Jaundice Due to Glucose-6-Phosphate Dehydrogenase Deficiency

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Educational Gaps

1. Does predischarge screening of bilirubin and glucose-6-phosphate dehydrogenase (G6PD) in otherwise healthy newborns prevent adverse consequences of newborn jaundice?
2. Are point-of-care assays for bilirubin and/or G6PD accurate and timely to determine the risk of subsequent severe hyperbilirubinemia?
3. Does identification of G6PD deficiency, clinician and parent education and targeted post-birthing care meet the safety and cost-effectiveness standards to prevent unnecessary risk of exchange transfusion?

Abstract

Glucose-6-phosphate dehydrogenase (G6PD) deficiency complicates the usually benign neonatal jaundice managed by existing prenatal and postnatal screening in the United States. Estimated at ~3.4% incidence, the condition ranges by infant race/ethnicity (12.2% in African American male infants to nearly 0% in white female infants). Oxidant stressors, sepsis, and delay in bilirubin elimination (such as co-inheritance with Gilbert's disease or persistent enterohepatic recirculation) add to total plasma or serum bilirubin (TSB) rise, need for phototherapy, and risk for exchange transfusion. Biology of G6PD deficiency, in the context of gender, race, ethnicity, enzyme concentration, and interaction with postnatal environment, affects clinical presentations. Mutation of the X-linked G6PD gene results in varying enzyme activity. A combination of clinical patterns are suggested: (1) early-onset hyperbilirubinemia (ie, TSB >75th percentile and increased bilirubin production); (2) predischarge TSB <75th percentile track exacerbated by starvation, unrecognized sepsis or late prematurity; (3) slow postnatal rise with natural decline; (4) slow postnatal rise with persistent prolonged unconjugated hyperbilirubinemia, >2 weeks age; and (5) complicated by acute-onset, dramatic hyperbilirubinemia with TSB rise >1 mg/dL per hour ("favism"). Absent G6PD deficiency diagnosis, postdischarge management for phototherapy requires expert assessment and triage for probable risk of favism. Screening as well as clinician and parental awareness of G6PD enzyme deficiency has been shown to reduce adverse neonatal consequences in several communities worldwide.

Abbreviations

ABE:	acute bilirubin encephalopathy
CI:	95% confidence interval
FST:	fluorescent spot test
G6PD:	glucose-6-phosphate dehydrogenase
NADPH:	reduced form of nicotinamide adenine dinucleotide phosphate
PCR:	polymerase chain reaction
TSB:	total plasma or serum bilirubin
UGT:	uridine diglucuronide transferase

Objectives

After completing this article, readers should be able to:

1. Define the biological and clinical expressions of neonatal hyperbilirubinemia associated with G6PD deficiency.
2. Describe the value and limitations of bilirubin screening to assess risk of severe hyperbilirubinemia in neonates with G6PD deficiency.
3. Define the clinical consequences of severe hyperbilirubinemia associated with G6PD deficiency.

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Introduction

Jaundice is observed in >80% of all healthy newborns during the first week after birth (1)(2), and glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most frequent human enzyme defect estimated in ~400 million individuals worldwide. (3)(4) The unique genetic, biochemical, and clinical significance of G6PD deficiency has been extensively investigated (Table 1). Its adverse physiologic manifestations are encountered globally due to migration, multiracial family structures, and diverse ethnic ancestries. (5) Both jaundice and G6PD deficiency individually are usually benign with limited, if any, lifelong impact. However, the incidence of neonatal jaundice is several-folds higher in G6PD-deficient infants compared with those who are sufficient. (6) Drug-sensitive or oxidant stress–mediated hemolysis, sepsis, ingestion or exposure to certain foods, dyes, and chemicals trigger acute manifestations. Chronic sequelae include

Table 1. Review of Evolution of Evidence of Neonatal G6PD deficiency

Time	Observation/Report	Reference
Greco-Roman Era	Ancient Greek cultural observations	Eating of fava beans (*Vicio faba*) forbidden by many community leaders
1905	Clinical description of favism	Fermi C, Martinetti P. Studio sul favismo. *Annali di Igiene Sperimentale.* 1905;15:76 (as listed in reference 8)
1941	Scientific description of favism	Luisada L. Favism: a singular disease affecting chiefly red blood cells. *Medicine.* 1941;20:229–231
1956	Discovery of G6PD deficiency	Carson PE, Flanagan CL, Ickes CE, Alving AS. Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science.* 1956; 124: 484–85
1958	X chromosome location of the G6PD gene	Childs B, Zinkham W, Browne EA, Kimbro EL, Torbert JV. A genetic study of a defect in glutathione metabolism of the erythrocytes. *Bull Johns Hopkins Hosp.* 1958;102:21–37
1959	Kernicterus: dangerous but preventable consequence	Beutler E. The hemolytic effect of primaquine and related compounds: a review. *Blood.* 1959;14:103–139
1960	Similarity between favism and primaquine-sensitivity reported in Sardinia	Panizon F. Icterus gravis neonatorum associated with a deficiency in glucose-6-phosphate dehydrogenase. *Biol Neonat.* 1960;2:167–177
1960	Malaya	Weatherall DJ. Enzyme deficiency in haemolytic disease of the newborn. *Lancet.* 1960;15;2:835–837
1961	New Guinea	Kidson C. Erythrocyte glucose-6-phosphate dehydrogenase deficiency in New Guinea and New Britain. *Nature.* 1961;190:1120–1121
1961	Greece	Doxiams SA, Fessas P, and Valaes T. *Lancet* 1961;1:297
1964	Chinese, Greek, and Italian immigrant infants in Canada	Naiman JL, Kosoy MH. Red cell glucose-6-phosphate dehydrogenase deficiency—a newly recognized cause of neonatal jaundice and kernicterus in Canada. *Can Med Assoc J.* 1964:91:1243–1252
1967	WHO standardization of procedures for the study of G6PD deficiency	Betke K, Beutler E, Brewer GJ, et al. Standardization of procedures for the study of glucose-6-phosphate dehydrogenase: report of a WHO scientific group. *World Health Organ Tech Rep Ser.* 1967;366:1–53
1966–1984	Definitive report of 140 biochemical variants of G6PD.	Beutler E. G6PD deficiency. *Blood.* 1984;84:3613–3636.

