Glucose-6-phosphate dehydrogenase deficiency in Tehran, Zanjan and Sistan-Balouchestan provinces: prevalence and frequency of Mediterranean variant of G6PD

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Abstract
Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an enzymopathy affecting about 400 million people worldwide. The distribution of G6PD deficiency and the molecular genetics of this enzyme vary widely among different ethnic groups. The aim of this study was to find out the frequency of G6PD deficiency and characterize the Mediterranean type mutation in deficient individuals in Turk, Balouch and Fars ethnic groups in Zanjan, Iranshahr and Tehran provinces. 1500 unrelated male individuals from Zanjan population. Genomic DNA was extracted from deficient individuals and also from 64 G6PD deficient individuals from Tehran city. PCR was used to amplify flanking regions of exons 6 and 7 of this gene. The PCR products were digested by the MboII enzyme and electrophoresed on 3% agarose gel. Thirty-three (2.2%) individuals were shown to be deficient for G6PD deficiency by fluorescent spot test. Genomic DNA was extracted from deficient individuals and also from 64 G6PD deficient individuals from Tehran city. PCR was used to amplify flanking regions of exons 6 and 7 of this gene. The PCR products were digested by the MboII enzyme and electrophoresed on 3% agarose gel. Thirty-three (2.2%) individuals were shown to be deficient for G6PD from Zanjan population. Twenty-four out of 33 (72.8%) of the deficient individuals showed a mutation at nt 563 which is characteristic of Mediterranean type of mutation. Nine individuals were negative for this mutation. At the molecular level, 50 (85%) of the individuals showed Mediterranean type of mutation and 15% were negative for this mutation. Our results from Tehran showed that 47/64 (73.4%) of deficient individuals had Mediterranean type mutation and 26.6% were negative for this mutation. Despite different frequencies exist for deficiency of G6PD in Turk, Balouch and Fars populations, the results of the present study and others have shown that the predominant mutation of G6PD in Iran is of Mediterranean type.

Keywords: PCR; G6PD deficiency; Iran; Mediterranean mutation

INTRODUCTION
Glucose-6-phosphate dehydrogenase (G6PD) deficiency was the first prevalent enzyme deficiency to have been described (WHO working group, 1989). Clinically it may manifest as neonatal jaundice, acute haemolytic anaemia and drug-induced haemolysis (Beutler, 1994). It was first established to be the genetic basis of fauvism in subjects from Sardinia. G6PD is an x-linked enzymopathy affecting about 400 million people worldwide (WHO working group, 1989). Around 7.5% of the world’s population are carriers for one or two deficient genes of G6PD, ranging from 0.1% in Japan to 35% in Africa and some European countries (Beutler, 1994). According to WHO the prevalence of G6PD deficiency in Iran has been
reported to be between 10-14.5% (Beutler, 1994) but, according on the studies that has been carried out in Iran the lowest and the highest frequency of G6PD deficiency was found to be between 1 to 22.8% (Noori-Daloii et al., 2007; Luzzatto et al., 1973). The lowest frequency was reported from Mako (northwest, Iran) and the highest incidence was reported from sought village Choreb (Mazandaran) (Noori-Daloii et al., 2007). So far more than 400 biochemical variants of G6PD have been identified (Beutler, 1994). Mediterranean type of G6PD is a variant of this enzyme that characterized by severe enzyme deficiency and B-like electrophoretic mobility (Beutler, 1994; Luzzatto et al., 1973). Between 140-160 different mutations have been reported in the G6PD gene worldwide (Cappellini et al., 2008). The molecular basis of Mediterranean type of G6PD is a single point mutation at nt. 536 (C→T) resulting in a serine to phenylalanine replacement at amino acid 188 (Tishkoff et al., 2001; Beutler, 1994; Kurdi-Haidar et al., 1990). This mutation creates an MboII site in exon VI of the G6PD gene. G6PD deficiency at the molecular level has not been studied in some regions of Iran. Therefore, we decided to find out the frequency of G6PD deficiency and the Mediterranean type of the mutations in southeast (Iranshahr, Balouch population), northwest (Zanjan, Turk population) and central (Tehran) regions of Iran.

MATERIALS AND METHODS

This research is a descriptive study that was carried out during 2002 to 2004. 1500 unrelated male individuals from Zanjan, 305 unrelated male subjects from Iranshahr and 64 unrelated subjects (58 males and 6 females) from Tehran were included in the study. All the 64 subjects from Tehran were referred to us from different clinics and had G6PD enzyme deficiency. None of the individuals had a history of haematologic disorders or were on medication.

