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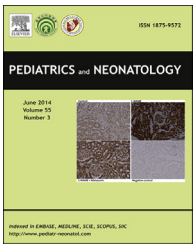

ORIGINAL ARTICLE

Is it Accurate to Separate Glucose-6-Phosphate Dehydrogenase Activity in Neonatal Hyperbilirubinemia as Deficient and Normal?

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Key Words
glucose-6-phosphate dehydrogenase activity;
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severe neonatal hyperbilirubinemia

Background: The aim of this study was to investigate glucose 6-phosphate dehydrogenase (G6PD) activity in term and late preterm babies with severe neonatal hyperbilirubinemia and its relationship to the severity and treatment of this disorder, regardless of level of G6PD activity (deficient/normal).
Methods: A total of 529 term and late preterm ($\geq$35 weeks) infants (228 female, 301 male) who were diagnosed with severe hyperbilirubinemia were included in this study. In each case, serum was collected to evaluate blood group, direct Coombs' test, complete blood cell count, total and direct bilirubin, thyroid-stimulating hormone, and G6PD activity. A partial correlation analysis was carried out to assess the relationship between G6PD activity and total bilirubin levels.
Results: A significant correlation was found between the severity of hyperbilirubinemia and G6PD activity in both males and females. Male neonates who had G6PD levels $<$12 U/g Hb required more phototherapy time than neonates who had G6PD levels $\geq$12 U/g Hb; and female neonates who had G6PD levels $<$16 U/g Hb required more phototherapy time than neonates who had G6PD levels $\geq$16 U/g Hb ($p < 0.0001$). When we analyzed only breastfed infants, a significant difference also emerged in both sexes. Decreased G6PD activity was associated with increased phototherapy time and the need for exchange transfusion.
Conclusion: Routine checks of G6PD level in hyperbilirubinemic neonates are very important in providing proper medical management to prevent bilirubin-induced neurological dysfunction.

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Appropriate identification of G6PD (<12 U/g Hb for male infants and <16 U/g Hb for female infants) raises awareness of the severity of the condition and the necessity for immediate care of severe hyperbilirubinemic infants.
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1. Introduction

The glucose-6-phosphate dehydrogenase (G6PD), which exists in every cell of the body, protects cell proteins from oxidative damage.[1,2] To control oxidative stress, G6PD converts nicotinamide adenine dinucleotide phosphate (NADP) into reduced nicotinamide adenine dinucleotide phosphate (NADPH) by the hexose monophosphate pathway. This pathway is the only source of NADPH in erythrocytes, and it is an important cofactor of glutathione metabolism. Hemolysis related to glutathione metabolism is a common problem that stems from G6PD deficiency.[3–5] G6PD deficiency, a very prevalent syndrome that affects an estimated 400 million people worldwide,[6] is an X-linked genetic defect that can cause hemolytic crisis or severe neonatal hyperbilirubinemia, which can lead to bilirubin encephalopathy and kernicterus.[7–10]

Neonatal hyperbilirubinemia is the result of an imbalance between the production and conjugation of bilirubin. Acute hemolysis, which leads to an increase in bilirubin, plays a relatively minor role in most cases of G6PD-associated neonatal hyperbilirubinemia. Decreased bilirubin conjugation with promoter polymorphism for the gene for the bilirubin-conjugating enzyme, uridine 5′-diphospho-glucuronoslytransferase 1A1, seems to be the major factor.[11–13]

During the neonatal period, oxidative stress is high and antioxidative functions are insufficient.[14,15] The NADPH level in erythrocytes is directly related to G6PD levels.[1,2] In babies at this precise physiological period, G6PD levels could be more consequential in terms of the antioxidative defense of erythrocytes. Therefore, even with normal levels of G6PD, lower enzyme activity might be the cause of the increased vulnerability to oxidative stress, which could increase the severity of hyperbilirubinemia.

We believe that only one cut-off level is not appropriate to explain the relationship between G6PD activity and hyperbilirubinemia. Thus, we hypothesized that in severe hyperbilirubinemic infants, G6PD levels have an important effect on the severity of the disease. Therefore, in this study, we investigated G6PD activity in term and late preterm babies who were admitted to a neonatal intensive care unit (NICU) due to severe neonatal hyperbilirubinemia and its relationship with the severity and treatment of this disorder, without separating G6PD as deficient or normal.

