

## The Prevalence of Mediterranean Mutation of Glucose-6-Phosphate Dehydrogenase (G6PD) in Zahedan

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### Abstract

**Background:** glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common genetic defects in the world, so that more than 400 million people in worldwide are affected with it, but its prevalence varies from 1-65% in different populations. Clinical manifestation of this defect is acute hemolytic anemia, neonatal hyperbilirubinemia and chronic nonspherocytic haemolytic anaemia. So far, almost 140 mutations have been identified in the gene of G6PD enzyme. Mediterranean is the most common mutation. The purpose of this study is to determine the prevalence of G6PD Mediterranean mutation in the deficient people in the city of Zahedan.

**Materials and Methods:** In this descriptive cross-sectional study, blood samples of 1440 male individuals, who were referred to Zahedan Reference Laboratory for premarital testing, were examined for G6PD deficiency using fluorescent spot test. Genomic DNA from blood of people with G6PD deficiency was extracted by DNA extraction kit. Mediterranean mutation was identified using PCR-RFLP method.

**Results:** 101 out of 1440 subjects had G6PD deficiency. Therefore prevalence of G6PD deficiency in Zahedan city was estimated about 7%. Mediterranean mutation frequency in patients with defect of G6PD was estimated 84.2% (85 out of 101 patients) and 15.8% (16 out of 101 patients) did not have mutation Mediterranean. The frequency of G6PD deficiency was expressed as a percentage of total cases and Mediterranean mutation prevalence was expressed as a percentage of total impaired individuals.

**Conclusion:** The result of this study showed that the frequency of G6PD deficiency in Zahedan city is lower than other cities of sistan and baluchestan province. Dominant mutation in present study was Mediterranean type and its frequency was very similar with prevalence of this mutation in other parts of Iran.

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### Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is the enzyme that transfers hydrogen from glucose-6-phosphate (G6P) to nicotinamide adenine dinucleotide phosphate (NADP) in pentose phosphate metabolic pathway. During this reaction NADP is reduced to NADPH<sub>2</sub>. NADPH<sub>2</sub> protects cells against oxidative damage by maintaining reduced glutathione (GSH) [1]. In the red blood cells, NADPH<sub>2</sub> is only produced through pentose phosphate pathway, while in other cells; this substance is produced in other pathways [2]. Thus, red blood cells are more sensitive to oxidants than other cells [3]. Therefore G6PD deficiency of red blood cells leads to

neonatal hyperbilirubinemia, acute hemolytic anemia, and chronic nonspherocytic haemolytic anaemia [4]. Many variants of G6PD have been identified based on activity and other biochemical characteristics of enzyme. In 1967, Committee of the World Health Organization (WHO) divided different types this enzyme into five groups based on enzyme activity [5]. The peoples whose enzyme are from class I and II, i.e. who their enzyme activity is less than 10% normal, in contact with oxidants will be affected by hemolysis. Different variants of G6PD are result of mutation in gene of this enzyme. So far about 140 mutations have been identified in G6PD gene. Most

of these mutations are caused by the displacement of one or two nucleotides and are missense mutation; it means that they cause displacement of an amino acid with another one. Some mutations have been identified which are produced by displacement of three nucleotides or elimination of 3 or 24 nucleotides [6]. The mutations so far identified, influence the entire gene and all exons except exon 3 and 13 [7]. The most common deficient variant of this enzyme is Mediterranean variant (G6PD Mediterranean) which was first reported in southern Italy and then in other countries around the Mediterranean Sea. But now it has been revealed that this variant exists in most parts of the world, including Saudi Arabia, Bahrain, Oman, Iraq, Turkey, Pakistan, Egypt, Italy, Spain, Greece and Iran as well [8]. Mediterranean variant is genetically heterogeneous; it means that several different mutations cause this variant. One of its different types is caused by the mutation that converts nucleotide 563C of G6PD gene to T, as a result of which at position 188 of the polypeptide chain of enzyme, phenylalanine substituted with serine amino acid. With this replacement, the enzyme activity will be reduced to less than 10 percent of normal [9]. Nucleotide substitution in G6PD gene creates some sites for cut restriction enzymes or removes the sites of effect of enzymes in normal gene. These cutting sites are used to identify G6PD mutations through PCR-RFLP method. This study aims to determine the prevalence of G6PD Mediterranean mutation in people with deficiency of this enzyme in the city of Zahedan. G6PD mutations have been studied in different regions of Iran and the most common has been reported to be Mediterranean mutation. Since there was no comprehensive report on the type of G6PD mutations in Sistan-Baluchistan province, this study was designed and conducted.

