Quantitative Neonatal Glucose-6-Phosphate Dehydrogenase Screening: Distribution, Reference Values, and Classification by Phenotype

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Objective To determine enzyme assay reference values for newborns in a Sephardic Jewish population at high risk for glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Study design Quantitative G6PD testing was performed on umbilical cord blood. The reduction of nicotinamide adenine dinucleotide phosphate to nicotinamide adenine dinucleotide phosphate-oxidase, reflecting G6PD activity, was measured spectrophotometrically. Hemoglobin (Hb) was measured on the same sample. G6PD activity was recorded as U/g Hb.

Results Males (N = 1502) were separated into 2 distinct groups: those <7 U/g Hb (n = 243 [16.2%], median 0.28 U/g Hb), designated G6PD deficient, presumably hemizygotes; and those ≥9 U/g Hb (n = 1256 [83.8%], 18.76 U/g Hb), designated G6PD normal, presumably hemizygotes. Female (n = 1298) values were a continuum and were categorized based on the male distribution: those <7 U/g Hb (n = 81 [6.2%], 4.84 U/g Hb), G6PD deficient, probably homozogytes; those ≥9.5 U/g Hb, equivalent to 50% of the male normal value, (n = 1153 (88.8%), 18.36 U/g Hb), G6PD normal, probably homozygotes; and those with intermediate values (n = 64 [4.9%], 8.61 U/g Hb), probable heterozygotes.

Conclusions Accurate identification of the male G6PD–deficient state was possible despite high normal neonatal G6PD values. Female values were presented as a continuum preventing accurate classification but were classified based on male phenotype for practical use. *(J Pediatr 2012;161:197-200)*.

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a commonly occurring genetic enzyme disorder known to affect hundreds of millions of individuals worldwide.[1-3] The most devastating potential complication of this in the newborn is an acute hemolytic crisis, causing extreme hyperbilirubinemia, which may result in acute bilirubin encephalopathy.[4]

Because G6PD deficiency is an X-linked condition, males may be either G6PD–normal or –deficient hemizygotes, whereas females may be either normal homozygotes, deficient homozygotes, or heterozygotes. Using biochemical testing, identification of the 2 male groups should be accurate and straightforward. Categorization of females, however, may be inaccurate. In any female cell, only 1 X chromosome is active.[5,6] If X chromosome inactivation were random, 50% of the cells would be G6PD normal and 50% would be deficient. G6PD enzyme activity, representing both cell components, would be intermediate between normal and deficient levels. However, because X chromosome inactivation is frequently nonrandom, varying proportions of red blood cells may have either G6PD–normal or –deficient activity. As a result heterozygotes will have a continuum of G6PD activity results.

A neonatal G6PD screening program at the Shaare Zedek Medical Center used a qualitative, fluorescent screening test in a targeted program that included male and female infants born to mothers of Sephardic Jewish background at high risk for G6PD deficiency. These infants can be expected to carry the G6PD Mediterranean mutation.[7,8] In the process of upgrading our screening program to a quantitative enzyme assay, we determined reference values for G6PD activity in both males and females of the target group. We compared values and distribution between the sexes and also compared values in males with a recent African-American cohort, who can be expected to have the G6PD A variant.[9] A classification of neonatal G6PD status for practical purposes, based on phenotype, is proposed.

Methods

Because of the routine, noninvasive, nonrandomized, and noncomparative nature of the project, the Institutional Review Board of the Shaare Zedek Medical

G6PD	Glucose-6-phosphate dehydrogenase
Hb	Hemoglobin

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Center approved the study without the need for individual parental permission. Healthy, term and late preterm (gestational age ≥36 weeks) infants who were cared for in the regular newborn nurseries were included.

Neonates whose mothers were fully or partly from Sephardic Jewish families who had immigrated to Israel from countries or geographic areas known to be associated with high risk for G6PD deficiency (Kurdistan, Iraq, Iran, Syria, Lebanon, Turkey, and Buchara[10,11]) were identified in the delivery room for G6PD testing. Umbilical cord blood was drawn routinely following delivery of the placenta, collected in ehylenediaminetetraacetic acid-containing tubes, and refrigerated until use. On the morning following delivery, umbilical cord blood was retrieved for G6PD testing. By noon, usually within 24 hours of the delivery and well before discharge, the results were made available to the newborn nursery. The parents were advised of their infant's condition, of the precautions to be taken, and of the increased risk of developing hyperbilirubinemia, and they received an explanatory sheet. Transcutaneous bilirubin testing was performed routinely on a daily basis, and a serum total bilirubin test performed should the transcutaneous bilirubin value be >75th percentile on the hour of life specific bilirubin nomogram. Any newborn who was discharged but had a plasma total bilirubin value >75th percentile was invited the following day for a bilirubin test on an outpatient basis. Phototherapy and exchange transfusion were performed according to the guidelines of the Israel Neonatal Society, which are based on the American Academy of Pediatrics 2004 guidelines.[12,13]