2. Methods

This prospective study was conducted between March 2002 and August 2007 at the Neonatology Department of Baskent University Hospital, Ankara, Turkey. The study protocol was approved by the institutional review board of the university. During this research period, 5300 babies were born at Baskent University, and 693 (299 females, 394 males) were diagnosed with severe hyperbilirubinemia. Of these, 45.5% were inborn, and the rest were all outborn. Phototherapy and exchange transfusion were performed according to the American Academy of Pediatrics (AAP) clinical practice guidelines.[16,17] The need for phototherapy and exchange transfusion according to the AAP guidelines was an accepted criterion as a definition of severe hyperbilirubinemia. The study population included inborn infants diagnosed with severe hyperbilirubinemia prior to discharge or rehospitalized following discharge from the birth facilities, and outborn infants transferred to our center for treatment of hyperbilirubinemia. All parents signed a mandatory written consent form. In each case, serum was collected to evaluate blood group, direct Coombs' test, complete blood cell count, total and direct bilirubin, thyroid-stimulating hormone, and G6PD activity. These assessments were performed to rule out a differential diagnosis. G6PD activity was measured when the infants were admitted to the NICU with severe hyperbilirubinemia. The samples were obtained from venous blood. Exclusion criteria were prematurity (<35 weeks), ABO incompatibility, direct Coombs' test positivity, hemoglobin level <12 g/dL, white blood cell count ≥25,000/mm^3, positive blood culture, congenital anomaly, maternal diabetes, infection, asphyxia, cephalohematoma, hypothyroidism, and metabolic diseases. Due to these criteria, 164 infants were excluded from the study, and the remaining 529 term and late preterm (≥35 weeks) infants (228 females, 301 males) who were diagnosed with severe hyperbilirubinemia were included in the study. The birth weight and gestational age of each infant were obtained from birth records. Postnatal age (hours) and body weight at admission, as well as type of feeding (breastfeeding, formula feeding, or mixed feeding), were recorded. Data regarding phototherapy start time (postnatal hours) and duration, as well as exchange transfusion, were also recorded. All infants received phototherapy, which continued until the total serum bilirubin level decreased to more than 2 mg/dL below the phototherapy starting threshold level. The phototherapy equipment and the level of irradiance were the same for all infants.

Bilirubin levels were measured by diazo reaction and photometric monitoring (Roche/Hitachi Modular P; Roche Diagnostics GmbH, Mannheim, Germany). G6PD activity was measured by spectrophotometry (Kornberg and Horacker, Lohr and Waller Trinity Biotech 345 UV, Dublin, Ireland). The principle of the test is that, in the process of the 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. In this study, hemoglobin was measured from the same sample, and G6PD activity was recorded as U/g Hb. G6PD activity is considered normal at 4.6–13.5 U/g Hb in our institution.

SPSS for Windows (version 15.0; SPSS Inc., Chicago, IL, USA) was used for statistical evaluation. Data are expressed as mean ± standard deviation (SD). The data were statistically analyzed by descriptive statistics, using the Student *t* test. Partial correlation analysis was used to assess the relationship between G6PD activity and total bilirubin levels. Hemoglobin, hematocrit, and birth weight were controlled for with correlation testing. Spearman's rank correlation was used to evaluate the relationship between total phototherapy time and G6PD activity. We used G6PD <12 U/g Hb in males and <16 U/g Hb in females as the cut-off values for each sex. These values had 90% specificity and 10% sensitivity for predicting longer phototherapy time. The Mann–Whitney *U* test was used to assess the difference in G6PD levels between the groups in terms of exchange transfusion requirement. Because of the strong relationship between breastfeeding and hyperbilirubinemia, we analyzed breastfed infants separately.

3. Results

The descriptive data of the babies are summarized in Table 1. Gestational age, age at the start of phototherapy, and percentage of distribution by feeding type are similar in male and female infants. Only birth weight was different – the males had higher birth weights than the females ($p = 0.001$). All infants in this study had severe hyperbilirubinemia, and all received phototherapy. Breastfeeding, formula, and mixed feeding rates were 72.2%, 6.4%, and 21.4%, respectively. We did not advise interruption of breastfeeding. There was a significant correlation between bilirubin levels and G6PD activity in females ($r = 0.215$, $p = 0.006$), but not in males ($r = 0.068$, $p = 0.288$). There was a significant correlation between bilirubin level and G6PD activity in the group of patients who started phototherapy after 72 hours (females: $r = -0.270$, $p = 0.001$; males: $r = -0.139$, $p = 0.049$) when controlled for birth weight and hemoglobin and hematocrit levels (Figure 1). Phototherapy was started after 72 hours in 237 males and 179 females.

A negative correlation was found between the duration of the phototherapy and G6PD activity in the group of patients who started phototherapy after 72 hours (females: $r = -0.301$, $p = 0.001$; males: $r = -0.157$, $p = 0.018$). This association is shown in Figure 2.

