Molecular Characterization of G6PD Deficient Variants in Nineveh Province, Northwestern Iraq

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Abstract Glucose-6-phosphate dehydrogenase (G6PD) deficiency considered to be the commonest inherited enzymopathies disorders worldwide including Iraq. Studies have addressed its prevalence and molecular characterization in several parts of the country, but no data were available from Nineveh province, northwestern-Iraq regarding molecular basis of this inherited enzymopathy. To determine the molecular basis of G6PD deficient variants in Nineveh province. A total of 61 G6PD deficient male individuals from Nineveh province were enrolled in this study. DNA from all enrolled individuals were extracted and analyzed for four deficient molecular variants using a polymerase chain reaction–restriction fragment polymorphism method. These deficient variants were G6PD-Mediterranean (563 C→T), G6PD-Chatham (1003 G→A), G6PD-A-(202 G→A) and G6PD-Cosenza (1376 G→C). Also enrolled individuals were screened for silent 1311 (C→T) mutation. It was found that 46 (75.41 %) were G6PD-Mediterranean, 1(1.64 %) were G6PD-Chatham, another 1(1.64 %) were G6PD-A-, and 13 (21.31 %) were remained uncharacterized. Also all G6PD-Mediterranean as well as one uncharacterized individuals were carriers of silent 1311 (C→T) mutation. This study documented that G6PD-Mediterranean constitute the bulk of G6PD deficient variants in this province and G6PD-Chatham and A- were encountered less frequently. Also that

silent 1311 (C→T) mutation were common among G6PD-Mediterranean deficient variants individuals.

Keywords G6PD · G6PD-Mediterranean · Nineveh · Iraq

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is one of the essential housekeeping enzymes that protect red blood cells from oxidant agents through generation of reduced NADPH, via the pentose phosphate shunt. The G6PD gene is X-linked and there are numerous mutations that cause a deficiency of this enzyme in the erythrocytes. G6PD deficiency usually results in intravascular hemolysis (anemia and jaundice) following the exposure to certain oxidant agents including: drugs, fava beans or following stress induced by infection. Functionally severe variants cause hereditary non-spherocytic hemolytic anemia [1].

Different mutations, each characteristic of certain populations had been characterized at the molecular level. The most common variants include: G6PD-Mediterranean (563 C→T) found in Southern Europe, the Middle East and in the Indian subcontinent and G6PD A-(202 G→A) found in Africa and in those of African origin, while other variants are common in Asia. Genetic variability of G6PD has played an important role in the understanding of a variety of development processes [1].

G6PD deficiency is quite prevalent in many countries in the Eastern Mediterranean areas, including Iraq [2–9] and several studies from Iraq had addressed the prevalence and the molecular basis of G6PD deficiency in Baghdad, Duhok, Sulaimania and middle Euphrates provinces; [6–10] however none are available from the northwestern province of Nineveh. The current study aimed at addressing the

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Table 1 The primer sequences, exons amplified and the restriction enzymes used in the PCR/RFLP procedures to detect five G6PD deficient mutations and the silent one

Variant	Primers	Exon amplified	Restriction Enzyme	Refs.
Mediterranean (563 C→T)	5' GGT GAG GCT CCT GAG TAC CA 3' 5' AGC TGT GAT CCT CAC TCC CC 3'	VI	MboII	[13]
Chatham (1003 G→A)	5' CAA GGA GCC CAT TCT CTC CCT T 3' 5' TTC TCC ACA TAG AGG AGG ACG GCT GCC AAA GT 3'	IX	BstXI	[14]
A-(202 G→A)	5' CGT GTC CCC AGC CAC TTC TA 3' 5' CAC GCT CAT AGA GTG GTG GG 3'	III–V	NlaIII	[14]
Cosenza (1376 G→C)	5' GCA GCC AGT GGG ATC AGC AAG 3' 5' GGC AAG GAG GGT GGC CGT GG 3'	XI–XIII	Bsu361	[14]
Silent (1311 C→T)	5' TGT TCT TTC AAC CCC GAG GAG T 3' 5' AAG ACG TCC AGG ATG AGG TGA TC 3'	X–XI	Bcl I	[15]

molecular basis of G6PD deficient variants in random males in the later province, and compares the results with those from other part from Iraq and surrounding countries.