**G6PD activity:** Three ml of EDTA anticoagulated peripheral blood was drawn from each individual (written informed consent was obtained from all individuals). Initial screening for G6PD deficiency was carried out on all peripheral blood samples using fluorescent spot test kit (Kimya Pajohan, Iran) (Beutler et al., 1968). DNA was extracted from peripheral blood cells using either Qiagen kit or boiling/proteinase k method (Goossens et al., 1981).

**Polymerase chain reaction (PCR):** Using primers forward 91 (5’-CCCGAAGAGGAATATCAAGGGGT-3’) and reverse 92 (5’-GAAGAGTACCCCTCGAGGGTGAGCT-3’) exons 6 and 7 of G6PD gene was amplified by polymerase chain reaction. For this, genomic DNA (100 ng) was used in a reaction containing 1 x manufacturer’s buffer, 200 μM each deoxynucleoside triphosphates (dATP, dCTP, dTTP, and dGTP), 15 pmol each of forward and reverse primers, and 1U Taq DNA polymerase (Cinnagen, Iran) in a total volume of 50 μl. Amplification was carried out for 35 cycles of 60 seconds at 94°C, 1 minute at 56ºC, and 1 minute at 72°C, with a final extension for 10 minutes at 72°C. A denaturation step at 94°C for 5 minutes at the beginning was applied to all DNA samples. PCR products were run on 3% Agarose gel followed by Ethidium bromide staining. A DNA fragment of 583 bp was produced by these primers.

**MboII enzyme digestion:** The amplified DNA fragments were digested by MboII (New England Biolabs, UK) at 37°C overnight according to supplier’s instructions. The digested PCR products were electrophoresed on a 3% agarose gel (Sigma, Germany) for 2-3 hours and the gels were photographed using Biorad Gel Documentation System. In normal individuals two bands of 379 bp and 120 bp was produced after digestion. If the Mediterranean type of mutation was present, the 379 bp band will be cut into 276 and 103 bp bands. The smaller bands of 60 and 24 bp were not seen properly and diffused on this gel.

**RESULTS**

**Frequency of G6PD deficiency:** One thousand and five hundred unrelated male individuals were screened for G6PD deficiency in Zanjan province. Thirty-three out of 1500 individuals were deficient for G6PD enzyme. Therefore, a frequency of 2.2% was obtained for this enzyme deficiency. Thirty-three out of 1500 individuals were deficient for G6PD enzyme. Therefore, a frequency of 2.2% was obtained for this enzyme deficiency in Zanjan. Thirty one out of 305 deficient individuals (94%) had a severe enzyme deficiency and 2/33 individuals (6%) had partial deficiency. Three hundred and five unrelated male individuals from Tehran were screened for G6PD deficiency. Fifty nine out of 305 (19.3%) individuals were shown to be deficient. Fifty two out of 59 deficient individuals (88%) had a severe enzyme deficiency and 7/59 (12%) had a partial deficiency. Since the case selection in Tehran was not a random process and all the studied subjects were referred to us from Tehran...
clinics with known G6PD enzyme deficiency, therefore, we could not discuss about the frequency of G6PD enzyme deficiency in Tehran population.

The frequency of Mediterranean type mutation in Zanjan: Genomic DNA was extracted from peripheral blood samples of 33 deficient subjects. Exons six and seven of G6PD gene was amplified by primers 91 and 92. A DNA fragment of 583 bp was obtained for all deficient samples (Fig. 1). In order to find out the Mediterranean type of mutation, PCR products were digested by MboII enzyme. In individuals with non Mediterranean type of mutation the 583 bp band was cut into four fragments: 379, 120, 60 and 24 bp. In subjects with the Mediterranean mutation an extra MboII site was created, so that 379 bp fragment was cut into 276 and 103 bp bands (Fig. 2).

Twenty four out of 33 (72.8%) deficient individuals showed a mutation at nt 563 of G6PD gene. Nine out of 33 individuals (27.2%) were negative for Mediterranean type of mutation.

The frequency of Mediterranean type mutation in Iranshahr: Using the same method we found that 50 out of 59 deficient individuals of Iranshahr (85%) had Mediterranean type of mutation and 9/59 (15%) showed non Mediterranean type of mutation.

The frequency of Mediterranean type mutation in Tehran: Forty seven out of 64 deficient individuals (73.4%) showed Mediterranean type of mutation and 17/64 (26.6%) were negative for this mutation. All six female individuals who had G6PD enzyme deficiency were heterozygote for Mediterranean mutation and produced 379, 276, 120, 103, 60 and 24 bp fragments (data not shown).

DISCUSSION

Our study confirmed that 2.2% of Zanjan population was deficient for G6PD enzyme. 94% had a severe enzyme deficiency and 6% had partial deficiency. 19.3% individuals of Iranshahr were also shown to be deficient for G6PD enzyme. Eighty eight percent had a severe enzyme deficiency and 12% had a partial deficiency.