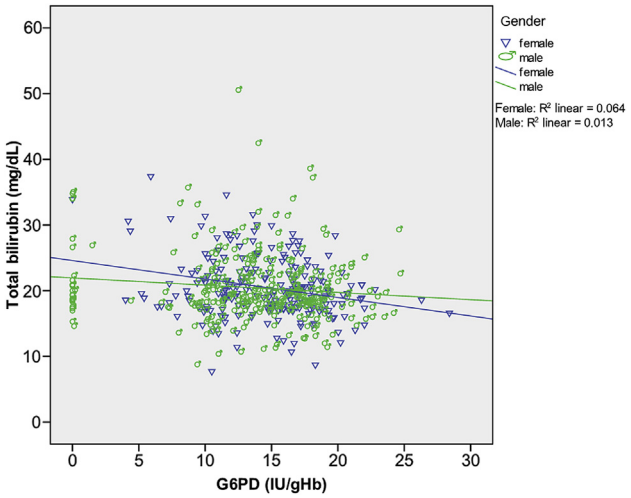

Figure 1 Correlation between G6PD activity and bilirubin levels.

In this study, the female neonates with G6PD levels <16 U/g Hb required more phototherapy time compared with the female neonates with G6PD levels ≥16 U/g Hb ($p < 0.0001$). The male neonates with G6PD levels <12 U/g Hb required more phototherapy time compared with the male neonates with G6PD levels ≥12 U/g Hb ($p < 0.0001$; Table 2). When we analyzed only breastfed infants, we observed a similar significant difference in both sexes ($p = 0.001$; Table 3).

Exchange transfusions were performed on 33 infants. G6PD activity (10.22 ± 5.98 U/g Hb) was significantly lower in the infants who underwent exchange transfusions than in those treated with phototherapy alone (14.12 ± 4.92 U/g Hb; $p = 0.009$).

Three male infants developed kernicterus; their G6PD levels were 12.8 U/g Hb, 9.4 U/g Hb, and 0.06 U/g Hb.

4. Discussion

Based on our analysis, we found a significant correlation between bilirubin levels and G6PD activity in infants of both

Table 1 Demographic features of female and male newborns.

No. of patients	Female 228 (43)	Male 301 (57)	*p*
Gestational age (wk)	38.82 ± 1.62 38.06–39.04	38.96 ± 1.53 38.79–39.14	0.38
Age at the start of phototherapy (h)	128.36 ± 59.28 120.41–136.31	134.94 ± 70.65 126.79–143.09	0.157
Birth weight (g)	3049.86 ± 514.37 2980.88–3118.84	3275.57 ± 515.11 3215.79–3335.34	<0.0001
Breastfed	160 (70.2)	222 (73.8)	0.63
Formula-fed	15 (6.6)	19 (6.3)	
Breastfed and formula-fed	53 (23.2)	60 (19.9)	

Data are presented as *n* (%) or mean ± SD, 95% CI.

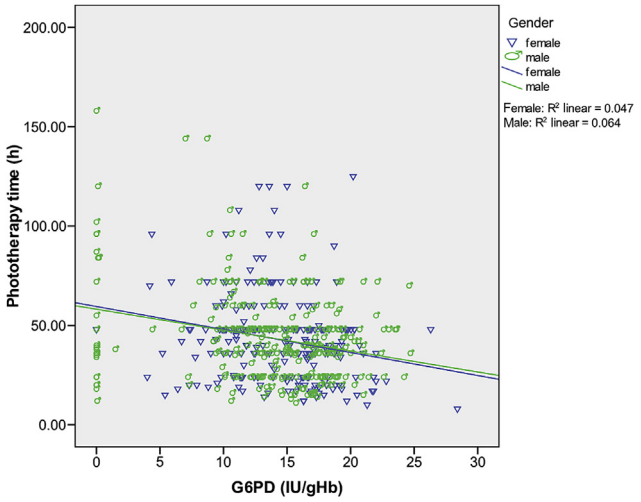

Figure 2 The relationship between G6PD activity and phototherapy duration.

sexes with severe hyperbilirubinemia. Infants with G6PD activity (males <12 U/g Hb; females <16 U/g Hb) who had severe hyperbilirubinemia underwent a longer period of phototherapy.

Owing to the wide distribution of G6PD activity and the difficulties in diagnosing G6PD-deficient female infants, we believe that only one cut-off level is not appropriate to explain the relationship between G6PD activity and hyperbilirubinemia.