Materials and Methods

Nineveh is a large province in Northwestern Iraq that borders on Syria. It has a population of about 3.5 millions consisting of different ethnic groups (Arab, Kurd, Turkmen and Assyrian) [11]. This study was conducted between May 2012 and May 2013.

Sixty one G6PD-deficient individual were enrolled in this study. Forty seven of the enrolled cases were detected following screening 806 males attending public health laboratories in Nineveh province for routine premarital investigations, the diagnosis of G6PD deficiency was based on methemoglobin reduction test followed by confirmation by a quantitative kinetic enzyme assay (Biolabo-France) [9]. The other 14 males presented with acute hemolytic process following fava bean ingestion as ascertained by history. No cases with hemolysis secondary to infections or oxidant drug exposure were found in our study.

Informed consent was taken from all enrollees and the study was approved by the appropriate ethical committees at the Mosul and Duhok colleges of Medicine, Iraq.

A 2 ml sample was collected in K2-EDTA tubes from each enrollee during screening and kept frozen at -40°C for DNA study. DNA was extracted by a modified salting out extraction (that yield high quantity with high purity DNA) [12] from specimens of patients determined to be G6PD deficient by the above procedures. The extracted DNA was then screened sequentially for four G6PD deficient mutations commonly encountered in the region depending on the available data from the surrounding countries namely: G6PD Mediterranean (563 C→T), G6PD Chatham (1003 G→A), G6PD A-(202 G→A) and G6PD Cosenza (1376 G→C) as well as for the presence of the

nucleotide (nt) 1311(C→T) polymorphism using restriction fragments length polymorphism technique following DNA amplification by polymerase chain reaction (PCR/RFLP). The primers, restriction enzymes and procedural details used for each of these reactions were as detailed elsewhere (Table 1) [13–15].

Finally statistical analysis performed with the Mann–Whitney test whenever appropriate. A $p < 0.05$ was considered significant.

Results

Enzyme levels among the 47 G6PD deficient subjects picked by premarital screening ranged from 0.11 to 1.38 IU/gHb (Mean $\pm$ SD = 0.53 ± 0.27 IU/gHb) (Normal range in our Laboratory 10.1–14.1 IU/gHb) and these levels were significantly lower than enzyme levels in those with favism (ranged from 0.56 to 3.38 IU/gHb (mean $\pm$ SD = 1.13 ± 0.32) ($p_{\text{value}} = 0.036$).

Following amplification with specific primers and digestion with specific enzymes, variable patterns seen among healthy (normal non deficient controls) and affected individuals as illustrated in Table 2 [13, 14, 16].

Based on the specific patterns, following amplification and enzyme digestion, G6PD Mediterranean (563 C→T) was the most common, being detected in 46/61 individuals (75.41 %) and G6PD Chatham (1003 G→A) and G6PD A-(202 G→A) were detected in 1/61 (1.64 %) each, while no cases with Cosenza were identified, leaving the remaining 13/61 (21.31) cases uncharacterized. The mean enzyme level of patient with G6PD-Mediterranean was lower (0.91 ± 0.33 IU/gHb) than those with other mutations including G6PD Chatham (1.38 IU/gHb), G6PD A-(1.29 IU/gHb) and the uncharacterized cases (1.87 ± 0.22 IU/gHb).

