

Molecular Characterization of Cosenza Mutation among Patients with Glucose-6-Phosphate Dehydrogenase Deficiency in Khuzestan Province, Southwest Iran

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common hereditary enzymatic disorders in human, increases the vulnerability of erythrocytes to oxidative stress. It is also characterized by remarkable molecular and biochemical heterogeneity. According to previous investigations, G6PD Cosenza (G1376C) is a common G6PD mutation in some parts of Iran. Therefore in the present study we have characterized Cosenza mutation among G6PD deficient individuals in Khuzestan province. In order to identify G6PD Cosenza, we analyzed the G6PD gene in 64 samples out of 231 deficient individuals who had not G6PD Mediterranean mutation, using PCR- restriction fragment length polymorphism (RFLP) method. G6PD Cosenza mutation was found in 6 males of 231 samples, resulting in the relative rate of 2.6% and allele frequency of 0.023 among Khuzestanian G6PD deficient subjects. A comparison of these results with previous findings in some parts of Iran suggests that G6PD Cosenza is a common mutation in Khuzestanian G6PD deficient individuals.

Iran J Med Sci 2011; 36(1): 40-44.

Keywords • G6PD deficiency • Cosenza mutation • molecular characterization

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common hereditary hemolytic disorders in human, affecting around 400 million people worldwide.¹ The majority of deficient individuals live in tropical and subtropical regions where malaria is still endemic or has been eradicated only recently. In fact, G6PD deficient individuals have been protected from severe forms of malaria in these areas.¹⁻³ Although most of individuals with G6PD deficiency are usually asymptomatic, deficient subjects may suffer from neonatal jaundice, severe non-spherocytic hemolytic anemia or acute hemolytic anemia induced by infection, ingestion of fava beans or some drugs.⁴ The severity of these serious consequences of G6PD varies based on different gene mutations which cause different levels of residual enzyme activity.⁴ Therefore to prevent the above complications, it is important to investigate their molecular bases.

Mutations in G6PD gene are responsible for G6PD deficiency disorders. This gene is located on the Xq28 region with

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Received: 9 February 2010
Revised: 15 May 2010
Accepted: 27 June 2010

a length of 18.5 Kb, which contains 13 exons and 12 introns.¹ Since G6PD deficiency is an X-linked recessive disorder, it is more frequent in males than females.⁴ Glucose-6-phosphate dehydrogenase enzyme, the product of G6PD gene, catalyzes the first step of the pentose phosphate pathway (PPP), which provides cells with pentoses and reduction power in the form of nicotinamide adenine dinucleotide phosphate (NADPH). Nicotinamide adenine dinucleotide phosphate cofactor is required for various redox reactions, and protects cells against oxidative stress via glutathione and catalase. Glucose-6-phosphate dehydrogenase is the only source of NADPH in erythrocytes, so any oxidative stress in G6PD deficient red blood cells may result in hemolytic anemia.^{1,4-5}

Approximately 140 mutations and 400 biochemical variants have been reported for this enzyme till now. Therefore G6PD deficiency has a remarkable molecular and biochemical heterogeneity.^{1,6}

The G6PD Cosenza mutation was described for the first time in the province of Calabria, southern Italy. This mutation belongs to the group of severe G6PD deficiencies often associated with hemolysis. Previous investigations have revealed that G6PD Cosenza (G1376C), which is a common G6PD mutation in some parts of Iran, has a variable frequency ranging from 0% to 12.33%.⁷⁻¹⁵ Given the variability and high frequency of G6PD Cosenza in Iran, in the present study we have characterized G6PD Cosenza among deficient individuals in the province of Khuzestan, which is located in the southwest of the country bordering Iraq and the Persian Gulf with a population of about five million mostly Iranian Arabs.

