PEDIATRICS®
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

Predischarge Bilirubin Screening in Glucose-6-Phosphate Dehydrogenase-Deficient Neonates

Michael Kaplan, Cathy Hammerman, Roselyn Feldman and Rachel Brisk

Pediatrics 2000;105;533-537
DOI: 10.1542/peds.105.3.533

Predischarge Bilirubin Screening in Glucose-6-Phosphate Dehydrogenase-Deficient Neonates

Michael Kaplan, MB, ChB*‡; Cathy Hammerman, MD*‡; Roselyn Feldman, RN, MPA*; and Rachel Brisk, MSc§

ABSTRACT. *Objective.* **To assess the validity of predischarge serum bilirubin values in determining or predicting hyperbilirubinemia in glucose-6-phosphate dehydrogenase (G-6-PD)-deficient neonates, and to facilitate appropriate discharge planning.**

Methods. **Serum total bilirubin values were determined between 44 and 72 hours of life in a cohort of term, healthy neonates at high-risk for G-6-PD deficiency but with no other risk factors for hyperbilirubinemia. Percentile-based bilirubin nomograms were constructed for G-6-PD-deficient infants and normal infants according to age at sampling. The incidence of hyperbilirubinemia (serum bilirubin value ≥256 μmol/L [15 mg/dL]) for each group was determined according to the percentiles for that group.**

Results. **In both G-6-PD-deficient neonates (n = 108) and control neonates (n = 215) with serum bilirubin values <50th percentile for age, the incidence of hyperbilirubinemia was low in the G-6-PD-deficient neonates, with no measurable incidence in the controls. The incidence of hyperbilirubinemia became clinically consequential, and significantly higher in the G-6-PD-deficient groups, when the percentiles were ≥50: for those in the 50% to 74% range the incidence was moderate (23%) for the G-6-PD-deficient and small (7%) for the control infants (relative risk, 3.29; 95% confidence interval, 1.01–10.67). Among those infants ≥75th percentile, 82% of the G-6-PD-deficient infants, compared with 25% of the control infants, were either already hyperbilirubinemic at the time of screening or subsequently developed hyperbilirubinemia (relative risk, 3.23; 95% confidence interval, 1.99–5.24).**

Conclusions. **Timed, predischarge serum bilirubin screening can be used to identify G-6-PD-deficient neonates at low, intermediate, or high-risk of developing severe neonatal hyperbilirubinemia, and thus offer a selective approach to the discharge and follow-up surveillance of these infants.** *Pediatrics* **2000;105:533–537;** ***bilirubin, neonatal jaundice, glucose-6-phosphate dehydrogenase deficiency, screening test.***

ABBREVIATIONS. G-6-PD, glucose-6-phosphate dehydrogenase; CI, confidence interval.

From the *Department of Neonatology, and the §Clinical Biochemistry Laboratory, Shaare Zedek Medical Center; and the ‡Faculty of Medicine of the Hebrew University, Jerusalem, Israel.

Received for publication May 7, 1999; accepted Aug 13, 1999.

Reprint requests to (M.K.) Department of Neonatology, Shaare Zedek Medical Center, Box 3235, Jerusalem 91031, Israel. E-mail: kaplan@cc. huji.ac.il

Kernicterus is a potentially severe complication of glucose-6-phosphate dehydrogenase (G-6-PD) deficiency-associated neonatal hyperbilirubinemia.[1,2] Recent reports of kernicterus arising from both Africa[3] and from America[4,5] emphasize the importance of G-6-PD deficiency in its etiology, even in modern times. Resurgence of kernicterus, a preventable condition, in recent years, concomitant with the era of early postnatal hospital discharge,[6] has made identification of infants at high-risk for this condition essential.

The predominant factor in the pathogenesis of neonatal hyperbilirubinemia associated with G-6-PD deficiency has been shown to be decreased bilirubin conjugation.[7,8] This is the result of a gene interaction between the G-6-PD deficiency and promoter polymorphism for the variant gene for the bilirubin conjugating enzyme UDP glucuronosyltransferase 1A1 seen in Gilbert syndrome.[9] Prediction of hyperbilirubinemia in G-6-PD-deficient neonates will therefore necessarily have to include a test reflective of bilirubin conjugation. Because the serum total bilirubin value at any point in time reflects the balance between bilirubin production and bilirubin elimination (hepatic uptake, conjugation, and excretion), the serum total bilirubin value can be regarded as a simple index of both these factors.

