Glucose-6-Phosphate Dehydrogenase Deficiency in Children from 0 to 14 Years Hospitalized at the Pediatric Hospital David Bernardino, Luanda, Angola

Miguel Brito1,2*, Chissengo Lucama Tchonhi3, Brigida Santos4 and Luisa Veiga1
1Lisbon School of Health Technology, Polytechnic Institute of Lisbon, Portugal
2CISA Project–Health Research Centre in Angola, Caxito, Angola
3Faculty of Medicine, University Agostinho Neto, Luanda, Angola
4Hospital Pediatrico David Bernardino, Luanda, Angola

Abstract

The Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymatic defect in the world. The most common clinical manifestations are acute hemolytic anemia associated with drugs, infections, neonatal jaundice and hemolytic non-spherocytic chronic anemia. The main aim of this study was to determine the frequency of major genetic variants of G6PD leading to enzyme deficiency in children from 0 to 14 years at a Pediatric Hospital in Luanda, Angola. A cross-sectional and descriptive analytical study covered a total of 194 children aged from 0 to 14 years, of both genders and hospitalized at the Pediatric Hospital David Bernardino, Luanda between November and December, 2011. The G202A, A376G and C563T mutations of the G6PD gene were determined by real-time PCR with Taqman probes. The disabled A-/A- genotype was detected in 10 girls (10.9%). Among the boys, 21 (20.6%) presented the genotype A-. Considering all the samples, the A- variant was observed in 22.4% of cases. The Mediterranean mutation was not detected in the Angolan sample. Furthermore, no association was found between genotype and anemia, nutritional state and mucosa color. A significant association, however, was observed with jaundice. Based on the results obtained, there is a clear need to identify those with the disabled genotype in the Angolan population in order to avoid cases of drug-induced anemia, particularly in the treatment of malaria, so prevalent in Angola.

Keywords: G6PD deficiency; Hemolytic anemia; Malaria; Drug interaction

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymatic red blood cell disorder in humans [1]. The G6PD enzyme catalyzes the first step in the pentose phosphate pathway, leading to antioxidants that protect cells against oxidative damage [2]. A G6PD-deficient patient lacks the ability to protect red blood cells against oxidative stresses produced by the taking of certain drugs, metabolic conditions, infections, and ingestion of some foods [3,4]. This disorder affects about 500 million people but seldom identified in populations where malaria is rare but may exceed 10% in prevalence where malaria is endemic, since individuals with G6PD deficiency have been related to greater levels of resistance to infection by Plasmodium falciparum. Therefore, the highest prevalence of G6PD deficiency is reported in Africa, Southern Europe, the Middle East, Southeast Asia, and the central and southern Pacific islands. However, due to migration, deficient alleles are now quite prevalent in North and South America and in some Northern European regions [5,6].

G6PD deficiency has been shown to exist due to enzyme variants that exhibit less than optimum enzyme activity. These variants reflect allelic genes located in the long arm of the X chromosome (Xq28) resulting in an X-linked recessive mode of inheritance [7,8]. More than 160 mutations have been identified as responsible for the deficiency, the majority of them consisting of single point mutations causing the replacement of amino acids [6]. Two of the most common G6PD variants are the Mediterranean G6PD B- C563T, mainly encountered in the Mediterranean areas, and the African G6PD A- 202A/376G largely found in African subjects [6]. Individuals with G6PD deficiency incur a higher risk of acute hemolysis when exposed to some medications such as antimalarial or antibiotics, antipyretics and analgesics with healthcare providers requiring caution when managing these patients [9]. Special care should also be taken in endemic malaria areas since this disease is treated with drugs that can cause severe hemolysis in G6PD-deficient individuals [10]. Frequently, these patients suffer from cyanosis, headache, fatigue, tachycardia, dyspnea, lethargy, lumbar/substernal pain, abdominal pain, splenomegaly, hemoglobinuria, and/or scleral icterus. Moreover, the broken down products of hemoglobin may accumulate in the blood, causing jaundice and excreted in urine, causing a dark brown discoloration [3,10-12]. In extreme cases, acute hemolysis can lead to permanent neurologic damage or death [8]. Strategies to reduce the above mentioned complications associated to G6PD deficiency may comprise the screening of newborns/children in regions with high frequencies of G6PD deficiency and the capacity building of clinical professionals and the awareness of the general population.

