Molecular Aspects of Glucose-6-Phosphate Dehydrogenase Deficiency in Iran

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Abstract

Background: G6PD deficiency is the most common hereditary enzyme deficiency that affected more than 400 million people worldwide. This enzyme deficiency is caused by a spectrum of mutations in the gene encoding G6PD on chromosome X. Epidemiologically; G6PD deficiency has been specially considered in Middle East countries including Iran, Oman and Saudi Arabia.

Materials and Methods: This study has reviewed more than 70 papers related to the epidemiological significance and various diagnostic strategies of G6PD deficiency from 1956 to 2010.

Results: The results showed a higher prevalence of Mediterranean variant followed by Chatham and Cosenza compared to other variants in Iran.

Conclusion: Accurate identification of G6PD deficiency variants in areas with high prevalence of this disease will help to screen patients and their families with risk level when faced with oxidant agents.

Introduction

G6PD (Glucose-6-phosphate dehydrogenase) deficiency is the most common hereditary enzyme deficiency reported so far [1]. This enzyme deficiency has affected more than 400 million people around the world. Due to the process of the natural selection, most cases of illness are observed in tropical regions of Africa, the Middle East, tropical and subtropical regions of Asia and the Mediterranean rim [2, 3]. Most cases of this enzyme deficiency have been reported in Kurd Jewish men, up to about 50% [4]. The first studies related to G6PD deficiency and Favism were conducted in 1950 through observing the relationship between administration of the antimalarial drug of primaquine and development of hemolytic anemia in American soldiers [5]. However, in 1926, there were also some scattered reports on hemolytic anemia development after taking antimalarial drug of pamakine (Plasmoquine). People with Favism often have no symptoms of hemolytic anemia and will suffer hemolysis only when exposed to oxidative stresses including some medications, infections, and oxidants such as Favas. The most important drugs and chemicals which can cause hemolysis in patients with Favism include acetalalide, furazolidone, methylene blue, nalidixic acid, naphthalene, niridazole, isobutyl nitrites, nitrofurantoin, phenazopyridine, primaquine, sulfa cetamide, sulfamethoxazole, sulfanilamide, tiazolsulfone, toluidine blue, trinitrotoluene (TNT), urate oxidase and phenyl hydrazine [6, 7]. However, some cases of spontaneous hemolysis have also been reported in patients with severe enzyme deficiency [8-11]. Overall, intensity of hemolysis in patients with Favism depends on the G6PD enzyme level in red blood cells [12-14]. At present, all hematologic and non-hematologic complications resulting from G6PD deficiency are known [15]. Favism will be often diagnosed with hemolysis, hemoglobinuria, anemia and jaundice. These people may also have headaches, nausea, back pain, feeling cold and fever [7]. The most common form of G6PD isoenzyme is type B. However, in blacks, isoenzyme type A has a higher incidence and a faster electrophoretic movement than type B [16].

Biochemical pathway

G6PD enzyme is the most important enzyme in pentose phosphate shunt. This allosteric enzyme in the first reaction of pentose phosphate shunt is able to convert glucose-6-phosphate to a composition called 6-phosphogluconolactone and NAPD+ is reduced and critical composition of NADPH is formed during this reaction. NADPH is an electron carrier combination which helps to preserve and create reduced form of glutathione inside the cell and ultimately protect the cell against oxidative damages [17-19].
Pentose phosphate shunt is the only source and pathway producing NADPH in red blood cells (RBC) which is due to the removal of cell nuclei and organelles during the erythroid differentiation pathway in RBC. However, there are other alternative pathways in most body cells to produce NADPH. Thus, G6PD deficiency can cause hemolysis, while the lysis of other body cells will not occur [20].

**Genetics of G6PD**

Gene coding G6PD is located on chromosome X (Xq28) [21, 22]. This gene includes 13 exons and 12 introns and approximately 20 kb base long. Exon 1 is a non-coding exon, while the other 12 next exons are coding. This gene, like other functional genes, has a promoter rich with bases C and G. The length of each exon is 120-236 bp and the enzyme encoding G6PD has 515 amino acids [23-25].

