (17.0 mg/dL) at > 72 hours. Criteria for readmission for treatment of hyperbilirubinemia included PTB concentrations of ≥ 342 $\mu\text{mol/L}$ (20.0 mg/dL), unless there were special risk factors, such as poor breastfeeding or parental anxiety, in which case an infant might be admitted with a PTB concentration of 308 to 342 $\mu\text{mol/L}$ (18.0–20.0 mg/dL). Infants were cared for in isolettes during phototherapy treatment, which was administered with 1 or 2 Draeger 4000 banks of phototherapy lights (Draeger Medizin-technik GmbH, Lubeck, Germany) placed ~20 cm above the infant. In addition, each neonate received phototherapy from a phototherapy mattress (Biliblanket Plus; Ohmeda Medical, Laurel, MD). The overhead light irradiance, measured at the level of the infants' skin, was at least 35 $\mu\text{W}/\text{cm}^2$ per nm. Postdischarge

follow-up monitoring of neonates was performed in the outpatient clinic of the University of Chicago Hospitals.

Laboratory Methods

For G-6-PD testing, 0.5 mL of umbilical cord blood was collected in an EDTA-containing tube immediately after delivery. Samples were shipped to the laboratories of the Mayo Clinic (Rochester, MN) for quantitative G-6-PD testing, as follows. The red blood cells were filtered through a cellulose slurry to remove leukocytes and platelets. Quantitative G-6-PD enzyme assays were performed according to the method described by Beutler.¹⁹ Briefly, G-6-PD oxidizes glucose-6-phosphate to 6-phosphogluconate, with concurrent reduction of nicotinamide adenine dinucleotide phosphate to reduced nicotinamide adenine dinucleotide phosphate. Production of the latter, absorbance of which can be measured spectrophotometrically at 340 nm, reflects enzyme activity. Absorbance measurements were performed with a Technicon RA 1000 spectrophotometer (Technicon Instruments, Tarrytown, NY), and enzyme activity was expressed as units per gram of hemoglobin (Hb). For reference, normal values for African American neonates have been reported as 287 ± 63 U/100 mL erythrocytes,¹¹ which correspond to $\sim 8.0 \pm 1.5$ U/g of Hb.²⁰

TcB determinations were performed by using Bilichek (Respi-ronics, Norcross, GA). PTB measurements were performed by using a photometrically monitored diazo reaction (Roche/Hitachi Modular P; Roche Diagnostics GmbH, Mannheim, Germany). The instrument was calibrated with a lyophilized control serum based on human serum (Precipath U plus; Roche Diagnostics).

The principle of the ETCOc test is that, for each molecule of biliverdin, and subsequently bilirubin, produced from the degradation of heme by the enzyme heme oxygenase, equimolar quantities of carbon monoxide are produced. This carbon monoxide is transported to the lungs in the form of carboxyhemoglobin. Measurement of ETCOc reflects endogenous carbon monoxide production. Because the majority of available heme is derived from Hb, accurate assessment of ETCOc provides an index of the rate of heme catabolism and therefore bilirubin production.^{15,21–24} ETCOc was measured with the CO-Stat end tidal breath analyzer (Natus Medical Inc, San Carlos, CA), which automatically corrects for ambient carbon monoxide levels. Results are expressed as parts per million. Because maternal cigarette smoking may increase blood carboxyhemoglobin values for both the mother and the fetus, ETCOc values may be increased among newborns born to smoking mothers for up to 48 hours after delivery.²⁵ Therefore, for ETCOc analyses, only the subgroup of newborns born to non-smoking mothers was included.

Definition

Hyperbilirubinemia was defined as any TcB or PTB value ≥ 95 th percentile for the hour of life in which the sample was drawn, according to the nomogram provided by Bhutani et al.¹⁷

Sample Size

With the assumption of an incidence of hyperbilirubinemia among the G-6-PD-deficient neonates of 20% and an incidence in the control group of 6%, with $\alpha = .05$ and power = .8, 52 patients in each group would be necessary. With the assumption of a 10% frequency of G-6-PD deficiency in the population studied, 500 neonates would need to be enrolled to yield the required 50 G-6-PD-deficient neonates.