Studies on G6PD deficiency indicate that infants with G6PD deficiency have higher rates of hemolysis, a higher prevalence of hyperbilirubinemia, and higher rates of phototherapy treatment than those with normal G6PD levels.[18–20] In a study of 757 male and 326 female newborns with G6PD deficiency, Weng et al[21] found that G6PD activity in G6PD-deficient male newborns with hyperbilirubinemia was significantly lower than that of newborns without hyperbilirubinemia. However, no significant difference was shown between G6PD-deficient female newborns with hyperbilirubinemia and those without hyperbilirubinemia. There are conflicting views in the literature regarding the association between hyperbilirubinemia and enzyme activity. Kaplan et al[22] compared enzyme activity between male infants with and without hyperbilirubinemia. The study included 64 male infants with G6PD deficiency and 436 male infants with normal enzyme activity; the researchers found no association between hyperbilirubinemia and enzyme activity among G6PD-deficient neonates. By contrast, infants who developed hyperbilirubinemia with a normal G6PD level had slightly higher enzyme activity than those without hyperbilirubinemia. Another study of male infants with G6PD deficiency showed no significant decrease in enzyme activity between those with and without hyperbilirubinemia.[23] In two studies, Kaplan et al[22,24] reported that there was no correlation between G6PD activity and total bilirubin levels in G6PD-deficient male newborns. We investigated the relationship between G6PD activity and hyperbilirubinemia severity without evaluating normal or deficient G6PD levels in severe hyperbilirubinemic infants, and we found a significant negative correlation between total bilirubin levels and enzyme activity. In studies involving G6PD deficiency and neonatal hyperbilirubinemia, the risk of developing hyperbilirubinemia is usually comparable in G6PD-deficient and normal male newborns. Some studies have shown no association between G6PD-deficient individuals with low or high enzyme activity and the severity and incidence of hyperbilirubinemia; however, female newborns were excluded from these studies.[22,24]

Owing to the wide range of G6PD activity and the difficulties in diagnosing G6PD deficiency in female infants, we conclude that only one cut-off level is not appropriate to explain the relationship between G6PD activity and hyperbilirubinemia. In a recent study, the researchers proposed that the range of borderline G6PD activity should be 2–10 U/g Hb rather than the currently accepted range of 2–7 U/g Hb.[25] Infants with low and borderline G6PD activity were more likely to require phototherapy and to be referred for exacerbation of jaundice. Other studies support the expansion of the upper limit of intermediate range G6PD activity.[26,27] It is evident that there is no consensus regarding the level of G6PD activity that results in the risk of neonatal hyperbilirubinemia. However, the tendency is to push the borderline higher. This is a view we also embrace, based on the findings of our study, which supports the overall correlation between G6PD activity and the severity of hyperbilirubinemia.

When compared with adults, low levels of glutathione/glutathione disulfide in newborns have shown that newborns have higher oxidative stress,[15] indicating that erythrocytes are exposed to increased free radicals after delivery.[28] The G6PD is necessary for the regeneration of the reduced form of glutathione, which is essential for the reduction of hydrogen peroxide and oxygen radicals and for the maintenance of hemoglobin and other hemoglobin proteins in the reduced state.[4,29,30] Under normal conditions, maintenance of this pathway protects erythrocytes from oxidative stress; however, reduced G6PD levels could lead to failure of this system and hemolysis. Particularly in the neonatal period, when oxidative stress is higher and the antioxidant system is diminished,[14,15] this situation could exacerbate the severity of hyperbilirubinemia. A study measuring heme destruction by corrected end tidal carbon monoxide indicated that heme catabolism might play an important role in the development of hyperbilirubinemia

Table 2 G6PD activity, bilirubin levels, and phototherapy time.

Male (n = 301)	G6PD <12 U/g Hb (n = 86)	G6PD ≥12 U/g Hb (n = 215)	p
Phototherapy time (h)	45.6 ± 20.5	41.6 ± 23.5	<0.0001
Female (n = 228)	G6PD <16 U/g Hb (n = 126)	G6PD ≥16 U/g Hb (n = 102)	p
Phototherapy time (h)	47.1 ± 23.7	36.4 ± 19.3	<0.0001

Data are presented as mean ± SD.

Table 3 G6PD activity, bilirubin levels, and phototherapy time in infants who were breastfed only.

