

Identification of Mutations in G6PD Gene in Patients in Hormozgan Province of Iran

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymatic disorder of red blood cell in human affecting more than 400 million people worldwide. G6PD is the key regulatory enzyme in the hexose monophosphate shunt (HMS) catalase the oxidation of glucose-6-phosphate (G6PD) to 6-phospho gluconolacton and the production of reducing equivalents in the form of NADPH to meet the cellular redox state and its deficiency cause hemolytic anemia , Favism and Neonatal jaundice. In this paper we have analyzed the G6PD gene in 73 patients with history of favism. The extracted DNA was analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) for known G6PD mutations including Mediterranean, Chatham and Cosenza. The results determined that, from the total 73 samples, 58 had G6PD Mediterranean (79.45%), 9 had G6PD Cosenza (12.33%) and 6 had G6PD Chatham (8.21%) while the frequency of G6PD Mediterranean in the state of Hormozgan was almost the same as in the state of Sistan and Balochestan (80.42%), the frequency of the other two mutations were significantly different in these two states. The significance of differences among these and other states by which we reported previously are discussed. G6PD Mediterranean was the most prevalent mutation in Iran and other countries in tropical and subtropical areas. In this paper we also try to document the commonly known mutations in patients with G6PD deficiency, with a history of favism.

Keywords: G6PD; Hormozgan province; Chatham; Cosenza; Mediterranean

Introduction

Glucose-6-phosphate dehydrogenase G6PD deficiency is one of the most common inherited disorders of

mankind, more than 400 million people being affected worldwide. Inheritance of (G6PD) deficiency is sex-linked [1-6]. G6PD gene is located on the Xq28 region of X chromosome. It contains 13 exons and 12 introns

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and is 18.5 kb in length. The active enzyme is composed variably of two or four identical 515 amino acid subunits; each monomer has a molecular weight of 59 kDa. Glucose-6-phosphate dehydrogenase (G6PD) enzyme is a highly polymorphic encoded by X-linked (Xq28) human gene [7-9] and expressed in several tissues in body. This enzyme catalyzes the first step in pentose phosphate pathway that converts glucose-6-phosphate to 6-phosphogluconate with production of NADPH₂ [7,8]. NADPH₂ is an important cofactor which is required for various redox reactions and for protecting the cell from oxidative stress [7,9-11].

G6PD catalyses the first step in the pentose phosphate pathway (PPP). This pathway involves the conversion of glucose into pentose sugars, necessary for a variety of biosynthetic reactions [2,12-16].

Although G6PD is a house keeping enzyme that is expressed in all tissues, clinical manifestations of its deficiency are seen almost exclusively in red cells. The most common manifestations are neonatal Jaundice and acute haemolytic anemia related to drugs infection or the ingestion of fava beans [17-19].

“Chatham” and “Cosenza” mutations are the most common variants in coastal provinces after Mediterranean mutation, respectively [21-23]. This has been already confirmed by DNA analysis on G6PD deficiency samples in Mazandaran state (North of Iran) [20,21]. The present study firstly looks into the Mediterranean mutation at nt 563 (C→T) in all of DNA samples while the remaining samples not exhibiting Mediterranean mutations were analyzed for Chatham mutation at nt 1003 (G→A). Those not showing both aforementioned mutations were analyzed for Cosenza mutation at nt 1376 (G→C).

Material and Methods

Seventy three peripheral blood samples (3-5 ml in 300 µEDTA 0.5 M) were collected from unrelated male and female children with Jaundice and hemolytic anemia from Bandarabas and Queshm Island, in Hormozgan State. In order to confirm G6PD deficiency in these blood samples, a fluorescent spot test was used (Beutler test).

Genomic DNA was extracted from peripheral blood leukocytes by salting out and DNA extraction kit. The DNA region from the G6PD gene encompassing each point mutation was selectively amplified by PCR using specific oligonucleotide primers, followed by digestion with restriction enzyme. Digestion products were analyzed on an acrylamid gel [22-25]. All DNA samples were screened for the C→T mutation at nt 563, which is characteristic of G6PD Mediterranean, using F-Med (5'-

CCC CGA AGA GGA ATT CAA GGG GGT-3'), R-Med (5'-GAA GAG TAG CCC TCG AGG GTG ACT-3') primers and PCR amplification followed by digestion by MboII restriction endo-nuclease. After testing all samples, the same samples which did not show Mediterranean mutation, 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 CTC CCT T-3') an R-chat(5'-TTC TCC ACA TAG AGG 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 mutation (G to C mutation at nt 1376) using F-cos (5'-GCA GCC AGT GGC 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 annealing temperature was 64°C [23,24].

Results and Discussion

All 73 samples were diagnosed as G6PD deficient. At first step, DNA samples were analyzed for Mediterranean mutation (Ser 188 Phe). Mediterranean mutation creates a new restriction site for MboII in exon 6 then five fragments (276bp, 120bp, 103bp, 60bp and 24bp) appear on the acryl amide gel (Fig. 1).

PCR-RFLP demonstrated that, 58 out of 73 DNA samples (79.45%) had Mediterranean mutation (Fig. 1).

Molecular characterization revealed that in Southeast Asia which is far from the Mediterranean Sea, G6PD Mediterranean is less common and other variants are prevalent [5,13,18,19].

Chatham mutation (Ala 335 Thr) is another variant that creates a new site for BstXI in exon 9 and products three fragments (100bp, 78bp, 30bp) in contrast with 130bp, 78bp fragments in normal (Fig. 2). The results showed that 8.21% of the samples had Chatham mutation.