*Corresponding author: Miguel Brito, Lisbon School of Health Technology, Polytechnic Institute of Lisbon, 1990-096 Lisbon, Portugal. Tel: +351 216980400; E-mail: miguel.brito@estesl.ipl.pt

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population about avoiding those drugs that precipitate attacks of hemolytic anemia.

Therefore, our main goal involved determining the frequencies of genetic variants of G6PD in children from 0 to 14 years attending the Pediatric Hospital in. To the best of our knowledge, this is the first time the frequencies of G6PD mutations have been published in Angola.

**Methods**

A cross-sectional and descriptive analytical study took place on 194 children from 0 to 14 years, of both genders, hospitalized at the Pediatric Hospital David Bernardino, Luanda, in November and December 2011.

Clinical and biochemical evaluations were performed. Weight, hemoglobin values, type of anemia, membrane mucosal coloration, diagnosis of malaria and jaundice condition were all taken into consideration.

Whole blood samples were collected and stored on 3 mm Cards Kit papers. DNA extraction was undertaken by Generation Capture Card Kit - DNA Purification; DNA Elution (Gentra Systems, Inc., Minneapolis). The G202A, A376G and C563T genotypes were determined through Real Time PCR methods with TaqMan Pre-Designed SNP Genotyping Assays (Applied Biosystems, USA). To perform the genotype analysis, the target fragments were amplified in 20 µl reaction mixture containing 10 µl TaqMan Universal PCR Master Mix, 1 µl primers, 5 µl Milliq water and 4 µl DNA. Real Time PCR, iCycler iQ® Multicolor Real-Time PCR Detection System (BIO-RAD) was conducted as follows: 10 min of the initial step at 95°C, 50 cycles of 15 sec and 1 min at 92°C and 60°C, respectively, with Taqman probes.

Statistical analysis made recourse to statistical package for the social sciences (SPSS) version 21.0 for Windows. One-way analysis of variance (ANOVA) was deployed to test for hemoglobin differences between anemia status. The exact test served to compare the mutation frequencies and the frequencies of clinical conditions associated to different genotypes or gender.

**Results**

A total of 194 children (92 girls and 102 boys) were analyzed. Ages ranged from two days to fourteen years of age but the majority (59%) were under two years old, 20% were between two and five, and 21% aged over five. Considering the total sample: 33% were underweight; anemia, anemia type (Anova, p>0.05), membrane mucous coloration, being underweight or malaria diagnoses were observed between boys and girls for the same genotype. On the other hand, significant differences between jaundice frequencies were observed, with male subjects presenting a higher frequency (Exact test p<0.02) (Table 3).

No significant differences between hemoglobin concentrations, anemia, anemia type (Anova, p>0.05), membrane mucous coloration, being underweight or malaria diagnoses were observed between boys and girls for the same genotype. On the other hand, significant differences between jaundice frequencies were observed, with male subjects presenting a higher frequency (Exact test p<0.02) (Table 3).

### Table 1: Summary of the clinical parameters for the studied population.

| Anemia type [n (%)]* | Overall |
|----------------------|---------|
| NH                   | 57 (14%)|
| MH                   | 138 (71%)|

| Membrane mucous coloration [n (%)] | Overall |
|-----------------------------------|---------|
| Normal                            | 310 (88%)|
| Discolored                        | 62 (32%)|

| Jaundice [n (%)]** | Overall |
|--------------------|---------|
| Yes                | 117 (33%)|
| No                 | 124 (36%)|

| Underweight (WAZ) [n (%)] | Overall |
|---------------------------|---------|
| Normal                    | 214 (63%)|
| Moderate                  | 23 (13%)|
| Severe                    | 35 (20%)|

| Malaria Diagnosis [n (%)] | Overall |
|--------------------------|---------|
| No                       | 129 (37%)|
| Yes                      | 65 (18%)|

*NN normocytic normochromic, NH normocytic hypochromic, MH microcytic hypochromic

