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# Erythrocyte glucose-6-phosphate dehydrogenase deficiency in male newborn babies and its relationship with neonatal jaundice

## *Deficiência de glicose-6-fosfato desidrogenase eritrocitária em recém-nascidos do sexo masculino e sua relação com a icterícia neonatal*

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*Glucose-6-phosphate dehydrogenase (G6PD) deficiency, the commonest red cell enzymopathy in humans, has an X-linked inheritance. The major clinical manifestations are drug induced hemolytic anemia, neonatal jaundice and chronic nonspherocytic hemolytic anemia. The incidence of neonatal hyperbilirubinemia is much greater in G6PD-deficient neonates than babies without this deficiency. The aim of this study was to ascertain the presence of neonatal jaundice in erythrocyte G6PD-deficient male newborns. Samples of umbilical cord blood from a total of 204 male newborns of the Januário Cicco School Maternity located in Natal, Rio Grande do Norte, Brazil were analyzed. The G6PD deficiency was identified by the methemoglobin reduction test (Brewer's test). The deficiency was confirmed by quantitative spectrophotometric assay for enzyme activity and cellulose acetate electrophoresis was used to identify the G6PD variant. Eight newborns were found to be G6PD deficient with four of them exhibiting jaundice during the first 48 hours after birth with bilirubin levels higher than 10 mg/dL. All deficient individuals presented the G6PD A- variant at electrophoresis. Our findings confirmed the association between G6PD deficiency and neonatal jaundice. Hence, early diagnosis of the deficiency at birth is essential to control the appearance of jaundice and to prevent the exposure of these newborns to known hemolytic agents.*

***Keywords:*** *Glucosephosphate dehydrogenase; infant, newborn; jaundice; hyperbilirubinemia.*

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## Introduction

Glucose 6-phosphate dehydrogenase (G6PD) is a constitutive enzyme that is expressed in all tissues. It catalyzes glucose-6-phosphate oxidation to 6-phosphogluconolactone and converts the coenzyme nicotinamide adenine dinucleotide phosphate to its reduced form (NADPH). The hexosemonophosphate pathway is the only source of NADPH in red blood cells.[1] NADPH is used to reduce oxidized glutathione to its reduced form (GSH), which is very important to reduce peroxide and other reactive oxygen species by glutathione reductase. NADPH also activates catalase, which, in turn, reduces peroxides.[2]

G6PD deficiency was first described in black individuals who were given primaquine to treat malaria and presented jaundice, thereby launching the hematological field of erythroenzymopathies. G6PD deficiency is by far the most prevalent erythroenzymopathy, affecting more than 400 million people throughout the world, in particular in Africa, Mediterranean countries and Southwest Asia. The G6PD gene is located in the long arm of chromosome X (Xq 28), with 13 exons and 12 introns.[1] As G6PD is X linked, its deficiency mainly affects men (hemizygosity) as they have just one X chromosome and thus suffer low enzyme activity and hemolysis when exposed to hemolytic agents. On the other hand, women may be heterozygotes, presenting with one normal and one deficient allele and thus with intermediary enzyme activity or homozygotes when both alleles are affected.[3]

Most of deficient individuals are asymptomatic and may exhibit neonatal jaundice or hemolysis when oxidative stress is triggered by drugs, infections or broad beans (fava). Chronic nonspherocytic hemolytic anemia is much less common.[1,3]

The incidence of neonatal hyperbilirubinemia is much greater in G6PD-deficient neonates than in babies without this deficiency. However, this incidence may vary between ethnic groups and geographic regions. The clinical manifestation of neonatal jaundice related to G6PD deficiency is not very common at birth but often appears from one to four days after birth and requirs phototherapy in order to avoid kernicterus. Nevertheless, neonatal jaundice can be very severe in G6PD-deficient neonates, especially when associated with prematurity, infection, maternal exposure to oxidant drugs and environmental factors such as naphthalene-camphor mothballs.[1,4]

In Brazil the relationship between G6PD deficiency and neonatal jaundice has been little studied, but even so controversial results have been published as the association was found in São Paulo State,[5,6,7] but not in the State of Bahia.[8]

The aim of this work was to ascertain the presence of neonatal jaundice in erythrocyte G6PD-deficient male newborns.