Another noteworthy point is that the intron part between exons 2 and 3 is too long with a length equal to 9857 bp [23]. There is a region rich with cytosine guanine dinucleotide (CpG Island) in the fifth head of this gene. This region exists in human and rat population as completely protected [14].

The major role of this region is to regulate the gene encoding G6PD which is performed through methylation and demethylation of dinucleotides [26]. After deletional analysis, the 2850bp part of fifth region of this gene was identified which is responsible for a 436 bp domain that control the gene expression [27]. The gene encoding G6PD is one of the most polymorphic genes of the human chromosome. About 442 of whose allelic variants are so far reported [6]. These variants are evaluated based on the three main indicators: the difference in the degree of enzyme activity, the differences in electrophoretic pattern and the difference in physical, chemical, and kinetic properties [28].

According to WHO criterion, 299 G6PD allelic variants which have been identified by various techniques are classified in five categories which have been shown in table 1 [29]. About 160 mutations of G6PD deficiency have been so far reported [28-30]. G6PD A genotype is a special variant which occurs due to mutations of A376G and G202A [31]. G202A mutation is the cause of 95% of the reported cases of G6PDA-variant in Africa. In addition, G6PD-Santamaria genotype, which is a class-2 variant with low prevalence, occurs due to two simultaneous mutations, in nucleotides 376 and 524 of exons encoding gene G6PD. This genotype was first detected in Costa Rica and has also been found with low prevalence in southern Italy areas [32].

Mutations such as large deletional, nonsense and framework change mutations, which completely delete the functional G6PD production, are fundamentally incompatible with human life [33]. In addition, no mutation has been so far reported in the coding region of enzyme activity region as well as the promoter region [34]. It is noteworthy that polymorphism in non-coding sequences of G6PD is also involved in the occurrence of G6PD deficiency. The precise mechanism of this enzyme deficiency is so far unknown [3].

Through the studies conducted in 2000, a precise relationship was identified between the mutations that cause the replacement of amino acids in G6PD structure and the evolutionary history of different geographical areas that explain mechanism of natural selection regarding this enzyme deficiency (especially in malarious areas) [35]. There is a need for analysis of different point mutations in the gene encoding this enzyme to study G6PD deficiency from molecular view as well as understanding its phenotypic and genotypic relationship [8].

At least 160 mutations and mutation fusion have been so far identified all of which can cause G6PD deficiency [28, 36]. It is important to note that since complete deletion of the G6PD production is incompatible with life, most mutations are point mutations and some are deletion mutations (3 cases of which are known) so that they would not change the gene as do the framework mutations. So far only one splicing mutation has been reported, while no mutation has been reported in the promoter region. There is an exception in which G6PD production will not be disturbed despite the existence of mutation. This mutation is known as “Georgia” in which the codon encoding tyrosine no. 428 (Tyr428) will change to the stop codon. During this mutation, 83% of the produced polypeptide chain of G6PD is incomplete and yet functional. However, this mutation was found in a heterozygous woman and it seems that the phenomenon of unbalanced X-chromosome inactivation has helped the occurrence of G6PD deficiency symptoms [37]. It is noteworthy that in women with malignant blood disorders such as myeloproliferative syndromes in conditions where there is no proper method to check for malignancy, it can be useful to assess polymorphic genes, including G6PD gene to study X-chromosome inactivation [38].

Point mutations that cause class 1 variants are limited to the two regions: one of these regions is close to the connection point of NADP+ or NADPH to G6PD enzyme and the other region is located at the glucose-6-phosphate connection region. 87% of the mutations that occur in these two regions involve only 28% of the polypeptide chain. The other notable point is that the change in a codon in the gene coding G6PD can cause classification of the variants resulted from different groups as well as various clinical symptoms. For example, if the change in the methionine no. 212 (Met212) is converted to Valine (Val), it will cause G6PD Santiago variant which is classified as the class 1 variant and if it is converted to cysteine (Cys), it will cause G6PD Coimbra variant which is classified as the class 2 variant [6].