hemolytic (nonspherocytic) anemia, cholelithiasis, splenomegaly, and protection against malaria. Co-inheritance with conditions such as thalassemia, pyruvate kinase deficiency, hereditary spherocytosis, and congenital dyserythropoietic anemia can exacerbate the chronic anemia. (7)(8)

Neonatal G6PD deficiency is a setup for a clinical "perfect storm" of hyperbilirubinemia. Increased bilirubin production, usual and variably high in most neonates, coupled with co-inheritance of G6PD and uridine diglucuronide transferase (UGT) gene polymorphisms can overwhelm the slow bilirubin elimination system in the newborn. Exacerbation of bilirubin production either by the most identifiable causes of neonatal hemolysis or unrecognized or unanticipated hemolytic disorders results in a bilirubin rate of rise that exceeds the 95th percentile track (>0.2 mg/dL per hour). Delays in recognition of these clinical risk factors, inability to adequately monitor the rapid progression of these signs, and limited therapeutic options to reverse the process in a timely manner leads to adverse lifelong consequences in some infants. These include risk of bilirubin-induced neurologic damage, rescue use of risk-laden exchange transfusion, and kernicteric mortality. This review focuses on the prevalence and the genetic, biochemical, and clinical perspectives of neonatal G6PD deficiency; the burden and risk of interventions; as well as the value and limitations of contemporary screening strategies needed for an evidence-based best practice to prevent adverse outcomes.

Prevalence in the United States

Approximately 3.2 million livebirths in the United States are healthy, >35 weeks in gestation, and should have benign outcomes with little or no threat of neurological compromise from medical conditions during the first year after birth. Proven preventive health measures provided in newborn nurseries have been effective in reducing infant mortality and morbidities in this group of infants. Jaundice is the commonest clinical diagnosis in neonatal medicine and is due to elevated unconjugated (indirect) and/or conjugated (direct) bilirubin levels. Standard definitions for severity of neonatal hyperbilirubinemia at age >72 hours are listed in Table 2. (9) Bilirubin is a known antioxidant at low levels (in vitro) and a potent neurotoxin at high levels (in vivo). Elevated bilirubin concentrations may occur as a result of increased bilirubin production (breakdown of heme moiety of hemoglobin in the red blood cell) and/or delayed bilirubin elimination (hepatic and intestinal) as well as by a unique neonatal phenomenon of enterohepatic reabsorption of bilirubin. (10)

G6PD deficiency is the most easily identified inherited disorder that causes newborn jaundice, severe hyperbilirubinemia, and bilirubin encephalopathy. (10)(11)(12)(13) Furthermore, acute bilirubin encephalopathy (ABE) and its posticteric chronic sequelae (kernicterus, in its classic form) are the most severe, life-threatening manifestations of neonatal G6PD deficiency that should be preventable. The overall US ethnic incidence of G6PD

Table 2. Progression of Newborn Jaundice to Severe Hyperbilirubinemia

Clinical Event	Incidence[a] (range)	Suggested Clinical Action	Analogy
Newborn jaundice	65%–84%	Bilirubin screening	Use of a seat belt
Bilirubin >75th percentile for age (hours)	25%–30%	Evaluate and manage	Use safety precautions
Bilirubin >15 mg/dL, age >72 h; Bilirubin >75th + risk factors (late preterm) or >95th percentile; or, crossing percentile tracks	8%–12%	Consider use of phototherapy; data on G6PD status should be available	Effective treatment, safety concerns; prevent risk of brain damage
Use of intensive phototherapy	4%–8%	For bilirubin rate of rise >5 mg/dL per 24 h	Crash-cart approach
Use of exchange transfusion	45–60/ 100,000 births	Emergency procedure for any neurologic signs or TSB >30 mg/dL	Crash landing
Bilirubin level >30 mg/dL[b]	Avoidable event	Intensive monitoring for life-saving interventions	Sentinel event

[a]Estimated range, based on cumulative review of literature.
[b]Risk of kernicterus is ~1 in 4 infants. At facilities with limited access to intensive newborn care, this threshold for referrals may be lowered to 20 to 25 mg/dL, based on local experiences. TSB = total plasma or serum bilirubin.

deficiency is estimated at 3.4% and ranges among race/ethnic cohorts from 0% to 12.2% (Table 3), with male African Americans being the most vulnerable. (14)(15) Clinical manifestations during the neonatal period include early-onset hyperbilirubinemia, persistent or delayed resolution of significant hyperbilirubinemia (due to UGT gene polymorphism as a comorbidity), acute onset of hazardous hyperbilirubinemia, risk of associated sepsis, and neonatal death. Manifestations of acute hemolysis, "favism," chronic anemia, abnormal drug reactions (such as exposure to certain antimalarials) are evident in infants, children, and adults. Single-site (16)(17) and global experiences in regions and nations at high risk for G6PD deficiency have led to identification of at-risk infants and have led to public health education programs to prevent or recognize favism crises following hemolytic triggers. (18)(19)(20)(21)(22)(23) This may not be unexpected in countries where there is a high background incidence of the deficiency, and it is therefore not surprising that recent reports of G6PD deficiency as a major etiological factor for kernicterus emanate from countries such as Turkey, Nigeria, Oman, Malaysia, Taiwan, China, Philippines, and India and from the Mediterranean nations. However, an association between G6PD deficiency and severe hyperbilirubinemia and/or bilirubin encephalopathy is now being noted in other countries because of changing demographics, including the United States, in which the overall incidence of G6PD deficiency is low and G6PD deficiency is not ordinarily recognized as being a health hazard.