## Materials and Methods

This descriptive cross-sectional study was conducted on 1440 men who referred to the reference laboratory for before marriage tests from February 2007 to the end of March 2008 and from June 2010 to the end of July 2010. Blood samples were kept in the refrigerator and were transferred on ice to the Biochemistry laboratory of Medical School to review the activity of G6PD.

**Qualitative and quantitative measurement of the activity of G6PD:** Quantitative measurement of enzyme activity was performed using fluorescent spot test. The result was recorded as normal (strong fluorescence) and deficient (low fluorescence or no fluorescence). Positive and negative controls were tested each day along with samples to verify the method. The enzyme activity was also measured quantitatively for the samples in which qualitative activity of the enzyme was deficient. Qualitative and quantitative measurements were performed using the kit of Shim enzyme Company, Iran.

**Extraction of genomic DNA:** The blood samples of people, whose G6PD deficiency was qualitatively and quantitatively confirmed, were kept in  $-20^{\circ}\text{C}$  until DNA extraction. DNA extraction from blood was performed using the kit of Roche company, Germany. The extracted DNA was electrophoresed on 0/8% agarose gel to review its quality.

**Amplification of nucleotide sequence containing Mediterranean mutation (exon 6 and 7):** Polymerase chain reaction (PCR) was performed using primer 1 (5'-CCCCGAAGAGGGGTTCAA GGGGCT-3') and primer 2 (5'-GAAGAGTAGCCCTGCAGGGTGACT-3') in thermal cycler of Applo, made in America. PCR conditions were as follows: Initial denaturation of DNA at  $94^{\circ}\text{C}$  for 5 min, 32 cycles each consisting of three steps of denaturation at  $94^{\circ}\text{C}$  for 1 min, Annealing at  $60^{\circ}\text{C}$  for 1 min, extension at  $72^{\circ}\text{C}$  for 1 minute and final extension at  $72^{\circ}\text{C}$  for 10 minutes. PCR products were electrophoresed on 1.5% agarose gel at presence of DNA size marker.

**Digestion of PCR products of Mediterranean site (RFLP):** PCR products of Mediterranean site were cut using MboII enzyme (Fermentas Co.) according to the manufacturer's protocol and digested products were electrophoresed on 3% agarose gel at presence of DNA size marker and negative control samples.

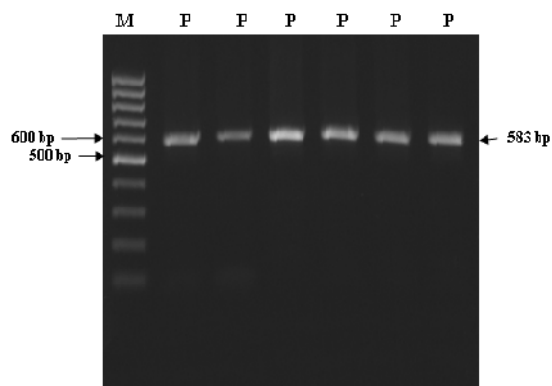
## Results

Evaluated individuals in this work were in the age range of  $27.5 \pm 3.4$  years old and they were all living in Zahedan. Of 1440 individuals that G6PD of blood samples were qualitatively evaluated, in 101(7% of the total samples) enzyme activity was deficient. According to hematology tests, all subjects were healthy and did not show signs of anemia and jaundice. Genomics DNA of all deficient peoples was extracted and genomic DNA two healthy individuals with no history of jaundice or hemolysis used as negative control. PCR product of Exon 6 and 7 using primers 1 and 2 was 583 base pairs, which was confirmed by electrophoresis in 1.5% agarose gel and comparing with DNA size marker (Fig. 1).