**Significant differences for boys (Exact test, p<0.008) and for girls (Exact test, p=0.028)

### Table 2: Glucose – 6 Phosphate Dehydrogenase genotypes and alleles found in the children of Luanda Pediatric Hospital. The Mediterranean mutation (563 C-T) was not detected in the sample.

| Genotypes | n | Frequency (%) |
|-----------|---|---------------|
| Boys      |   |               |
| B         | 59 | 57.8          |
| A         | 22 | 21.6          |
| A-        | 21 | 20.6          |
| Girls     |   |               |
| B/B       | 41 | 44.6          |
| A/A       | 2  | 2.2           |
| A/A-      | 6  | 6.5           |
| B/A       | 16 | 17.4          |
| B/A-      | 17 | 18.5          |
| A/A-      | 10 | 10.9          |
| Alleles   |   |               |
| B (376A)  | 174| 60.8          |
| A (A376G) | 48 | 16.8          |
| A- (A376G/G202A) | 64 | 22.4 |

**G6PD deficiency n Frequency (%)**

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|---------|---|---------------|
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| A- (A376G/G202A) | 64 | 22.4 |

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Discussion

Although there are several G6PD deficiency studies done in other African populations, none have been published concerning the Angolan population [13]. This preliminary result, carried out in a Pediatric Hospital population, addresses the public health problem of G6PD deficiencies. However, the epidemiological study of the Angolan population is essential to defining a strategy for the implementation of population screening.

The results obtained, in children from 0 to 14 years attending the Pediatric Hospital in Luanda, in November and December 2011, demonstrated a high frequency of A- variant. A total of 15.9% of children were A-, with 10.9% of girls A- homozygotes and 20.5% of boys hemizygotes A-.

In general, the G6PD A- allele, which contains two mutations, A376G and G202A, is the most common G6PD deficiency variant in Africa, with a frequency that ranges from 0–25% [13,14]. These frequencies are in accordance with those reported by this study. Lower frequencies were observed in other African populations namely in the 4th Nile cataract region in Sudan [15] or in the Republic of Guinea [16]. Higher frequencies were detected among Palestinians in the Gaza Strip [8] and in Tunisia [6]. Moreover, we searched for the Mediterranean allele in the Angolan sample since there are historical references to European populations having settled in Angola, in particular the Portuguese, where this mutation is prevalent [7]. However, we did not encounter the allele in our sample. More studies are needed to confirm the presence of this allele in Angola.

Our data suggest a poor relation between genotypes and the clinical G6PD characteristics, except for a significant association with jaundice. These results may stem from only making one collection of clinical data, in a single moment and not having been able to follow patients throughout their hospital stay.

A total of five children with A-genotype were medicated with drugs potentially hemolytic, including antibiotics and antimalarials. Since this study was retrospective and the children were not followed, there are no associations between anemia or jaundice and the medication.

Haemolysis associated with G6PD deficiency has long since been described as a serious adverse event for a number of antimalarials [17]. However, we did not observe significant differences in Hb values among children with a full G6PD defect (hemi- and homozygous), with or without treatment with antimalarials. Several possible explanations arise, in particular, the fact that we carried out only one determination of biochemical parameters, and some children were at the beginning of their treatment process while other children were at different phases in their treatment courses. That information was not obtained. Another explanation is that these children already have very low Hb values prior to treatment. In a previous study done in Angola, the prevalence of anemia was higher than 40% [18]. Finally, some drugs are only capable of provoking crises in patients with the more severely deficient polymorphic variants, such as G6PD Mediterranean, but not in the mild variant G6PD A- [9].

The geographic correlation between malaria endemicity and G6PD deficiency suggests that these mutations confer resistance to malaria. Clinical data have supported these observations however doubts still remain about whether this resistance is just a feature of heterozygous females or also hemizygous males [2]. In our results, although not significantly, we detected a decrease in malaria infections in females or also hemizygous males [2]. In our results, although not significantly, we detected a decrease in malaria infections in females A-, but not in males. No differences were observed in heterozygous females. Monteiro [19] also did not find evidence for malaria protection in carriers of G6PD (A-) allele (hemizygous or heterozygous). A larger sample and the follow-up of a cohort of children would be required to test the hypothesis of resistance to malaria in the Angolan population.