The USA Pilot Kernicterus Registry report highlighted the disproportionate representation of G6PD deficiency in a population that was screened by contemporary prenatal care, maternal Rh blood type screening, and cared for as well babies by practicing pediatricians in 1992 to 2004. (24) The most striking report is that of the Pilot Kernicterus Registry, in which 20.8% of newborns cared for as healthy infants readmitted within a week of discharge with ABE were subsequently diagnosed to have G6PD deficiency. This association is many-fold the estimated general frequency of the condition in the United States. Jaundice and hyperbilirubinemia were not usually identified. The G6PD diagnosis was confirmed by either laboratory diagnosis of deficiency using quantitative kinetic G6PD assay interpreted for age and gender or by assay of blood collected after the age of 3 months. DNA analysis was sometimes available but not required.

Of the 125 infants in the registry, 6 infants died during their first year, and 26 infants were confirmed to be G6PD deficient. Twenty-four of these 26 infants were male infants; 73% were African American; 15.4% Asian, 7.7% Hispanic, and 3.9% White; all but 1 were breastfed. First week mortality was 5 of 125 (4%) with hyperbilirubinemia (TSB >30 mg/dL). Mortality included two infants who died earlier to exchange transfusion and one who died before any treatment could be initiated. G6PD deficiency appeared to be a confounding risk factor for both mortality and morbidity Additional risk factors were late prematurity and sepsis. Four of the five deaths were in infants of <37 weeks' GA. However, the severity was evident by uniformly excessive levels >35 mg/dL for infants readmitted between 2.5 and 5 days of age. TSB levels ≤35 mg/dL were noted in six infants (range: 28–33 mg/dL) with ABE; of whom, two had severe sequelae, one died and one responded to "crash-cart" approach). Twenty infants had TSB >35 mg/dL; 19 had advanced ABE. Also from the United States, several case reports of kernicterus in G6PD-deficient female neonates have recently been published. (25)(26) Other examples emanate from Canada, where even fewer individuals in the entire population (<0.5%) are likely to be affected. (19) Of 12 newborns with kernicterus encountered in Toronto between 1990

Table 3. G6PD Deficiency Among US Military Recruits

		Gender Differences of G6PD Deficiency		
Ethnicity of Recruits Tested for G6PD deficiency	Total Recruits Tested	Male Recruits Tested	Percent Male With G6PD Deficiency	Percent Female With G6PD Deficiency
African American	11,276	8,513	12.2	4.1
Asian	2,123	1,658	4.3	0.9
Hispanic	5,304	4,462	2.0	1.2
Caucasian	42,126	38,108	0.3	0.0
American Indian/Alaskan	604	492	0.8	0.9
Other	1,869	1,643	3.0	1.8
Total	63,302	54,876	2.5	1.6

Adapted from Chinevere et al. (15)

and 2000, 7 (58%) were G6PD deficient. More recently, 258 cases of severe neonatal hyperbilirubinemia (TSB >25 mg/dL or having had an exchange transfusion) were collected through the Canadian Paediatric Surveillance Program: of 93 newborns in which a cause could be found for their severe hyperbilirubinemia, 20 (21.5%) were G6PD deficient. (27) Similar findings from a survey from the United Kingdom and Ireland (28) report 108 newborns with extreme neonatal hyperbilirubinemia (30 mg/dL) in whom G6PD deficiency independently increased the risk of encephalopathy many fold (odds ratio = 28.2; 95% confidence interval [CI]: 2.6–307.7).

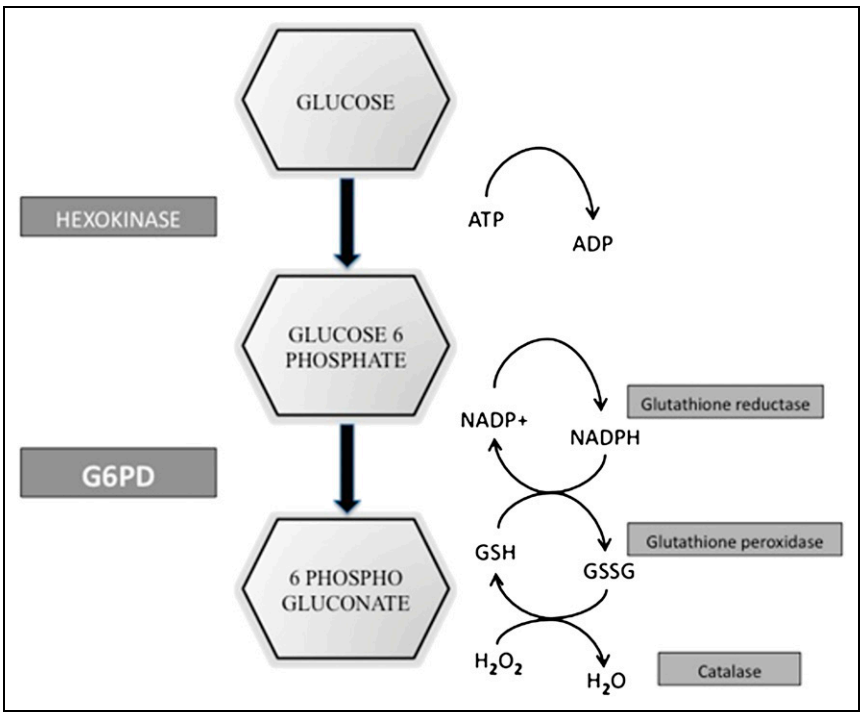

Figure 1. Role of G6PD enzyme action in pentose pathway in red blood cell (RBC) metabolism. G6PD catalyzes NADP+ to its reduced form, NADPH, in the pentose phosphate pathway. ADP=adenosine diphosphate; ATP=adenosine triphosphate; GSH=reduced glutathione; GSSG=oxidized glutathione; NADP+ =nicotinamide adenine dinucleotide phosphate (oxidized form).

