Glucose-6-Phosphate Dehydrogenase Deficiency
Correlation between Genotype and Phenotype

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Abstract. In Saudi Arabia, Glucose-6-phosphate dehydrogenase
deficiency exists at variable frequency in different regions in the
Kingdom. The aim of this study was to investigate the mutations and
clinical significance of the Glucose-6-phosphate dehydrogenase gene
among the population in this area.

Keywords: Glucose-6-phosphate dehydrogenase deficiency, Genotype,
Phenotype.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the
most common human enzyme deficiencies and is estimated to affect
more than 400 million people worldwide[1]. G6PD is a housekeeping
enzyme critical in the redox metabolism of all aerobic cells. G6PD
deficiency has been a prototype of hemolytic anemia due to
enzymopathy, i.e., to a primary abnormality of a red cell enzyme.
Although most of the enzyme-deficient subjects are asymptomatic,
deficient individuals may show episodic hemolytic anemia induced by infections or certain drugs and spontaneous chronic nonspherocytic hemolytic anemia (NSHA)[2]. G6PD is determined by a gene on the X chromosome, Xq28[3]. Therefore, diseases involving this enzyme occur far more frequently in males than in females. To date, more than 130 different molecular abnormalities and 400 biochemical variants have been described in G6PD-deficient subjects, with a considerable variation in the defect among various racial groups[4]. A high incidence (ranging from 5% to 40%) of a variant designated G6PD B− or G6PD Mediterranean was found in Italians, Greeks, Middle Eastern, African, and Asian ethnic groups[5-7]. Several G6PD-deficient variants have been reported among Arabs of the Arabian Gulf region. The most common of these variants is G6PD Mediterranean type[8-12].

A distinction must be made between the prevalence of G6PD deficiency as a genetic abnormality and the incidence of hemolytic anemia associated with G6PD deficiency.

In this study, we have analyzed the molecular variants of the G6PD deficiency with its clinical significance in western Saudi Arabia.

**Materials and Methods**

A total of 600 unrelated Saudi volunteers of both sexes (336 males, 264 females) age ranging from 18-42, mean 24, were screened for the presence of G6PD deficiency. Blood samples were collected from blood donors, students, and health-care workers. The study was conducted at the Hematology Research Laboratory in King Fahd Medical Research Center (KFMRC) at King Abdulaziz University (KAU) in Jeddah, Saudi Arabia. Because Jeddah is the largest city in the western region of Saudi Arabia, the population of the study was from this city and other neighboring towns and villages on the west coast of Saudi Arabia.

G6PD deficiency was diagnosed using a standard method for quantitative assays via G6PD quantity kit (Sigma Diagnostics, USA) with a cutoff point as 4.6 U/g Hb. Values less than 4.6 U/g Hb were accepted as being G6PD deficient[13,14].

DNA was extracted from 42 individuals who were G6PD-deficient (36 males and 6 females). These subjects were screened for gene mutations using polymerase chain reaction/restriction fragment length
polymorphism (PCR-RFLP). Screening included Mediterranean $^{563\text{C} \rightarrow \text{T}}$, and Aures $^{143\text{T} \rightarrow \text{C}}$, and African $^{202\text{G} \rightarrow \text{A}, 376\text{A} \rightarrow \text{G}}$ [9,15].

Specific G6PD regions were selected for amplification by PCR using primers flanking (the sites of the gene)$^{[15]}$.

G6PD Mediterranean mutation was identified by means of MboII digestion of PCR amplified fragment, including exons 6 and 7 of the G6PD gene converting codon (188 from Ser to Phe).

G6PD Aureus was identified from T- to C transition at nucleotide 143, converting codon 48 from ATC (ile) to ACC (thr) by Mbo I digestion of PCR amplified product, including exon 3 and 4.

G6PD A+ gene has an A-> G transition resulting in the substitution of Aspartic acid for Asparagines as 142nd amino acid from the N terminal of the enzyme.

G6PD A-. An allele that predominates in some African populations (including African Americans) occurs in individuals carrying the A+ mutation. Substitution of adenine for guanine at nucleotide 202 is found in this mutation, which was responsible for in vivo instability of the enzyme protein.

All PCR amplified samples for exon 3 & 4 were sent to Bioscientia Laboratory GmbH (Germany) for gene sequencing.