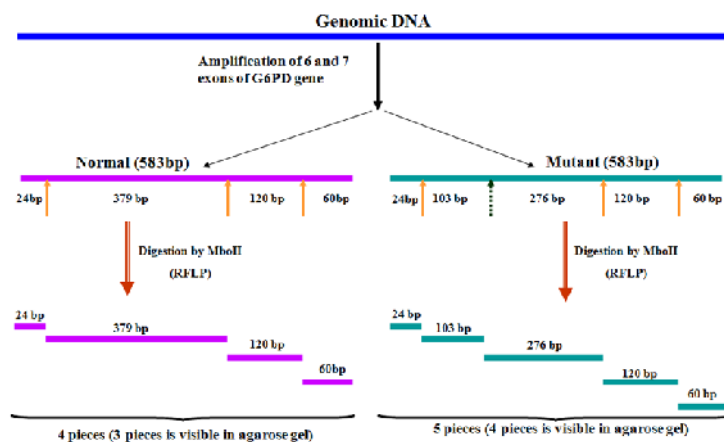
After ensuring that sequence of 583 base pairs has been well amplified in all samples, their PCR products were cut by MboII enzyme. The pattern of amplification and cutting of the 583 base pairs fragment of for normal and mutant genes is shown in figure 2. In the normal gene, the enzyme will cut PCR product in 3 regions and creates the 24, 60, 120 and 379 base pairs fragments. Mediterranean mutation creates an additional cut position in the amplified sequence, and 24, 60, 103, 120 and 276 base pair fragments will produce using MboII enzyme. Since 24bp fragment is not observed in agarose gel, in the electrophoretic pattern of digested PCR product of normal gene 3 bands were observed and four bands were observed in the electrophoretic pattern of the digested product of mutant gene. The electrophoretic

pattern of a few digested samples is shown in figure 3. Mediterranean mutation was diagnosed in 85 out of 101 samples with deficient G6PD. Thus, the prevalence of this mutation was estimated to be

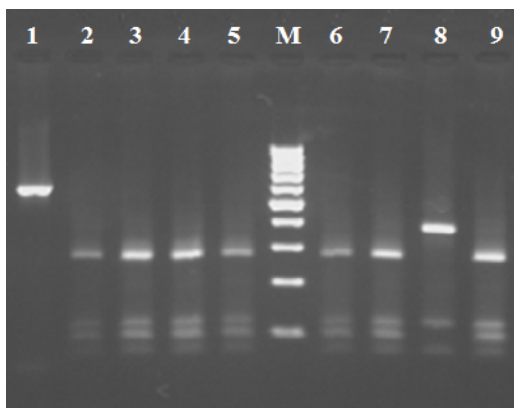
84.2%. Sixteen samples have a mutation other than Mediterranean mutation, which should be examined for other G6PD mutations.



**Figure 1.** The results of amplification of nucleotide sequence containing Mediterranean mutation (M: 100bp marker, P: PCR product)



**Figure 2.** Shows the cutting pattern of 583 nucleotide fragment of Mediterranean mutation by enzyme MboII in normal and mutant samples (upward arrows indicate the cutting position by the MboII enzyme).



**Figure 3.** Shows the electrophoretic pattern of digested products of 583 base pair fragment containing Mediterranean mutation. Lane 1 shows uncut PCR product, lane 8 shows the pieces result from cutting 583 base pairs fragment of normal G6PD. Lanes 2-7 and 9 show pieces result from cutting 583 base pairs fragment of samples with defective G6PD. Lane M indicates 100bp size marker. In lane 8, pieces of 60, 120 and 379bp can be respectively seen from bottom to top, which shows the pattern of a normal gene. In lanes 2-7 and 9, pieces of 60, 103, 120 and 276bp can be respectively seen from bottom to top, which is related to Mediterranean mutation pattern.

