Molecular Identification of the Most Prevalent Mutation of Glucose-6-Phosphate Dehydrogenase Gene in Deficient Patients in Sistan and Balochestan Province of Iran

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) in humans is an X-chromosome-linked disorder and housekeeping enzyme, vital for the survival of every cell. It catalyses the oxidation of glucose-6-phosphate to 6-phospho gluconate in the first committed step of the pentose phosphate pathway, which provides cells with pentoses and reducing power in the form of NADPH. NADPH is required to protect the cells (via glutathione and catalase) against oxidative damage. In this paper we have analyzed the G6PD gene in 92 patients with history of favaism. The extracted DNA was analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) for known G6PD mutations such as: Mediterranean, Chatham and Cosenza. The results determined that, from the total 92 samples, 74 had G6PD Mediterranean (80.42%) and 2 had G6PD Chatham (2.17%), and Cosenza mutation was not observed (17.43%). G6PD Mediterranean was the most prevalent mutation in Iran and other countries in tropical and subtropical areas. The frequency of Chatham was low in the Sistan and Balochestan province in comparison with other provinces of Iran. In this paper we also try to document the commonly known mutations in patients with G6PD deficiency, with a history of favaism.

Keywords: G6PD; Sistan and Balochestan provinces; Chatham; Cosenza; Mediterranean

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency in an x-linked disorder and housekeeping enzyme, vital for the survival of every cell [16,37,38]. G6PD is known to be highly polymorphic from the biochemical characterization of enzyme variants, and more than 380 variants have been

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found[34-36]. It catalyses the oxidation of glucose 6-phosphate to 6-phospho gluconate in the first committed step of the pentose phosphate pathway, which provides cells with pentoses and reducing power in the form of NADPH [8,10,11,14,22,37]. In red blood cells this is the only source of NADPH required to protect the cell (via glutathion and catalase) against hydrogen peroxide and other oxidative damage [7,10,30,37]. G6PD deficiency is a genetic abnormality associated with a range of clinical conditions. Some of the deficient subjects are asymptomatic, whereas others are at risk of neonatal jaundice and severe acute hemolytic anemia following the ingestion of certain drugs, during some infection and notably through eating fava beans (favidism) [1,24,36]. G6PD deficiency is one of the most common inherited disorders of mankind, more than 400 million people being affected world wide [16,36]. Epidemiological and in vitro studies indicate that this result from the selective advantage provided by G6PD deficiency during Plasmodium falciparum infection [17,25,30].

One of the most common G6PD variants is Mediterranean, causes class2 abnormality and often associated with favidism [27,28].

Favidism is a common disorder in province Sistan and Balochestan, southern-east of Iran, especially in southern towns such as: Iranshahr, Sarbaze and Chabahar. In this area Malaria is one of the common disorder which limited in patients with G6PD deficiency. Sistan and Balochestan is one of the big provinces in Iran. It covers an area of 187500 square kilometers. This province is located in southern-east of Iran. The Khurasan province and Afganistan in the north, Afganistan and Pakistan in the east, Kerman and Hormozgan provinces in the west surround the Sistan and Balochestan. On the south Sistan and Balochestan border the sea of Oman.

In this paper we try to document the commonly known mutations in patients with G6PD deficiency, with a history of favidism.

Materials and Methods

We selected 92 G6PD deficient subjects. All of the samples had a history of favidism and hospitalized at the Khatam Alanbia Hospital (in Iranshahr). The total samples 92, showed G6PD deficiency when the dye reduction test was used.

DNA Extraction

Genomic DNA was extracted from white blood cells from the G6PD deficient subjects by standard methods such as salting out and kit methods [25-28].

Amplification

The total samples 92, were amplified for C to T mutation at nt 563, which is characteristic of G6PD Mediterranean. PCR reaction was preformed using F-Med (5’…CCC CGA AGA GGA ATT CAA GGG GGT…3’) and R-Med (5’…GAA GAG TAG CCC TCG AGG GTG ACT…3’) primers. Cinagen Taq DNA polymerase and biotech apparatus amplification was carried out for 35 cycles (one cycle consist of one minute at each of following temperatures: 95°C, 60°C, 72°C. In samples by which C to T mutation was absent, we looked for G to A mutation at nt 1003, which is characteristic of G6PD Chatham. For PCR 2 reaction we used F-chat (5’…CAA GAA GCC CAT TCT CCT CCT T…3’) an R-chat(5’…TTG CCA AAG TAG AGG ACG GCT GCC AAA GT…3’) primers, (10 cycles 95°C, 30° and 70°C 1 min and 20 cycles 95°C, 65°C, 72°C each temperature 1 min) and PCR amplification under the above mentioned conditions. Remaining samples were examined for Cosenza (G to C mutation at nt 1376) using F-cos (5’…GCA GCC AGT GCC ATC AGC AAG…3’) and R-cos (5’…GGG AAG GAG GGT GGC CGT GG…3’) primers and PCR amplification under the same conditions mentioned above unless the aneling temperature was 64°C [27,28].

Digestion

Mediterranean PCR products were digested with MboII (a restriction endonuclease enzyme) for 4h at 37°C, and the digestion products were analyzed on 8% acryl amide gel. The G6PD-Med mutation at base position 563 creates an Mbo II site in exon 6 of the G6PD gene.

Chatham PCA products were digested with Bst XI for overnight at 55°C, and the digestion products were analyzed on 12% acryl amide gel.