**The most common variants and their geographical distribution**

Malarious areas are highly regarded in terms of high prevalence of G6PD deficiency. Nigeria has a 28% incidence of G6PD deficiency and all of these cases are G6PD A-(376G, 202A) variant [39]. In Asia, Cambodia has 15.8% incidence of enzyme deficiency that 82.4% of which is related to Viangchan variant, 3% of which is related to Union variant and 3% is related to the variant Coimbra [40]. Also in countries such as Myanmar and
India, the prevalence of 10.8% and 10.5% of the enzyme deficiency has been reported respectively. In Myanmar, all cases of reported G6PD deficiency are Mahidol variants [41]. In India, 60.4% of cases are related to the Mediterranean variant, 24.5% of cases are related to the Kerala-Kalyan variant and 13.3% of cases are related to the Orissa variant [42]. It has been revealed that in the two countries of Brazil and Mexico, most cases of G6PD deficiency are related to the variant G6PD A- (376G-202A) and a small percentage is also related to the variants G6PD A- (376G/968C) and Seattle [43-45].

In the Middle East, Iran, Oman and Saudi Arabia have the most significant risk of G6PD deficiency [46-48]. In Oman and Saudi Arabia, Mediterranean variants of Chatham and G6PDA- are considered as dominant variants [20]. Various cities and provinces of Iran have also been studied in terms of incidence of G6PD deficiency variants. In Golestan, 69% of the variants are related to the Mediterranean variant and 26.7% of the variants were Chatham [46]. A descriptive study which was conducted to determine the G6PD deficiency and review the deficiency type at the molecular level in males living in Zanjan from 2001 to 2003 revealed that the prevalence of G6PD deficiency in Zanjan was lower compared to some other provinces. Also, the incidence of malaria in Zanjan is low and it can be justified through the prevalence of enzymatic deficiency in this city. The study showed that most people in this region have Mediterranean mutation at position 563 of gene G6PD [49]. Through other studies that were conducted in Tehran from 2001 to 2002, the dominance (73.4%) of Mediterranean mutation was revealed compared to the other mutations. Therefore, the prevalence of Mediterranean mutation in Iran is similar to neighboring countries [50]. Table 2 reviews and compare the frequency and types of G6PD mutations in the some cities and provinces of Iran.

<table>
<thead>
<tr>
<th>Type</th>
<th>Enzyme activity</th>
<th>Clinical Symptoms</th>
<th>Abundance and geographic distribution</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Less than 1% or undetectable</td>
<td>Chronic hemolytic anemia</td>
<td>Very rare with no precise geographic distribution</td>
<td>G6PD-Buenos Aires</td>
</tr>
<tr>
<td>2</td>
<td>Less than 10%</td>
<td>Acute hemolytic Anemia mediated drugs and fava beans</td>
<td>Abundant in all parts of the world</td>
<td>G6PD-Durham</td>
</tr>
<tr>
<td>3</td>
<td>10-60%</td>
<td>Acute or chronic hemolytic anemia</td>
<td>Abundant in malarious area</td>
<td>G6PD-Mediterranean</td>
</tr>
<tr>
<td>4</td>
<td>60-90% with normal activity</td>
<td>Asymptomatic</td>
<td>Not specified</td>
<td>G6PD-Argentina</td>
</tr>
<tr>
<td>5</td>
<td>More than 110% with increasing of activity</td>
<td>Asymptomatic</td>
<td>Not specified</td>
<td>G6PD-Cassano</td>
</tr>
</tbody>
</table>

