

G6PD DEFICIENCY, DISTRIBUTION AND VARIANTS IN SAUDI ARABIA: AN OVERVIEW

Arjumand S. Warsy, PhD; Mohsen A.F. El-Hazmi, MBBChir, PhD, FRCPath, FACB

Background: The first report of glucose-6-phosphate dehydrogenase (G6PD) deficiency in the Saudi population of the Eastern Province paved the way for extensive investigations to determine the distribution and molecular pathogenesis of G6PD deficiency in Saudis in different parts of the country.

Materials and Methods: During a national study lasting from 1982 to 1993, 24,407 Saudis in 31 different areas of Saudi Arabia were screened for G6PD deficiency using spectrophoretic estimation of the enzyme activity and electrophoretic separation of the phenotypes.

Results: The results in the males and females were separately analyzed, and showed a statistically significant difference in the frequency in the male (0.0905) and female (0.041) population ($P < 0.05$). The frequency in the male varied from 0 to 0.398, and in the female from 0 to 0.214. The phenotypes identified included G6PD-A⁺, G6PD-Mediterranean and G6PD-A⁻, and G6PD-Med-like with G6PD-B⁺ as the normal phenotype in all areas.

Conclusion: This study shows that G6PD deficiency is a frequently identified single-gene disorder in Saudi Arabia and G6PD-Mediterranean is the major variant producing the severe deficiency state in this population. *Ann Saudi Med* 2001;21(3-4):174-177.

Key Words: G6PD deficiency, hemolytic anemia, polymorphism.

Glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) is the first enzyme of the hexose monophosphate shunt and catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconolactone, with the concomitant reduction of NADP⁺ to NADPH.¹⁻³ The gene for G6PD is located on the long arm of the X-chromosome and is inherited as an X-linked recessive trait.⁴ The G6PD gene is highly polymorphic, and over 300 variants resulting from single-point mutations are known to exist in the different populations of the world.⁵ Several variants have significantly reduced activity and result in a condition referred to as "G6PD deficiency."³⁻⁶ The state is inherited as an X-linked recessive state, and presents one of the most frequently encountered red-cell enzymopathies. The major interest in the G6PD-deficient state results from the associated hemolytic anemia resulting from oxidative stress.⁶

The first report of G6PD deficiency in Saudi Arabia dates back over three decades when, in 1965, Gelpi⁷ reported the presence of this state in different villages in the Eastern Province of Saudi Arabia. In the following years, reports of G6PD deficiency in other provinces of Saudi Arabia were published,⁸⁻²¹ and showed the occurrence of

G6PD deficiency at a high though variable frequency. We conducted a nationwide study in different regions in an attempt to investigate the distribution pattern in different parts of the Kingdom. This paper summarizes our findings on the distribution and molecular aspects of G6PD deficiency in Saudis.

Materials and Methods

The study group included randomly selected males and females living in different provinces of Saudi Arabia, with ages ranging from 2-70 years. There were 13,796 males and 13,613 females, from whom 5.0 mL blood samples were collected by venipuncture in acid-citrate dextrose (ACD) tubes. The whole blood was used to determine the hematological parameters using Coulter Counter ZF6, with a hemoglobinometer attachment. The blood was centrifuged to separate the plasma from the cells. The red cells taken from the bottom of the tube were hemolyzed with cold distilled water and the fresh hemolysate was used to determine the G6PD activity spectrophotometrically, using kits from Boehringer Mannheim Diagnostica. The G6PD unit was defined as millimoles of substrate converted to product per minute at the specified pH and temperature. The activity was expressed as mU/10⁹ erythrocytes.⁵

Another sample of the fresh hemolysate was subjected to cellulose acetate electrophoresis using Titan III Plates (Helena Cat. No. 3023) and Supra Heme buffer at pH 8.6 for 20 min. at 350V (Helena Cat. No. 5802). Visualization of G6PD band was carried out by specific staining for G6PD using G6PD staining reagent (Helena

From the Departments of Medical Biochemistry (Prof. Dr. El-Hazmi), College of Medicine and King Khalid University Hospital, and Biochemistry (Prof. Warsy), College of Science, King Saud University, Riyadh, Saudi Arabia.

Address reprint requests and correspondence to Prof. Dr. El-Hazmi: Department of Medical Biochemistry & WHO Collaborating Center for Hemoglobinopathies, Thalassemias and Enzymopathies, King Khalid University Hospital, P.O. Box 2925, Riyadh 11461, Saudi Arabia.

Accepted for publication 18 May 2001. Received 18 February 2001.

TABLE 1. Frequency of G6PD deficiency in different provinces of Saudi Arabia.