Neonatal G6PD Deficiency

Enzyme Function

The enzyme is threefold the size of the hemoglobin molecule and has a functional defect and altered stability due to mutation, rendering it partially active because of loss of molecular conformation or increased susceptibility to proteolytic enzymes. Red blood cells (RBC) devoid of mitochondria rely on glycolysis energy metabolism through the Emden-Meyerhof pathway to convert glucose to lactate and generate adenosine triphosphate (Fig 1). Approximately 10% of the glucose utilization is shunted through the pentose pathway leading to a key metabolic consequence: G6PD enzyme is the rate-limiting enzyme for oxidation of glucose-6-phosphate to 6-phosphogluconic acid with concurrent release of NADPH (reduced form of nicotinamide adenine dinucleotide phosphate). This cofactor serves as a proton donor for many essential enzymatic reactions and is crucial for cells to counterbalance oxidative stress. RBC source of NADPH is solely dependent on the pentose pathway cascade such that glutathione can be maintained in a reduced state to facilitate the conversion of methemoglobin to oxyhemoglobin. The stability of catalase and the ability to regenerate reduced forms of glutathione are integral for detoxification of hydrogen peroxide (H_2O_2). Thus, G6PD serves as the dominant cellular defense against oxidative stress. The enzyme has 515 amino acids and comprises two major domains: (1) amino acids 27–200 at the N-terminal with a β-α-β dinucleotide binding site (amino acids 38–44) and (2) a larger $\beta + \alpha$ domain. Both are linked by an α helix that serves as a binding site (amino acids 198–206). The enzyme is located in all cells in varying concentrations. In neonates with G6PD deficiency, the enzyme activity is heterogeneous and has been classified to five levels of activity: class I: severe deficiency with no or minimal detected enzyme activity, II: severe deficiency with 1% to 10% residual enzyme activity, III: moderate deficiency with 10% to 60% residual enzyme activity, IV: normal enzyme activity at 60% to 150%; and V: increased enzyme activity at >150%.

Molecular Basis for Enzyme Deficiency

The G6PD gene is located on the telomeric region of the long arm of X-chromosome (band Xq28). Mutation of the X-linked G6PD gene (~127 have been reported with a single base substitute leading to amino acid replacements) results in many variants of protein with varying enzyme activity that result in different patterns of clinical manifestations. Oxidative stress and exogenous agents usually appear to serve as triggers for increased bilirubin production. These mutations appear to be de novo, are

not geographic-specific, and do not point to a common ancestor inheritance. Male cases are overrepresented compared with female cases. Males are hemizygous for the G6PD gene; therefore the expression is either normal or deficient. In contrast, in females who have two copies of the gene on each chromosome, the gene expression can be normal or heterozygous. Homozygous inheritance in females can occur; whereas, heterozygous females have genetic mosaicism secondary to X-chromosome inactivation and can have similar manifestation as male neonates. DNA screening allows characterization of different population groups and importantly identifies the female heterozygote. However, not all heterozygotes have phenotypic manifestations of G6PD deficiency.

Biological Risk Factors

Most frequent biological risk factors for severe hyperbilirubinemia and kernicterus are late prematurity, undiagnosed hemolytic disease, genetic abnormalities (such as G6PD deficiency, congenital spherocytosis, galactosemia, Crigler-Najjar syndrome and others that are undiagnosed) and concurrent complications of dehydration, sepsis, or acidosis, hypoalbuminemia, or poor feeding. (1)(2) Other than hemolysis, late preterm infants and G6PD deficiency are among the more frequent reasons for excessive hyperbilirubinemia. (1)(10)(29)(30) Late preterm infants have delayed maturation, a lower concentration of UGT, and immature gastrointestinal function and exhibit feeding difficulties that predispose them to dehydration, increased enterohepatic circulation, decreased stool frequency, and hyperbilirubinemia. G6PD deficiency is a major risk factor for kernicterus because of both increased bilirubin production (which may or may not have been exacerbated by a hemolytic process (10)(11)(12) and concurrent diminished bilirubin conjugation. Exacerbation with coexpression of genetic mutations involved with bilirubin conjugation and elimination and/or a triggered increase in bilirubin production (such as exposure to oxidants, infections and drugs) may lead to progressive or hazardous hyperbilirubinemia.

Risk of Sepsis

Infection acts as a significant trigger for hemolysis in individuals with G6PD deficiency. (7)(8)(31)(32)(33) These agents include bacterial sepsis, urinary tract infections, pneumonia, cytomegalovirus, hepatitis A and B, and intercurrent viral infections. A Saudi Arabian study of 33,943 newborns documented an 18% incidence of G6PD deficiency. Culture-proven sepsis was threefold more likely, as documented in 314 of 100,000 G6PD-deficient infants compared with 100 of 100,000 infants with normal G6PD activity. (34) In the Pilot Kernicterus Registry cohort, (24) out of 26 infants with a confirmed enzyme deficiency, 4 (15.4%) died with proven or presumed sepsis. Of those tested and confirmed to have G6PD deficiency, 8 (30.7%) had proven or presumed sepsis. In the remaining 99 infants (in whom G6PD status was often not investigated), 1 infant died of presumed sepsis (1%); only 10 (10.1%) surviving had proven or presumed sepsis. Total serum bilirubin ranged from 21.5 to 54 mg/dL on readmission and at least 42% (11 of 17 infants tested) had associated cholestasis with conjugated bilirubin >2 mg/dL. Increased risk of sepsis has also been reported among preterm infants with G6PD deficiency. (35) Using DNA analysis for G6PD variants including A376G, A202G, A376G, C563T, G1376T, and G1388A, the prevalence of G6PD deficiency among 170 infants with birthweight <2.0 kg admitted to a neonatal intensive care nursery was 5.3%. Stage 2 necrotizing enterocolitis was 6.9-fold higher (95% CI: 2–23.5) compared with matched cohort. In the same nursery, the prevalence among the 675 universally screened term and preterm infants was 4.3% such that preterm infants with NEC were 8.6 times (95% CI: 2.9–25.6), more likely to have G6PD deficiency.