Data Analyses

For analysis, patients were divided into the study group, ie, those who were G-6-PD-deficient, and the control group, with normal G-6-PD activity. The cohort was also arbitrarily subdivided into 3 subgroups according to the degree of bilirubinemia, ie, 1) neonates whose PTB concentrations for the hour of life did not exceed the 74th percentile, 2) neonates whose PTB concentrations were in ≥ 75 th percentile for the hour of life, and 3) neonates designated hyperbilirubinemic (PTB concentrations in ≥ 95 th percentile for the hour of life). The 75th percentile was chosen as the cutoff point to comply with nursery protocol, which mandated additional bilirubin testing for neonates with PTB concentrations in the upper quartile. Continuous variables were compared by using Student's *t* test or the Mann-Whitney rank sum test, as appropriate, for data with or without normal distribution. Cate-

TABLE 1. Demographic Data for G-6-PD-Deficient Versus G-6-PD-Normal Male Neonates

Category	G-6-PD-Deficient (n = 64)	G-6-PD-Normal (n = 436)
Birth weight, g	3232 ± 470	3271 ± 476
Gestational age, wk	38.9 ± 1.5	38.9 ± 1.5
Caesarian section, no.	19 (29.7%)	82 (18.8%)
Group B streptococcal colonization, no.	21/53 (39.6%)	133/356 (37.4%)
Breastfeeding (exclusive and mixed), no.	22 (34.4%)	174 (39.9%)
Maternal smoking, no.	5 (7.8%)	74 (16.9%)
Maternal diabetes mellitus, no.	2 (3.1%)	8 (1.8%)
Cephalhematoma or bruising, no.	2 (3.1%)	12 (2.6%)

No significant differences were found for any of these categories between the 2 groups.

gorical variables were compared by using χ^2 analysis or Fisher's exact test, as appropriate. Relative risks (and 95% confidence intervals) were used to compare the additional risk of G-6-PD-deficient neonates developing hyperbilirubinemia or requiring phototherapy with that for control subjects. Parametric data are presented as mean ± SD, and nonparametric data are presented as median and interquartile range. To determine whether, among the G-6-PD-deficient neonates, decreasing residual enzyme activity had any effect on exacerbating jaundice and to determine the contribution of increased hemolysis to the mechanism of the jaundice, multivariate regression analysis was used to correlate residual G-6-PD activity and ETCOc concentrations with bilirubin grading, as described above. Significance was defined as $P < .05$ or, for relative risk determinations, a 95% confidence interval that did not include the value 1.

RESULTS

Enrollment

Study subjects were enrolled as consecutive births between September 2002 and May 2003. No mothers refused enrollment. One G-6-PD blood sample clotted, necessitating enrollment of 501 infants, although only 500 were included in the analysis.

G-6-PD Distribution

Of the 500 neonates, 64 (12.8%) were G-6-PD-deficient (mean enzyme activity: 2.76 ± 1.15 U/g Hb), whereas the remainder had normal enzyme activity (21.81 ± 2.88 U/g Hb). There was no overlap between the 2 groups.

Demographic Data

Demographic data for the neonates in both groups are summarized in Table 1. No significant differences were noted between the groups for any of the categories listed. Of 14 G-6-PD-deficient neonates who underwent direct Coombs' testing, 1 exhibited positive results, compared with 3 of 60 G-6-PD-normal neonates (not significant).

Jaundice and Hyperbilirubinemia

Predischarge PTB concentrations (with the exclusion of 4 G-6-PD-deficient and 7 control neonates who began receiving phototherapy before 36 hours) were higher in the G-6-PD-deficient group than in the control group (157 ± 43 $\mu\text{mol/L}$ vs 139 ± 48 $\mu\text{mol/L}$, $P = .006$ [9.2 ± 2.5 mg/dL vs 8.1 ± 2.8 mg/dL]). Within the G-6-PD-normal group, the distribution of PTB concentrations generally adhered to the expected results; 102 of 436 neonates (23.4%) developed PTB concentrations in ≥ 75 th percentile for the hour of life, whereas 29 (6.7%) became hyperbilirubinemic (≥ 95 th percentile). In contrast, 31

