

Prevalence of Three Common Glucose-6-Phosphate Dehydrogenase Gene Mutations in Neonates in Province of Mazandaran, North of Iran, 2012

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ABSTRACT In northern provinces of Iran high rates of incidence of glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency have been reported and most of these patients carry one of the three common G6PD gene mutations: Mediterranean, Chatham or Cosenza. The aim of this study was to investigate prevalence of each of these mutations among neonates in Mazandaran, a northern province of Iran. Four hundred and twelve blood samples were collected and using standard protocols DNA was extracted. In order to detect the above mutations PCR-RFLP method was applied. Fifty-three of neonates had G6PD gene mutation (12.9%, CI 95%: 9.66-16.14). About 17% of female and 9% of male newborns were carriers for one of the three common G6PD gene mutations. The Mediterranean type had the highest gene frequency (0.0607) among the three examined mutations. The present study shows around 17% (CI 95%: 11.97-22.03) of Mazandarani female population is carrier for one of the three mutations and since the likelihood of having an affected child in a carrier woman is 1 in every 4 child births, the researchers recommended all women to be screened for the presence of three common G6PD gene mutations prior to pregnancy.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency is the most common enzymopathy in the world and affects more than 400 million people worldwide. It is a sex linked, recessively inherited disorder (Cappellini and Fiorelli 2008; Mehta et al. 2000). Affected individuals are usually asymptomatic but in some cases, oxidative stress may lead to acute hemolysis. The hemolytic event is usually triggered by ingestion of fava bean. Other presentation of the disorder would be neonatal jaundice which is not hemolytic in nature (Beutler and Vulliamy 2002; Youngster et al. 2010; Lai et al. 2013; Taki et al. 2001).

The gene involved in the disease is located on Xq28, containing 13 exons that encode a protein with 515 amino acids (Beutler 1994). It is estimated that 7.5% of the world population carry one or two mutated G6PD gene, with a prevalence spectrum ranging from 0.1% in Japan and some European countries to 35% in African continent (Beutler 1994; Noori-Dalooi

et al. 2007). G6PD gene is a highly polymorphic one, and till now about 140 different mutations of the gene have been reported (Cappellini and Fiorelli 2008).

G6PD gene mutation distribution rates differ from one geographical area to another. G6PD A- variant, a certain G6PD enzyme deficiency responsible for 90% of the world affected cases is quite common in Italy, the Canary Islands, Spain, Portugal, and in some parts of the Middle East. G6PD A- 202 (G→A) / 376 (A→G) is the most prevalent mutation in African continent (Cappellini and Fiorelli 2008; Oppenheim et al. 1995; Dallol et al. 2012; Laouini et al. 2013).

High incidences of G6PD enzyme deficiency have been reported in some parts of Iran, and the prevalence of the disease varies markedly among different provinces. In northern provinces of Iran, fava bean is a common ingredient of various dishes, and therefore it is not unexpected to have high incidence rate of the disease (8.7%–16.4%) in that region (Mazandaran and Gilan provinces) (Noori-Dalooi et al. 2007).

A G6PD gene mutation called “Mediterranean” has a wide distribution range, from Mediterranean and Middle Eastern countries to Indian subcontinent. It is the most common mutation among patients from Northern provinces of Iran. Following this mutation, Chatham and

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Cosenza mutations, the two other common G6PD gene mutations, have the highest frequency rates in that area (Beutler 1994; Beutler and Vulliamy 2002; Mesbah-Namin et al. 2002).

Since G6PD enzyme deficiency is prevalent in north of Iran and until now no concentrated study on general population of that region has been done, the aim of this study is to determine the frequencies of three common G6PD gene mutations among neonates in Mazandaran.

MATERIAL AND METHODS

Four hundred and twelve samples of umbilical cord blood were collected at Amir Mazandarani hospital, Sari, Iran, 2012. After receiving consent from infant's parents, the samples were frozen and stored at -80°C up to the time of DNA extraction.

Genomic DNA was extracted from leukocytes using standard *phenol-chloroform* method. DNA samples were evaluated for the presence of three G6PD common gene mutations, namely Mediterranean (563 C→T), Chatham (1003 G→A), and Cosenza (1376 G→C), using polymerase chain reaction (PCR) method with specific primers (Table 1), followed by digestion with specific restriction enzymes. MboII, BstXI, and Bsu36I enzymes were applied to detect Mediterranean, Chatham, and Cosenza mutations, respectively. Then, digestion products were subject to electrophoresis on 3% concentrated agarose gel (Noori-Dalooi et al. 2004).

In order to detect Mediterranean type of G6PD gene mutation, PCR product was digested and analyzed. After digestion of the original 583bp PCR product with MboII restriction enzyme, wild type cases had 4 bands of 24, 60, 120 and 379bp length, detectable on electrophoresis agarose gel, while in mutant alleles, beside three former segments, two fragments of 276bp and 103bp, instead of a single 379bp fragment, were detected. (Noori-Dalooi et al. 2004)

For detecting Chatham mutation, after digestion of the original PCR product by BstXI restriction enzyme, samples from wild type cases were defragmented into 130bp and 78bp length pieces on 3% agarose gel, while in mutant cases the 130bp fragment was cut into two segments (100bp and 30bp) (Noori-Dalooi et al. 2004).