Cosenza mutation (G1367C, Arg 459 Pro) is another variant in G6PD gene in exon 11-13. The size of this fragment after amplification is 548 bp length, this PCR product appears as two fragments with 232bp and 316bp length after digestion by Bsu36I on agarose gel in deficient patients (Fig. 3). Remaining nine samples were analyzed for Cosenza mutation. The result showed that 12.33% of the samples had Cosenza mutation. In other provinces in Iran such as Gillan, Golestan, Khorasan (except Mazandaran 6.75%) G6PD-Cosenza was not seen [20-25].

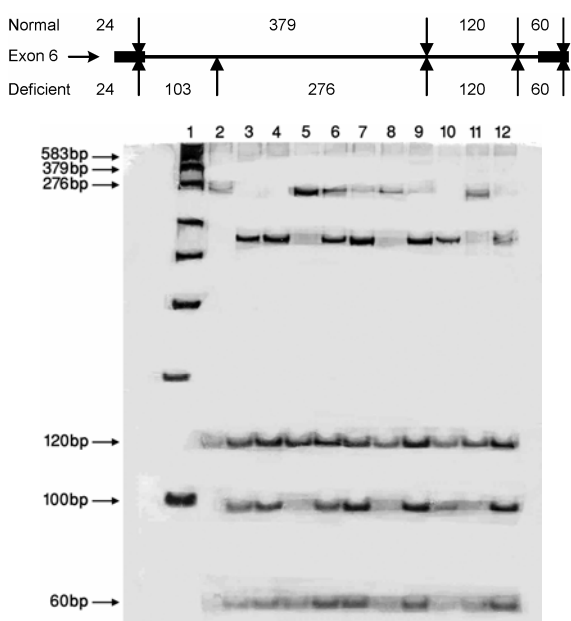


Figure 1. Restriction sites for MboII in Mediterranean G6PD (upper panel) and restriction digestion analysis of PCR products related to G6PD Mediterranean mutation with MboII enzyme on 12% acryl amide gel from left to right (lower panel). Arrows show restriction sites in normal and deficient patients. Lane 1: 50 bp DNA ladder marker; Lane 2: PCR product; Lane 3: G6PD-Med Positive control; Lane 5: G6PD-Med negative control; Lanes 4, 7, 9, 10, 12: G6PD Mediterranean mutation; Lane 6: A heterozygote sample; Lanes 8, 11: normal samples.

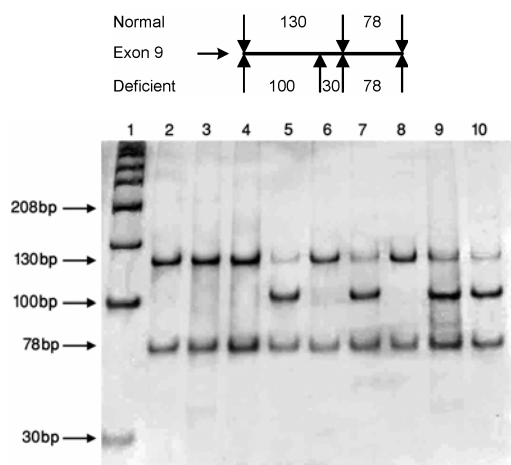


Figure 2. Restriction sites for BstXI in Chatham G6PD (upper panel) and restriction digestion analysis of PCR products related to G6PD Chatham mutation with BstXI enzyme on 12% Acryl amide gel (PAGE) from right to left (lower panel). Arrows show restriction sites in normal and deficient DNAs. Lane 1: Molecular weight marker (50 bp); Lane 2: G6PD-Chatham negative control, Lane 3: G6PD-Chatham positive control, Lanes 4, 6, 8: normal samples, Lanes 5, 7, 9, 10: G6PD Chatham mutation.

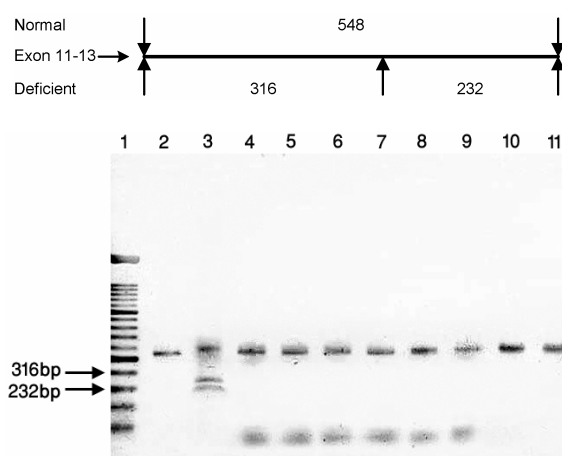


Figure 3. Restriction sites for Bsu36I in Cosenza G6PD (upper panel) and restriction digestion analysis of PCR products related to G6PD Cosenza mutation with Bsu36I enzyme from left to right (lower panel). Arrows show restriction sites in normal and deficient patients. Lane 1: molecular weight marker (100 bp); Lane 2: G6PD-Cosenza negative control; Lane 3: G6PD-Cosenza positive control, Lanes 4-11: normal samples.

The results compared to Mazandaran indicate that Mediterranean mutation and Chatham mutation are dominant variants in these areas (18-25). It is probable that a new mutation or ancestral origin caused Cosenza mutation in Hormozgan and Mazandaran.

In Gillan, Mazandaran and Khorasan provinces the prevalence of Mediterranean and Chatham mutations are nearly the same. For example, in Khorasan province 16% of patients had Mediterranean mutation, 12% had Chatham mutation and Cosenza mutation was not detected. In contrary to the three Northern provinces where most of the patients suffer from one of the three mentioned mutations, in Khorasan province 22% of patients did not show the three investigated mutations, therefore search for other mutations is required [19-25]. Comparison of present results with our previous reports indicates a great difference between the frequencies of Mediterranean mutations. This may suggest the different origin of ethnic groups, more detailed population survey may clarify this possibility.

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