Bhutani et al[10] recently used this principle to successfully evaluate the predictive value of universal predischarge serum bilirubin measurements. They determined that a percentile-based, timed bilirubin nomogram for the first week of life was useful for classifying neonates at high- or low-risk for developing significant hyperbilirubinemia (defined as serum bilirubin value ≥95th percentile for age in hours). Because a serum bilirubin value at the beginning of the second day may be at a much higher point on the percentile scale than the identical value at the end of that day,[11] the nomograms were constructed based on hour-specificity. In their study, Bhutani et al[10] included primarily infants at low-risk for hyperbilirubinemia. We therefore asked whether this principle would be of use in the high-risk, G-6-PD deficiency situation.

The aim of this study was thus to determine if age-specific serum total bilirubin values, plotted on a percentile-based nomogram adapted from Bhutani and colleague's[10] model, would be useful in determining the risk of developing hyperbilirubinemia in

G-6-PD-deficient neonates with no other obvious cause for hyperbilirubinemia.

METHODS

The population studied was drawn from newborns consecutively delivered at the Shaare Zedek Medical Center to mothers of the Sephardic Jewish community at high-risk for G-6-PD deficiency[12,13] and its associated hyperbilirubinemia.[14] Both females and males were included. The females in this group were drawn from previously published databases[9,15] whereas the males were collected as part of an ongoing study. According to routine nursery protocol, all neonates of this high-risk subset of our population are routinely tested for G-6-PD deficiency on the first day of life. The results are available to us the same day. Blood group typing and direct Coombs' testing are performed on infants born to blood group O or Rh-negative mothers, or on any infant whose bilirubin levels are high enough to warrant phototherapy. Infants are routinely observed visually for the development of significant clinical jaundice, and serum bilirubin testing is performed as clinically indicated. G-6-PD-deficient infants are observed in-hospital for a minimum of 72 hours. Subsequent to discharge, infants are followed by our medical staff, as outpatients, until stabilization of the serum bilirubin values or the need for therapy is determined. Phototherapy is commenced in the G-6-PD-deficient neonates if the serum bilirubin values exceeds 254 μmol/L (14.9 mg/dL), and exchange transfusion performed should the serum bilirubin value exceed 342 μmol/L (20 mg/dL) and be unresponsive to a trial of phototherapy for up to 3 hours. Breastfeeding is encouraged, although the mothers are warned of the dangers of eating fava beans while nursing or ingesting any drugs known to be triggers of hemolysis in G-6-PD deficiency.

For the purpose of this study, the only deviation from routine treatment was a mandatory serum bilirubin test performed between 44 and 72 hours of life, concurrent with routine predischarge metabolic screening. The infants' ages were recorded at the time of this bilirubin sampling. Additional serum bilirubin determinations were performed as clinically required. Only healthy, inborn neonates admitted to the well-baby nursery, with gestational age ≥37 weeks, free of any condition which might exacerbate hyperbilirubinemia, such as cephalhematoma, maternal diabetes, sepsis (ruled out by clinical evaluation and blood cultures as deemed necessary), Down syndrome, or Coombs'-positive hemolytic anemia, were included in the survey. The Institutional Review Board of the Shaare Zedek Medical Center approved this bilirubin testing.

Laboratory Analysis

Initial G-6-PD screening was performed using a commercial visual qualitative color reduction kit (Kit No. 400, Sigma Diagnostics, St Louis, MO) which has previously been shown to be accurate in determining the G-6-PD status in male neonates.[16] Because of the inaccuracy of this test in identifying heterozygotes,[15] and to confirm those males with a G-6-PD-deficient screening test result, molecular classification of the G-6-PD genotype was performed by Dr Ernest Beutler at the Scripps Research Institute, La Jolla, California, on DNA obtained from umbilical cord blood. Polymerase chain reaction followed by allele-specific oligonucleotide hybridization was used to determine the presence or absence of nt 563, the nucleotide mutated in G-6-PD Mediterranean.[17] Details of the procedure have been published elsewhere.[9]

Serum total bilirubin values were determined by reflectance spectrophotometry using an Ektachem analyzer (Vitros 700c/750XRC Chemistry System, Johnson and Johnson Clinical Diagnostics, Rochester, NY). Blood group determinations and direct Coombs' testing were performed by routine laboratory techniques.