(48.4%, $P < .001$) and 14 (21.9%, $P < .001$) of the G-6-PD-deficient neonates developed PTB levels in ≥ 75 th and ≥ 95 th percentiles for the hour of life, respectively (Fig 1). The risk of G-6-PD-deficient neonates developing PTB concentrations in ≥ 75 th percentile for the hour of life, relative to the G-6-PD-normal control subjects, was 2.07 (95% confidence interval: 1.53-2.81), whereas that of developing hyperbilirubinemia was 3.27 (95% confidence interval: 1.83-5.86). Among the neonates with PTB levels in ≥ 95 th percentile, the highest noted PTB levels were similar between the 2 groups (245 ± 58 $\mu\text{mol/L}$ vs 238 ± 53 $\mu\text{mol/L}$, $P = .65$ [14.3 ± 3.4 mg/dL vs 13.9 ± 3.1 mg/dL]), although these values do not represent the natural peak, because phototherapy had been instituted in many instances. Similarly, the ages at which the highest PTB levels were noted among those with PTB levels in ≥ 95 th percentile were not significantly different between the groups (64 ± 27 hours vs 55 ± 23 hours, $P = .3$). No G-6-PD-deficient neonates were readmitted for treatment of hyperbilirubinemia, compared with 6 neonates (1.4%) in the control group ($P = .7$).

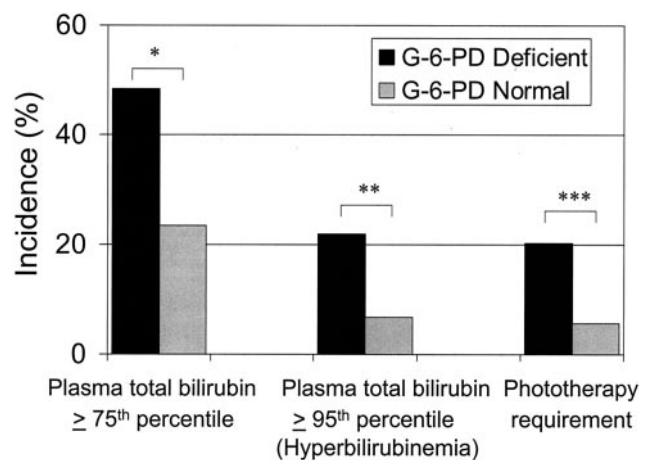


Fig 1. Percentages of neonates with PTB concentrations of ≥ 75 th percentile on the bilirubin nomogram timed for the hour of life,¹⁷ hyperbilirubinemia (≥ 95 th percentile), and requirements for phototherapy, in the G-6-PD-deficient and control groups. For the former 2 parameters, the percentages of G-6-PD-normal neonates were similar to the expected distribution; however, within the G-6-PD-deficient group, the percentages of affected neonates were significantly higher than those of the G-6-PD-normal neonates (*relative risk: 2.07; 95% confidence interval: 1.53-2.81; **relative risk: 3.27; 95% confidence interval: 1.83-5.86; ***relative risk: 3.54; 95% confidence interval: 1.91-6.56).

TABLE 2. Data Relating to Phototherapy for the G-6-PD-Deficient and G-6-PD-Normal Neonatal Groups

Category	G-6-PD-Deficient (<i>n</i> = 64)	G-6-PD-Normal (<i>n</i> = 436)	Significance (<i>P</i> Value)
No. requiring phototherapy	13 (20.3%)*	25 (5.7%)	
Age at onset, h	50 ± 26	47 ± 26	.6
Age at discontinuation, h	82 ± 26	81 ± 20	.8
Duration of phototherapy, h	25 (17–33)	24 (20–50)	.5
PTB level at onset, μmol/L	245 ± 58	238 ± 53	.65
PTB level at onset, mg/dL	13.9 ± 3.4	13.2 ± 3.2	
Highest noted PTB level, μmol/L†	263 ± 46	251 ± 50	.5
(Highest noted PTB level, mg/dL	15.4 ± 2.7	14.7 ± 2.9)	
PTB level at discontinuation, μmol/L	162 ± 38	166 ± 21	.8
(PTB level at discontinuation, mg/dL	9.5 ± 2.2	9.7 ± 1.2)	
Rate of bilirubin decrease, μmol/h	3.1 ± 2.1	2.7 ± 2.9	.6
(Rate of bilirubin decrease, mg/h	0.18 ± 0.12	0.16 ± 0.17)	

Data are presented as mean ± SD or median (interquartile range), as appropriate.

* Relative risk: 3.54; 95% confidence interval: 1.91–6.56.

† Not the natural peak, because PTB concentrations might have been tempered by the institution of phototherapy.

Phototherapy

Significantly more G-6-PD-deficient neonates (13 neonates, 20.3%) required phototherapy, compared with 25 (5.7%) of the G-6-PD-normal group ($P < .001$). The relative risk of G-6-PD-deficient neonates requiring phototherapy, compared with G-6-PD-normal control subjects, was 3.54 (95% confidence interval: 1.91–6.56). Additional details regarding phototherapy are summarized in Table 2, in which it can be seen that, despite the greater number of G-6-PD-deficient neonates who required phototherapy, no significant differences between the groups with respect to any of the factors noted were observed.