The G6PD Chatham mutation at base position 1003 creates a BstXI site in exon 9 of the G6PD gene. Cosenza PCR products were digested with EcoS811 for 2 h at 37°C, and digestion products were analyzed on 1% agarose gel. The G6PD Cosenza mutation at base position 1376 creates an EcoS811 site in exon 11, 12 and 13 of G6PD gene [27,28,30].

Results

All of 92 subjects with G6PD deficiency were first screened by PCR-RE analysis for G6PD-Med. A 583 bp fragment encompassing exon 6 and 7 was amplified from genomic DNA by PCR with F-Med and R-Med primers.
After Mbo II digestion the normal samples showed 60 bp, 120 and 379 fragments, and in mutant samples 276 bp and 103 bp fragments were seen in place of normal fragment of 379 bp (Fig. 1). In Heterozygote samples we can see 60 bp, 120 bp, 276 bp, 103bp and 379 bp fragments (Fig. 2).

The G6PD Mediterranean genotype was observed in 74 cases of 92 deficient subjects (80.43%), see Figure 2 (a sample of this result).

The 18 remaining samples were then examined for G6PD Chatham (A 208 bp fragment). After BstXI digestion the normal samples showed two fragments, 78 bp and 130 bp, and in mutant samples, 100 bp and 30 bp fragments were seen in place of normal fragment of 130 bp (Fig. 3).

The G6PD Chatham genotype was seen only in 2 cases of 18 remaining samples (2.17%), see Figure 4 (a sample of this results).

The 16 remaining samples were then examined for G6PD-Cosenza (A 548 fragment). No one of the 16 remaining samples were shown G6PD-Cosenza mutation.

**Discussion**

Among the 92 sample with G6PD deficiency screen for the most prevalent mutations of G6PD gene (Mediterranean, Chatham and Cosenza, in province Sistan and Balochestan (Iran), 74 samples (80.43%) showed G6PD Mediterranean, 2 samples (2.17%) showed D6PD Chatham and the remaining16 samples were not showed G6PD Cosenza remaining to be analyzed by other methods such as SSCP (single strand conformational polymorphism) and DNA sequencing for other mutations.

In other provinces in Iran such as Gilan, Golesthan, Khorasan and Hormozgun (except Mazandaran 6.75%) G6PD-Cosenza was not seen [25-28, and unpublished data for Hormozgun].

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**Figure 1.** Oligonucleotide primers F-Med and R-Med amplify a 583pb fragment across exon 6 and 7 of the G6PD gene. They were shown MboII digested sites.

**Figure 2.** In spec of G6PD Mediterranean mutation on 8% acryl amide gel. Lane 1: 50 bp DNA ladder Marker; Lane 2: G6PD-Med Negative control; Lane 3: G6PD-Med Positive control; Lanes 4, 8, 7, 9, 11 and 12: The samples have G6PD Mediterranean mutation.; Lane 10: A Heterozygote sample.

**Figure 3.** Oligonucleotide primers F-chat and R-chat amplify a 208 bp fragment, exon 9 of the G6PD gene.

**Figure 4.** Inspect of D6PD Chatham mutation on 12% acrylamide gel. Lane 1: G6PD-Chatham positive control; Lane 2: 50pb DNA Ladder Marker; Lane 3: A sample with G6PD Chatham mutation; Lane 4: G6PD-Chatham Negative control. The other lanes have no shown G6PD-Chatham mutation.
Table 1. Prevalence of Mediterranean and Chatham mutations in some provinces in Iran

<table>
<thead>
<tr>
<th>Province</th>
<th>Khorasan</th>
<th>Mazandaran</th>
<th>Golestan</th>
<th>Hormozgan</th>
<th>Sistan and Balochestan</th>
<th>Gilan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of Med mutation</td>
<td>66</td>
<td>69</td>
<td>62.2</td>
<td>79.45</td>
<td>80.42</td>
<td>86.4</td>
</tr>
<tr>
<td>Percent of Chatham mutation</td>
<td>12</td>
<td>27</td>
<td>26.8</td>
<td>8.21</td>
<td>2.17</td>
<td>9.7</td>
</tr>
</tbody>
</table>

Table 2. Prevalence of Mediterranean and Chatham mutations in some countries in the world

<table>
<thead>
<tr>
<th>Country</th>
<th>Percent of Med mutation</th>
<th>Percent of Chatham mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>85</td>
<td>15-20</td>
</tr>
<tr>
<td>India</td>
<td>82</td>
<td>–</td>
</tr>
<tr>
<td>Oman</td>
<td>75</td>
<td>10</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>60</td>
<td>–</td>
</tr>
<tr>
<td>Emarat</td>
<td>55</td>
<td>–</td>
</tr>
<tr>
<td>Aljazair</td>
<td>22</td>
<td>–</td>
</tr>
<tr>
<td>Iraq</td>
<td>&gt;22</td>
<td>13</td>
</tr>
<tr>
<td>Philippine</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>Spain</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

G6PD Mediterranean is the most common mutation in many countries such as Turkey [3,4], Italy [21], Pakistan [23], India [18], Bahrain [13], Kuwait [6], Oman [12], Saudi Arabia [5,9], Iraq [2], Greece and the countries around the Mediterranean area [15,19, 20,29,34]. In Iran, Mediterranean variant shows a high prevalence, the results in some provinces are presented in Table 1. The prevalence of Mediterranean and Chatham mutations in some countries in the world are shown in Table 2 [2,9,12,18-21,24,33].

The results also showed that the G6PD Chatham have the lowest prevalence in Sistan and Balochestan in comparison with other provinces in Iran, see Table 2 [25-28, and unpublished data].

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