Province	Male	Female
Northwestern	0.0850	0.0527
Southwestern	0.1240	0.0504
Central	0.0270	0.0125
Eastern	0.2546	0.1246
Northern	0.0147	0.0078

TABLE 2. Frequency of G6PD deficiency in different areas of Saudi Arabia.

Province	Male	Female
Northern		
Hail	0.0171	0.0073
Tabuk	0.0146	0.0126
Arar	0.0064	0
Al-Jouf	0.0127	0.140
Northwestern		
Al-Ula	0.080	0.032
Khaiber	0.220	0.160
Yanbu	0.0179	0.0064
Makkah	0.0569	0.0423
Central		
Riyadh	0.071	0.025
Qasim	0.018	0.015
Buraidah	0.0305	0.0085
Al-Russ	0.0109	0.010
Al-Unaiza	0.0035	0
Al-Mesnab	0	0
Bakeria	0	0
Southwestern		
Qunfuda	0.1275	0.1015
Bisha	0.0767	0.054
Najran	0.057	0.006
Jizan	0.204	0.048
Sabya	0.107	0.045
Samta	0.091	0.066
Abu Areesh	0.106	0.0332
Farasan	0.027	0.029
Baish	0.026	0.0306
Fifa	0.122	0.111
Al-Baha	0.1275	0.1158
Mahayel	0.1579	0.0352
Abha	0.1597	0.0685
Eastern		
Al-Qatif	0.398	0.214
Al-Hofuf	0.2325	0.125
Hafr Al-Batin	0.084	0.043

Cat. No. 5620) for 20 min. at 20°C. After color development, the plates were fixed for 2-3 min. in 7.5% trichloroacetic acid, washed with 5% acetic acid, dried in air and stored. Scanning was carried out before fixing, using a Quick Scan Densitometer (Helena).

Results

A wide range of G6PD activity was encountered in the male and female samples. The normal reference range of G6PD was used as 60-130 mU/10⁹ erythrocytes, with a

mean of 95 for Saudi males, and 60-140 mU/10⁹ erythrocytes with a mean of 100 mU/10⁹ erythrocytes for Saudi females.²³ Individuals with severe G6PD deficiency were considered as those with G6PD activity <20% of the lower normal. Of the total 13,796 males, 1249 were deficient and among the 13,613 females screened, 558 were deficient, giving a frequency of G6PD deficiency of 0.0905 and 0.041, respectively, in the males and females. The samples from different provinces were separated and the frequency of G6PD deficiency was calculated in each province. The results are presented in Table 1. The Eastern Province had the highest frequency of G6PD deficiency, while the Northern Province had the lowest. Within each province the samples were further separated on the basis of different areas and frequency of G6PD deficiency calculated. Table 2 presents the results and shows significantly variable frequency in different areas. The phenotyping showed the normal enzyme as G6PD-B⁺ and four of its variants: 1) G6PD-Mediterranean, which was most severely deficient, with activity ranging from 10.5±4.6 mU/10⁹ erythrocytes. It had a similar mobility to G6PD-B⁺, but in most cases it was very faintly visible or invisible; 2) G6PD-A⁺ moved faster and had almost normal activity (81.3±15 mU/10⁹ erythrocytes); 3) G6PD-A⁻ moved at the same place/pace as G6PD-A⁺ but had low activity (22.6±10.6 mU/10⁹ erythrocytes); and 4) a variant with the same mobility as G6PD-B⁺, but activity ranging between 20%-60% of normal (37.5±8.1 mU/10⁹ erythrocytes) was also identified. The frequency of the different variants in the various provinces and total frequency are presented in Table 3. In all provinces, the G6PD-B⁺ was the normal enzyme, and the most frequently encountered severely deficient variant was G6PD-Med, while G6PD-A⁺ and A⁻ occurred at low frequency.

Discussion

This study is a comprehensive investigation conducted over almost 10 years to determine the frequency of G6PD deficiency and its variants in Saudis. The activity of G6PD was determined spectrophotometrically using mature red cells obtained from the bottom of the tube containing the red-cell sediment after centrifugation. This step is essential in order to avoid using reticulocytes and young red cells, which are usually rich in G6PD levels, and hence can mask G6PD deficiency. All samples with G6PD activity less than 15 mU/10⁹ RBC were classified as severely deficient. On electrophoresis, either no G6PD band was seen for these samples or a very faint band was observed. Partially deficient variants had between 12-35 mU/10⁹ RBC.