ETCOc Studies

ETCOc values (median and interquartile range) were significantly higher in the G-6-PD-deficient group ($n = 59$) than in the control group ($n = 363$) (2.4 [2.0–2.9] ppm vs 2.1 [1.7–2.5] ppm, $P < .001$).

Contribution of G-6-PD Activity and Hemolysis to Bilirubin Grading

For the G-6-PD-deficient neonates, multivariate analysis ($r = .2$) demonstrated no significant correlation of either ETCOc values ($P = .11$) or residual G-6-PD enzymatic activity ($P = .61$) with bilirubinemia grading (defined in Data Analyses).

Prevalence of G-6-PD Deficiency and Hyperbilirubinemia

The prevalence of G-6-PD deficiency was greater among neonates with bilirubin values in ≥ 75 th percentile (31 of 133 neonates, 23%) and ≥ 95 th percentile (14 of 43 neonates, 32%), compared with the entire cohort (64 of 500 neonates, 12.8%; $P = .004$ and $P = .001$ for the 75th and 95th percentile comparisons, respectively).

DISCUSSION

Severe neonatal jaundice, with the potential for bilirubin encephalopathy, is a possible complication of G-6-PD deficiency that has been noted for many affected population groups, as reviewed previously.^{26,27} In Nigeria, where the G-6-PD A- variant is

encountered, a high incidence of associated hemolysis and kernicterus has been noted.²⁸ It is not surprising that G-6-PD A- is now encountered in geographic regions inhabited by descendants of original Africans. It has been estimated that 30 000 to 40 000 G-6-PD-deficient African American male infants are born each year in the United States.²⁹ In a recent report on the United States-based Pilot Kernicterus Registry,² G-6-PD deficiency was shown to affect 31.5% of 61 patients with kernicterus (of a total of 90) who were readmitted in the first 7 days of life. Although the incidence of G-6-PD deficiency in the full complement of 90 neonates in that study is not known and may be lower than 31.5% (although not lower than 21%), we do not know by which methods G-6-PD was tested, and the population included in the registry was gathered in a nonsystematic manner, there does seem to be a connection between G-6-PD deficiency and the development of kernicterus within the registry. G-6-PD deficiency has been emphasized as a major cause of severe hyperbilirubinemia in general^{12,30} and among African American neonates in particular.^{29,31} The Centers for Disease Control and Prevention recently included the enzyme deficiency in a mnemonic regarding the causes of hyperbilirubinemia, designed to increase physician awareness of the condition.³² It is remarkable that, despite apparent awareness on the part of some authorities, to date there have been no prospectively designed, cohort studies definitively evaluating the risk of hyperbilirubinemia in this population subgroup.

Some reasons for the lack of concern regarding G-6-PD deficiency among African American neonates may include early reports that hyperbilirubinemia rates were not significantly higher among G-6-PD-deficient neonates, compared with those with normal enzyme activity.^{11,33,34} More recent statements suggested that, with A- G-6-PD deficiency, spontaneous hemolysis may occur among premature but not term infants.³⁵ Furthermore, as a group, black neonates in the United States (the majority of whom are not G-6-PD-deficient) have a lower incidence of hyperbilirubinemia than do their

white or Asian counterparts.³⁶ In addition, only some G-6-PD-deficient neonates develop hyperbilirubinemia. However, other reports^{1-8,37-40} and the data reported here provide sufficient evidence to offset these conceptions. Our data establish an increased frequency of hyperbilirubinemia requiring phototherapy among G-6-PD-deficient infants, but not increased bilirubin levels, compared with control subjects. Our study clearly documents a prevalence of G-6-PD deficiency among neonates for whom hyperbilirubinemia was documented that was higher than that for the entire cohort. The rate of hemolysis was higher in the G-6-PD-deficient group than in the control group. In this survey, no cases of severe hemolysis (akin to favism) or kernicterus were encountered. The latter observation is not surprising, because kernicterus is an extremely rare, although highly important, condition and the chance of a case occurring among 64 neonates is very small. Furthermore, vigilant neonatal nursery care was combined with cautious postdischarge follow-up monitoring, the specific aim of which was to prevent severe hyperbilirubinemia.