Patterns of Clinical Presentation (36–41)

Bilirubin Levels During the First 24 hours

A preliminary report of G6PD deficiency diagnosed by umbilical cord blood sample using fluorescent spot test (FST) in 44 infants (>34 weeks and >1.8 kg) compared with 88 randomly cross-matched G6PD sufficient infants noted similar bilirubin values. (40) Cord blood mean TSB in G6PD deficient newborns was 1.18 ± 0.6 mg/dL compared with control values of 1.26 ± 0.8 mg/dL. Kaplan et al studied infants at age 3 hours and observed that the TSB in G6PD deficient neonates was 2.9 ± 0.7 g/dL and significantly higher than 2.6 ± 0.6 g/dL in control infants. (41) By day 3, the mean TSB in G6PD deficient infants was 10.2 ± 3.1 mg/dL compared with 8.9 ± 3.0 mg/dL. This early rate of bilirubin rise was higher in G6PD deficient infants: 0.15 ± 0.05 mg/dL per hour versus 0.13 ± 0.05 mg/dL/h. Significant hyperbilirubinemia occurred in 30.8% of G6PD deficient infants compared with 6% in the control cohort (relative risk = 5.1; 95% CI: 2.5–11). Evaluation of jaundice during the first 24 hours after birth should probably include G6PD deficiency. (1)(2)

Predischarge Bilirubin

In a Chicago-based African American male neonatal cohort, more G6PD-deficient neonates developed hyperbilirubinemia, TSB >95th percentile, than did G6PD-normal

control subjects (14 of 64, 21.9%, vs 29 of 436, 6.7%; relative risk = 3.27; 95% CI: 1.83–5.86). (42) None required exchange transfusion or developed kernicterus. Reanalysis of this cohort identified exclusive breastfeeding (adjusted odds ratio=3.15; 95% CI: 1.39–7.14), G6PD deficiency (4.96; 95% CI: 2.28–10.80) and predischarge TSB ≥75th percentile (7.47; 95% CI: 3.50–15.94) as significant risk factors for developing hyperbilirubinemia. (43)

Rate of Bilirubin Rise

On the basis of the clinical reports and review of data from the Kernicterus Registry, (24) five patterns are suggested (Fig 2):

1. Slow postnatal rise that does not exceed the 95th percentile bilirubin track during the first week and resolves with a maturing bilirubin elimination system;
2. Predischarge TSB <75th percentile track with exacerbation by starvation, dehydration, unrecognized sepsis, or late prematurity and progression to TSB >95th percentile;
3. Early-onset hyperbilirubinemia: TSB >75th percentile, increased bilirubin production, rapid rise (>0.2 mg/dL per hour), which usually responds to timely intensive phototherapy;
4. Prolonged unconjugated hyperbilirubinemia (>2 weeks of age); and
5. Dramatic, acute-onset excessive hyperbilirubinemia with TSB rise >1 mg/dL per hour (Fig 3, often concurrent to patterns described above).

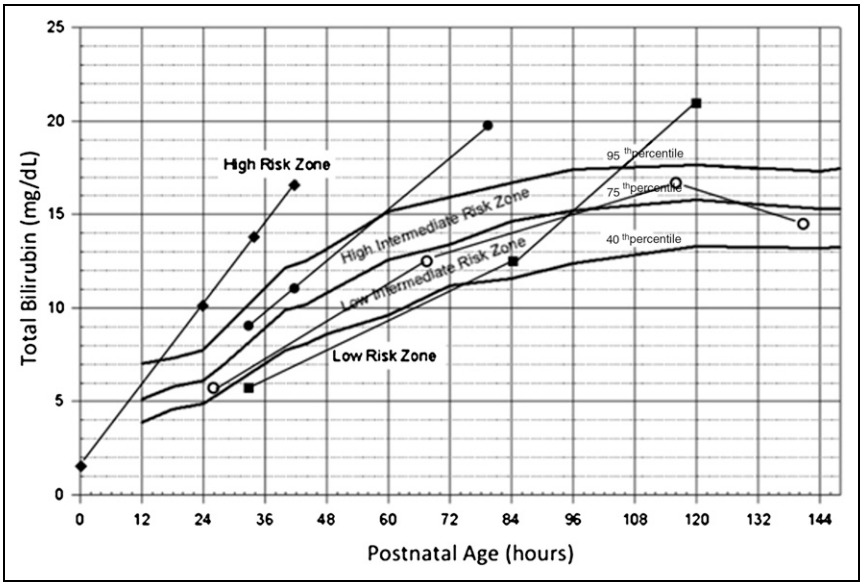

Figure 2. Schematic of clinical presentations of neonatal G6PD deficiency. Serial TSB levels (until intervention with phototherapy or natural decline) for (1) early-onset hyperbilirubinemia: increased bilirubin production (◆) and TSB >75th percentile (•); (2) predischarge TSB <75th percentile track exacerbated by starvation, unrecognized sepsis, or late prematurity (■); (3) slow postnatal rise with natural decline (○); or (4) slow postnatal rise with persistent prolonged unconjugated hyperbilirubinemia, >2 weeks age (not illustrated).