Patients and Methods

Screening study was performed on 1064 randomly selected blood samples from volunteer male donors referring to Ahvaz Blood Transfusion Center from February to April 2008. Screening test for the diagnosis of G6PD deficiency was done by fluorescent spot method (Sigma). Eighty-one (7.6%) of them were found to be severely G6PD deficient.¹⁶ However blood sample were taken only from 79 deficient male blood donors for next studies.

In order to identify G6PD molecular characterization, 231 G6PD deficient blood samples were collected from 79 screened male blood donors and 152 individuals (116 males and 36 females) who were referred to hospitals of Khuzestan province with a history of favism,

acute anemia or neonatal jaundice. G6PD deficiency was diagnosed based on the fluorescent spot test in all individuals.¹⁷ All samples were collected into 0.5 M EDTA (Becton Dickinson, Plymouth, UK) solution, carried on ice and kept at -70 °C for DNA extraction. Because G6PD Mediterranean (C563T) is reported as the most common mutation in Middle East and some provinces of Iran, at first we analyzed all samples for this mutation.¹⁶ Finally 64 (55 males and 9 females) out of 231 samples were recognized without Mediterranean mutation, which were then analyzed to identify Cosenza mutation. Genomic DNA was extracted from leukocytes by using "PicoPure" DNA extraction kit from Molecular Devices (San Diego, CA). Cosenza mutation site is located in exon 12 of G6PD gene. For detection of the Cosenza mutation, exon 11-13 of G6PD gene was selectively amplified by PCR method 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.¹⁴ Polymerase chain reaction (PCR) assay was performed in final volume of 25 µl. PCR reaction mix contained 10X PCR buffer, 10 mM of each deoxynucleotide triphosphate, 25 pmol of each primer, 0.5 µg genomic DNA, 2 U/ml of Taq DNA polymerase and 50 mM MgCl₂. The PCR reaction was carried out for 30 cycles as follows: 10 cycles (94 °C for 30 seconds, 68 °C for 1 min and 72 °C for 30 seconds) and 20 cycles (95 °C, 65 °C, 72 °C each temperature for 1 min).

In order to certify the fidelity of PCR, amplified segments were run on 1.5% agarose gel (figure 1). Since the Cosenza mutation creates an Eco81I recognition site (figure 2), this endonuclease was used to perform Restriction fragment length polymorphism (RFLP) analysis. Cosenza PCR products were digested by Eco81I enzyme (Fermentas GmbH, Germany) at 37 °C, overnight. The digested fragments were tested on 2% agarose gel.

Results

Among the 231 G6PD deficient individuals (a total of 267 alleles), 195 (84.1%) were males and 36 were females. Only 64 samples (55 males and 9 females) out of 231 deficient subjects did not have G6PD Mediterranean. They were analyzed to characterize G6PD Cosenza Mutation, using PCR-RFLP method. Cosenza PCR product was a 548 bp fragment, which appeared as two fragments with 232 bp and 316 bp lengths after digestion by Eco81I on 2% agarose gel in mutant subjects (figure 3).