One possible reason for the apparent contrast between the current data and those of the earlier studies^{11,33,34} may be that, in the older studies, bilirubin values were analyzed by using mean values during a particular 24-hour period. In contrast, we plotted bilirubin values on the hour-of-life nomogram¹⁷ and defined hyperbilirubinemia according to the highest noted bilirubin percentile for the hour of life, which is a much more sensitive approach. Another advantage of our method was that we were able to identify hyperbilirubinemia among neonates who developed early jaundice and for whom phototherapy was instituted before the routine predischarge bilirubin screening.

In this study, we used ETCOc measurements as our primary means of evaluating hemolysis. The median ETCOc value for the G-6-PD-deficient neonates was ~14% higher than that for the control population. However, because the pathogenesis of bilirubinemia is multifactorial, increased heme catabolism or bilirubin production does not necessarily indicate that the plasma bilirubin concentrations, or total bilirubin load, should be 14% higher. Heme catabolism is only 1 factor contributing to plasma bilirubin concentrations and does not reflect subsequent steps in the elimination of bilirubin, such as its uptake into hepatocytes or its conjugation, deficiencies of which may also contribute to the plasma total unconjugated bilirubin concentrations. Indeed, a tendency for decreased bilirubin conjugation among G-6-PD-deficient neonates was reported,^{41,42} the result of an interaction between G-6-PD deficiency and TA promoter polymorphism of the gene encoding the bilirubin-conjugating enzyme uridine diphosphate-glucuronosyltransferase 1A1.⁴³ Because the PTB level at any time point represents a balance between the production and elimination of bilirubin, an apparent modest increase in hemolysis may have a major effect on PTB concentrations under conditions of diminished bilirubin conjugation. Conversely, severely increased hemolysis may have minimal effects

on PTB levels under conditions of excellent bilirubin conjugation and excretion. Superimposition of additional icterogenic factors increasing the bilirubin load, such as hemolysis attributable to recognized or unidentifiable chemical triggers or infection or decreased bilirubin conjugation secondary to prematurity or uridine diphosphate-glucuronosyltransferase 1A1 promoter polymorphism, as seen in Gilbert's syndrome,⁴³ may further upset the equilibrium between the production and elimination of bilirubin, with the potential for extreme hyperbilirubinemia and kernicterus. For these reasons, the moderately increased ETCOc values are of concern, especially because increased hemolysis has been recognized as a risk factor for kernicterus. As stated above, neonates born to smoking mothers were not included in the ETCOc analyses. However, the number of smoking mothers in the G-6-PD-deficient group was approximately one-half of that in the control group (not statistically significant) and only 5 G-6-PD-deficient infants were excluded from these analyses, which likely had a minimal effect on the results.

In this study, we did not routinely assess hematologic parameters associated with hemolysis among older pediatric patients or adults. Among newborns, there may be overlap between the profiles associated with hemolytic and nonhemolytic states.^{44,45} The reticulocyte count is frequently used as a reflection of hemolysis, because it is regarded as a measure of the bone marrow's compensation for anemia. However, this count may be inaccurate among newborns, because it depends on the bone marrow's capacity to respond, the lag time between the hemolytic event and the response, and the effect of nonhemolytic causes of anemia stimulating a reticulocytic reaction.⁴⁶ Although no attempt was made to fractionate the PTB levels into direct and indirect components, it was presumed that PTB levels represent primarily unconjugated bilirubin. Conditions leading to direct bilirubinemia are extremely rare and, even in cases of biliary atresia, the direct fraction is unlikely to become manifest in the first days of life.¹²

It is intriguing that, despite the increased hemolysis and higher incidence of hyperbilirubinemia in the G-6-PD-deficient group, the severity of hyperbilirubinemia, once it developed, was similar in the study and control groups. Not only did all neonates who met the criteria for phototherapy respond to this treatment, but the age at onset, duration of treatment, age at the termination of treatment, and hourly rate of bilirubin decrease were comparable. Similarly, PTB levels at the onset of treatment and the maximal noted PTB levels were in the same ranges for the 2 groups. Although it is possible that there was more efficient hepatic handling of bilirubin among the G-6-PD-deficient neonates who were receiving phototherapy, compared with control subjects, this study was not designed to evaluate bilirubin conjugation or excretion. In previous studies that demonstrated a tendency for diminished bilirubin conjugation in the mechanism of jaundice among G-6-PD-deficient neonates,^{41,42} sampling was performed before the institution of phototherapy; therefore, results should not be extrapolated to neonates

in the current study, who were receiving phototherapy.