Data Analysis

The G-6-PD genotype as determined by molecular means was used to classify the infants into study and control groups. The study group comprised those who had been tested for the condition and found to have G-6-PD deficiency, whereas the control population comprised those of the same population group who had been tested and were found to be G-6-PD normal. Because female G-6-PD-deficient homozygotes and heterozygotes had a similar incidence of hyperbilirubinemia,[15] neonates with both these genotypes were included in the G-6-PD-deficient study group. Females and males were pooled in both the G-6-PD-deficient and normal groups, because the incidence of hyperbilirubinemia was not significantly different between the sexes. Hyperbilirubinemia was defined as a serum bilirubin ≥256 μmol/L (15 mg/dL) during the first week of life.

Percentile-based bilirubin nomograms according to the age of life at which the mandatory serum bilirubin values were drawn, were calculated for the G-6-PD-deficient and normal control infants, respectively. Each infant was represented by only 1 bilirubin point on the nomogram—that drawn at the time of the metabolic screening. Previous or subsequent serum bilirubin values, determined for clinical reasons, were not included in the nomogram. For the purpose of the nomogram, infants were divided into groups at 4-hour intervals, starting at 44 hours and continuing until 72 hours. For each 4-hour interval, the percentiles of the serum bilirubin values taken during that time interval were calculated and smoothed percentile curves were drawn. The number of study and control infants who developed hyperbilirubinemia in each percentile range was noted. Relative risk was calculated to estimate the risk of developing hyperbilirubinemia in the G-6-PD-deficient groups relative to that of the control group. Ninety-five percent confidence intervals (CIs), with hyperbilirubinemia as dependent, and G-6-PD status as an independent variable, were used as a measure of the statistical precision of each relative risk, and were regarded as significant when the 95% CIs were wholly more than or less than 1. If the 95% CI straddled 1, the results were regarded as not significant. Categorical variables were compared using χ^2 analysis, whereas continuous variables were analyzed using Student's t test, significance being defined as $P < .05$.

RESULTS

Timed serum bilirubin values were obtained in 323 neonates (172 males, 151 females), of whom 108 were G-6-PD-deficient and 215 were G-6-PD normal. Demographic and clinical details of these infants are summarized in Table 1. The mean (plus/minus standard deviation) age of sampling was 56 ± 9 hours and 55 ± 9 hours for the study and control groups, respectively. Thirty-one (28.7%) of the G-6-PD-deficient neonates developed hyperbilirubinemia, compared with 18 (8.4%) of the control population ($P < .0001$). At the time of the mandatory bilirubin determination, some infants already had serum bilirubin values which met our definition of hyperbilirubinemia: 14 of the G-6-PD-deficient neonates already had a serum bilirubin value ≥256 μmol/L (15 mg/dL), compared with 6 of the control infants ($P = .002$). Phototherapy was not commenced in any patient before the testing. All infants who did require phototherapy responded to this treatment. No infant required exchange transfusion and no infant developed kernicterus. The mean (standard deviation) peak serum bilirubin value of those neonates with a value ≥256 μmol/L (15 mg/dL) was 316 ± 22 μmol/L (18.5 ± 1.3 mg/dL) (upper limit, 371 μmol/L [21.7 mg/dL]) and 284 ± 15 μmol/L (16.6 ± 0.9 mg/dL) (upper limit, 320 μmol/L [18.7 mg/dL]) for the G-6-PD-deficient and control infants, respec-

TABLE 1. Demographic Details of Neonates Studied

Parameter	G-6-PD Deficient	G-6-PD Normal	Significance
n	110	215	
Gestational age (wk)	39.5 ± 1.3	39.8 ± 1.5	NS
Birth weight (g)	3192 ± 396	3307 ± 424	$P = .02$
Vaginal delivery (%)	90	95	NS
Exclusively breastfed (%)	70	77	NS

NS indicates not significant.

