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Short Communication

Prevalence and Molecular Identification of Mediterranean Glucose-6-Phosphate Dehydrogenase Deficiency in Khuzestan Province, Iran

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Abstract

Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most frequent genetic enzymatic disorder in human, which is inherited as an X-linked gene. It encodes a housekeeping enzyme, which is vital for cell survival. According to previous investigations, Mediterranean mutation (C563T) of *g6pd* gene is the most prevalent mutation in some provinces of Iran and neighboring countries. We aimed to study the Mediterranean mutation of g6pd gene in Khuzestan province of Iran.

Methods: A total of 1064 randomly selected male blood samples were selected in Ahvaz, Khuzestan Province, in 2008 and screened for G6PD deficiency using fluorescent spot test method. In order to determine the frequency of G6PD Mediterranean variant, 144 G6PD deficient samples were analyzed by PCR-RFLP method.

Results: Eighty-one out of 1064 random selected screened samples were G6PD deficient, so a 7.6% frequency was obtained for G6PD deficiency. In addition, 105 out of 144 collected deficient samples had Mediterranean mutation that resulted in a 72.91% allel frequency.

Conclusion: Corresponding to other investigations in Middle East countries and some provinces of Iran, we found that the Mediterranean mutation of *g6pd* gene was the most prevalent variant and G6PD deficiency occurred in a high frequency.

Keywords: *G6PD deficiency, Mediterranean mutation, Iran*

Introduction

Glucose 6-phosphate dehydrogenase deficiency (G6PD-D) is one of the most common inherited disorders in human so that more than 400 million people are affected by this deficiency worldwide (1). G6PD-D is caused by defects in *g6pd* gene and results in a number of different hemolytic anemias due to exposing to some oxidative agents (2). G6PD catalyses the first step of the pentose phosphate pathway and provides cells with required NADPH for biosynthesis and protecting them against oxidative stress, therefore is vital for cell survival (3). G6PD is the only NADPH generating enzyme of the erythrocytes and the most important function of this enzyme is detoxification of oxidative agents, so erythrocytes are much more sensitive to the lack or deficiency of this enzyme rather than other tissues (4).

G6pd gene located at Xq28, consists of 13 exons (exon 1 does not code any protein) and encodes a housekeeping enzyme expressing in all body tissues (5). Since G6PD-D is an X-linked recessive disorder therefore it is more frequent in males than females. The distribution of G6PD-D is highly correlated with the distribution of current or past malaria endemicity, because, G6PD-D confers a reduced risk of infection by the *Plasmodium* parasites (6).

This enzyme is known as one of the most polymorphic enzymes in human with respect to its biochemical and genetic characterizations, so about 380 different variants have been identified so far (7, 8). Approximately all *g6pd* mutations occur in coding region and mainly result in single amino acid substitutions (5). No large deletion or frameshift mutations have been reported in this gene till now.

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This indicates that a total lack of G6PD is incompatible with life. The G6PD Mediterranean is among the most important and prevalent variants of the enzyme, that is a result of C to T transition at nucleotide 563 (exon 6), causing acute hemolytic anemia (3).

Some clinical manifestations of G6PD-D are: chronic non-spherocytic hemolytic anemia, neonatal jaundice and acute hemolytic anemia related to infection, ingestion of fava beans (Favism) or some chemical agents or medicine (2).

Since most deficient variants of the G6PD enzyme cause little health hazards, therefore, the donor's bloods are not routinely screened for G6PD-D in blood banks. Considering potential hazards of severe hemolysis, the present study was conducted to determine the prevalence of G6PD-D in male donors referred to Ahvaz Blood Transfusion Center. Furthermore, the Mediterranean mutation was previously reported as the most frequent variant of different provinces of Iran (9-14), so in the present study we used PCR-RFLP method to analyze G6PD-D samples and to characterize the incidence rate of Mediterranean mutation and establish the mutations underlying G6PD-D in Khuzestan Population.

Materials and Methods

Screening study was performed on 1064 randomly selected blood samples from volunteer male donors referred to Ahvaz Blood Transfusion Center from February until April 2008. Also In order to determine the frequency of Mediterranean mutation, 144 deficient male blood samples including 81 samples selected by screening test from blood transfusion center and 63 samples from the hospitals of Khuzestan Province, were analyzed. Peripheral bloods were collected with written informed consent. All samples were collected in 0.5 M EDTA (Becton Dickinson, Plymouth, UK) solution and kept at -70 °C. All deficient blood donors from hospitals had a history of favism, neonatal jaundice or hemolytic anemia.

G6PD deficiency diagnostic test for screening was done by fluorescent spot method (Sigma). This semi-quantitative assay is reliable and highly sensitive which classifies a sample simply as "normal" or "deficient" (15).