Because only some G-6-PD-deficient neonates develop hyperbilirubinemia, additional icterogenic factors have been sought. It has been questioned whether, within the G-6-PD-deficient subset, those with very low enzyme activity levels may have a higher incidence of, or more severe, jaundice, compared with those at the upper end of the spectrum. Studies to date have been inconclusive, and the level of enzyme activity in G-6-PD-deficient erythrocytes does not bear a consistent relationship to either the degree of hemolysis or the development of hyperbilirubinemia.^{33,47-49} Correlation analyses in the current study demonstrated no effect of residual G-6-PD activity on the severity of bilirubinemia grading. Similarly, the lack of correlation of ETCOc values with bilirubin grading confirms observations showing that the increased hemolysis found in association with G-6-PD deficiency may not be the primary factor involved in the pathogenesis of jaundice, in contrast to G-6-PD-normal populations, in which increased hemolysis plays a direct role in the mechanism of neonatal jaundice.⁴⁷

In most G-6-PD A- cases, a 202A mutation is encountered; 968C and 680T mutations have also been found, however.^{16,50} We did not attempt to perform genotypic analysis in this study, and it is possible that 1 of these G-6-PD A- genotypes was over-represented in the hyperbilirubinemic group. The aim of the study was to determine, in a practical clinical setting, whether African American neonates with G-6-PD deficiency were at increased risk for hyperbilirubinemia. Although practicing pediatricians could safely presume that almost all G-6-PD-deficient African American neonates they would encounter would have the A- variety, information regarding genotypes would not be available on a daily basis. In future studies, consideration should be given to addressing the issue of the various genotypes and their possible effects on the development of hyperbilirubinemia.

CONCLUSIONS

Our data have highlighted a subgroup of neonates within the African American community who may be at risk for hyperbilirubinemia and its complications. Fortunately, kernicterus seems to be a rare condition; to eliminate it completely, however, there is a need to monitor these neonates vigilantly, to detect hyperbilirubinemia and treat it without delay. The World Health Organization has recommended screening for G-6-PD deficiency in geographic areas where the incidence of the condition among male subjects exceeds 3 to 5%.⁵¹ Although the issue was not addressed in the current study, future studies and health planning programs should consider assessment of the cost-benefit ratio for screening in this group. The degree to which female neonates in this population are affected remains to be ascertained. A high degree of awareness is necessary to minimize the consequences of G-6-PD deficiency.

