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Glucose-6-phosphate-dehydrogenase deficiency and its correlation with other risk factors in jaundiced newborns in Southern Brazil

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ABSTRACT

Objective: To evaluate the correlation between glucose-6-phosphate-dehydrogenase (G6PD) deficiency and neonatal jaundice. **Methods:** Prospective, observational case-control study was conducted on 490 newborns admitted to Hospital de Clínicas de Porto Alegre for phototherapy, who all experienced 35 or more weeks of gestation, from March to December 2007. Enzymatic screening of G6PD activity was performed, followed by PCR. **Results:** There was prevalence of 4.6% and a boy-girl ratio of 3:1 in jaundiced newborns. No jaundiced neonate with ABO incompatibility presented G6PD deficiency, and no Mediterranean mutation was found. A higher proportion of deficiency was observed in Afro-descendants. There was no association with UGT1A1 variants. **Conclusions:** G6PD deficiency is not related to severe hyperbilirubinemia and considering the high miscegenation in this area of Brazil, other gene interactions should be investigated.

1. Introduction

Hyperbilirubinemia once was considered as a benign condition. Due to the potential toxicity of bilirubin, neonates should be monitored for higher risk of unfavorable outcomes such as kernicterus. Some risk factors are widely known, such as preterm birth, blood incompatibility, glucose-6-phosphate-dehydrogenase (G6PD) deficiency, breastfeeding, dehydration, asphyxia and infection[1]. The association of these factors with limited hepatic conjugation[2,3] was later proposed.

G6PD deficiency is an X-linked recessive enzymopathy, involving in the pentose phosphate pathway and protection of the cells from oxidative damage. People with deficiency in this enzyme are more susceptible to developing severe hemolytic anemia, which is associated with neonatal jaundice pathogenesis[4]. Hyperbilirubinemia also appears in the absence of hemolysis-triggering factors, increasing production of bilirubin in jaundiced and non-jaundiced individuals[5].

In the 1980s, it is reported around 7% had G6PD deficiency in Brazil[6]. In Rio Grande do Sul, 8% had combined G6PD deficiency, with predominance of African variant[7].

Association of G6PD deficiency with other risk factors is well described in the etiology of severe hyperbilirubinemia, as in the case of UGT1A1 polymorphisms, with limited conjugation capacity[2]. Some investigators did not find this genetic interaction regarding to jaundice[8].

This study is conducted to investigate the frequency of G6PD deficiency as a risk factor for severe hyperbilirubinemia in one Neonatal Unit of Southern Brazil. It also aims to estimate the prevalence of most typical mutations of G6PD in this sample and the possible interactions with other risk factors, such as the polymorphic variants of UGT1A1.

2. Materials and methods

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A prospective observational case-control study was conducted on all newborns admitted to the Service of Neonatology, Hospital de Clínicas de Porto Alegre for phototherapy, who all had a gestational age of greater than 35 weeks and weighted more than 2 000 g, from March to December 2007. Patients with cholestasis, cephalohematoma or sepses were excluded. The study was approved by the Institution's Ethics Committee, and the informed consent was obtained from eligible patients' guardians. Residual blood samples were collected from newborns in the laboratory, and anicteric newborns were selected from the neonatology as the control.

G6PD activity was quantified through the hemoglobin normalization procedure, using Neolisa G-6-PD kit (Interscientific Corporation, 2700 North 29th Avenue-Suite 220, Hollywood, FL - USA, Cat. Nr 3570-050) and the cutoff point was 8 Ug/Hb[9]. These patients with reduced activity had their DNA extracted by an adapted procedure according to Lahiri and Nurnberger[10]. The most frequent mutations in southern Brazil were A- (G202A, A376G) and Mediterranean (C563T). The regions analyzed were amplified by polymerase chain reaction (PCR) in a thermal cycler (Eppendorf Personal Cycler) and cleaved with Nla III, Fok I and Mbo II endonucleases[7]. The polymorphic genotypes of UGT1A1 of all neonates were treated by capillary electrophoresis and analyzed by Gene Mapper software[11].

The statistical analysis was performed using SPSS statistical software. The baseline demographic data between ill cases and controls were compared using *Chi*-square test, with or without Yates' correction, for categorical variables and Student's *t* test for continuous variables. The various jaundice parameters were compared using ANOVA and Mann-Whitney (as applicable). The statistical significance level considered for any case was a two-sided α of 0.05 and 80% power.