Prolonged Unconjugated Hyperbilirubinemia

Elevated unconjugated hyperbilirubinemia during infancy and adulthood are seen with co-inheritance of G6PD deficiency and Gilbert's syndrome. In these individuals, the bilirubin concentration is affected by the presence of the $TA_{(7)}$ allele of UGT gene, which is G6PD dose-dependent. (8) Often mislabeled as "breast-milk jaundice," infants with persistent unconjugated hyperbilirubinemia have underlying and often undiagnosed genetic issues. In a Scottish population, increased incidence of homozygosity of variant UGT promotor gene among breastfed infants with prolonged jaundice was 31% compared with 6% among those with acute jaundice. (44) In a northern Indian observational study (45) of infants with clinical jaundice at age 2 weeks, 93% of the 71 had unconjugated hyperbilirubinemia (TSB 11.6 ± 3.7 mg/dL), and 24% were G6PD deficient (odds ratio = 4; 95% CI: 1.1–14.1). Jaundice resolved by age 5 to 8 weeks. In a similar group of infants, Gourley et al diagnosed Gilbert's disease (46) and observed potential therapeutic benefits of casein hydrolysate. (47)

Risk of Need for Intervention

Limited contemporary US data assess the burden of adverse consequences of newborn jaundice in the G6PD-deficient population. However, a few US and international studies provide a worrisome perspective. Following the establishment of a selective G6PD screening program at a birthing facility in Cleveland, 1,102 babies were screened from 1,578 consecutive male births. Of these, 122 (11.1%) were G6PD deficient, using the FST assay, and represented 7.7% of all male newborns (95% African American). This cohort exhibited a sevenfold higher risk of readmission for phototherapy. (18) Risk of adverse outcomes and need for interventions also appears to depend on the interaction between G6PD deficiency and UGT co-inheritance and

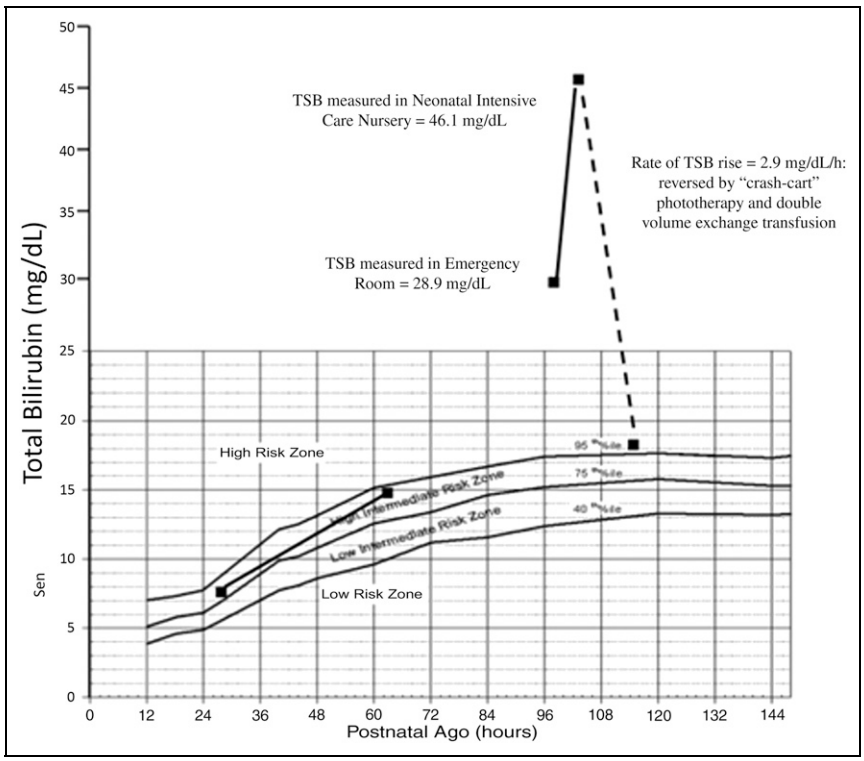

Figure 3. Composite schematic representation of favism manifestation.

particularly manifests among infants who are readmitted for rising TSB levels (Table 4).

Using data from a selective FST assay for G6PD screening in 12,000 deliveries at a Jerusalem hospital, (44) 2548 (21%) were deemed at risk (universal screening for ethnicity, gender, TSB >75 percentile, late preterm). Of these, 276 (10.6%) infants from this selective cohort were G6PD deficient. Among the G6PD deficient newborns, 12 of 276 (4,374 per 100,000 births) needed exchange transfusion compared with 8 of 11,724 (68/100,000 births) in the low-risk, nontested, and G6PD-sufficient cohort ($P < .001$). Mean bilirubin levels ranged from 19.9 to 35 mg/dL and none manifested kernicterus (one had isolated hearing loss). These data were compared with 34,000 deliveries in Saudi Arabia. (45) All infants screened for G6PD screening and 6248 male and female subjects (18%) were considered deficient. Exchange transfusion was used in 42 infants at a rate of 672 per 100,000 births of G6PD-deficient infants. There was no reported occurrence of kernicterus. These observations contrasted to three of 4755 unscreened home deliveries (63/100,000 births) that resulted in kernicterus. Similarly, screening programs in Sardinia led to 75% decrease in incidence of favism (20). In Greece, kernicterus virtually disappeared. (21) Studies from Taiwan, Hong Kong, the Philippines, Malaysia, Vietnam, and Singapore (22)(23) also report decline in occurrence of kernicterus. Available data suggest that screening, in combination with parental and caretaker education, has been instrumental in limiting morbidity and mortality from severe hyperbilirubinemia and bilirubin encephalopathy.

