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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