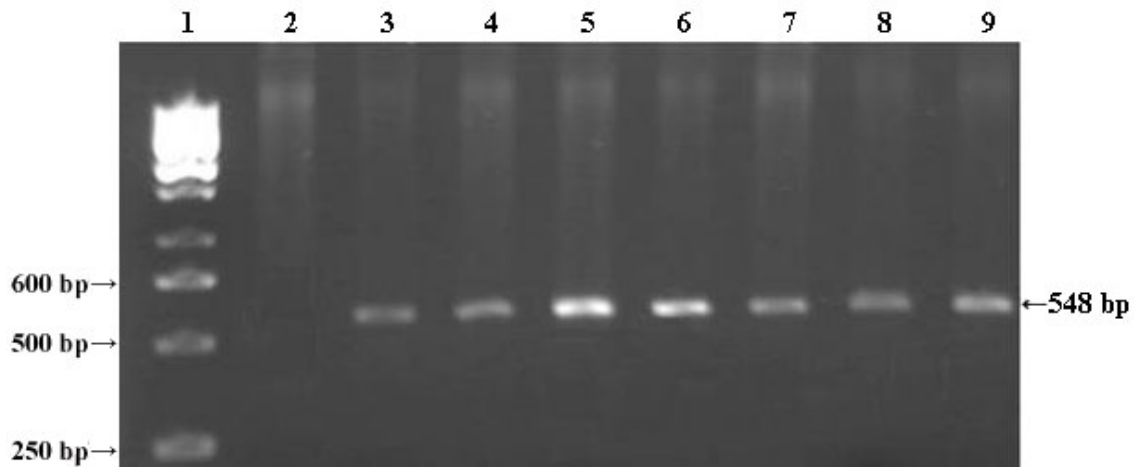


Figure 1: Polymerase chain reaction (PCR) products related to glucose-6-phosphate dehydrogenase Cosenza mutation on 1.5 % agarose gel. Lane 1: Size Marker 1 Kbp, Lane 2: negative control, Lanes 3, 4, 5, 6, 7, 8 and 9: Cosenza PCR products with 548 bp length

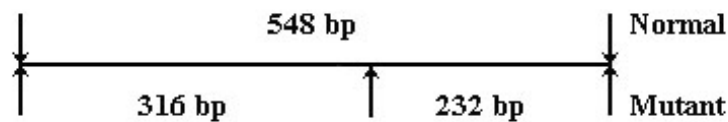


Figure 2: Oligonucleotide primers F-cos and R-cos amplify a 548bp fragment across exon 11 and 13 of the glucose-6-phosphate dehydrogenase gene that after digestion by Eco811 appeared as two fragments with 232 bp and 316 bp

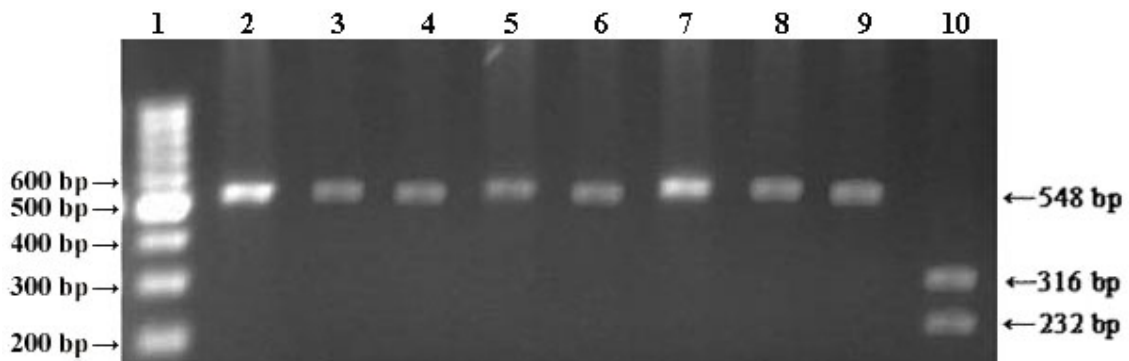


Figure 3: Restriction digestion analysis of polymerase chain reaction (PCR) products related to glucose-6-phosphate dehydrogenase Cosenza mutation with Eco811 enzyme on 2% agarose gel. Lane 1: Size Marker 100 bp, Lane 2: PCR product, Lanes 3, 4, 5, 6, 7, 8 and 9: samples without Cosenza mutation, Lane 10: hemizygote male with Cosenza mutation

The result showed that 6 males out of 231 samples had the Cosenza mutation. Therefore the Cosenza mutation relative rate and allele frequency in Khuzestanian deficient subjects are 2.6% and 0.023, respectively. Fifty eight samples did not have Mediterranean and Cosenza mutations.