The data generated during this study showed that G6PD deficiency occurs in all provinces of Saudi Arabia, though at a variable frequency. In the Eastern Province, both males and females have the highest frequency of G6PD deficiency, followed by those in the Southwestern Province. The number of deficient females was more than the number calculated using Hardy-Weinberg equilibrium. This is believed to be due to a high rate of consanguinity in

TABLE 3. Frequency of G6PD variants in Saudis.*

Province	Male	Female
Northwestern		
B ⁺	0.8586	0.8325
A ⁺	0.0192	0.01145
A ⁻	0.0106	0.0062
Med	0.0337	0.0177
Med-like	0.0780	0.1072
G6PD*	0	0
Hetero	-	0.0249
Southwestern		
B ⁺	0.788	0.793
A ⁺	0.019	0.0086
A ⁻	0.0056	0.0043
Med	0.0994	0.0718
Med-like	0.0865	0.107
G6PD*	0.0017	0.0009
Hetero	0	0.0145
Central		
B ⁺	0.8737	0.9142
A ⁺	0.0325	0.0242
A ⁻	0.0074	0.0009
Med	0.039	0.0075
Med-like	0.0427	0.0345
G6PD*	0.0046	0.0039
Hetero	-	0.0149
Eastern		
B ⁺	0.598	0.6992
A ⁺	0.016	0.025
A ⁻	0.0107	0.015
Med	0.3551	0.1692
Med-like	0.0187	0.064
G6PD*	0.004	0.0013
Hetero	-	0.0263

*Adapted from ref. 21.

Saudis,²⁴ or a higher inactivation of normal X-chromosome in heterozygous females, thus increasing the number of deficient females and disturbing the Hardy-Weinberg equilibrium.

The normal G6PD, as in every population of the world investigated to date, is G6PD-B⁺ in the Saudis in each region, but significant interregional variations are obvious in its frequency.²⁵ In addition, the frequency of G6PD-B⁺ differs in males and females. The other normal variant found in Saudis is G6PD-A⁺, an African variant. This is a variant with a higher mobility than G6PD-B⁺, but the activity is about 90% of the normal. It occurs in Saudis but at a nonpolymorphic level in most areas. G6PD-A⁻, a deficient African variant with the same mobility as G6PD-A⁺, is also found in Saudis, but at a lower prevalence.

G6PD-Mediterranean was the most frequently encountered deficient variant in all areas of Saudi Arabia. This is the variant frequently encountered in the countries around the Mediterranean and produces a severe deficiency, resulting in hemolytic anemia under oxidative stress. It is also the variant producing favism. A variant with the same mobility as G6PD-B⁺, but activity between 20%-60%, is encountered at a high frequency in some areas

and needs to be further characterized.

The frequency of G6PD deficiency was correlated with past or present history of malaria endemicity.²⁶ In each area of Saudi Arabia where malaria had been encountered in the past or was presently endemic, the frequency of G6PD deficiency was high. This strongly supports the malaria hypothesis.²⁷⁻³¹

In conclusion, G6PD deficiency occurs frequently in several areas of Saudi Arabia, and G6PD-Mediterranean is the most frequently encountered variant producing severe G6PD deficiency. The frequency correlates with malaria endemicity, and thus it may provide a natural protection against malaria, as is the situation in several populations of the world where sickle cell gene (HbS), α - and β -thalassemias and G6PD deficiency provide a strong inborn resistance against malaria.