The prevalence of G6PD deficiency varies greatly in different countries and also among different ethnic groups within a country. The frequency of G6PD deficiency in Saudi Arabia has been reported to be in the range of 2-26% (El-Hazmi et al., 1990; Gelpi, 1965). Its prevalence in United Arab Emirates was 11% (Bayoumi et al., 1996), in Kuwait 19% (Bayoumi et al., 1996), in Bahrain 21% (Mohammed et al., 1992), in Oman 27% (White et al., 1993) and in Pakistan was between 2.3-8% (Saha et al., 1994).

Iran has a population of more than seventy millions with different ethnic groups. According to WHO, the prevalence of G6PD deficiency in Iran has been estimated to be in the range of 10-14.5% (Beutler, 1994),
Although some other reports pointed out different prevalence ranging from 1 to 22.8%. The lowest frequency (1%) was reported from Mako (northwest) and the highest frequency (22.8%) has been reported from sought village Choreb (Mazandaran) (Noori-Daloii et al., 2007). The prevalence around the Caspian Sea in north of Iran is reported to be 12.5% (Noori-Daloii et al., 2007; Mesbah et al., 2000). Ohkura et al. (1984) also reported an incidence of 8.6% of G6PD deficiency in Mazandaran and Gilan provinces (Ohkura et al., 1984). Karimi et al reported that the incidence of G6PD deficiency is around 12% in males and 0.9% in females in two Fars ethnic groups living in northern and southern Iran (Karimi et al., 2003). Rahimia et al. (2006) have found an overall frequency of G6PD deficiency of 5.3% for males in the Kurdish population of western Iran (Rahimia et al., 2006). Farahani and co-workers have been shown an overall frequency of 2.2% for Arak province (central region) of Iran (Farahani et al., 2002).

A strong correlation has been shown between G6PD deficiency and malaria in areas in which malaria is prevalent. Since the frequency of malaria is very low in Zanjani, therefore, the low rate of G6PD deficiency was predictable for this region. Conversely, the high prevalence of malaria in Iranshahr may justify the high prevalence of G6PD deficiency in this region.

At the molecular level, twenty-four G6PD deficient individuals (72.8%) from Zanjani (northwest Iran) had the Mediterranean type mutation. Nine individuals (27.2%) were negative for this mutation. We also found that 50 out of 59 (85%) Iranshahr (southeast Iran) individuals had the Mediterranean type mutation. 9 out of 59 (15%) showed non-Mediterranean type of mutation. Our results from Tehran (central region of Iran) showed that the prevalence of Mediterranean type of mutation is 73.4%. Since Tehran has a mixed population therefore the obtained results can not be attributed to a specific ethnic group.

Mesbah et al. (2000) reported that the frequency of Mediterranean type of mutation in Mazandaran region (north part) was 66.2%. Studies of Noori-Daloii et al. (2007) in Golestan, Gilan and Mazandaran provinces (around the Caspian sea in north of Iran) showed an overall frequency of 70.78% for this mutation (Noori-Daloii et al., 2007; Noori-Daloii et al., 2004). Studies of Noori-Daloii et al. (2006 a) in East provinces of Iran showed that 66% of patients of Khorasan province (northeast) and 80.4% of individuals of Sistan and Balouchestan province (southeast) had Mediterranean mutation respectively (Noori-Daloii et al., 2006 a; Noori-Daloii et al., 2005). The highest prevalences (91.2%) of Mediterranean mutation has been reported from Kurdish population in Western region of Iran (Rahimia et al., 2006). Some reports from southern provinces of Iran showed that 79.4% patients of Hormozgan and 84.6% patients of Fars province had Mediterranean mutation (Noori-Daloii et al., 2006 b; Karimi et al., 2003). There are also reports that Mediterranean type of mutation is prevalent in neighbouring countries of Persian Gulf and Middle East countries such as United Arab Emirates (55.5%), Saudi Arabia (80%) (Niazi et al., 1996), Pakistan (76%) (Saha et al., 1994) and Oman (74%) (Daar et al., 1996).

Despite different frequencies exist for deficiency of G6PD enzyme in Turk, Fars and Balouch and other ethnic groups in different regions of Iran (central, north, northwest and Southeast) the results of this study and other studies have shown that the incidence of Mediterranean type of G6PD mutation in all the above regions are approximately the same. Therefore, it can be concluded that the gene frequency of G6PD deficiency has been distributed equally among different populations of Iran. This may indicate that the predominant G6PD mutation in Iran is of Mediterranean type. These findings also suggest that the Mediterranean type of mutation may be quite ancient and may have spread by migrations that have been taken place perhaps over millennia.

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