## Material and Methods

From May 2004 to December 2005, a cross-sectional study analyzed 204 umbilical cord blood samples of male newborns from the Januário Cicco School Maternity located in Natal, Rio Grande do Norte, Brazil. The survey was approved by the Ethics Committee of the Federal University of Rio Grande do Norte and all mothers signed an informed consent form.

Newborns weighing more than 2500g, born from non-diabetic pregnant women were studied. The gestational age was assessed by the records of prenatal care units and confirmed after birth according to Capurro method.[9] Preterm newborns were also excluded. Reports have shown that newborns of diabetic mothers and premature newborns may suffer from hyperbilirubinemia.[10,11,12]

Weight and Apgar score were noted as was the maternal ethnic group based upon skin color, hair, and nose and lips shape. Special attention was paid to any medicines given to the mother and her newborn as well as any naphthalene or nitrites impregnation of the mother's clothes.

Umbilical cord blood samples were collected after umbilical cord clamping in tubes containing ACD (citric acid, citrate and dextrose) and kept at 4°C for a maximum of 24 hours before performing laboratory tests. All samples were tested by the methemoglobin reduction test (Brewer's test).[13] G6PD deficiency screened by the Brewer's test was confirmed in blood obtained by venous puncture and analyzed by quantitative spectrophotometric assay for enzyme activity.[14] G6PD activity was expressed in international units per gram of hemoglobin per minute at 37ºC (IU/gHb/min at 37°C). A mean of 12.9 ± 2.4 IU/gHb/min at 37°C was considered to be the normal reference value. Cellulose acetate electrophoresis was performed in order to identify the G6PD variant.[15]

All newborns were examined at 12-hour intervals by a neonatologist for any signs of jaundice and serum bilirubin testing was performed when deemed necessary by clinical evaluation. Jaundice was classified according to Kramer system.[16] Blood and reticulocyte counting were also performed in all G6PD-deficient jaundiced neonates and, when necessary, the Coombs test was employed in order to detect any materno-fetal alloimmunization. G6PD activity was assayed in both parents.

Although the deficient neonates were not followed up after hospital discharge, their mothers received an identification card indicating G6PD deficiency and containing basic information about the clinical trait, possible clinical situations and a list of drugs and medicines which may trigger hemolysis.

## Results

Eight (3.9%) G6PD-deficient individuals were identified among the 204 male newborns who participated in this study.

The sample comprised newborns from 78 white women and 126 black women (Table 1); these figures are not representative of the general population, as the aim of this study was just to disclose the presence of jaundice in G6PD-deficient male newborns.

Four out the eight newborns (50%) evolved with jaundice within the first two days after birth, exhibiting bilirubin levels higher than 10 mg/dL which affected dermal zones 3 and 4 with progressive cephalo-pedal icterus (Table 2). Among the four deficient newborns who presented with jaundice, only two had low hemoglobin levels (Table 3). Interestingly one of them was positive for the direct Coombs test and reticulocytosis, showing an association between materno-fetal ABO blood group incompatibility and G6PD deficiency.

All G6PD-deficient individuals presented the African G6PD A- variant at electrophoresis. Their mothers showed intermediary G6PD activity, except for one for whom it was impossible to obtain a blood sample.

Table 1. Ethnic extraction and frequency of glucose-6-phosphate dehydrogenase (G6PD) deficiency in newborns

| Ethnic maternal group | Number of samples | Blood group | | | |
|---|---|---|---|---|---|
| | | Normal activity | | Deficient | |
| | | n | % | n | % |
| White | 78 (38.2%) | 76 | 97.4 | 2 | 2.6 |
| Negroid | 126 (61.8) | 120 | 95.2 | 6 | 4.9 |
| Total | 204 | 196 | 96.1 | 8 | 3.9 |