3. Results

A total of 490 neonates were selected from March to December 2007 (Table 1), including 243 newborns admitted to phototherapy and 247 normal babies. Twenty-two patients had G6PD deficiency (4.6%), including 13 jaundiced patients (5.5%) and 9 in control group (3.7%), with the odds ratio as 1.5 (P=0.35 and CI=0.63-3.6). The mean activity of G6PD was 5.81 U/gHb in jaundice group and 5.54 U/gHb in control group.

Sixteen (6.2%, 16/258) boys and 6 (2.7%, 6/222) girls had G6PD deficiency (P=0.06), with the male to female ratio as 2.2:1. One G6PD deficiency girl's result was missing. Out of 138 hyperbilirubinemic boys, 10 (7.2%) had G6PD deficiency, while among 99 hyperbilirubinemic girls, 3 (3%) had the deficiency, with the male to female ratio as 3:1.

None of the ABO incompatible patients in jaundice group presented the G6PD deficiency (P=0.045).

All patients were sorted into three groups based on gestational age: 12% at 35-(36+6) wk, 11.4% at 37-(37+6) wk and 76.3% at ≥38 wk. No correlation was observed between gestational age and the G6PD deficiency. There were 2 late-preterm neonates in jaundice group and only 1 in the control group; the case group had 4 neonates under 38 wk and the control group had 1 (P=0.56).

Table 1

Demographic data of the 490 neonates analyzed between March and December 2007, at the HCPA [*n* (%)].

Characteristics		Ill cases	Controls
Gender	Male	141 (58.0)	122 (49.4)
	Female	102 (42.0)	125 (50.6)
Ethnic group	White	185 (76.1)	191 (77.3)
	Black	29 (11.9)	27 (10.9)
	Mulatto	29 (11.9)	29 (11.7)
Weight for gestational age	Adequate	187 (77.0)	192 (77.7)
	Small	44 (18.1)	40 (16.2)
	Large	12 (4.9)	15 (6.1)
Incompatibility*	ABO incompatibility	73 (30.0)	47 (19.0)
	Rh incompatibility	23 (9.5)	26 (10.5)
Delivery type	Vaginal delivery	164 (67.5)	160 (64.8)
	Caesarean	79 (32.5)	87 (35.2)
	Resuscitation at birth	18 (7.4)	28 (11.3)
	G6PD deficiency	13 (5.5)	9 (3.7)
	Weight at birth (g)*	3 073.0±530.0	3 217.0±460.0
	Gestational age (weeks)*	38.4±1.7	39.5±1.3
	Mother's age (years)	25.4±6.6	26.0±6.8
	Number of prenatal visits	7.0±3.1	6.5±3.3
	Apgar score at 5′	9.0±1.0	9.0±1.0
	G6PD (Ug/Hb) activity	17.2±5.9	17.2±5.6

* P<0.05.

Regarding the ethnic groups, 15 neonates (4%, 15/368) in the white group, 4 (7%, 4/56) in the black group and 3 (5.4%, 3/56) in the mulatto group presented the deficiency (P=0.5). Among these 237 jaundice neonates, it was found 6 (3.2%) neonates with the deficiency in the white group, 3 (10.3%) in the black group and 3 (10.3%) in the mulatto group (P=0.046).

PCR for 202/376 mutation showed 6 cases with homozygote in jaundice group and 3 in control group; 2 with heterozygote in jaundice group and 3 in control group; 5 jaundice cases and 3 normal cases had no detectable mutation.

No neonate presented the Mediterranean mutation and 8 patients did not present either of the studied mutations, although they showed reduced enzymatic activity.

Two (2.8%) patients with UGT1A1 risk genotypes also presented the G6PD deficiency: one hyperbilirubinemic neonate and one control neonate. The hyperbilirubinemic

newborn presented the African mutation in homozygosis and the control neonate did not present mutation.

4. Discussion

G6PD deficiency has been considered as one of 10 most important etiologies of non-hemolytic neonatal jaundice[12], accounting for 22% cases in the US Pilot Kernicterus Registry[13].

The prevalence of G6PD deficiency in our study is 4.6%. It is reported a frequency of 2%-2.5% in Sicilia, 13% in Sardinia[14], and 8% in southern Brazil[7]. The low prevalence in this study may be because of the inclusion of jaundiced neonates with reticulocytosis, which could led to false negative results. So it is suggested selecting neonates older than 3 months old, when the turnover of erythrocytes has stabilized[15]. Another possible reason is because our study excluded patients with sepsis, which may be a hemolysis-triggering factor.