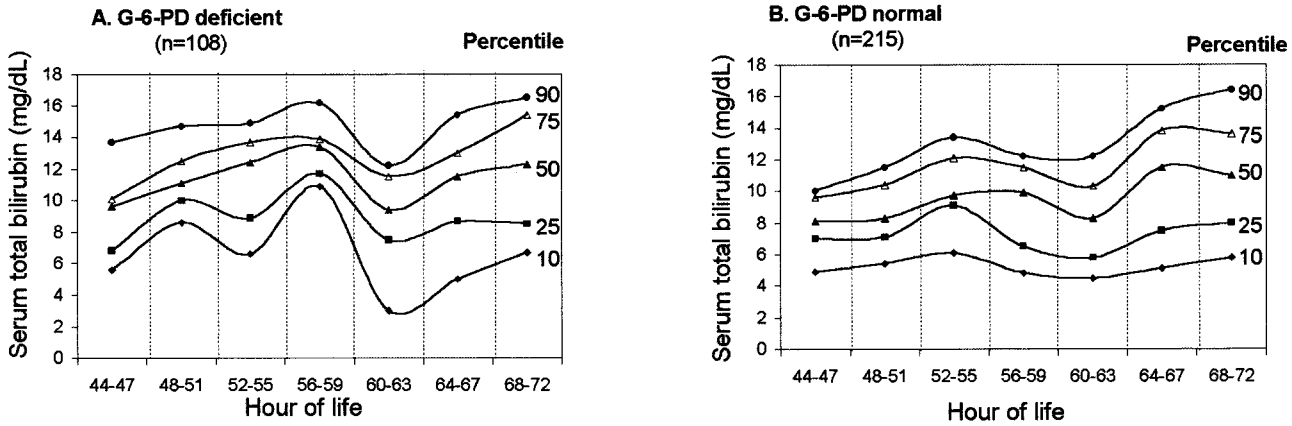

Fig 1. Smoothed percentile curves for serum total bilirubin values, in the G-6-PD-deficient (A) and control neonatal populations (B), respectively, according to the hour of life of sampling.

tively. However, institution of phototherapy in many of these patients most likely prevented these values from reaching their natural peak.

Smoothed percentile nomograms for timed serum bilirubin values for the G-6-PD-deficient and control neonates are shown in Fig 1A and B, respectively. The incidence of hyperbilirubinemia in neonates of each group, according to these timed percentiles is illustrated in Fig 2. It will be noted that, of those with a timed bilirubin in the ≤50th percentile group, there was a minimal risk of hyperbilirubinemia in the G-6-PD-deficient neonates, and no measurable incidence in the control group. G-6-PD-deficient infants in the 50% to 74% group had a 23% incidence of hyperbilirubinemia, whereas in the control neonates this was only 7% (relative risk, 3.29; 95% CI range, 1.01–10.67). In the 75% to 89% group, the respective incidences of hyperbilirubinemia were 76.5% and 22.6% (relative risk, 3.37; 95% CI range, 1.68–6.84; $P = .0009$), while in the percentile range ≥90, respective values were 90.9% and 29.2% (relative risk, 3.12; 95% CI range, 1.63–5.98; $P = .003$) for the G-6-PD-deficient and control neonates.

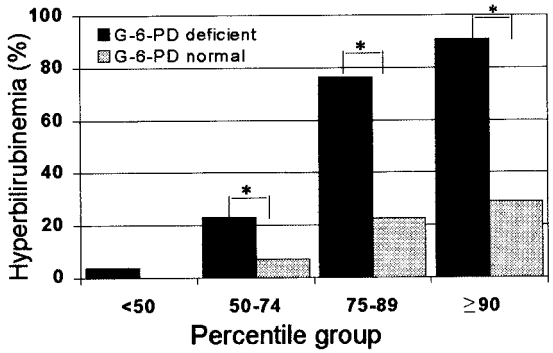

Fig 2. Incidence (percentage) of neonatal hyperbilirubinemia (defined as serum total bilirubin value ≥256 μmol/L [15 mg/dL] in the first week of life) for the G-6-PD-deficient and control infants, according to each group's respective percentile range for timed, predischarge bilirubin value. *, percentile range 50 to 74; relative risk: 3.29; 95% confidence interval (CI): 1.01 to 10.67; percentile range, 75% to 89; relative risk: 3.37; 95% CI: 1.68 to 6.84; percentile range: ≥90; relative risk: 3.12; 95% CI: 1.63 to 5.98

DISCUSSION

In the current study, we have confirmed the usefulness of percentile-based nomograms for timed bilirubin values in predicting hyperbilirubinemia in infants at low-risk for developing that condition, and demonstrated its efficacy in identifying infants in jeopardy in the extremely high-risk G-6-PD-deficient population as well. More than 80% of G-6-PD-deficient neonates with a timed serum bilirubin in the ≥75th percentile developed hyperbilirubinemia, a threefold increased risk compared with the control population. In contrast, both in the study and control populations, those with bilirubin values ≤50th percentile were at minimal risk of developing hyperbilirubinemia. Predischarge serum bilirubin screening is therefore a useful way of assessing the risk of developing hyperbilirubinemia in G-6-PD-deficient neonates.