Results

Forty-two (7%) of 600 Saudis screened were found to be G6PD-deficient, with an overall frequency of G6PD-mutated alleles being 0.265 as previously reported$^{[16]}$.

The G6PD Mediterranean is the most common mutation in western Saudi Arabia followed by Aureus mutation.

Table 1 summarizes the mutation detected in G6PD deficient Saudi subjects.

Sixteen (38%) of 42 G6PD deficient subjects have the Mediterranean mutation.
Seven (17%) have Aureus type; 5 (12%) carry the novel mutation; 2 (5%) have a double heterozygous Mediterranean plus novel mutation; and 12 (28%) have an unidentifiable type of mutation.

Table 1. Summary of the clinical classifications with correlation of molecular characterization in 42 Saudi patients.

<table>
<thead>
<tr>
<th>G6PD variants</th>
<th>Number (%)</th>
<th>Mean of the enzyme activity</th>
<th>Clinical classifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR/RFLP</td>
<td></td>
<td>g/g Hb.</td>
<td>NJ&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mediterranean</td>
<td>16 (38.1)</td>
<td>1.78</td>
<td>±</td>
</tr>
<tr>
<td>Aureus</td>
<td>7 (16.67)</td>
<td>1.3</td>
<td>+</td>
</tr>
<tr>
<td>Novel</td>
<td>5 (11.9)</td>
<td>2.98</td>
<td>–</td>
</tr>
<tr>
<td>Med/Novel</td>
<td>2 (4.76)</td>
<td>2.8</td>
<td>±</td>
</tr>
<tr>
<td>African A-</td>
<td>NIL (0)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Unidentified</td>
<td>12 (28.57)</td>
<td>3.3</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>42 (100.00)</td>
<td>3.3</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>a</sup> = Neonatal jaundice; <sup>b</sup> = Oxidative stress induced hemolysis; <sup>c</sup> = Chronic hemolytic anemia.

None of the subjects showed the African A-<sup>202, 376</sup> mutations by PCR/RFLP and was confirmed by gene sequencing.

G6PD quantitative methods showed a significant prognostic indicator for clinical detection of the severity of G6PD deficiency. In this study, Aureus mutation had a severe clinical course among patients with the lowest G6PD red cell activity, while the unidentified and the new variant have a milder clinical manifestation with higher levels of red cell enzyme activity as seen in Table 1.

Table 2 summarized the mutation pattern of G6PD variants.

The typical patterns of G6PD Mediterranean and Aureus found in this study can be seen in Fig. 1 and 2.
Table 2. Summary of G6PD mutation by PCR and endonuclease cleavage.

<table>
<thead>
<tr>
<th>G6PD variant</th>
<th>Mutation</th>
<th>Amplified exon</th>
<th>Enzyme</th>
<th>Fragment size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Name</td>
<td>Restriction site</td>
</tr>
<tr>
<td>Mediterranean</td>
<td>563 C-&gt;T</td>
<td>6+7</td>
<td>Mbo II</td>
<td>GAAGA/CTTCT</td>
</tr>
<tr>
<td></td>
<td>376 A-&gt;G</td>
<td>5</td>
<td>Fok I</td>
<td>GGATG/CCTAC</td>
</tr>
<tr>
<td>Aureus</td>
<td>143 T-&gt;C</td>
<td>3+4</td>
<td>Mbo I</td>
<td>GATC/CTAG</td>
</tr>
<tr>
<td>New</td>
<td>C-&gt;A</td>
<td>3+4</td>
<td>Fok I</td>
<td>GGATG/CCTAC</td>
</tr>
</tbody>
</table>

Fig. 1. PCR amplified fragments showing Mediterranean mutation in G6PD deficient Saudi patients.


Discussion

High frequencies of G6PD deficiency have been reported in most countries in the region. G6PD Mediterranean is the highest documented molecular variant found (188 Ser->Phe) in this study, as well as previously reported by several investigators in Saudi Arabia \cite{10,17-19} (Table 1, Fig. 1).

G6PD Mediterranean is also reported to be the most frequently detected variants among individuals with G6PD deficiency in the Middle East and Gulf area \cite{5,6,8,9,11,12}.