WHO[16] suggests screening in areas where the prevalence is 3% to 5% in boys. Our study showed 6.2% of prevalence among boys with the deficiency, similar study was conducted in Kuwait (6.5%)[17].

The boy-girl ratio with the G6PD deficiency was 2.2:1 in this study, and Atay *et al* found 3:1 in Turkey[18]; both studies agree with the observations of higher prevalence in boys by Kaplan *et al*[19]. It should be noted that our study might have lost girls' result, as the screening employed a quantitative method, which is known to present some risk of false negative results[20].

In our study, 6.2% of all Afro-descendants (all black and mulatto neonates combined) presented the G6PD deficiency. In North America, a prevalence of 11% to 13% was found in Afro-descendants[21]. Compri *et al*[22] demonstrated prevalence in male Afro-descendants as 10%. It might have happened due to the ethnic classification in these studies, and perhaps because that the skin color is not a true indicator of ethnic origin in Brazil[23].

No statistical difference is observed between the jaundiced patients and the controls regarding G6PD deficiency. Kaplan *et al*[5] reported increased bilirubin production in both jaundiced and non-jaundiced patients. In our study, the bilirubin levels of control patients are not measured as they were chosen based on visual estimation. Our odds ratio of 1.5 about the development of severe hyperbilirubinemia is lower than that found by Kaplan *et al* in 1997, which was 2.4[2]. The prevalent G6PD mutation in studies of Kaplan is the Mediterranean mutation, which is not found in our study. A study conducted in Afro-Americans[21] showed the odds ratio of 3.2. It's possible that in our study the newborn controls with the deficiency were not exposed *in utero* to any agent, which can triggered hemolysis or conjugation defect, they didn't show high bilirubin levels to be considered for a treatment.

The study conducted by Compri *et al*[22] also found no Mediterranean mutation and a nearly exclusive presence of the African mutation, even in Euro-descendants. Some neonates of our study presented undetectable mutation, which might be due to the presence of some mutation that have not been investigated. And at present more than 140 mutations have been described for G6PD[24].

None of the ABO incompatible patients in the jaundiced neonates group presented the deficiency. A study conducted by Sephardic Jews[25] showed that the neonates with G6PD deficiency and ABO incompatibility did not show greater evidence of hemolysis than those with the incompatibility only.

No correlation was observed between the gestational age and G6PD deficiency. It is reported that more neonates with G6PD deficiency had serious hyperbilirubinemia than term neonates[26]. Near-term neonates are also susceptible because of the delayed conjugation and difficult feeding, leading to increased enterohepatic circulation[27]. Low albumin fraction is another risk factor, and neonates with low albumin fraction are vulnerable to bilirubin neurotoxic effects due to more permeable hematoencephalic barrier[28].

Only 2.8% of the patients with UGT1A1 risk genotypes had G6PD deficiency, which is different from the results obtained by Kaplan *et al*[2]. He found neonatal jaundice is related to the coexistence of G6PD deficiency and the addition of allele (TA)7 in hetero or homozygosis. A study conducted in the United States[29] demonstrated a correlation of the UGT1A1 variants and G6PD deficiency with other genes, such as those involved in organic anion transporting polypeptide (OATP) polymorphisms.

AAP practice guideline in 2004[15] recommends screening for G6PD deficiency in cases of strong suspicious: hyperbilirubinemia above 95% within the first 24 h of life, poor response to phototherapy, and history of jaundice in siblings. Considering our findings and the recommendations of the WHO, a screening for G6PD deficiency could be considered in all jaundiced patients without blood incompatibility, confirming the results at 3 months of age, when the hemoglobin stabilization occurs.

In conclusion, G6PD deficiency and UGT1A1 polymorphic variants are not associated with severe hyperbilirubinemia in newborns from Porto Alegre with $\geqslant$ 35 wk gestation. The prevalence of G6PD deficiency in our population is lower than in other studies, and no severe Mediterranean mutation is found. Due to the high miscegenation in this area of south Brazil, other factors and gene interactions could explain the severe neonatal hyperbilirubinemia, as well as the study of other polymorphisms in hepatic conjugation.

Conflict of interest statement

We declare that we have no conflict of interest.

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