Molecular Homogeneity of G6PD Deficiency

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Objectives: To study the molecular basis of G6PD deficiency in the Kingdom of Bahrain. Further emphasis will be presented on the genetic polymorphism at nucleotide 1311 for both normal and deficient subjects.

Methods: DNA extraction was done for 83 G6PD-deficient subjects and 80 normal controls. A combination of PCR-RFLP and PCR-DGGE procedures were employed to uncover the sequence variations at nt 563 (C/T) (G6PD Mediterranean) and at nt 1311 (C/T) polymorphism in both subjects with deficient and normal G6PD activity.

Results: Ninety-one percent (93/102) of the X chromosomes from G6PD deficient patients had nt 563 (C-T) mutation (G6PD Med), whereas ~9% of the X chromosomes from G6PD deficient subjects might have other G6PD variant(s) (or normal X chromosomes in heterozygote females). Niney-six percent (89/93) of the G6PD Med-bearing X chromosomes showed thymine (T) at nucleotide position 1311. In contrast, 70% (82/117) of the normal X chromosomes showed cytosine (C) at nucleotide position 1311, while it was thymine (T) in 30% (35/117) of the normal X chromosomes.

Conclusion: The vast majority (91%) of X chromosomes from G6PD-deficient subjects in Bahrain are harboring nt 563 (C-T) mutation (G6PD Mediterranean). The G6PD Med variant in Bahrain is in tight linkage disequilibrium with thymine (T) at nt 1311. These data, collectively, revealed high moleculer homogeneity of G6PD deficiency in Bahrain.

Further studies are needed to uncover factor(s) contributing to heterogeneous phenotypic expression of the disease in Bahrain.

Glucose-6-phosphate dehydrogenase (G6PD) is the key enzyme in the pentose monophosphate shunt and provides the NADPH (reduced form of Nicotinamide Adenine Dinucleotide Phosphate), which is essential for a number of biosynthetic and detoxifying reactions[1]. Indeed, NADPH has an essential function in protecting red

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blood cells (RBCs) against oxidative stress and, therefore, the G6PD has a pivotal role in protecting red blood cells from oxidative damage[2].

G6PD deficiency is the most common enzymopathy in man and it is estimated to affect over 400 million people worldwide[3]. The frequency of G6PD deficiency varies widely among world population with high frequency being reported from some regional countries. For instance, it has been found at a frequency of 25% in Omanis male population, while it has been reported with the highest frequency in Qatif oasis of Eastern Saudi Arabia, in which 65% of the male population is affected[4,5]. In the Kingdom of Bahrain, a previous phenotypic screening study showed high prevalence of G6PD deficiency involving up to 26.4% amongst male blood donors[6].

Interestingly, this enzymopathy has been found to correlate geographically with global distribution of falciparum malaria (a virulent form of malaria due to infection with *Plasmodium falciparum* parasite). A previous study gave further evidence for the protective role played by G6PD deficiency against malaria in endemic regions in both heterozygote females or hemizygote males[7].

Favism is an acute hemolytic anemia induced by ingestion of fava bean (broad bean). It is by far the most common clinical manifestation of G6PD deficiency. Patients with favism are always G6PD deficient, however, not all G6PD-deficient individuals would develop hemolysis by ingesting fava beans. This indicates that G6PD deficiency is necessary, but is not a sufficient cause of favism. Presumably some other factor(s), including genetic, might be involved in this phenotypic heterogeneity[8]. Another clinical aspect of G6PD deficiency is neonatal jaundice (NNJ), which is one of the life-threatening consequences in neonates with G6PD deficiency. This clinical manifestation seems likely to be attributed to impairment of liver function, rather than hemolysis of red blood cells (RBCs), in the G6PD-deficient neonates[8].

The G6PD enzyme exists, in its active form, as a dimer (or tetramer) each of which consists of 515-amino acid polypeptide subunits (indeed 514 amino acids since the first N-terminal Met is not present in the mature protein)[2,9]. Each dimer contains tightly bound NADP that plays both structural and functional roles. The enzyme is encoded by the G6PD gene, which is located on the long arm of the X-chromosome (Xq28) and spans over 18 kilobases (kb) consisting of 13 exons (coding sequences) and 12 introns (non-coding sequences) (Fig 1). The first exon contains no coding sequences, while intron number 2 is extraordinarily long extending for about 10 kb[10,11].