In order to evaluate Cosenza mutation (1376 G→C), Bsu36I restriction enzyme was applied and 548bp PCR product remained intact in wild type cases, while in mutant alleles two fragments with 316bp and 232bp length were detected (Noori-Dalooi et al. 2004). Allelic frequencies of the mutations were calculated using Hardy-Weinberg equilibrium.

RESULTS

Four hundred and twelve neonates, consisting of 198 male (48%) and 214 female (52%) cases, whose parents were of Mazandarani ethnicity, were included in this study. In 626 studied X chromosomes, 38 chromosomes (25 in females and 13 in males) contained Mediterranean mutation and the gene frequency among Mazandarani neonates was 0.0607. Ten X chromosomes consisting of 7 female and 3 male cases had Chatham (1003 G→A) mutation (gene frequency: 0.0160) and 5 X chromosomes had G6PD Cosenza mutation (Table 2). This mutation had a gene frequency of 0.0080.

The researchers' finding indicated that 12.9% (CI 95%: 9.66-16.14) of Mazandarani population (16.8% CI95%11.79-21.81) of females and 8.6 % (CI 95%: 4.69-12.51) of males have at least one of the three investigated mutations. Fifty-three out of 626 investigated X chromosomes had one of the three evaluated mutations, and overall gene frequency of these mutations is 0.0847. Moreover, in our study no female was found to have mutant allele on both X chromosomes (homozygote state).

Table 1: Sequences of primers used in the amplification of specified segments of G6PD gene for the detection of three common gene mutations.

<i>Mediterranean</i>	F 5'-CCC CGA AGA GGA ATT CAA GGG GGT-3' R 5'-GAA GAG TAG CCC TCG AGG GTG ACT-3'
<i>Chatham</i>	F 5'-CAA GGA GCC CAT TCT CTC CCT T-3' R 5'-TTC TCC ACA TAG AGG ACG ACG GCT GCC AAA GT-3'
<i>Cosenza</i>	F 5'-GCA GCC AGT GGC ATC AGC AAG-3' R 5'-GGG AAG GAG GGT GGC CGT GG-3'

Table 2: Distribution of G6PD gene mutations among neonates (Mazandaran, Iran) and gene frequency of the evaluated mutations

<i>Mutation Group</i>	<i>Mediterranean</i>	<i>Chatham</i>	<i>Cosenza</i>
Female (214 Cases)	25	7	4
Male (198 Cases)	13	3	1
Whole group (412)	38	10	5
Gene frequency (in whole group)	0.0607	0.0160	0.0080
Gene frequency (in mutated alleles)	72%	19%	9%

DISCUSSION

Mazandaran province located on the southern coastline of Caspian Sea, with about 4 million inhabitants, has the highest prevalence of G6PD enzyme deficiency cases in Iran (Noori-Dalooi et al. 2007). The high prevalence of G6PD enzyme deficiency in the region can be explained by its historical relation with malaria outbreaks.

G6PD enzyme deficiency has considerable biochemical and molecular heterogeneity and its incidence in Middle Eastern countries varies from 1% in Egypt to 11.5% in Iran (Usanga and Ameen 2000). The researchers' previous report showed that 1 in 25 male and 1 in 69 female persons are at risk of G6PD enzyme deficiency (Kosaryan et al. 2011). Several molecular studies in Iran have shown that Mediterranean, Chatham and Cosenza mutations are the most common types of G6PD gene mutations in that region (Noori-Dalooi et al. 2004). Although the frequencies of three common G6PD gene mutations among G6PD patients in Mazandaran have been investigated, the exact frequencies of these mutations in the whole population were unknown. This is the first study to evaluate the frequencies of these mutations in Mazandarani neonates, reflecting distribution of G6PD deficient alleles in general population.

Mesbah-Namin et al. reported the frequency of three common G6PD variants among G6PD patients in Mazandaran as follows: Mediterranean, 66.2%; Chatham, 27%; and Cosenza, 6.8%. This does not precisely reflect the distribution of G6PD deficient alleles in the entire population (Mesbah-Namin et al. 2002). According to that study, Chatham mutation in Mazandarani G6PD deficient patients has the highest prevalence in the world. Our data showed this mutation has a gene frequency of 0.0160 in the population of Mazandaran province.

