Glucose-6-Phosphate Dehydrogenase Deficiency in Filipino Neonates with Jaundice

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ABSTRACT

Objectives**.** Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common of all clinically significant enzyme defects of red blood cells. It has a high rate of prevalence in the Philippines. Concern about hemolytic anemia and jaundice due to unrecognized G6PD deficiency led us to determine the prevalence of G6PD deficiency among jaundiced neonates in the Philippine General Hospital, a tertiary referral hospital in the Philippines. It was hypothesized that G6PD deficiency was more prevalent in neonates with jaundice than in the normal population. We also compared the clinical presentation and course (hospital stay and days of phototherapy requirement) for G6PD deficient and G6PD normal neonates.

Materials and Methods**.** We studied 102 clinically jaundiced neonates admitted to the nursery of the Philippine General Hospital. Blood samples in individual microtainers were quantitatively tested for G6PD activity using a commercial G6PD assay kit. The clinical presentation and hospital courses of patients were statistically compared using the t-test for single proportions.

Results. G6PD deficiency was diagnosed in 17 of 102 cases[16.7% (95% CI: 10.0 to 25.3)], which is significantly higher than the normal population (p<0.001). In all G6PD-deficient neonates, no evidence of other factors known to cause hyperbilirubinemia were detected. There was no significant difference on phototherapy requirement and length of hospitalization in G6PD- deficient and other jaundiced neonates.

Conclusion. The prevalence of G6PD deficiency among jaundiced neonates was found to be higher than the normal population thus, early detection of this enzymopathy, regardless of sex, and close surveillance of the affected newborns is important in reducing the risk of severe hyperbilirubinemia.

Key Words: Glucose-6-phosphate dehydrogenase (G6PD) deficiency, hyperbilirubinemia, jaundice, neonates

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is a cytosolic enzyme present in all cells. It catalyzes the first step in the pentose phosphate shunt, oxidizing glucose-6-phosphate (G-6-P) to 6-phosphogluconate (6-PG), reducing nicotinamide adenine dinucleotide phosphate (NADP) to NADPH. This reaction generates NADPH, which is required as an electron donor in various biosynthetic pathways and for the regeneration of reduced glutathione, which helps protect cells against oxidative damage. The production of NADPH by G6PD is of particular importance in red blood cells, which are highly susceptible to oxidative damage where other NADPH-producing enzymes are lacking.[1]

G6PD deficiency is the most common known enzyme deficiency in humans. It is estimated to affect 400 million people worldwide, with the highest prevalence rates recorded in tropical Africa, in the Middle East, in tropical and subtropical Asia, in some areas of the Mediterranean, and in Papua New Guinea. The project update of the Philippine Newborn Screening Program as of May 2008 pegs the crude incidence to be 1 in 53 out of 905,000 patients. G6PD deficiency is an X-linked inherited condition associated with an increased incidence of neonatal hyperbilirubinemia, especially in the more severe type affecting the Asian and Mediterranean groups.[2,3] Common clinical manifestations of G6PD deficiency include neonatal jaundice and acute hemolytic anemia.

Neonatal jaundice is one of the most life- and health-threatening consequences of G6PD deficiency, and kernicterus or hemolysis may occur in these infants. It has been found that G6PD-associated neonatal jaundice is occasionally severe enough to cause death or permanent neurological damage.[4]

The finding of G6PD deficiency in a jaundiced infant does not in itself prove that the jaundice was caused by the enzyme defect, thus other causes of jaundice must be excluded. It is still, however, of utmost importance to establish the prevalence of this disorder in Filipino neonates since hyperbilirubinemia may have been precipitated by the unrecognized exposure of these patients to certain chemicals and drugs.

There is concern about jaundice due to unrecognized G6PD deficiency. Many pediatricians do not take G6PD into consideration when evaluating likely causes of neonatal jaundice; thus the concern about jaundice due to unrecognized G6PD deficiency. The primary aim of this study is to report the prevalence of G6PD among neonates with jaundice in a setting where a high prevalence rate of

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G6PD deficiency is known. We also describe the clinical presentation and course of G6PD-deficient and non-deficient patients. To our knowledge this is the first local report of the prevalence of G6PD deficiency among patients with neonatal jaundice in a G6PD high prevalence setting.

Methods

Subjects

This one year prospective study involved 102 neonates with neonatal jaundice (bilirubin levels of 10 mg/dL or more) born in the Philippine General Hospital, a tertiary teaching hospital in Manila, Philippines. Informed consent was obtained prior to inclusion to the study.

Materials and Methods

Birth weight, history of formula feeding, gestational age, sex, the time of onset of jaundice, serum bilirubin levels, reticulocyte levels, hematocrit levels, phototherapy duration, duration of hospital stay and need for exchange transfusion were abstracted from the patients' medical records. Laboratory evaluations included blood group typing of mother and newborn, complete blood count, direct Coombs test and erythrocyte G6PD levels.