Figure 1. Scehmatic diagram showing chromosomal locus of the G6PD gene at Xq28. Black boxes represent exons (coding sequences) and introns (non coding sequences) represented by interconnected lines. Positions are indicated of nt 563 (C-T) mutation (G6PD Med), and nt 1311 (C-T) in exons 6 and 11, respectively

Molecular analysis of G6PD gene revealed more than 100 different mutations or combination of mutations that cause G6PD deficiency, and the vast majority of these variants are sporadic, rather than polymorphic. However, each population has a characteristic profile of polymorphic G6PD variant(s)[12]. For example, G6PD A⁻ (nt 202 G-A and nt 376 A-G; V68M and N126D) is almost exclusively the only cause of G6PD deficiency in African populations, whereas G6PD Mediterranean variant (nt 563 C-T; S188F) predominates in the Mediterranean, Middle East, and parts of India[1,13].

Study of the complete DNA sequence of the G6PD gene revealed a number of additional "silent" polymorhic sites, for example, not changing amino acid sequences of the enzyme. These polymorhic sites (altogether) create haplotypes that have been useful in establishing origin and homogeneity in which various mutations have arisen. One such silent polymorphism is at nucleotide (nt) position 1311 (C-T) in exon 11 which would not produce any substitution at the amino acid level and has been found to be quite prevalent in various populations[14].

To study the molecular basis of G6PD deficiency in the Kingdom of Bahrain. Further emphasis will be presented on the genetic polymorphism at nucleotide 1311 for both normal and deficient subjects.

METHODS

Total of 83 G6PD-deficient subjects (64 males and 19 females) and 80 normal controls (43 males and 37 females) were enrolled in this study. All subjects were Bahraini natives and had been recruited through a Student Screening Project for hemoglobinopathies and G6PD deficiency[15].

G6PD activity was assessed in red blood cells by a semi-quantitative method using a dye decolourisation test procedure as described before[16].

Detection of nt 563 (C-T). DNA was prepared from peripheral blood leukocytes by standard procedures. The method for detection of G6PD Mediterranean variant was, principally, as described before[17]. Modifications of the method included the following: 330-bp fragment encompassing merely exon 6 of the G6PD gene was PCR-amplified from genomic DNA by using the newly designed primers: G6PD 6.1 (5'GTCTGAATGATGCAGCTGTGA3') and G6PD 6.2 (5'TTCTGGAGGAATTCGTCCTC3').

RESULTS

Enzyme activity. Eighty-three G6PD-deficient subjects (64 males and 19 females) enrolled in this study showed severely reduced enzyme activity in their red blood cell lysates, while eighty normal subjects (43 males and 37 females) revealed normal activity of the enzyme.

DNA analysis for nt 563 (C-T) mutation. This mutation was identified in 93 of the 102 of the X chromosomes (91.2%) from G6PD-deficient subjects. In addition, five G6PD-deficient males showed normal gel profile for nt 563 (C-T) mutation, whereas four deficient females revealed heterozygote gel profile for this mutation. Hence, a

total of 9 of the 102 of the X chromosomes studied from G6PD-deficient subjects (8.8%) revealed normal gel profile for this mutation (Table 1).

DNA analysis for nt 1311 (C-T). Nucleotide 1311T silent polymorphism was found to be associated with nt 563 (C-T) mutation in 89 of the 93 X chromosomes (95.7%) that harbor this mutation. Nucleotide 563 (C-T) mutation was found to be associated with cytosine in two X chromosomes (2.15 %), however two other X chromosomes (2.15 %) could not be ascertained whether having cytosine or thymine at nt position 1311 (Table 2).

Table 1. Prevalence of G6PD Mediterranean variant (nt 563 C-T) in Bahraini patients having G6PD deficiency phenotype.

No. of Patients	Sex	G6PD Activity	No of X Chromosomes	
			G6PD Med variant	Other G6PD variant(s)
64	M	Reduced	59	5
19	F	Reduced	34	4*
		Total (%)	93 (91.2)	9 (8.8)

* 4 G6PD deficient females showed heterozygosity for G6PD Med variant; either they might be compound heterozygous for G6PD Med and other variant, or just a simple heterozygous for the G6PD Mediterranean variant.

Table 2. **Linkage of the mutation at nt position 563 (C-T) (G6PD Med) with the silent polymorphism at nt position 1311 (C-T) in Bahrain**

G6PD Med Variant (No. of Chromosomes)	Nt 1311 (C/T)	%
89	T	95.7
2	C	2.15
2*	C/T	2.15
Total: 93		100

*Heterozygous females for G6PD Med variant, family studies are needed to ascertain linkage with "C' or "T".