G6PD Aureus was the second highest variant identified in this study in 17% of subjects (Table 1, Fig. 2). In Saudi Arabia, Niazi described G6PD Aureus in 7 (35%) of 20 children with severe G6PD deficiency and in a 16-year old boy with a history of passing dark urine after eating fava beans \cite{20}. G6PD Aureus was reported previously in Saudi Arabia \cite{2} and in neighboring countries including Kuwait \cite{11} and the United Arab Emirates \cite{8}. Nafa et al., identified a T- to C transition at nucleotide 143 converting codon 48 from ATC (ile) to ACC (thr) \cite{21}. This mutation has also been found in the native population of Algeria and Spain \cite{22}. The relative frequency of this mutation in the various populations is low and the origin of the mutation remains uncertain.
A "de novo" point mutation with transition G-> A in exon 2 and 3 amplified product giving rise to Fok1 site. This mutation occurs in the same exon where African A- mutation occurs. The occurrence of this novel mutation was discovered at a frequency of 0.110 in this study.

However, the most pertinent part of this study is the absence of G6PD African A- variant which is frequently reported in many studies\cite{15,18-20}.

None of the samples showed African A- mutation as revealed by PCR/RFLP and also was confirmed by gene sequencing carried out at Bioscientia Laboratory (Germany)\cite{23}.

An increase in sample size is needed for the verification of the absence of G6PD African A-mutation.

G6PD deficiency may cause neonatal jaundice, hemolytic anemia (favism) following consumption of broad beans, and stress oxidative hemolysis which, occasionally, can cause severe hemolytic anemia following treatment with specific drugs or participated by infection.

Preventive measures should be taken in all G6PD deficient subjects. From the clinical point of view, G6PD deficiency in western Saudi Arabia is of a milder category except for Aureus mutation. Of the 16 G6PD Mediterranean, only 2 showed acute Favism while 3 had clinical manifestation with oxidative stress.

However, G6PD Aureus showed more severe clinical manifestation. All subjects reported a history of neonatal jaundice, while 6 of 7 patients had a history of oxidative stress induced hemolysis. 3 cases had Favism and one deficient female patient showed continuous chronic hemolysis with a hemoglobin level less than 10.0 g/dl (Table 1).

The novel and the unidentified mutations were on the milder clinical side. Significant proportions of individuals with G6PD deficiency were asymptomatic.

G6PD quantitative method showed a significant correlation with clinical manifestations and had prognostic values. G6PD Aureus had severe clinical manifestations associated with lowest G6PD red cell activity followed by Mediterranean type (Table 1).
We have shown that the overall frequency of G6PD-mutated alleles among the Saudi population in this area to be 0.265. This is in concordance with previous reports\cite{16,24}.

**Conclusion**

We strongly confirm the importance of molecular characterization of G6PD variants along with electrophoretic mobility and biochemical activity to test the candidate mutation.

This study has characterized the molecular heterogeneity of G6PD variants among Saudis in the western region of Saudi Arabia, suggesting significant gene flow.

The majority of G6PD deficient individuals were asymptomatic, except for G6PD Aureus which is associated with severe clinical course.

G6PD quantitative assay methods showed significant correlations with the clinical manifestations and had prognostic values.

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**References**


المقارنة بين الصفات الجينية والحالات السريرية لمرض عوز نازعة الهيدروجين - 6 - فوسفات

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المستخلص: إنّ مرض عوز نازعة الهيدروجين - 6 - فوسفات
بسبب نقص إنزيم 6 في الدم، يؤدي إلى مضاعفات مختلفة، تبدأ
من مرحلة ما بعد الولادة، ويتخللها تكسّر بالدم، بسبب تعرض
المريض لبعض الأدوية والالتهابات. وعلى فـإن تشخيص هذا
المرض مهم لعمل الإجراءات الوقائية اللازمة. ولذلك فقد قمنا
بعمل دراسة مخبرية لتحديد مدى انتشار هذا المرض في المنطقة،
ودراسة الصفات الجينية ومقارنتها بالحالات السريرية. حيث
جّمعت حوالي ٦٠٠ عينة دم من متبرعين لا توجد بينهم صلة
قرابة. أثبتت الدراسات أنّ نسبة انتشار المرض كانت حوالي ٢٧٪
بين السعوديين، وأن هناك تواصّفاً بين الصفات الجينية الأكثر انتشاراً
في المملكة مقارنةً بالدراسات المحلية، وقد تم الكشف عن صفة جينية
جديدة لهذا المرض في منطقة جدة. كما أثبتت الدراسة أهمية
مقارنة الصفات الجينية لهذا المرض بالحالات السريرية.