Table 2. Clinical and laboratory data of the glucose-6-phosphate dehydrogenase (G6PD) deficient newborns

| Register number | Maternal ethnic group | Blood group | | G6PD activity (UI/gHb/min at 37º C) | Jaundice | | |
|---|---|---|---|---|---|---|---|
| | | Mother | NB | | Onset | Dermal Zone | Peak total bilirubin (mg/dL) / age |
| 15 | N | A+ | nd | 6.0 | 37-48 h | Zone 4 | 18.1/120 h |
| 61 | N | O+ | O | 1.8 | 24-36 h | Zone 3 | 12.7/48 h |
| 63* | N | O+ | O+ | 2.0 | - | - | - |
| 86* | W | Nd | A+ | 1.8 | - | - | - |
| 103* | N | O+ | Nd | 3.1 | - | - | - |
| 113* | N | O+ | O+ | 2.0 | - | - | - |
| 146 | N | O+ | A+ | 2.4 | < 24 h | Zone 4 | 20.5/64 h |
| 180 | W | O+ | A+ | 3.8 | 37-48 h | Zone 3 | 10.9/72 h |

Non-jaundiced; NB = newborn; N = negroid; W = white; nd = not determined.
Reference values: Total bilirubin: 0.7 to 1.2 mg/dL; G6PD activity: 12.9 ± 2.4 UI/gHb/min at 37 ºC

Table 3. Hematological data of the glucose-6-phosphate dehydrogenase (G6PD) deficient newborns jaundiced

| Number register | Hematological data | | | Reticulocyte count (%) | Direct Coombs test |
|---|---|---|---|---|---|
| | Red blood cells (x10 12/L) | Hematocrit (%) | Hemoglobin (g/dL) | | |
| 15 | 4.64 | 49.1 | 17.1 | 5.2 | not determined |
| 61 | 4.82 | 48.5 | 16.4 | 4.1 | not determined |
| 146 | 4.40 | 39.0 | 13.3 | 3.5 | Negative |
| 180 | 4.12 | 40.2 | 13.8 | 10.8 | Positive |
| Mean | 4.50 | 44.2 | 15.2 | 5.9 | - |
| SD | 0.30 | 5.3 | 1.9 | 3.3 | - |

SD = standard deviation
Reference values (3rd day after birth) [25] : Red blood cell count: 5.3 ± 1.3 x $10^{12}$/L; hematocrit: 56 ± 11%; hemoglobin: 18.5 ± 4.0 g/dL; reticulocytes: 1-4.5%.

## Discussion

In Brazil, several surveys have been carried out regarding the populational prevalence of G6PD deficiency, but few have studied the presence of jaundice in G6PD-deficient newborns. In the current study, all G6PD-deficient individuals detected by the screening test were subsequently checked by quantitative spectrophotometric assay.

The African variant (G6PD A-) observed in all G6PD-deficient individuals reflects its high frequency in Brazil. Other studies based on electrophoretic mobility[6,7,17] and DNA analysis[18] from deficient individuals have confirmed the frequency of the A- variant and low frequencies of the Mediterranean variant.

Among the eight deficient newborns, four presented with jaundice with two of them also exhibiting ABO alloimmune materno-fetal incompatibility. All icteric newborns were submitted to phototherapy.

The G6PD-deficient newborn who presented ABO incompatibility also had a low hemoglobin level. Although he had signs of hemolysis (anemia, reticulocytosis and positive direct Coombs test), he did not exhibit bilirubin levels higher than the other newborns with jaundice suggesting that the combination of traits does not express any additional hemolytic effect as has previously been reported.[19] Another aspect about this newborn is that he was identified by the Brewer's test in spite of presenting with reticulocytosis; it is well known that reticulocytes, even in G6PD-deficient individuals, exhibit higher G6PD activity. Moderate G6PD

deficiency of 3.8 IU is detected by the Brewer's screening test, which helps to ascertain the etiology in many cases of neonatal hyperbilirubinemia, thereby enabling early interventions soon after the onset of jaundice. Moreover, this warns neonatologists to prescribe non-oxidative drugs for neonates as well as for their puerperal mothers as maternal milk may contain the chemical agent. It is also interesting to notice that the Brewer's test was able to detect G6PD deficiency at a level of 6.0 IU.

The data obtained in the current work identified an association between neonatal jaundice and the African G6PD A-variant. This variant may present an inconclusive diagnosis soon after a hemolytic crisis due to the high enzyme activity found in reticulocytes.[3] However a recent study reported that the A-variant may be detected during a hemolytic crisis even when there is low enzyme activity, thus a diagnosis is possible.[20] Neither low blood counts nor reticulocytosis was observed in the G6PD-deficient individuals without ABO or Rh blood group incompatibility.