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REFERENCES

1. Johnson LH, Brown AK. A pilot registry for acute and chronic kernicterus in term and near term infants. *Pediatrics*. 1999;104(suppl):736A
2. Johnson LH, Bhutani VK, Brown AK. System-based approach to management of neonatal jaundice and prevention of kernicterus. *J Pediatr*. 2002;140:396-403
3. Penn AA, Enzmann DR, Hahn JS, Stevenson DK. Kernicterus in a full term infant. *Pediatrics*. 1994;93:1003-1006
4. Washington EC, Ector W, Abboud M, Ohning B, Holden K. Hemolytic jaundice due to G6PD deficiency causing kernicterus in a female newborn. *South Med J*. 1995;88:776-779
5. Carpentieri U, Moore RL, Nichols MM. Kernicterus in a newborn female with G-6-PD deficiency. *J Pediatr*. 1974;89:854-855
6. Moore AM, Perlman M. Symptomatic hypoglycemia in otherwise healthy, breastfed, term newborns. *Pediatrics*. 1999;103:837-839
7. MacDonald MG. Hidden risks: early discharge and bilirubin toxicity due to glucose-6-phosphate dehydrogenase deficiency. *Pediatrics*. 1995;96:734-738
8. Ahlfors CE, Herbsman O. Unbound bilirubin in a term newborn with kernicterus. *Pediatrics*. 2003;111:1110-1112
9. Tarlow AR, Brewer GJ, Carson PE, Alving AS. Primaquine sensitivity. *Arch Int Med*. 1962;109:209-234
10. Heller P, Best WR, Nelson RB, Becktel J. Clinical implications of sickle-cell trait and glucose-6-phosphate dehydrogenase deficiency in hospitalized black male patients. *N Engl J Med*. 1979;300:1001-1005
11. O'Flynn MED, Hsia DY-H. Serum bilirubin levels and glucose-6-phosphate dehydrogenase deficiency in newborn American Negroes. *J Pediatr*. 1963;63:160-161
12. Maisels MJ. Jaundice. In: Avery GB, Fletcher MA, MacDonald MG, eds. *Neonatology: Pathophysiology and Management of the Newborn*. Philadelphia, PA: Lippincott Williams & Wilkins; 1999:765-819
13. Maisels MJ, Newman TB. Jaundice in full-term and near-term babies who leave the hospital within 36 hours. *Clin Perinatol*. 1998;25:295-302
14. Brown AK, Damus K, Kim MH, et al. Factors relating to readmission of term and near term neonates in the first two weeks of life. *J Perinat Med*. 1999;27:263-275
15. Stevenson DK, Fanaroff AA, Maisels MJ, et al. Prediction of hyperbilirubinemia in near-term and term infants. *Pediatrics*. 2001;108:31-39
16. Beutler E. G6PD deficiency. *Blood*. 1994;84:3613-3636
17. Bhutani VK, Johnson L, Sivieri EM. Predictive ability of a predischarge hour-specific serum bilirubin for subsequent significant hyperbilirubinemia in healthy term and near-term newborns. *Pediatrics*. 1999;103:6-14
18. American Academy of Pediatrics, Provisional Committee for Quality Improvement and Subcommittee on Hyperbilirubinemia. Practice parameter: management of hyperbilirubinemia in the healthy term newborn. *Pediatrics*. 1994;94:558-565
19. Beutler E. *Red Cell Metabolism: A Manual of Biochemical Methods*. 3rd ed. New York, NY: Grune & Stratton; 1984:68-71
20. Kaplan M, Leiter C, Hammerman C, Rudensky B. Enzymatic activity in glucose-6-phosphate dehydrogenase-normal and -deficient neonates measured with a commercial kit. *Clin Chem*. 1995;41:1665-1667
21. Vreman HJ, Mahoney JJ, Stevenson DK. Carbon monoxide and carboxyhemoglobin. *Adv Pediatr*. 1995;42:303-334
22. Stevenson DK, Vreman HJ. Carboxyhemoglobin and bilirubin production in neonates. *Pediatrics*. 1997;100:252-254
23. Stevenson DK, Vreman HJ, Oh W, et al. Bilirubin production in healthy term infants as measured by carbon monoxide in breath. *Clin Chem*. 1994;40:1934-1939
24. Vreman HJ, Stevenson DK, Oh W, et al. Semiportable electrochemical instrument for determining carbon monoxide in breath. *Clin Chem*. 1994;40:1927-1933
25. Alden ER, Lynch SR, Wennberg RP. Carboxyhemoglobin determination in evaluating neonatal jaundice. *Am J Dis Child*. 1978;127:214-217
26. Valaes T. Severe neonatal jaundice associated with glucose-6-phosphate dehydrogenase deficiency: pathogenesis and global epidemiology. *Acta Paediatr Suppl*. 1994;394:58-76
27. Kaplan M, Hammerman C. Severe neonatal hyperbilirubinemia: a potential complication of glucose-6-phosphate dehydrogenase deficiency. *Clin Perinatol*. 1998;25:575-590
28. Slusher TM, Vreman HJ, McLaren DW, Lewison LJ, Brown AK, Stevenson DK. Glucose-6-phosphate dehydrogenase deficiency and carboxy-