Subjects with normal phenotype for G6PD activity revealed different distribution of the polymorphism at nt position 1311. Eighty-two of the 117 normal X chromosomes (70%) showed cytosine at nt position 1311, while it was thymine in 35 of the X chromosomes (30%) (Table 3).

Table 3. **Polymorphism at nucleotide position 1311 (C/T) in G6PD-normal X chromosomes in various populations**

Population Origin	No. of X Chromosomes	No. of 1311C	No.of 1311T	1311T (%)	Reference
Bahrain	117	82	35	30	Present study
Saudi Arabia	14	14	2	14.3	17
Oriental	59	56	3	5.1	14
Central/South American	30	27	3	10	14
White non-Jewish	68	59	9	13.2	14
White Jewish	41	32	9	22	14
Sicilians	18	15	3	16.7	14
African	20	15	5	25	14
Indian (Asian)	20	11	9	45	14

Table 4. **Prevalence of G6PD deficiency in various Middle Eastern countries.**

Country	Prevalence (%)	References
Bahrain	26.4	6
Saudi Arabia	2-65	3,18
Kuwait	19	19
Iraq	6-13	20
Jordan	2	21
Lebanon	3	22
Egypt	1-26.4	25,23
Oman	25-27.3	4,24
Iran*	11.5	25

Iranian living in Kuwait.

DISCUSSION

G6PD deficiency is quite prevalent in the Middle East and exists in several forms throughout the region (Table 4). Moreover, the highest frequency of G6PD deficiency has been reported from Saudis (Qatif oasis) where 65% are afflicted[5]. Bahrain is ranking high at 26.4% among blood donors (Table 4). A previous phenotype study from Bahrain presumed, on the basis of very low enzyme activity and B-like electrophoretic mobility, that severe G6PD deficiency in this country would most likely to be due to G6PD Mediterranean variant[26]. This is the first study to uncover

the molecular basis of G6PD deficiency in Bahrain. In this study we found marked molecular homogeneity of G6PD deficiency in Bahrain. Ninety-one percent of the X chromosomes from subjects having G6PD deficiency phenotype revealing that they have nt 563 (C-T) (G6PD Med) mutation as the sole molecular lesion causing G6PD deficiency (Table 1). In addition, polymorphism at nt position 1311 was found to be thymine in the vast majority (96%) of G6PD Med-bearing X chromosomes from Bahraini patients (Table 2). This indicates an overwhelming homogeneity of G6PD Med mutation in Bahrain is known to exist in linkage disequilibrium (refers to the fact that particular alleles at nearby sites can co-occur on the same haplotype more often than expected by chance) with two haplotype background; one of them represented by cytosine at nt 1311, whereas the other haplotype showing thymine at nt 1311[14,17]. However, this silent mutation at nt 1311 was shown to be quite prevalent even on X chromosomes with normal G6PD activity in various populations (Table 3). The fact that this polymorphism was represented in 30% of G6PD-normal chromosomes, but in nearly 96% of G6PD-Mediterranean chromosomes here in Bahrain implies, as it was observed before for other Middle Eastern populations, a marked linkage disequilibrium between G6PD Med mutation and thymine at nt 1311[17]. This might indicate, in its simple interpretation, a unicentric origin of the G6PD-Med mutation onto a G6PD gene that already had nt 1311 mutation[17]. Absence of nt 1311 mutation (that is cytosine at nt 1311; for example, 2.15% of our G6PD-Med chromosomes) might be explained by intragenic recombination event or population admixture. Alternatively, existence of G6PD Mediterranean mutation on two haplotype background might suggest a recurrence of this mutation, independently, on two different genetic background.

CONCLUSIONS

This study revealed high molecular homogeneity of G6PD deficiency in Bahrain attributed mainly to the G6PD Mediterranean variant (nt 563 (C-T)). Further studies are needed to uncover factor(s) contributing to heterogeneous phenotypic expression of the disease.

REFERENCES

1. Luzzatto L, Mehta A. Glucose-6-phosphate Dehydrogenase. In: Scriver CR, Stanbury JB, Besudet AL, eds. The Metabolic and Molecular Bases of Inherited Disease. New York: McGraw-Hill, Inc. 1995:3367-98.
2. Mason PJ. New Insights into G6PD Deficiency. Br J Haematol 1996;94:585-91.
3. Beutler E. The Genetic of Glucose-6-phosphate Dehydrogenase Deficiency. Semin Hematol 1990;27:137-164.
4. Al-Riyami A, Ebrahim GJ. Genetic Blood Disorders Survey in the Sultanate of Oman. J Trop Pediatr 2003;49[Suppl 1]:11-20.
5. Gelpi AP. Glucose-6-phosphate Dehydrogenase Deficiency, the Sickling Trait and Malaria in Saudi Arabian Children. J Pediat 1967;71:138-46.
6. Bhagwat GP, Bapat JP. Glucose-6-phosphate Dehydrogenase Deficiency in Bahrain Blood Donors. Bah Med Bull 1987;9:120-2.
7. Ruwende C, Khoo SC, Snow RW, et al. Natural Selection of Hemi- and Heterozygotes for G6PD Deficiency in Africa by Resistance to Severe Malaria. Nature 1995;376:246-9.