We did not evaluate our data on the previously published healthy infant nomogram of Bhutani et al,[10] but preferred to construct our own which would better reflect the geographic, ethnical, and cultural characteristics distinctive to our population as opposed to that of Bhutani and colleague's population. We believed that this would be more accurate in that environmental or other genetic influences might affect the distribution of the bilirubin values. Furthermore, as the G-6-PD-deficient neonates have such a high incidence of hyperbilirubinemia, we believed it justified to construct a separate nomogram especially for this study group. Because G-6-PD status is usually available at our institution by the time of the predischarge bilirubin screening, each individual infant can be plotted on the appropriate graph before discharge.

In Israel, by order of the government Ministry of Health, infants are kept hospitalized for a minimum of 48 hours after delivery. Because of the especially high-risk of G-6-PD-deficient neonates developing severe hyperbilirubinemia, at this hospital we keep such neonates in-hospital for a minimum of 72 hours. Therefore the time period during which the routine bilirubin screening was performed, between 44 and 72 hours, was appro-

priate for our population in this setting. In other countries where earlier discharge may be the rule, construction of percentile curves for that population at an earlier period in the infants' lives may be warranted.

For the purpose of this study, consistent with our other studies, we defined hyperbilirubinemia as a serum bilirubin of ≥256 μmol/L (15 mg/dL). This value is equivalent to the 97th percentile of both the Collaborative Perinatal Project,[18] and a subsequent study of >2000 neonates studied in the 1980s.[19] It is also the cutoff point at which we routinely begin phototherapy in G-6-PD-deficient newborns, a measure which has enabled us to keep the need for exchange transfusion to a minimum. Therefore our definition of hyperbilirubinemia differs from that of Bhutani et al,[10] and, as a result, the number of neonates who became hyperbilirubinemic in the control group are necessarily different from the numbers in that study. However, despite differences in definition, the underlying concept of very low-risk for hyperbilirubinemia in those neonates whose serum bilirubin values were <50th percentile, and high-risk in those >75th percentile, was shown in both studies. The G-6-PD-deficient neonates also fall into this pattern.

Percentile curves in both study (Fig 1A) and control populations (Fig 1B) show an apparent peak between 52 and 59 hours, with a fall from 60 to 63 hours, and a subsequent rise toward 72 hours. We do not think that this is a specific pattern inherent to our population, but rather a combination of the relatively small numbers from which these curves were constructed, and the coincidence of several infants who had particularly low bilirubin values at the time of screening, who happened to fall into this time frame. Although it would obviously be preferable to include a patient cohort of the size used by Bhutani et al,[10] the Sephardic Jewish population at-risk for G-6-PD deficiency comprises only a small percentage of the population delivering at this hospital. Because we encounter only a few G-6-PD-deficient patients weekly, it would take an excessively long time period to collect a number of patients similar to that of Bhutani et al. Despite the smaller cohort size, our results clearly show that the concept of universal bilirubin screening for the determination or prediction of hyperbilirubinemia is valid also in a population subgroup with a high incidence of hyperbilirubinemia.

Although these high-risk neonates were followed-up as outpatients after discharge, we do not believe that this system resulted in many patients being lost to follow-up. Patient (parental) compliance in our population is excellent. Parents of all G-6-PD-deficient neonates receive both a written explanatory sheet and oral instruction enumerating the risks of their infants developing severe jaundice and the implications thereof. There is no cost to the family for the first bilirubin test as an outpatient. Furthermore, because of the Jewish religious injunction not to perform ritual circumcision should a infant be jaundiced, there is a greater awareness of neonatal jaundice in the community. We therefore feel confident that certainly all G-6-PD neonates who developed a serum bilirubin value ≥256 μmol/L (15 mg/dL), and virtually all control infants, were brought to our attention.