A commercial kit was used to measure spectroscopic absorbance at 340 nm due to reduction of $NADP^+$ to NADPH at 37°C, reflecting G6PD enzyme activity (Sentinal Diagnostics, Milan, Italy). The principle of the test is that, in the process of conversion of glucose-6-phosphate to 6-phosphogluconate, a reaction catalyzed by G6PD, $NADP^+$ is reduced to NADPH. The amount of NADPH produced is an index of G6PD activity. Formation of NADPH is measured over a set period of time. Hemoglobin (Hb) was measured spectrophotometrically at 540 nm with a commercial kit using the cyanmethemoglobin method, on the same sample (Pointe Scientific, Inc, Canton, Michigan). G6PD activity was recorded as U/g Hb.

Data Analysis

G6PD values were stratified by activity levels and the number of infants per activity units plotted (**Figure**). Males and females were classified by phenotype into probable genotypes. Males were expected to separate into 2 distinct groups, those with low reading G6PD activity, deficient hemizygotes, and those with normal enzyme activity, normal hemizygotes. Females, on the other hand, were expected to present as a continuum of G6PD activity and were classified arbitrarily, based on male distribution, as suggested by Luzzatto (L. Luzzatto, personal communication): (1) G6PD activity within the male deficient range: G6PD deficient, probably homozygote; (2) G6PD activity >50% of the median male normal range: G6PD normal, probably homozygote; and (3) G6PD activity greater than the male deficient value but <50% of the normal male median value: G6PD intermediate, probably heterozygote. This 50% cutoff point was chosen to comply with the standard definition of G6PD deficiency.[2]

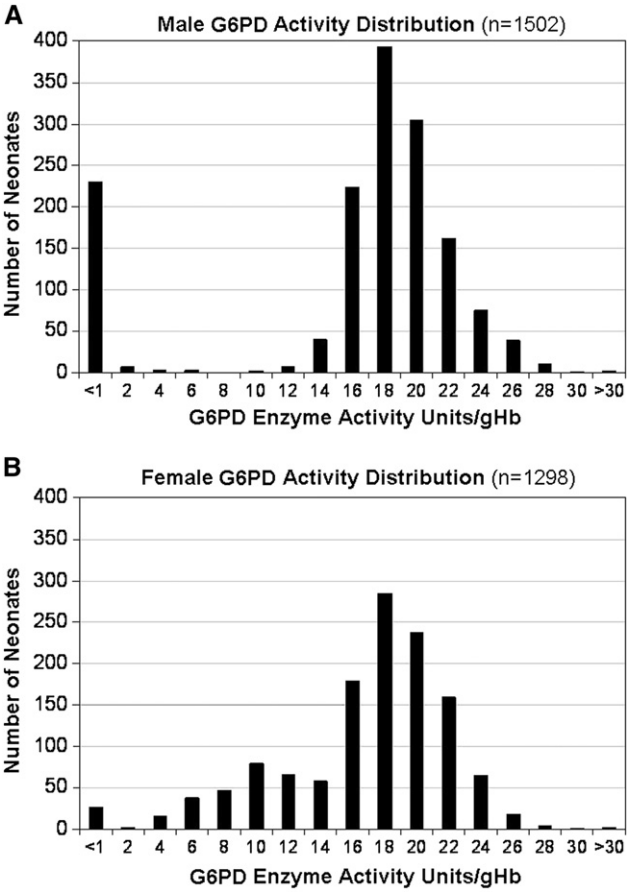

Figure. Distribution of G6PD activity among the **A,** male neonates and **B,** the female neonates. Note that the males separate into 2 subgroups, G6PD deficient and G6PD normal, with no overlap between the groups. In contrast to the male distribution, there was no clear distinction between the 3 subgroups (G6PD–deficient homozygotes, normal homozygotes, and heterozygotes) based on phenotype.

Finally, the database of a recent study of African-American male neonates,[9] in which some of the current authors were involved, was used to compare G6PD deficient values between that group and the male G6PD–deficient subset of the present study. Although several years separated the 2 studies, different laboratories processed the samples, and the currently used commercial kit was not used in the African-American study, the tests were processed according to the same principle (see earlier).[14] Enzyme values between groups were compared using the Mann-Whitney rank sum test. Significance was defined as at $P < .05$.