To determine the erythrocyte G6PD levels, 1.0 mL of fresh, whole blood was extracted from each subject and placed in a microtainer containing EDTA as an anticoagulant. Blood (0.5 ml) from this sample was placed immediately onto a filter paper card (Schleicher and Schuell 903C, Keene, NH, USA). A trained research assistant performed the procedure and labeled the specimens accordingly. These samples were then transported immediately to the Institute of Human Genetics at the National Institutes of Health – Philippines, which is located physically nearby.

The filter paper cards were air-dried for four (4) hours and analyzed for G6PD activity using the Neonatal G6PD Kit (PerkinElmer Wallac Inc., Norton, OH, USA).

Blood samples contained in individual microtainers were tested using the G6PD assay kit (Trinity Biotech, St. Louis, MO 63114, USA) to confirm neonates with a positive G6PD screen. G6PD activity levels < 146-376 U/10^{12} RBC were considered deficient.

Statistical Analysis

The prevalence of G6PD deficiency was estimated at a 95% confidence level using the Exact Binomial Method of Stata 9.0 (StataCorp LP, Texas). It was also compared with the expected prevalence of G6PD deficiency among the normal neonates using the Z-test for single proportions. The neonates were described according to demographic features, laboratory values, etiology of jaundice, duration of phototherapy and hospital stay, which were statistically compared using the two sample independent T-test. Fisher's exact test was used to compare the clinical outcomes of the patients. All statistical tests were done using OpenEpi verion 2.2.1 (www.OpenEpi.com).

Results

Of the 102 samples collected from June 2003 to June 2004, G6PD deficiency was detected in 17 patients [16.7% (95% CI: 10.0 to 25.3)]. This was significantly higher than the general population of screened newborns ($p<0.001$). There were more males with G6PD deficiency than females but this was not statistically significant (18.9% vs 13.6%, $p=0.56$). All 17 found to be G6PD positive were born before 40 weeks (data not shown). While infection is the second most common cause of hyperbilirubinemia in our patients, other common causes of hyperbilirubinemia are seen in a small proportion of patients (Table 1).

Table 1. Etiologic distribution of hyperbilirubinemia

ETIOLOGY	n	(%)
G6PD deficiency	17	16.67
Infection	15	14.71
ABO incompatibility	3	2.94
Cephalhematoma	2	1.96
Down Syndrome	1	0.98
Hemolytic anemia	1	0.98
Gastric Outlet Obstruction	1	0.98
Anemia of prematurity	1	0.98
Infant of diabetic mother	1	0.98
Thickly meconium stained	1	0.98
Unknown	59	57.84
TOTAL	**102**	**100**

In all 17 G6PD deficient neonates, no evidence of other factors known to cause hyperbilirubinemia were detected. None of the G6PD deficient neonates had infection while 14 (16.5%) of the normal subjects had infection during their admission. Of the 17 neonates found to be G6PD deficient, 14 were breastfed (exclusive and mixed) and 3 were exclusively formula-fed. Both G6PD deficient and normal subjects had normal TSH levels and none of the subjects were reported to have Respiratory Distress Syndrome.

There was no statistically significant difference between the two groups in terms of the clinical parameters and course of the patients (Table 2). However, the total bilirubin and indirect bilirubin levels were significantly higher in patients with G6PD deficiency than those with normal G6PD activity. The G6PD activity was not significantly different between the males and females with deficient enzyme activity ($p=0.23$).

Overall, 100 (98%) patients underwent phototherapy. Only 2 patients, both from the G6PD normal group, did not require any intervention for their hyperbilirubinemia. Three (2.9%) patients required exchange transfusion, all from the G6PD normal group. There was no statistically significant difference among male and female patients in relation to clinical course.

Discussion

Inherited defects of erythrocyte metabolism, membrane function, and hemoglobin synthesis may all manifest in

Table 2. Clinical presentation and course of G6PD deficiency and G6PD normal patients

PARAMETER	G6PD Deficient (n=17)		G6PD Normal (n=85)		p-value
	Mean (SD)	Min-Max	Mean (SD)	Min-Max	
Birth weight (g)	2291.18 (485.49)	1600-3500	2317.94(654.84)	1200-3900	0.87
Onset of jaundice (day)	2.65 (1.06)	1-5	2.99(1.34)	1-8	0.33
Total bilirubin (mg/dL) (NV= 0-1.0)	16.03(4.69)	10.55-27.30	13.38(3.78)	1.69-24.33	0.02
Direct bilirubin (mg/dL) (NV=0-0.29)	0.45(0.29)	0.21-1.38	0.37(0.29)	0.06-2.12	0.36
Indirect bilirubin (mg/dL)(NV=0.2-0.8	16.44(5.94)	10.30-27.63	13.22(4.01)	1.45-26.21	0.01
Reticulocyte (NV=0.005-0.015)	0.02(0.01)	0.01-0.03	0.04(0.04)	0.00-0.10	0.26
Hematocrit (NV=0.37-0.54)	0.45(0.12)	0.24-0.61	0.44(0.09)	0.17-0.63	0.84
Phototherapy (days)	6.53 (4.42)	2-21	6.05(3.28)	2-18	0.61
Hospitalization (days)	11.88 (6.46)	3-25	14.60(12.03)	2-83	0.37
G6PD activity (N >146U/1T RBC)	50.10(38.38)	2.10-127.02	533.56(234.70)	148-1185.10	<0.001