Clinically all 14 patients presenting with favism as well as 10/47 individuals with G6PD deficiency detected during screening, who gave a previous suggestive history of

Table 2 Restriction patterns and PCR of normal and mutant subjects

G6PD variant	Fragment size (bps)		Refs.
	PCR product	Restriction patterns	
		Wild type mutant type	
Mediterranean (563 C→T)	267	267 151 116	[13]
Chatham (1003 G→A)	208	130 100 78 78 30	[14]
A-202 (202 G→A)	1130	419 419 349 226 180 180 102 123 80 102 80	[13]
Cosenza	548	548 316 232	[14]
nt 1311 polymorphism (C→T)	202	202 180 22	[16]

Table 3 Results of 5 PCR/RFLP variants

Variant	No. (%)	nt 1311 (T) polymorphism
G6PD-Mediterranean	46 (75.41 %)	46/46 (100 %)
G6PD-Chatham	1 (1.64 %)	0
G6PD-A ⁻	1 (1.64 %)	0
Uncharacterized	13 (21.31 %)	1/13

hemolysis, turned out to be G6PD-Mediterranean, while the remaining 22 patients with G6PD-Mediterranean and all individuals with other mutations, including uncharacterized cases, gave no history of hemolysis and they were completely unaware of their deficiency state.

Regarding nucleotide (nt) 1311(C→T) polymorphism, all cases with G6PD Mediterranean (563 C→T) as well as 1 uncharacterized case were carriers for thiamine (T) at nt 1311 (Table 3), while the remaining 14 cases were carriers of cytosine(C) at nt 1311.

Discussion

G6PD deficiency has been recognized as one of the commonest inherited hematological disorder throughout the world including Iraq [1–3]. Previous data from Nineveh province revealed that frequency of G6PD deficiency is 5.8 % among males [9]. Despite the high frequency of this

disorder in the region, no study had ever focused on its molecular basis. The latter maybe helpful for population studies, consideration of some therapeutic drugs especially anti-malarial drugs, investigating patients with variable degrees of hemolysis and complicated cases like combined G6PD deficiency and sickle cells diseases that are common in the region, as well as for prenatal diagnosis as they required smaller sample in comparison to biochemical enzyme assay; [17] the current study is the first to tackle such an issue in the province.

The Mediterranean variant (563C→T) was found to be the most frequent variant in many parts of the world including Mediterranean countries, Middle East and Indian subcontinent with figures reaching to 97 % in Kurdish Jewish, [16–21]. This variant is the most common variants in other parts of Iraq [6–8]. Our figures were similar to those reported in Baghdad and Sulaymaniyah, but lower than those of Duhok and the middle Euphrates provinces [6–8, 10]. The wide distribution of the Mediterranean mutation within this region suggests that this mutation is quite ancient and may have spread by migrations that had taken place over millennia [16].

G6PD Chatham (1003, G→A) was found in only 1(1.64 %) of our deficient individuals and this figure is lower than those from other parts of Iraq [6–8] and also lower than many neighboring countries including Iran and most Eastern Mediterranean countries [15, 22].

The African A- variant (202 G→A) was also found in only 1 (1.64 %) of our deficient individuals and this is comparable to that from Baghdad, but lower than those reported from the middle Euphrates provinces and most of the Arab Mediterranean countries where rates up to 46 % were reported in Algeria [10].

Although G6PD-Cosenza had been reported at polymorphic frequency in some Middle East countries [15], however it was not identified in any case in the current study and the remaining 13 cases remained uncharacterized, and this may be due to some other rare mutations or may be due to a novel mutation that is unique to this area. The uncharacterized cases require further studies using sequencing techniques.

Clinical hemolysis was documented more commonly among patients with G6PD-Mediterranean variant and this is mainly attributed to the lower enzyme levels and to some extent to the larger number of patients detected with this variant.

All individuals with G6PD-Mediterranean variant as well as one case who remained uncharacterized were carriers for nt 1311 (T) polymorphism and this is comparable to that reported from Duhok, Baghdad and from some nearby countries as 1311 (C→T) nucleotide polymorphism may be found at polymorphic frequency in otherwise normal G6PD alleles and in nearly 100 % of G6PD-

Mediterranean chromosomes amounts to a non-random association of the two mutant loci, that descend from single ancestral chromosome (linkage disequilibrium) [16, 23].

In conclusion, this study has confirmed that G6PD Mediterranean is the most common G6PD deficient variant, followed by G6PD-Chatham and G6PD-A-, which is consistent with other studies in the country and most neighboring countries. Further studies on uncharacterized variants should be the next step and may uncover novel G6PD deficient molecular variants.

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