In an era of prescribed early neonatal discharge, universal predischarge timed bilirubin screening in G-6-PD-deficient neonates may enable these infants to be categorized into those at high-risk, and those at low-risk, for developing neonatal hyperbilirubinemia. A selective approach to follow-up and surveillance of G-6-PD-deficient neonates may enable those with serum bilirubin values in the <50th percentile range to be discharged at 48 hours along with their G-6-PD normal counterparts, provided adequate follow-up arrangements are available. Delayed discharge could be limited to those at high-risk for developing hyperbilirubinemia. It is possible that prediction could be even further enhanced if the risk based on percentile oriented bilirubin values was further modified by taking into consideration additional factors known to exacerbate the degree of neonatal jaundice, such as prematurity, maternal diabetes mellitus, breastfeeding, and ecchymoses or cephalhematoma.

ACKNOWLEDGMENT

We thank Ernest Beutler, MD, for the G-6-PD genotyping and review of the manuscript.

REFERENCES

1. Beutler E. G6PD deficiency. *Blood.* 1994;84:3613–3636
2. Valaes T. Severe neonatal jaundice associated with glucose-6-phosphate dehydrogenase deficiency: pathogenesis and global epidemiology. *Acta Paediatr Suppl.* 1994;394:58–57
3. Slusher TM, Vreman, HJ, McLaren DW, Lewison LJ, Brown AK, Stevenson DK. Glucose-6-phosphate dehydrogenase deficiency and carboxyhemoglobin concentrations associated with bilirubin-related morbidity and death in Nigerian infants. *J Pediatr.* 1995;126:102–108
4. MacDonald MG. Hidden risks: early discharge and bilirubin toxicity due to glucose-6-phosphate dehydrogenase deficiency. *Pediatrics.* 1995; 96:734–738
5. Wahington 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
6. Brown AK, Johnson L. Loss of concern about jaundice and the reemergence of kernicterus in full-term infants in the era of managed care. In: Fanaroff AA, Klaus MH, eds. *The Year Book of Neonatal and Perinatal Medicine*. St Louis, MO: Mosby Year Book; 1996:17–28
7. Kaplan M, Rubaltelli FF, Hammerman C, et al. Conjugated bilirubin in neonates with glucose-6-phosphate dehydrogenase deficiency. *J Pediatr.* 1996;128:695–697
8. 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). URL: http://www.pediatrics.org/cgi/content/full/102/3/e37
9. Kaplan M, Renbaum P, Levi-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
10. 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
11. Maisels MJ, Newman TB. Predicting hyperbilirubinemia in newborns: the importance of timing. *Pediatrics.* 1999;103:493–495
12. Sheba C, Szeinberg A, Ramot B, Adam A, Ashkenazi I. Epidemiologic

surveys of deleterious genes in different population groups in Israel. *Am J Public Health.* 1962;52:1101–1105
13. Kaplan M, Hammerman C, Rudensky B, Kvit R, Abramov A. Neonatal screening for glucose-6-phosphate dehydrogenase deficiency: sex distribution. *Arch Dis Child.* 1994;71:F59–F60
14. Kaplan M, Abramov A. Neonatal hyperbilirubinemia associated with glucose-6-phosphate dehydrogenase deficiency in Sephardic-Jewish infants: incidence, severity and the effect of phototherapy. *Pediatrics.* 1992;90:401–405
15. Kaplan M, Beutler E, Vreman HJ, et al. Neonatal hyperbilirubinemia in glucose-6-phosphate dehydrogenase deficient heterozygotes. *Pediatrics.* 1999;104:68–74
16. Kaplan M, Leiter C, Hammerman C, Rudensky B. Comparison of commercial screening tests for glucose-6-phosphate dehydrogenase deficiency in the neonatal period. *Clin Chem.* 1997;43:1236–1237
17. Vives-Corrons J-L, Kuhl W, Pujades MA, Beutler E. Molecular genetics of G6PD Mediterranean variant and description of a new G6PD mutant, G6PD Andalus ^{1361A}. *Am J Hum Genet.* 1990;47:575–579
18. Hardy JB, Drage JS, Jackson EC. *The First Year of Life: The Collaborative Perinatal Project of the National Institutes of Neurological and Communicative Disorders and Stroke*. Baltimore, MD: Johns Hopkins University Press; 1979:104
19. Maisels MJ, Gifford K. Normal serum bilirubin levels in the newborn and the effect of breastfeeding. *Pediatrics.* 1986;78:837–843

Predischarge Bilirubin Screening in Glucose-6-Phosphate Dehydrogenase-Deficient Neonates

Michael Kaplan, Cathy Hammerman, Roselyn Feldman and Rachel Brisk

Pediatrics 2000;105;533-537
DOI: 10.1542/peds.105.3.533