- hemoglobin concentrations associated with bilirubin-related morbidity and death in Nigerian infants. *J Pediatr*. 1995;126:102–108
29. Kaplan M, Hammerman C. Glucose-6-phosphate dehydrogenase-deficient neonates: a potential cause for concern in North America. *Pediatrics*. 2000;106:1478–1480
 30. Stoll BJ, Kliegman RM. Jaundice and hyperbilirubinemia in the newborn. In: Behrman RE, Kliegman RM, eds. *Nelson Textbook of Pediatrics*. Philadelphia, PA: WB Saunders; 2004:592–596
 31. Brown AK. Hyperbilirubinemia in black infants: role of glucose-6-phosphate dehydrogenase deficiency. *Clin Pediatr*. 1992;31:712–715
 32. Centers for Disease Control and Prevention. Kernicterus in full-term infants: United States, 1994–1998. *MMWR Morb Mortal Wkly Rep*. 2001; 50:491–494
 33. Zinkham WH. Peripheral blood and bilirubin values in normal full-term primaquine-sensitive Negro infants: effect of vitamin K. *Pediatrics*. 1963;31:983–995
 34. Wolff JA, Grossman BH, Paya K. Neonatal serum bilirubin and glucose-6-phosphate dehydrogenase deficiency. *Am J Dis Child*. 1967;113: 251–254
 35. Segel GB. Glucose-6-phosphate dehydrogenase (G-6-PD) and related deficiencies. In: Behrman RE, Kliegman RM, eds. *Nelson Textbook of Pediatrics*. Philadelphia, PA: WB Saunders; 2004:1636–1638
 36. Linn S, Schoenbaum SC, Monson RR, Rosner B, Stubblefield PG, Ryan KJ. Epidemiology of neonatal hyperbilirubinemia. *Pediatrics*. 1985;75: 770–774
 37. Eshaghpour E, Oski FA, Williams M. The relationship of erythrocyte glucose-6-phosphate dehydrogenase deficiency to hyperbilirubinemia in Negro premature infants. *J Pediatr*. 1967;70:595–601
 38. Lopez R, Cooperman JM. Glucose-6-phosphate dehydrogenase deficiency and hyperbilirubinemia in the newborn. *Am J Dis Child*. 1971; 122:66–70
 39. Karayalcin G, Acs H, Lanzkowsky P. G6PD deficiency and hyperbilirubinemia in black American full-term infants. *NY State J Med*. 1979;79: 22–25
 40. Gibbs WN, Gray R, Lowry M. Glucose-6-phosphate dehydrogenase deficiency and neonatal jaundice in Jamaica. *Br J Haematol*. 1979;43: 263–274
 41. Kaplan M, Rubaltelli FF, Hammerman C, et al. Conjugated bilirubin in neonates with glucose-6-phosphate dehydrogenase deficiency. *J Pediatr*. 1996;128:695–697
 42. Kaplan M, Muraca M, Hammerman C, et al. Bilirubin conjugation, reflected by serum conjugated bilirubin fractions, in glucose-6-phosphate dehydrogenase-deficient neonates: a determining factor in the pathogenesis of hyperbilirubinemia. *Pediatrics*. 1998;102(3). Available at: www.pediatrics.org/cgi/content/full/102/3/e37
 43. Kaplan M, Renbaum P, Levy-Lahad E, Hammerman C, Lahad A, Beutler E. Gilbert syndrome and glucose-6-phosphate dehydrogenase deficiency: a dose-dependent genetic interaction crucial to neonatal hyperbilirubinemia. *Proc Natl Acad Sci USA*. 1997;94:12128–12132
 44. Doyle JJ, Schmidt B, Blanchette V, Zipursky A. Hematology. In: Avery GB, Fletcher MA, MacDonald MG, eds. *Neonatology: Pathophysiology and Management of the Newborn*. Philadelphia, PA: Lippincott Williams & Wilkins; 1999:1045–1099
 45. Matsunaga AT, Lubin AT. Hemolytic anemia in the newborn. *Clin Perinatol*. 1995;22:803–828
 46. Javier MC, Krauss A, Nesin M. Corrected end-tidal carbon monoxide closely correlates with the corrected reticulocyte count in Coombs' test-positive term neonates. *Pediatrics*. 2003;112:1333–1337
 47. Kaplan M, Vreman HJ, Hammerman C, Leiter C, Abramov A, Stevenson DK. Contribution of haemolysis to jaundice in Sephardic Jewish glucose-6-phosphate dehydrogenase-deficient neonates. *Br J Haematol*. 1996;93:822–827
 48. Lo YS, Lu CC, Chiou SS, et al. Molecular characterization of glucose-6-phosphate dehydrogenase deficiency in Chinese infants with or without severe neonatal hyperbilirubinemia. *Br J Haematol*. 1994;86:858–862
 49. Meloni T, Cutillo S, Testa U, et al. Neonatal jaundice and severity of glucose-6-phosphate dehydrogenase deficiency in Sardinian babies. *Early Hum Dev*. 1987;15:317–322
 50. Beutler E. Glucose-6-phosphate dehydrogenase deficiency and other red cell enzyme abnormalities. In: Beutler E, Lichtman MA, Coller BS, Kipps TJ, Seligsohn U, eds. *Williams Hematology*. New York, NY: McGraw-Hill; 2001:527–545
 51. WHO Working Group. Glucose-6-phosphate dehydrogenase deficiency. *Bull World Health Org*. 1989;67:601–611

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