## Discussion

The prevalence of G6PD deficiency in this study was estimated 7%. G6PD deficiency is the most common enzyme deficiency in the world [10]. This deficiency has been reported with different prevalence in various parts of Iran. In Iran, the lowest and highest prevalence have been reported 1% in Maku and 24% in Iranshahr (Baluch tribe), respectively [11]. Considering that G6PD deficiency is associated with resistance to the *Plasmodium falciparum* parasite [12], its high prevalence in is not unexpected in malaria-breeding region of Iranshahr. The present study showed that the prevalence of this deficiency in Zahedan is lower than the other parts of the province, because demographic composition of Zahedan includes different ethnic groups (Zabol, Baluch, Birjand, Kerman, and etc.) that usually have lived in areas where malaria have been less common. G6PD deficiency has been also investigated in other parts of Iran and different prevalence has been reported. The prevalence of this deficiency is reported 6.2% in males in Arak [13], 6.4% in Rasht [14], 5.3% in Kermanshah [15], 12% in Fars Province [16] and 8.6% in the northern provinces of the Iran [17]. The overall prevalence of G6PD deficiency throughout Iran is estimated 11.5% [16]. In the areas where G6PD deficiency is highly prevalent, it is important to consider this deficiency during the treatment of other diseases. For example, doctors should be aware from G6PD status of patients when prescribing drugs, especially anti-malarial drugs, and not to prescribe antioxidant drugs to peoples with G6PD deficiency. On the other hand, since enzyme deficiency can be caused by different mutations and taking some drugs and foods in some known mutations can cause hemolysis [18], it seems essential to determine the type of mutation at the molecular level. So far, almost 140 different mutations have been identified for G6PD at molecular level, the most of which result from replacement of a nucleotide and thus replacement of one amino acid [6]. A number of these mutations may exist in every region of the world. In this study, G6PD Mediterranean mutation was studied in Zahedan. In 84.2% of deficient people, G6PD deficiency was due to Mediterranean mutation. Mediterranean mutation is the most common mutation of this enzyme in all parts of the world. Its prevalence is also investigated in many region of Iran.

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The prevalence of the mentioned mutation has been reported 91.2% in Kordestan [15], 66.2% and 83.8% in the north and south of Fars province, respectively [16], 70.8% in the Northern provinces (Gilan, Mazandaran and Golestan) [17], 79.5% in Hormozgan [19], 66% in Khorasan [20] and 72.7% in Zanjan [21]. Despite the differences in the prevalence of this mutation in different parts of the country, the common mutation is from Mediterranean type in all of these studies. Our results are in agreement with results of other studies. The common mutation is also Mediterranean in regional countries such as Pakistan, Turkey, Bahrain, Oman, Saudi Arabia, Iraq, India, Italy and Greece [8].

Mediterranean mutation in 84.2% of deficient peoples in Zahedan city in the present study indicates a common origin and genetic homogeneity of the studied population. Considering that the in classification of World Health Organization Mediterranean G6PD placed into class II and enzymatic activity of this class is less than 10 % of normal [5], its existence will provide an inappropriate environment for the growth of malaria parasites. Thus, it seems to be a natural selection for the survival of patients with this type of deficiency in the malaria-breeding area. When a genetic mutation has the ability to reduce the health of a community, but it is highly prevalent among the population, it can be concluded that the existence of this mutation in this population is an advantage for survival. In present study, G6PD deficiency of 15.8% of deficient people was not due to Mediterranean mutation. Given that many of G6PD mutations cannot be identified through PCR-RFLP method used in this study, the samples should be evaluated through other methods such as Single-Strand Conformation Polymorphism (SSCP).

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