References

1. Beutler E. G6PD historical perspective and current status: In: Weatherall DJ, Fiorelli G, Gorin S, editors. *Advances of red blood cell biology*. New York: Raven Press, 1991:297-308.
2. Beutler E. Red cell enzyme defects. *Haematologic Pathology* 1990; 4:103-14.
3. Beutler E. Glucose-6-phosphate dehydrogenase deficiency. *N Engl J Med* 1991;324:109-74.
4. Beutler E. The molecular biology of G6PD variants and other red cell enzyme defects. *Ann Rev Med* 1992;43:47-59.
5. Beutler E, Vulliamy T, Luzzatto L. Hematologically important mutations: glucose-6-phosphate dehydrogenase. *Blood Cells Mol Dis* 1996;22:49-56.
6. Beutler E. Hemolytic anemia in disorders of red cell metabolism. New York: Plenum Press, 1978:23-168.
7. Gelpi AP. Glucose-6-phosphate dehydrogenase deficiency in Saudi Arabia. *Blood* 1965;25:486-93.
8. Gelpi AP, King MC. New data on glucose-6-phosphate dehydrogenase deficiency in Saudi Arabia. *Hum Hered* 1977;27: 285-91.
9. El-Hazmi MAF. Haemoglobin disorders: a pattern for thalassaemia and haemoglobinopathies in Arabia. *Acta Haematol* 1982;68:43-51.
10. El-Hazmi MAF. Abnormal hemoglobin and allied disorders in the Middle East - Saudi Arabia. In: Bowman JE, editor. *Distribution and evolution of hemoglobin and globin loci*. New York: Elsevier Science Publishing Co. Inc., 1983:239-49.
11. Bayoumi RA, Omer A, Samuel APW, Saha N, Sebai ZA, Sabaa HMA. Hemoglobin and erythrocytic glucose-6-phosphate dehydrogenase variants among selected tribes in Western Saudi Arabia. *Trop Geogr Med* 1978;31:245-52.
12. El-Hazmi MAF, Warsy AS. The frequency of glucose-6-phosphate dehydrogenase phenotypes and sickle cell gene in Al-Qasim. *Ann Saudi Med* 1992;38:106-12.
13. El-Hazmi MAF. Haemoglobinopathies, thalassaemias and enzymopathies in Saudi Arabia: the present status. *Acta Haematol* 1987;78 130-4.
14. El-Hazmi MAF. Red cell genetic abnormalities and environmental interactions: a study in Tehamat Aseer. *J Trop Med Hyg* 1987;90: 61-7.
15. El-Hazmi MAF, Al-Swailem AR, Al-Faleh FZ, Warsy AS. Frequency of glucose-6-phosphate dehydrogenase, pyruvate kinase and hexokinase deficiency in the Saudi population. *Hum Hered* 1986;36:45-9.
16. El-Hazmi MAF, Warsy AS. Frequency of glucose-6-phosphate dehydrogenase variants and deficiency in Arabia. *Gene Geogr* 1990; 4:15-20.
17. El-Hazmi MAF, Warsy AS. Frequency of glucose-6-phosphate dehydrogenase phenotypes and deficiency in Al-Baha. *Hum Hered* 1990;39:313-7.
18. Warsy AS, El-Hazmi MAF. Glucose-6-phosphate dehydrogenase

- deficiency in Saudi Arabia: a review. Saudi Med J 1987;8:12-20.
19. El-Hazmi MAF, Warsy AS. Glucose-6-phosphate dehydrogenase deficiency in Saudi Arabia: a study in Al-Ula. Hum Hered 1988;38: 317-22.
 20. El-Hazmi MAF, Warsy AS. The frequency of glucose-6-phosphate dehydrogenase phenotypes and sickle cell genes in Al-Qatif oasis. Ann Saudi Med 1994;14:491-4.
 21. El-Hazmi MAF, Warsy AS. Epidemiology of G6PD in Saudi Arabia. Saudi Med J 1996;18:255-60.
 22. Beutler E. Glucose-6-phosphate dehydrogenase: new perspectives. Blood 1989;73:1397-401.
 23. El-Hazmi MAF, Warsy AS. A normal reference range for erythrocyte glucose-6-phosphate dehydrogenase in a Saudi population. Med Lab Sci 1987;44:125-9.
 24. El-Hazmi MAF, Al-Swailem AR, Warsy AS, et al. Consanguinity among the Saudi Arabian population. J Med Genet 1995;32:623-5.
 25. El-Hazmi MAF, Warsy AS. Phenotypes of glucose-6-phosphate dehydrogenase in different regions of Saudi Arabia: a comparative assessment. Saudi Med J 1997;18:393-9.
 26. Malaria Control Programme in the Kingdom. General Directorate of Preventive Medicine, Ministry of Health, Kingdom of Saudi Arabia. Riyadh: Bahr Al-Alum Press, 1983:7-38.
 27. Livingston FB. The malaria hypothesis: In: Bowman JE, editor. Distribution and evolution of hemoglobin and globin loci. New York: Elsevier Science Publishing Co. Inc. 1983:15-43.
 28. Livingston FB. Malaria and human polymorphism. Ann Rev Genet 1971;5:33-64.
 29. Bienzle Y, Ayeni O, Lucas AO, Luzzatto L. Glucose-6-phosphate dehydrogenase deficiency and malaria: greater resistance of female heterozygotes for enzyme deficiency and of males with non-deficient variant. Lancet 1972;I:107-10.
 30. Luzzatto L. Genetics of red cells and susceptibility to malaria. Blood 1979;54:961-76.
 31. Gilles HM, Fletcher KA, Hendrickse RG, Lindner R, Reddy S, Allan N. Glucose-6-phosphate dehydrogenase deficiency, sickling and malaria in African children in Southwestern Nigeria. Lancet 1967;1: 138-40.
 32. Edington GM, Laing WN. Relationship between haemoglobin C and S and malaria in Ghana. Br Med J 1957;2:143-5.