8. Beutler E. G6PD Deficiency. Blood 1994;84:3613-36.

9. Persico MG, Viglietto G, Martini G, et al. Isolation of Human Glucose-6-phosphate Dehydrogenase (G6PD) cDNA Clones: Primary Structure of the Protein and Unusual 5-Prime Non Coding Region. Nucleic Acids Res 1986; 14: 2511-22.

10. Martini G, Touiolo D, Vulliamy T, et al. Structural Analaysis of the X-linked Gene Encoding Human Glucose-6-phosphate Dehydrogenase. EMBO J 1986; 5:1849-55.

11. Chen EY, Cheng A, Lee A, et al. Sequence of Human Glucose-6-phosphate Dehydrogenase Cloned in Plasmids and a Yeast Artificial Chromosome. Genomics 1991;10:792-800.

12. Beutler E, Vulliamy T, Luzzatto L. Hematologically Important Mutations: Glucose-6-phosphate Dehydrogenase. Blood Cells, Molecules, and Diseases 1996;22:49-56.

13. Al-Ali AK, Al-Mustafa ZH, Al-Madan M, et al. Molecular Characterization of Glucose-6-phosphate Dehydrogenase Deficiency in the Eastern Province of Saudi Arabia. Clin Chem Lab Med 2002;40:814-6.

14. Beutler E, Kuhl W. The Nt 1311 Polymorphism of G6PD: G6PD Mediterranean Mutation May Have Originated Independently in Europe and Asia. Am J Hum Genet. 1990;47:1008-12.

15. Al Arrayed SS, et al. Student Screening for Genetic Blood Disorders in Bahrain. Eastern Mediterranean Health Journal. In press.

16. Motulsky AG, Campbell-Kraut JM. Population Genetics of Glucose-6-phosphate Dehydrogenase Deficiency of the Red Cells. In: Blumberg BS (ed). Proceedings of Conference on Genetic Polymorphisms and Geographic Variations in Disease. New York: Grune & Stratton Inc, 1961:159-180.

17. Kurdi-Haidar B, Mason PJ, Berrebi A, et al. Origin and Spread of the Glucose-6-phosphate Dehydrogenase Variant (G6PD-Mediterranean) in the Middle East. Am J Hum Genet 1990;47:1013-9.

18. Warsy AS, El-Hazmi MAF. Glucose-6-phosphate Dehydrogenase Deficiency in Saudi Arabia-A Review. Saudi Med J 1987;8:12-20.

19. Shaker Y, Onsi A, Aziz R. The Frequency of Glucose-6-phosphate Dehydrogenase Deficiency in the Newborns and Adults in Kuwait. Am J Hum Genet 1966;18:609-13.

20. Amin-Zaki L, El-Din ST, Kubba K. Glucose-6-phosphate Dehydrogenase Deficiency Among Ethnic Groups in Iraq. WHO Bull 1972; 47:1-5.

21. Banerjee B, Saha N, Daoud ZF, et al. A Genetic Study of the Jordanians. Hum Hered 1981;31:65-9.

22. Der Kaloustian VM, Naffah J, Loiselet J. Genetic Diseases in Lebanon. Am J Med Genet 1980;7:187-203.

23. Ragab AH, El-Alfi OS, Abboud AM. Incidence of Glucose-6-phosphate Dehydrogenase Deficiency in Egypt. Am J Hum Genet 1966;18:21-5.

24. White JM, Christie BS, Nam D, et al. Frequency and Clinical Significance of Erythrocyte Genetic Abnormalities in Omanis. J Med Genet 1993;30:396-400.

25. Usanga EA, Ameen R. Glucose-6-phosphate Dehydrogenase Deficiency in Kuwait, Syria, Egypt, Iran, Jordan and Lebanon. Hum Hered 2000;50:158-61.

26. Ardati KO, Bajakian KM, Mohammed AK, et al. Glucose-6-phosphate Dehydrogenase Phenotypes in Bahrain: Quantitative Analysis and Electrophoretic Characterization. Saudi Med J 1995;16:102-4.