Our study has some limitations. The sampling time was very short, comprising just two months. We were not able to determine G6PD activity levels due to technical problems, namely the blood obtained on FTA cards that enabled us to extract the enzyme to determine activity.

Table 3: Summary of the clinical parameters for Angolan children suffering from G6PD hemolytic disorder.

|                  | Boys                  | Girls                 |
|------------------|-----------------------|-----------------------|
|                  | B and A               | A-                    | Without A- | A/A- and B/A- | A-/A-     |
| Hemoglobin (g/L) | Mean                  | 7.8                   | 7.3        | 7.9           | 7.5        | 7.8        |
|                  | 95 CI Mean            | 7.3-8.4               | 6.3-8.3    | 7.3-8.5       | 6.5-8.4    | 5.9-9.5    |
|                  | Median                | 7.4                   | 7.2        | 7.4           | 7.4        | 6.7        |
|                  | Range                 | 3.4-16.8              | 2.8-12.8   | 3.2-16.9      | 3.5-13.8   | 5.4-14     |
| Anemia [n (%)]   | Normal                | 11 (14%)              | 2 (10%)    | 3 (5%)        | 1 (4%)     | 1 (10%)    |
|                  | Mild                  | 0 (0%)                | 0 (0%)     | 5 (9%)        | 2 (9%)     | 0 (0%)     |
|                  | Moderate              | 32 (39%)              | 8 (38%)    | 26 (44%)      | 9 (39%)    | 4 (40%)    |
|                  | Severe                | 38 (47%)              | 11 (52%)   | 25 (42%)      | 11 (48%)   | 5 (50%)    |
| Anemia type [n (%)] | NN                  | 12 (15%)              | 5 (24%)    | 9 (15%)       | 2 (9%)     | 1 (10%)    |
|                  | NH                    | 12 (15%)              | 0 (0%)     | 11 (19%)      | 3 (13%)    | 1 (10%)    |
|                  | MH                    | 57 (70%)              | 16 (76%)   | 39 (66%)      | 18 (76%)   | 8 (80%)    |
| Membrane mucous coloration [n (%)] | Normal | 56 (70%)              | 14 (67%)   | 40 (68%)      | 15 (65%)   | 5 (56%)    |
|                  | Discolored            | 24 (30%)              | 7 (33%)    | 19 (32%)      | 8 (35%)    | 4 (44%)    |
| Jaundice [n (%)] | No                    | 81 (100%)             | 18 (86%)   | 55 (93%)      | 23 (100%)  | 6 (60%)    |
|                  | Yes                   | 0 (0%)                | 3 (14%)    | 4 (7%)        | 0 (0%)     | 4 (40%)    |
| Underweight (WAZ) [n (%)] | Normal | 49 (65%)              | 12 (60%)   | 39 (75%)      | 16 (72%)   | 5 (56%)    |
|                  | Moderate              | 7 (9%)                | 4 (20%)    | 5 (10%)       | 3 (14%)    | 4 (44%)    |
|                  | Severe                | 20 (26%)              | 4 (20%)    | 8 (15%)       | 3 (14%)    | 0 (0%)     |
| Malaria Diagnosis [n (%)] | No       | 52 (64%)              | 14 (67%)   | 41 (69%)      | 14 (81%)   | 8 (80%)    |
|                  | Yes                   | 29 (36%)              | 7 (33%)    | 18 (31%)      | 9 (39%)    | 2 (20%)    |

*NN normocytic normochromic, NH normocytic hypochromic, MH microcytic hypochromic
**Significant differences for boys (Exact test, p= 0.008) and for girls (Exact test, p=0.028)
Finally, the present study was carried out on a pediatric population seeking hospital treatment and the results cannot be generalized to the broader Angolan population, however, they do provide important preliminary indications of the genotypes frequencies.

Alerting clinical professionals about how frequent the genetic variants of G6PD are should contribute to raising awareness about the need to screen for G6PD deficiency in children. The potential association of G6PD mutations with clinically significant hemolysis requiring hospital admission justifies nationwide programs of newborn screening for G6PD deficiencies.

Based on the results obtained, it is clearly important to identify those with the disabled genotype in the Angolan population in order to avoid cases of drug-induced anemia, particularly treating malaria, such a frequent condition in Angola.

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