Other studies investigating the frequency of G6PD gene mutations in different provinces of Iran have shown G6PD Mediterranean mutation is the most prevalent one in those patients, ranging from 63% (Kerman province) to 91.2% (Kermanshah province) (Noori-Dalooi et al. 2003, 2009). G6PD Chatham mutation has the second highest frequency, but G6PD Cosenza mutation was not identified in several provinces including Fars, Isfahan, Hormozgan, Khorasan, Kurdistan, Sistan and Baluchestan, Zanjan, Yazd, Kerman and Gilan (Noori-Dalooi et al. 2003, 2009). All the above mentioned works followed a certain protocol to investigate G6PD enzyme deficiency and subsequently related gene mutations: in order to identify G6PD enzyme deficient patients, at first qualitative and quantitative evaluations, namely fluorescent spot and enzyme assay tests, respectively, were applied. Following that, molecular examinations were performed to detect G6PD common gene mutations in affected individuals. Apparently, this approach is unable to identify healthy females who carry the gene mutation in heterozygote state, and thus cannot be a reliable approach to recognize all the cases. Taking this matter into consideration, all the previous works simply reported frequency of G6PD gene mutation only among male patients, and rare cases of homozygote females. In our study the gene frequency of the three common mutations was investigated in the general population of Mazandaran Province, regardless of enzyme deficiency state of the examined cases. Using this approach, all the carries of each and every one of the three common G6PD gene mutations can be detected, and therefore a better knowledge on prevalence of these mutations can be achieved.

Population study of G6PD common gene mutations in Kuwait on 278 investigated chromosomes indicated that Mediterranean mutation with gene frequency of 0.0503 has the highest prevalence, and following that, 376A→G and 202G→A mutations have gene frequencies of 0.0215 and 0.0108, respectively. In comparison to this study, frequencies of Mediterranean mutation in the two populations are close to each other, but the two most common mutations following Mediterranean one in Kuwait (376A→G and 202G→A) and Iran (Chatham and Cosenza) are different. Other studies in Iran have also shown those mutations (that is, 376A→G and 202G→A), which are commonly reported from

Arab States of Persian Gulf, to be absent in Iranian population of G6PD deficient patients. The researchers' result confirms previous reports and may lead to the conclusion that G6PD mutations in Arab and Iranian ethnicities have different origins (Noori-Dalooi et al. 2009; Samilchuk et al. 1999).

Karimi et al. also compared the frequency of G6PD enzyme deficiency between Muslims and Jews (201 Muslims and 187 Jewish subjects) in Shiraz, a southern city of Iran, and their findings showed that in both groups Mediterranean mutation had the highest prevalence, and just in one Muslim case, Chatham (1003 G→A) and in two Jews Cosenza (1376 G→C) and G6PD A-mutations were detected. Comparing to this study and other studies in Iran, the mutations in Muslim population are similar to other parts of Iran, while in Jews a different mutation was observed (Karimi et al. 2008).

In Egypt, in a study on 70 neonates with high or prolonged jaundice, Settin et al. (2006) found a prevalence rate of 4.3% (3 cases including 2 males and 1 female) for G6PD Mediterranean gene mutation. Other studies in North African countries showed that G6PD Mediterranean gene mutation has the highest frequency in Egypt and Libya, while in Algeria this mutation has the second highest frequency (23%) after G6PD A- variant (46%) among enzyme deficient patients. Although this percentage (4.3%) indicates a high prevalence of this certain mutation in a North African country, yet this is almost half of its equivalent (8.5%) in this study (Nafa et al. 1994; Settin et al. 2006).

In two other Caspian Sea coastal provinces of Iran, Golestan and Gilan, presence of the G6PD Mediterranean, Chatam and Cosenza mutations among patients with G6PD deficiency has been previously studied. The results showed the highest rate of G6PD Mediterranean mutation (86.4%) in Caspian Sea coastal line belonging to Gilan while no patient with Cosenza mutation was found there. In Golestan, the prevalence of Cosenza mutation (11%) is higher than the other two provinces. These studies alongside with ours showed that moving from east side to west side of the southern Caspian Sea border, the occurrence of Mediterranean mutation rises, while at the same time prevalence rate of Cosenza mutation decreases (Noori-Dalooi et al. 2003; Noori-Dalooi et al. 2007).

Based on the current research and other similar studies, G6PD Mediterranean mutation is

present in all provinces of Iran, which themselves cover a spectrum of different ethnicities, while Cosenza mutation which is a common mutation in Mazandaran, is absent in several provinces of Iran. Mediterranean mutation is also reported from several Middle Eastern countries. Considering widespread distribution of this mutation one may conclude that it was initiated prior to other examined common mutations and spread in the entire region during an extended period of time. Similar to other studies in Iran, our finding showed that distribution pattern of these mutations in Mazandaran has more similarity to Mediterranean countries rather than Arab states of Persian Gulf (Noori-Dalooi et al. 2009).

CONCLUSION

This study showed that up to 12.9% of Mazandarani population has at least one of the three common G6PD gene mutations. This rate among male population is 8.6% and in females it reaches to 16.8%. Since G6PD enzyme deficiency is inherited through a sex-linked recessive pattern, the likelihood of having an affected child for a carrier mother in heterozygote state is 25%. As a result, a large proportion of the newborns are at risk of having G6PD enzyme deficiency.

RECOMMENDATIONS

Considering the high risk of bearing an affected child with G6PD enzyme deficiency, hereby we recommend a program to screen all newborns for mutations related to this deficiency.

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