Intervention Strategies

Progression of newborn jaundice to severe hyperbilirubinemia and suggested clinical action, based on this

Table 4. Jaundice and TSB >18 mg/dL at Readmission of Infants ≥35 Weeks' Gestation in Northern Indian Cohort

G6PD Status	Normal	Deficient	Normal	Deficient	Normal	Deficient
Peak TSB (mg/dL)	22.6	26.7	23	24	22.3	21.6
Jaundice onset (d)	3	4	4	3	3	4
Day of peak TSB	6	6	5	5	4	5
TA repeat numbers	TA_6/TA_6 wild		TA_6/TA_7 heterozygous		Multiples homozygous	
ABE	0/4	2/4	3/31	5/17	2/15	1/3

Data from a nested control study: Agrawal SK, Kumar P, Rathi R, et al. UGT1A1 gene polymorphisms in North Indian neonates presenting with unconjugated hyperbilirubinemia. *Ped Res* 2009;65:675–680. Cohort size = 127, infants with TSB >18mg/dL = 77 and control infants = 50. TA(n) polymorphism was higher (odds ratio = 8.6; 95% CI: 3.2–24.1) in this population. No cases of Gycine71Arginine mutation were identified. G6PD deficiency was an independent risk factor for TSB >18 mg/dL (odds ratio = 20.6; 95% CI: 3.6–117).

evidence review, are tabulated in Table 2. Parent and clinician educational resources are available on Web sites (eg, www. g6pddeficiency.org; www.nlm.nih.gov/medlineplus/ency/article/000528.htm) and provided in a "frequently asked questions" format. Use of effective phototherapy and exchange transfusion should be consistent with existing American Academy of Pediatrics guidelines, (1)(46) technical reports, (47) and recent reviews. (48, 49) A single dose of intramuscular tin-mesoporphyrin, a potent inhibitor of heme oxygenase activity, ameliorated jaundice in G6PD-deficient newborns and shifted the peak bilirubin distribution to lower values to entirely eliminate the need for phototherapy. (50) Chemopreventive agents have yet to be approved for safety and clinical use.

Newborn Screening for G6PD Deficiency

Newborn screening relies on the accurate (phenotypic) identification of deficient enzyme activity (Table 5). However, variations due to partial genotypic manifestations, postnatal age, and population of younger, high-enzyme-activity RBCs are significant confounding factors. The definitive biochemical test is the spectrophotometric quantitative enzyme assay based on rate of NADPH formation (μmoles/min/gHb) with absorbance at 340 nm wavelength and expressed as IU/gHb. A useful semi-quantitative screening test (FST) relies on the ability of NADPH to fluoresce intensely with exposure to long wave ultraviolet light. It lacks the sensitivity to diagnose infants with partial enzyme activity (20%–60%) including female heterozygotes. Other tests that detect NADPH such as methylene blue reduction, cresyl blue dye decolorization, cytochemical staining are also available. Identification of specific mutations by DNA/polymerase chain reaction (PCR) screening, ideal for identification of female heterozygotes, is limited by the diversity of known mutations, time-intensive process and occasional mismatch with phenotype expression of enzyme activity. In a recent study of healthy term infants of Chinese ethnicity in Singapore (74 with significant hyperbilirubinemia and 125 without), the qualitative enzyme screening test (FST) was compared to the definitive enzyme assay (using a cut-off of 8.5 IU/gHb) and single nucleotide polymorphism (DNA) analysis of the G6PD gene for seven common variants of this population. (50) FST identified 13.1% infants as deficient when compared to 19.6% by the quantitative enzyme assay and 13.6% by mutation screening. Of the 39 infants diagnosed by enzyme assay (Odds ratio = 5.3; 95% CI: 2.4–11.1), 13 infants had no mutation detected. In view of a wide array of mutations, not all are easily identified and the process can be time-intensive, PCR screening is not a current useful strategy. Female heterozygotes identifiable by PCR screening remain at significant risk for hazardous hyperbilirubinemia and kernicterus. In fact normal FST test levels or intermediate enzyme levels may be falsely reassuring. Thus, a two-step approach to measure enzyme functional assay with concomitant DNA verification seems to be the most accurate and practical approach to screen, monitor and diagnose neonatal G6PD deficiency. US population-based studies to determine the optimal and risk-based strategies for targeted routine newborn screening have not been conducted. Screening tests are available based on enzyme assay but point-of-care application has yet to be developed. Whether neonatal G6PD screening leads to reduction in the incidence of severe neonatal hyperbilirubinemia has not been prospectively tested in the ethnically diverse US population.

Implications for Clinical Practice

The 2004 American Academy of Pediatrics guidelines (1) and the updated clarifications (46) to manage neonatal hyperbilirubinemia provide a reasonably safe approach to identify an infant who is likely to be G6PD deficient to anticipate an aberrant manifestation. Rapid access to a diagnostic screening test for G6PD or a point of care device that allows for a dual risk assessment of bilirubin and G6PD-related hyperbilirubinemia would substantially target who would benefit from parental awareness and predischarge use of phototherapy.

In summary, in the absence of confirmatory G6PD-deficiency diagnosis, the probability of unpredictable favism presentations guides the postdischarge management for use of neonatal phototherapy based on expert assessment and need for urgent triage. Current clinical evidence suggests that most infants at risk for severe neonatal hyperbilirubinemia can be identified and managed before discharge from birthing facilities. However, infants with G6PD deficiency can follow diverse manifestations that can be dramatic and seemingly unpredictable. Thus, in communities that have not yet implemented G6PD screen or rapid testing and serve high-risk populations, direct readmission to neonatal intensive care nurseries is the safest and most prudent option. These infants are not only candidates for "crash-cart" phototherapy, most are also at risk for exchange transfusion that should be safe, timely, and with immediate parental consent.