For further studies, genomic DNA was extracted from white blood cells of the samples by standard method of DNA extraction kit (High Pure) from Roche Molecular Biochemicals, Switzerland. DNAs of total samples were amplified for C to T mutation at nucleotide 563, which is characteristic of G6PD Mediterranean variant. PCR reaction was performed using FMed (5́- AGC TCT GAT CCT CAC TCC CC-3́) and RMed (5́-GGC CAG GTG AGG CTC CTG AGT A-3́) primers to amplify the exon 6 and flanking regions, involving Mediterranean mutation. The PCR reaction was carried out for 35 repeats (each repeat consisted of 30 seconds with the following temperatures: denaturation: 94 °C, annealing: 64 °C and extension: 72 °C) using 0.5 unit of Taq DNA polymerase in a final volume of 25 µl. Then the PCR products were run on 1.5% agarose gel to verify the fidelity of PCR reaction.

As the Mediterranean mutation creates a new Mbo II enzyme (Fermentas GmbH, Germany) recognition site, so PCR products were digested with one unit of this enzyme for 16 h at 37 °C and were run on 2% agarose gel to detect the mutation.

Results

Using fluorescent spot method, G6PD-D was found in 81 samples out of 1064 screened blood samples resulting in a 7.6% frequency. Subsequently, all of 144 G6PD deficient males (63 patients and 81 out of 1064 donors) were analyzed by PCR-RFLP method to characterize G6PD Mediterranean. A 286 bp fragment involving exon 6 was amplified from genomic DNA by PCR with FMed and RMed primers. Then, products were digested with Mbo II enzyme to detect the Mediterranean mutation. The DNA with this mutation will be cleaved at the new enzyme recognition site and two fragments will be produced. After Mbo II digestion the normal samples showed 286 bp fragments (uncleaved PCR product) but mutant samples identified by 133 bp

and 153 bp fragments were observed instead of one 286 bp fragment.
Therefore 2 bands for hemizygotes and 1 for normal subjects appeared on agarose gel (Fig. 1). Consequently, the total frequency of the G6PD Mediterranean allele among male individuals was 72.91% (105 out of 144 samples) and accordingly, frequency of other mutations was 27.08%.

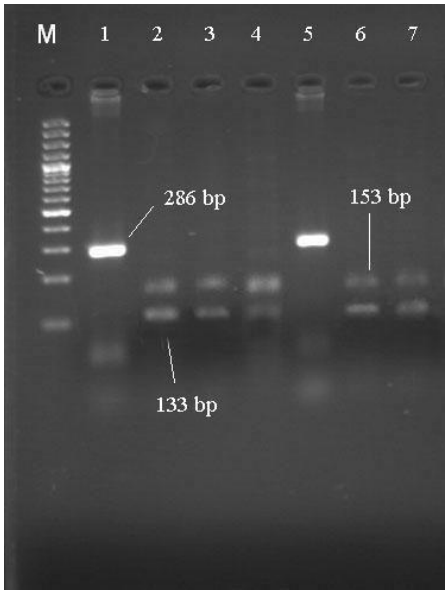

Fig. 1: RFLP results on 2% agarose gel: Lane 1& 5: subjects without Mediterranean mutation. Lanes 2,3,4,6 & 7: hemizygote subjects with Mediterranean mutation. M: 100 bp size marker

Discussion

More than 130 different mutations have been described for the *g6pd* gene (9). The frequency of G6PD-D in the Middle East varies widely, ranging from 1% for Egyptian to 11.5% for some ethnical groups of Iran (9). Agreeable to the report of WHO, the overall incidence of G6PD-D among the Iranian population was 10%-14.9% (16). Although screening of the donor's blood is not routinely performed, because there are no deleterious consequences in recipients of G6PD deficient blood, but transfusion of G6PD deficient red cells especially to premature infants sometimes has been associated with hemolysis and severe hyper-bilirubinemia, requiring, exchange transfusion (17). The malaria parasite has been prevalent and endemic in some regions of Iran in the past or currently. On the other hand some *g6pd* variants such as G6PD Mediterranean decrease infection risk of malaria, thus this disorder has a high incidence rate in these regions. Regarding high prevalence of G6PD-D in Iran and as we revealed in Khuzestan Province (7.6%), the prevalence of this defect should be considered noteworthy.
G6PD Mediterranean is the most prevalent G6PD deficient variant in many Middle East and Iran neighboring countries such as Turkey (18), Pakistan (19), India (20), Bahrain (21), Kuwait (22), Oman (23), Iraq (24), Saudi Arabia (25) and the countries around the Mediterranean Sea (3). According to previous studies of *g6pd* gene in different provinces of Iran G6PD Mediterranean has the most frequency among the other variants.
The high incidence of G6PD Mediterranean in Iran, which is the most severe variant of G6PD deficiency, encouraged us to investigate the prevalence of G6PD Mediterranean. In conclusion, our study shows that the incidence of G6PD Mediterranean among Khuzestan G6PD-D population is 72.91%, which is compatible with the previously performed studies in Iran, and we found that in this province, like the other provinces of Iran, the most prevalent mutation of *g6pd* gene was Mediterranean mutation. In comparison with the other provinces of Iran, frequency of G6PD Mediterranean mutation in Khuzestan is higher than Khorasan (66%), Mazandaran (69%) and Golestan (69%) but lower than Sistan and Baluchestan (80.42%), Gilan (86.4%) and Kermanshah (91.2%) (9-14).
Conclusively we found that the Mediterranean mutation of *g6pd* gene was the most prevalent variant and G6PD deficiency occurred in a high frequency in this province.

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