the newborn period. Defects of erythrocyte metabolism include G6PD deficiency and less common disorders such as pyruvate kinase deficiency. The major function of the erythrocyte is the delivery of oxygen to the tissues. The cell is constantly exposed to oxygen, and the erythrocyte membrane and cytoplasm are subjected to oxidative damage. Oxidation causes the formation of precipitates of denatured hemoglobin which appear to be associated with shortened erythrocyte life span. The erythrocyte has a metabolic system that prevents oxidative damage. G6PD is an enzyme in this system. If it is absent, there is risk of oxidative damage to the erythrocyte, particularly when the cell is stressed by chemicals or drugs capable of oxidative damage.[2]

Neonatal jaundice is a major clinical problem especially in Asian populations. G6PD deficiency, ABO incompatibility, low birth weight and sepsis are common causes of neonatal jaundice. This condition may cause acute hemolysis during oxidative stress, severe hyperbilirubinemia in the newborn and sometimes kernicterus in some populations, often in the absence of any identifiable trigger or hematological evidence of hemolysis.

Since G6PD deficiency is prevalent in the Philippines, we studied the prevalence of G6PD deficiency relative to neonatal jaundice in Filipino neonates. G6PD assays (and screening for other disorders included in the newborn screening panel) were performed on blood from 102 Filipino newborn babies born at the Philippine General Hospital. Observation of jaundice and determination of bilirubin levels as well were done for all patients.

G6PD deficiency was detected in 17 (16.7%) of patients while the latest national data presents 1 in 53 among 905,000 (1.9%) of the total newborn screened. This is consistent with studies in other countries that confirm G6PD as a common cause of neonatal hyperbilirubinemia. In Iran,[5] Nigeria,[6] India,[7] Saudi Arabia,[8] Singapore,[9] Indonesia[10] and Malaysia,[11] studies revealed the G6PD prevalences of 4.4%, 40%, 12.2%, 18.4%, 1.62%, 1.57% and 3.5%, respectively in jaundiced patients without hemolysis. The incidence of G6PD deficiency in jaundiced neonates was higher in males compared to females. The present study reports 18.9% incidence of G6PD deficiency among unrelated males and 13.6% among females and a male: female ratio of 2:1. It is also important to note that during the time of G6PD confirmation with the Trinity Biotech G6PD assay kit, normal ranges and cutoff values for newborns were not yet fully established. Thus, the presented prevalence may be an underestimation of the actual prevalence of G6PD deficiency in this jaundiced population.

Table 3. Comparison of G6PD normal and deficient groups in relation to phototherapy and need for exchange transfusion

	G6PD deficient (n=17)		G6PD normal (n=85)		
	Male(%)	Female(%)	Male(%)	Female(%)	p-value
Phototherapy	11(65)	6(35)	46(55)	37(45)	0.59
Exchange transfusion	0	0	2(2.4)	1(1.2)	1

An investigation of the prevalence of G6PD deficiency in neonatal hyperbilirubinemia which compared the clinical presentation and course of G6PD-deficient and normal Turkish patients indicated that no statistically significant difference was detected between G6PD-deficient and normal groups relative to: the time of jaundice onset; reticulocyte count; hematocrit level; phototherapy duration; and, duration of hospitalization. Serum bilirubin at admission, maximum serum bilirubin level and the need for exchange transfusion were higher in G6PD-deficient group.[12] However, in our study, all three (3) neonates who underwent exchange transfusion were G6PD normal (Table 3). None of the patients included in our study had hemolysis nor kernicterus. Day of onset of jaundice was 2-3 days in both G6PD deficient neonates and G6PD normal neonates thus, showing the importance of early newborn screening.

Hyperbilirubinemia in G6PD-deficient neonates is thought to be secondary to reduced hepatic conjugation and excretion of bilirubin, rather than increased bilirubin production resulting from hemolysis.[12,13] In this study, we found no cases of severe hemolysis or kernicterus among G6PD neonates, which is consistent with what has been previously reported in neighboring countries. Although the data among jaundiced G6PD neonates seem to be reassuring,

this may indicate that detection may have prevented occurrence of these critical endpoints. Furthermore, the collective result highlights the importance of continuation of such screening programs in the neonatal period.

The prevalence of G6PD deficiency among jaundiced neonates was found to be relatively high at 16.7%, thus early detection of this enzymopathy regardless of sex and close surveillance of the affected newborns is important. Early detection reduces the risk of complications secondary to hyperbilirubinemia such as kernicterus and hemolytic anemia. Implementation of newborn screening of neonates to identify G6PD deficient individuals will avoid extensions of hospital stays for affected newborns through timely counseling of their patients and caregivers. Counseling should be aimed at increasing awareness of hemolytic triggers.

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