Clinician and parental awareness of a possible G6PD enzyme deficiency has been shown to reduce adverse neonatal consequences in several communities worldwide. Significant research gaps exist to better understand the life-long population burden of G6PD deficiency.

Table 5. Definitions for Ranges of G6PD Enzyme (Quantitative Test)

Enzyme activity	Measure	Population Profile
Normal	7–10 IU/gHb[a]	Adult
Deficient	<50% of normal value	Presumed hemizygote-deficient male or heterozygote-deficient female
	<20% of normal value	Observed with most variants
	Undetectable activity	
Intermediate	Activity with large variation	Homozygote-deficient female
Accepted definition of G6PD deficiency	<30% enzyme activity	Above these levels, clinical manifestations are unlikely
Newborn screening	8.5 IU/gHb as a cutoff	Single center report (Singapore)
Umbilical cord blood	Enzyme concentration	Range
Normal subjects (n = 436)	21.8 ± 2.2 IU/gHb	14.5–33.8 IU/gHb
Deficient male subjects (n = 64)	2.7 ± 1.1 IU/gHb	0.04–6.6 IU/gHb

Definitions based on Luzzatto L. Glucose 6-phosphate dehydrogenase deficiency: from genotype to phenotype. *Hematology* 2006;2:63–68. Data in shaded box from Kaplan et al. Neonatal bilirubin production-conjugation imbalance: effect of glucose-6-phosphate dehydrogenase deficiency and borderline prematurity. *Arch Dis Child Fetal Neonatal Ed* 2005;90(2):F123–F127.
Limitations: (1) During or immediately after a hemolytic event, younger RBC may have higher enzyme content; (2) Neonates may have younger red cell population or higher reticulocyte count; (3) Leukocyte G6PD activity may confound assay; (4) Exposure to heat inactivates enzyme activity (sample needs to be protected); and (5) Qualitative screening tests likely to miss deficiency among heterozygote females.
[a]IU/gHb measured at 30°C.

These include a national evidence-based inquiry to define a neonatal G6PD screening strategy for targeted intensive follow-up for a safer management of a jaundiced neonate with G6PD deficiency in the United States.

American Board of Pediatrics Neonatal-Perinatal Content Specifications

- Know the factors, including genetic and increased red cell destruction, associated with an increase in bilirubin production.
- Know how to use a pre-discharge bilirubin measurement to predict the risk of severe hyperbilirubinemia.
- Know the factors that increase the risk of the development of kernicterus.
- Know the differential diagnosis, evaluation, and approach to management of infants with indirect hyperbilirubinemia.

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NeoReviews Quiz

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1. The overall incidence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in the United States is estimated at 3.4%, with a range among race/ethnic cohorts from 0 to 12.2%. Neonates with G6PD deficiency may be asymptomatic or have clinical findings ranging from mild jaundice to bilirubin encephalopathy. Of the following, the percentage of newborns in the USA Kernicterus Registry (1992-2004) with a subsequent diagnosis of G6PD deficiency is closest to:

   A. < 1%
   B. 3.4%
   C. 12.2%
   D. 20%
   E. 40%

2. Glucose-6-phosphate dehydrogenase (G6PD) protects erythrocytes from oxidative stress. This enzyme converts glucose-6-phosphate to 6-phosphogluconic acid. Of the following, the most significant anti-oxidant role of G6PD is the conversion of:

   A. Adenosine triphosphate (ATP) to adenosine diphosphate (ADP)
   B. Flavin adenine dinucleotide (FAD) to reduced flavin adenine dinucleotide (FADH2)
   C. Nicotinamide adenine dinucleotide phosphate (NADP+) to reduced NADP (NADPH)
   D. Oxidized glutathione (GSSG) to reduced glutathione (GSH)
   E. Water to hydrogen peroxide

3. A 3-day old infant has severe hyperbilirubinemia and a family history of glucose-6-phosphate dehydrogenase (G6PD) deficiency. The neonatologist measures this infant's G6PD activity and it is normal. However, 3 months later, repeat testing shows that the infant is deficient in G6PD. Of the following, the most likely explanation for this infant's initial false-negative test is:

   A. Dehydration
   B. Excessive heat to the blood sample
   C. Greater half-life of the infant's red blood cells
   D. Hemolysis leading to a greater number of younger red blood cells
   E. Higher amount of leukocyte G6PD activity

4. An infant has clinical findings consistent with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Of the following, the most reliable method to diagnose G6PD deficiency is:

   A. Amount of chemiluminescence produced by the enzymatic reaction
   B. Calorimetric assessment by measuring amount of heat released
   C. Identification of specific deoxyribonucleic (DNA) mutations by polymerase chain reaction
   D. Quantification of glucose 6-phosphate production
   E. Spectrophotometric assay based on rate of NADPH formation

5. Infants with ABO incompatibility or Rh disease typically follow a similar pattern of elevated indirect bilirubin. In contrast, infants with glucose-6-phosphate dehydrogenase (G6PD) deficiency exhibit 5 distinct patterns of bilirubin rise. Of the following, the pattern that is most inconsistent with G6PD deficiency is:

   A. Cyclical changes in bilirubin concentration in the first 2 months of life requiring intermittent phototherapy
   B. Early-onset hyperbilirubinemia that usually responds to timely phototherapy
   C. Normal pre-discharge bilirubin concentration followed by a severe exacerbation associated with starvation, dehydration, or sepsis
   D. Prolonged unconjugated hyperbilirubinemia at greater than 2 weeks of age
   E. Slow postnatal increase in bilirubin that resolves as the infant's bilirubin elimination system matures