Discussion

G6PD deficiency is a very prevalent disorder in Africa, Southern Europe, South East Asia, Oceania and Middle East especially neighboring

Persian Gulf countries including Iran,¹⁸ Kuwait,¹⁸ United Arab Emirates,¹⁹ Iraq,²⁰ Bahrain,²¹ and Oman,²² with a prevalence of 11.55, 5.51, 8.7, 6.1, 26.45 and 26-29%, respectively.

Khuzestan province is located in the south west Iran, bordering Iraq and the Persian Gulf. The prevalence of G6PD deficiency among male khuzestanian blood donors was reported to be 7.6%.¹⁶ This prevalence is obviously higher than the 6 % reported earlier for male blood donors in Fars province of southern Iran.²³ However, one other recent study showed that the overall prevalence of G6PD

deficiency among male and female children in the city of Shiraz (Fars province) was 11%.²⁴

Since G6PD deficiency is so frequent in Khuzestan, it is very important and desirable to fully identify the molecular basis of this disorder. Our Previous study revealed that G6PD Mediterranean (C563T, Ser188Phe) was the most common mutation in Khuzestan,¹⁶ like the other provinces of Iran.⁷⁻¹⁵ To pursue our investigation, we did analyze Cosenza mutation among G6PD deficient individuals in the present study.

Cosenza mutation, which was initially described in the north of Calabria, Southern Italy, by frequency of 1.9%, is caused by 1376 G → C (459 Arg → Pro) substitution. Its phenotype is associated with a severe enzyme deficiency (enzyme activity less than 10 %).²⁵ Thus far, G6PD Cosenza has been identified in some parts of Italy,^{26,27} Dalmatian region of south Croatia,²⁸ and some parts of Iran including Mazandaran,⁷ Kermanshah,^{11,12} and Hormozgan.¹⁴ However, it hasn't been found in Gilan,⁸ Golestan,⁹ Sistan and Balochestan,¹⁰ Khorasan,¹³ and Fars provinces.¹⁵

The highest incidence (37.5%) of G6PD Cosenza has been reported in Dalmatian region of south Croatia. It not known whether this distribution of G6PD Cosenza is the result of a common ancestry or an independent origin in Mediterranean basin and Middle East.²⁸

We have detected Cosenza mutation in 6 of 231 samples, resulting in a prevalence of 2.6% and allele frequency of 0.023. Therefore the incidence of Cosenza mutation in Khuzestan is higher than that in Kermanshah, but lower than those in Hormozgan and Mazandaran. Kermanshah, Hormozgan and Mazandaran are provinces that are respectively located in the western, southern and northern parts of Iran (table 1). The great difference between the incidences of G6PD Cosenza in some parts of Iran could be explained by immigration issues, which might have induced a flow of gene from Persian Gulf countries. Or alternatively, it might be due to the origin of ethnic groups, which may be clarified

Table 1: The distribution of Cosenza mutations in some provinces of Iran

Province	Cosenza (%)	Reference
Mazandaran	6.75	7
Gilan	0	8
Golestan	0	9
Khorasan	0	13
Sistan and Balochestan	0	10
Kermanshah	1.5	11
Fars	0	15
Hormozgan	12.33	14
Khuzestan	2.6	Present study

by studying additional markers in populations. The variation in the prevalence on Cosenza mutation may be partially comparable to that of thalassemia in the south of the country.

We also studied the association of Mediterranean and Cosenza mutation with (1311C→T) haplotype. The haplotype analysis revealed that unlike Mediterranean G6PD, Cosenza mutation was associated with (1311C) haplotype. Fifty eight samples, which have not Mediterranean and Cosenza mutations, are kept for identification of other G6PD mutations.

Conclusion

The findings of the present study indicate that G6PD Cosenza is a common mutation in Khuzestanian G6PD deficient individuals.

Acknowledgment

This study was financially supported by a grant (no. 3587) from the Shahid Chamran University of Ahvaz, Iran.

Conflict of Interest: None declared

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