Very recently new developments have been published on the pathophysiology of neonatal jaundice in G6PD-deficient individuals with several investigators believing that hemolysis is not the cause of jaundice in G6PD-deficient neonates.[21,22,23] Indeed, the decreased ability of the neonate liver to conjugate bilirubin seems to be the most important factor, in particular when G6PD deficiency is coinherited with mutations of UDP-glucuronosyltransferase 1 as in Gilbert syndrome.[20] Bacterial and viral infections, acidosis and hypoxia seem to be other factors involved in neonatal jaundice. The combination of G6PD deficiency and prematurity is a risk factor that is highly associated with severe hyperbilirubinemia and kernicterus. UDP-glucuronosyltransferase presents increasing activity during fetal development, and its decreased activity in premature neonates leads to ineffective bilirubin conjugation that leads to hyperbilirubinemia.[12] Environmental factors such as maternal exposure to oxidant drugs, herbal medicines and naphthalene used in clothing may precipitate or exacerbate neonatal jaundice in G6PD-deficient neonates.[1,3]

In this survey only full-term neonates were studied, that is those from more than 37 weeks of gestation. No clinical signs suggestive of infections, acidosis or hypoxia were observed among the G6PD-deficient neonates who exhibited jaundice. No oxidizing medicines that are associated with jaundice, such as synthetic vitamin K or nitrites, were given to the materno-fetal pair. All newborns were given natural vitamin K. The mean G6PD activity in G6PD-deficient male newborns was 2.9 ± 1.5 IU/gHb/min at 37°C. No correlation was observed between enzyme activity and the severity of the jaundice; although neonate nº 15 presented moderate enzyme deficiency, severe hyperbilirubinemia was observed. On the other hand, the bilirubin concentration was not so high for neonate nº 61 who showed severe enzyme deficiency. Interestingly, G6PD-deficient individuals who did not develop jaundice exhibited the lowest G6PD activity. No correlation has been observed between the levels of G6PD activity and the presence of jaundice in any other published works.[21] However, other investigators have demonstrated that enzyme activity in G6PD deficient male newborns with hyperbilirubinemia is significantly lower than those without hyperbilirubinemia.[24] It was also observed that even in the absence of putative hemolytic agents, these G6PD-deficient individuals may present moderate jaundice.

This study demonstrated a high rate of jaundice among G6PD deficient newborns, all of whom presented the African A- variant.

***Resumo***

*A deficiência de glicose-6-fosfato desidrogenase (G6PD) é a anormalidade enzimática hereditária mais frequente. É transmitida como caráter recessivo ligado ao cromossomo X e as principais manifestações clínicas são hemólise induzida por fármacos, icterícia neonatal e anemia hemolítica não esferocítica. O objetivo do estudo foi determinar a presença de icterícia neonatal em recém-nascidos do sexo masculino deficientes de glicose-6-fosfato desidrogenase. Foram analisadas 204 amostras de sangue umbilical de recém-nascidos do sexo masculino provenientes da Maternidade Escola Januário Cicco em Natal, Rio Grande do Norte. A deficiência da glicose-6-fosfato desidrogenase foi determinada através do método qualitativo da redução da metahemoglobina (teste de Brewer) e confirmada mediante determinação espectrofotométrica quantitativa da atividade da G6PD e pela eletroforese da enzima em acetato de celulose. Oito recém-nascidos apresentaram deficiência da G6PD, e quatro deles exibiram icterícia nas primeiras 48 horas depois do nascimento, com valores de bilirrubina maiores de 10 mg/dL. Todos os deficientes apresentaram a variante A-. Os dados encontrados confirmam a associação da deficiência da G6PD e a icterícia neonatal. Assim sendo, o diagnóstico precoce da deficiência logo após o nascimento é essencial ao controle do aparecimento da icterícia e para evitar o contato destes recém-nascidos com conhecidos agentes hemolíticos.*

***Palavras-chave:*** *Glucosefosfato desidrogenase; recém-nascido; icterícia neonatal; hiperbilirrubinemia.*

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