**Table 1.** Classification of G6PD according to WHO standards

**Table 2.** Frequency and types of G6PD mutations in different populations of Iran

<table>
<thead>
<tr>
<th>Mutation type and relative frequency</th>
<th>Population</th>
<th>City or Province</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6% (type of mutation has not reviewed)</td>
<td>1900 infant</td>
<td>Rasht [51]</td>
</tr>
<tr>
<td>82.3% carrier of Mediterranean variant, 8.3% carrier of Chatham variant</td>
<td>96 patients with Favism</td>
<td>Fars and Isfahan [52]</td>
</tr>
<tr>
<td>79.4% carrier of Mediterranean variant, 12.3% carrier of Cosenza variant, 8.2% carrier of Chatham variant</td>
<td>73 patients with Favism</td>
<td>Hormozgan [53]</td>
</tr>
<tr>
<td>6.02% (type of mutation has not reviewed)</td>
<td>332 infant</td>
<td>Arak [54]</td>
</tr>
<tr>
<td>66% carrier of Mediterranean variant, 12% carrier of Cosenza variant, 5% carrier of Chatham variant</td>
<td>76 patients with Favism</td>
<td>Khorasan [55]</td>
</tr>
<tr>
<td>72.8% in Zanjan, 85% in Iranshahr and 73.4% in Tehran carrying the Mediterranean variant, 5.3% in Tehran</td>
<td>732 students aged 7 to 11</td>
<td>Aron [56]</td>
</tr>
<tr>
<td>78.2% in Zanjan, 85% in Iranshahr and 73.4% in Tehran</td>
<td>1500 men in Zanjan, 305 men in Iranshahr and 64 person in Tehran</td>
<td>Tehran, Zanjan and Sistan &amp; baloochestan [57]</td>
</tr>
</tbody>
</table>

**Enzymatic evaluation of G6PD**

According to the WHO criterion, G6PD deficiency diagnostic tests are mainly performed based on production of NADPH from NADP+:

**Semi-quantitative assessment**

Fluorescent spot test: the advantages of this test include high speed, ease of performance, high sensitivity and low cost. This test can be used in areas with high prevalence of G6PD deficiency as well as endemic malaria, before prescribing antimalarial drugs such as primaquine. One type of this test can be used via visual assessment without UV lamps which can be used as a screening test in the tropics [58].

Tests to measure Matt levels of reduced hemoglobin: These tests are screening tests which NADPH concentration can indirectly be measured. These tests are based on NADPH oxidation [59]. This technique is able to directly assess G6PD activity in the intact red blood cells of healthy people with accuracy of about 75%. Tests to evaluate stability of reduced glutathione and Heinz bodies’ assessment test also help to screen the cases with G6PD deficiency.
Quantitative evaluation

The quantitative amount of G6PD is reviewed by spectrophotometry. This test is based on the measurement of the level of absorption at wavelength of 340nm, which represents the formation of NADPH. This test is conducted by mixing hemolysate containing glucose-6-phosphate, with cofactor NADP+ at 37°C and through the investigation of NADPH production. The activity of G6PD is reported as IU/RBCs or IU/Hb. While, measurement of G6PD activity will be performed after or during acute hemolysis or sudden hemolysis, a false increase may occur in G6PD activity followed by an increase in the number of reticulocytes that will cause false negative results. In fact, it has been seen that the activity of G6PD in the reticulocytes is 5 times as much as its value in the old RBC. In mature RBC, the synthesis of enzymes and proteins will not be performed due to the lack of nuclei and organelles, and concentration and activity of these enzymes gradually decreases with increase of RBC age.

These conditions also occur in measurement of the activity G6PD in RBC of infants at the presence of G6PD deficiency. Here, it is necessary to explain the phenomenon of X-chromosome inactivation. Men can be healthy or sick (hemizygous) in terms of the G6PD deficiency. Women, like men, have either phenotypic states of healthy or sick (homozygous), but individuals in the heterozygous state are asymptomatic carriers due to the phenomenon of X-chromosome inactivation. Such women show an intermediate state of the patients with enzyme deficiency and healthy individuals in terms of enzymatic activity [14]. In fact, these individuals have two cell populations a number of which are normal cells and some of which has enzyme deficiency [60].