Only breastfed male (n = 222)	G6PD <12 U/g Hb (n = 77)	G6PD ≥12 U/g Hb (n = 145)	p
Phototherapy time (h)	53.9 ± 30.1	40.3 ± 18.1	0.001
Only breastfed female (n = 160)	G6PD <16 U/g Hb (n = 97)	G6PD ≥16 U/g Hb (n = 63)	p
Phototherapy time (h)	48.5 ± 24.4	36.0 ± 20.5	0.001

Data are presented as mean ± SD.

during the first 4 days of life.[24] According to that study, the association between neonatal hyperbilirubinemia and lower G6PD activity might be related to moderate hemolysis.[24]

It is difficult to demonstrate mild hemolysis with routine laboratory evaluations. No decrease was detected in the hemoglobin levels of these patients, nor was there an increase in the reticulocyte count or hemolysis in peripheral blood samples. In the first days of life, bilirubin conjugation and elimination are markedly low, and minor increments of bilirubin production could contribute to the development of severe hyperbilirubinemia.[31]

Another important point which was emphasized in our study was phototherapy duration, which was significantly longer when G6PD activity decreased, in both male and female newborns. Viewed from this perspective, this study, to our knowledge, seems to be the first in the literature to compare overall G6PD activity and phototherapy duration. We found that when G6PD activity was <12 U/g Hb in males and <16 U/g Hb in females, there was a strong association between prolonged phototherapy time and G6PD activity.

Previous reports have shown that G6PD deficiency leads to early occurrence of hyperbilirubinemia, which requires phototherapy or exchange transfusion.[32,33] In different populations, this could be concomitant with kernicterus or death.[34,35] In our study, G6PD activity in the group treated with exchange transfusion was significantly lower. Three male infants whose G6PD levels were 12.8 U/g Hb, 9.4 U/g Hb, and 0.06 U/g Hb developed kernicterus during the study period.

Breastfeeding, G6PD deficiency, and ABO incompatibility are common etiologies in severe hyperbilirubinemia.[36] Jaundice in breastfed infants is commonly of undetermined etiology, and breastfed infants have higher bilirubin levels than formula-fed infants.[37] To demonstrate the effect of G6PD activity on severe hyperbilirubinemia, we analyzed breastfed infants separately, and our analysis indicated a statistically significant relationship between prolonged phototherapy time and G6PD activity. Although we found a significant correlation between G6PD activity and severity of hyperbilirubinemia in female newborns who were fed mother's milk alone, we did not find this correlation in male newborns. Breast milk is a modifier for selected genotypes,[37,38] and thus it can potentially affect development of severe obvious neonatal jaundice.

The increase in hyperbilirubinemia severity when G6PD activity decreases may be related to physiological features. The baby moves from the intrauterine environment, where partial oxygen pressure is 20—25 mmHg, to the extrauterine environment, where partial oxygen pressure is 100 mmHg. This increase in oxygen pressure may lead to increased production of reactive oxygen molecules. In addition, a lower antioxidant capacity, coupled with the stress of the delivery, may stimulate hypoxia and oxidative stress.[6] Therefore, newborns need even higher G6PD levels to combat higher oxidative stress.[15]

In a study comparing hyperbilirubinemia in 64 male G6PD-deficient newborns and 436 normal male infants, the G6PD-deficient group had a phototherapy requirement 3.54-fold higher than that of the other group.[24] In another study, which compared 24 neonates with G6PD deficiency and indirect hyperbilirubinemia with 624 term neonates with normal G6PD and indirect hyperbilirubinemia according to serum bilirubin on admission, maximum serum bilirubin level and the need for exchange transfusion were higher (33.3% vs. 15%) in the G6PD-deficient group.[20] Katar[39] reported on 56 male infants with hyperbilirubinemia who received exchange transfusions, 10 (18%) of whom were G6PD deficient. The increase in necessity of exchange transfusion and phototherapy duration in the G6PD-deficient group is due to the increased severity of hyperbilirubinemia. G6PD deficiency should be considered in neonates who develop hyperbilirubinemia within the 2nd day of life, with two peaks seen on Day 4 and Day 7.[33] In our study, most of the infants needed phototherapy after 72 hours of life.

In conclusion, the results of this study demonstrate that there is a significant correlation between the severity of hyperbilirubinemia and G6PD activity, regardless of G6PD level (deficient/normal), in both male and female infants. Breastfed females exhibited a significant correlation between G6PD activity and hyperbilirubinemia severity. Another important aspect of this study was the duration of phototherapy, which significantly increased as G6PD activity decreased, in both male and female newborns.

Routine monitoring of G6PD levels in hyperbilirubinemic neonates is very important for providing proper medical management to prevent bilirubin-induced neurological dysfunction. Appropriate identification of G6PD (<12 U/g Hb for male infants and <16 U/g Hb for female infants) raises awareness of the severity of this condition, as well as the necessity for immediate care of severe hyperbilirubinemic infants.

Conflict of interest

The authors state that there is no conflict of interest regarding the publication of this article.

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