In fact, heterozygous women are biochemically and clinically important for G6PD deficiency. During sudden and acute hemolysis that occurs in carrier women, the results of both quantitative and fluorescence tests will be normal, despite the presence of G6PD deficiency, because these tests only measure the enzymatic activity in live RBC. In conditions where intravenous acute hemolysis is observed, and we suspect of the presence of G6PD deficiency, the use of any antioxidant drugs should be discontinued immediately and laboratory tests should be performed 10-15 days later. Also in conditions where blood was transfused to the patient, tests must be conducted several days after blood transfusion. In these cases, genetic analysis and study of family tree can help the final diagnosis [28]. G6PD activities should be measured on blood without leukocytes due to the involvement of G6PD of leukocytes with the desired results, especially in leukocytosis conditions. The best anti-coagulation to conduct G6PD activity measurement test is K2-EDTA. However, ACD, CPD, Heparin and sodium citrate can also be used. In the presence of these anticoagulants, G6PD activity will remain stable at 4°C for 72 hours. To prevent reduction of G6PD activity, the tested samples should be carried in heat insulation cover and within ice container. In situations where blood is collected in tubes containing ammonium oxalate, the G6PD activity measurement will be reliable for 12 hours at 4°C [61]. Some laboratory parameters can affect the measurement of G6PD activity, including RBC count and reticulocyte, indirect bilirubin and total plasma, plasma iron, lactate dehydrogenase, serum ferritin, serum haptoglobin and urinary hemoglobin concentrations. Also, consideration of factors such as normal spectrum, consistency of results with age, sex and demographics can be useful for testing [28].

Molecular diagnosis

Molecular analysis can be used for population screening, family studies and prenatal diagnosis. The greatest importance of molecular tests to analyze G6PD is in heterozygous females. Advanced molecular techniques that are being used today include amplification refractory mutation system (ARMS), gel electrophoresis, probe melting curve, Micro array, HPLC, matrix-assisted laser desorption/ionization-time of flight mass spectrometry, Reverse dot blot, the single base extension assay and analysis of fragment length obtained from RFLP by microcapillary electrophoretic chips [62-70]. By investigating specific G6PD mutations, a good description of clinical phenotypes and epidemiological information can be presented regarding the different geographical distribution of genetic variants. For the molecular diagnosis of G6PD deficiency in the laboratory, two analytical stages should be used: 1. PCR with RFLP helps accurate screening of common mutations in certain geographical areas [70, 2]. Sequencing of the gene helps to identify new mutations with low prevalence [28].

Discussion

This article has tried to review biochemistry, genetics and molecular diagnostics solutions of G6PD deficiency. A comparison was also conducted between the prevalence of the allelic variants of G6PD gene in different regions of Iran and other countries. Various studies suggest the significant incidence of G6PD deficiency in Iran, particularly the Mediterranean variants and Chatham. While in Cambodia Viangchan Variant, and in Myanmar Mahidol variant is considered as the most dominant variant. However, in Brazil and Mexico, G6PDA- variant is the most common variant.

In India, Mediterranean variant is more prevalent than other variants [40-45]. This significant prevalence of G6PD deficiency in Iran necessitates for more attention of health and medical officials to this disease. In this regard, genetic counseling and health trainings are important to prevent the birth of sick babies, especially in families with a history of this disease [71]. No doubt, this high prevalence also suggests the importance of consideration of planning for early diagnosis and treatment of affected individuals. It seems that despite various diagnostic studies in recent years, many cases of G6PD deficiency have not been yet diagnosed. In this regard, the subject of diagnosis for early treatment and prevention from the birth of sick babies should be a priority. In residents of
areas which are composed of different races due to economic, political and cultural factors, such as South and South West provinces of the country, variants will be more diverse and this will face the treatment with several problems [57]. The use of molecular diagnostic methods in screening asymptomatic women who are carriers of the disease genes, contributes substantially to prevention from the birth of sick baby. Therefore, it is recommended to use molecular diagnostic methods in areas where there is high prevalence of the disease.

Also it is important to consider some points to control the disease complications. In case of clinical complications related to G6PD deficiency, the disease should be confirmed by enzymatic quantitative assessment using spectrophotometry. If there is a need to implement a population screening program, a semi-quantitative fluorescent spot test can be used before performing spectrophotometry. If G6PD deficiency was determined, the patient should avoid exposure to any drug or chemical oxidants. Patients should also be trained to take the necessary measures at occurrence of acute hemolysis. It is recommended to use diagnostic tests to identify and screen babies who born in families with a history of disease or in certain geographic areas or show symptoms of acute hemolysis [2].

**Authors’ Contributions**

Ali Dehghani Fard: Gathering data, review and writing
Yousef Mortazavi: Review, writing and editing
Najmaldin Saki: Review and writing
Majid Farshdusti Hagh: Review and writing

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