

High Incidence of Glucose-6-phosphate Dehydrogenase Deficiency in Croatian Island Isolate: Example from Vis Island, Croatia

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Aim To determine the prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in the population of the town of Komiža on the island of Vis, which has previously been reported as a place with several cases of favism.

Methods We screened 302 randomly selected men, using the fluorescent spot test. Fluorescence readings were performed at the beginning and 5, 10, and 20 minutes after incubation, and were classified into three groups: bright fluorescence, weak fluorescence, and no fluorescence. All men found to be G6PD deficient were tested with a quantitative spectrophotometric UV method.

Results Of the 302 tested blood samples, 36 (11.9%) samples showed weak fluorescence or no fluorescence spots. Spectrophotometric UV test showed that 18 (5.96%) men were G6PD deficient. The prevalence of G6PD deficiency in the population of Komiža is significantly higher ($P < 0.001$) than the prevalence in the whole population of Dalmatia in the south of Croatia (0.75% in men).

Conclusion On the basis of these findings, we recommend including the newborns from the island of Vis into a screening program for G6PD deficiency. Our results indicate that G6PD deficiency should be determined for all the island isolates in the Mediterranean basin and they warrant further studies.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common inherited disorders, with approximately 400 million people affected worldwide (1,2). More than 140 different mutations of the G6PD gene have been identified (3). Molecular analysis of the inhabitants of the Dalmatian region in the south of Croatia identified the G6PD Cosenza (1376 G→C) as the most frequent mutation, followed by G6PD Mediterranean mutation (563 C→T) (4). All individuals with G6PD Mediterranean mutation had concomitant silent C→T transition at the position 1311, which is often found in Mediterranean basin, but not in Asia (5). Association of the factor V Leiden and G6PD deficiency has been observed in Dalmatian population (6).

Glucose-6-phosphate dehydrogenase deficiency is unrecognized in most affected individuals. However, it may have a clinical expression such as acute acquired hemolytic anemia, chronic nonspherocytic hemolytic anemia, favism, and neonatal hyperbilirubinemia (7). Different variants of the enzyme are found in high frequency in African, Mediterranean, and Asian populations (8). Heterozygote advantage against malaria was found to account for the high frequency of distinct alleles in particular populations (9).

Komiža is a small town on the isolated island of Vis, in the Adriatic Sea, a part of the Mediterranean basin. Although the prevalence of G6PD deficiency has not previously been determined in Komiža, there were several cases of favism originating from Komiža which were treated in our hospital. Therefore, we hypothesized that there could be a significantly higher incidence of G6PD deficiency than in other parts of Dalmatia.

The aim of this study was to determine the prevalence of G6PD deficiency in the population of Komiža and compare it with the prevalence of G6PD deficiency in the whole population of Dalmatia. Being an X-linked genetic condition, the prevalence of G6PD deficiency in any given population is determined by the number of men with the deficiency (10).

Participants and methods

Participants

The study included 302 adult men from Komiža (median age 47 years; range 5.3-89.8 years) and was performed from December 2002 to February 2003. With the assistance of local general practitioners, we made a list of the names and addresses of all 750 men in Komiža, and assigned a number from 1 to 750 to each name. Estimating that the clinically significant prevalence of G6PD deficiency is 5%, and knowing the prevalence among men in Dalmatian region, we calculated that we needed 320 patients to detect a difference with 90% certainty and a 5% significance level. The patients were selected using a random number generator. The response rate was 94.4%. The Ethical Committee of the Split University Hospital approved the study and all studied individuals signed the informed consent.

Fluorescent spot test

International Committee for Standardization in Hematology recommended the fluorescent spot test as the most acceptable method for screening control (11). This method detects the fluorescence of NADPH under long-wave (365 nm) UV light in complete darkness. Reduction of NADP to NADPH occurs in the presence of G6PD, ie, the rate of NADPH formation is proportional to G6PD activity. Blood used for the test was anticoagulated in KT3-EDTA (Becton Dickinson, Plymouth, UK) and stored at 4°C up to 7 days. Commercially available kits (Cat. No. 203, Sigma Diagnostics, Taufkirchen, Germany) were used. Ten microliters of the whole blood was incubated with 200 µL of the reagent mixture. Spots were made on the filter paper at the beginning (zero-time) and 5, 10, and 20 minutes after blood incubation with reagent mixture. Normal samples fluoresce brightly, whereas deficient samples show little or no fluorescence. The results were classified into three groups: bright

fluorescence (BF), weak fluorescence (WF), and no fluorescence (NF).

Quantitative spectrophotometric method

Enzyme activity was determined by measuring the rate of absorbance change at 340 nm, due to the reduction of NADP to NADPH when a sample was incubated with G6P. Glucose-6-phosphate dehydrogenase activity was calculated in relation to erythrocyte count. Commercially available kits (Cat. No. PD 410, Randox Laboratories Ltd, Crumlin, Great Britain) were used. Results were interpreted as the percentage of normal G6PD activity. Enzyme activity less than 10% of the normal activity was classified as a severe deficiency, whereas the activity between 10 and 60% of the normal activity was classified as a moderate deficiency.

Statistical analysis

The prevalence of G6PD deficiency in Komiža and prevalence among men in the whole region were compared by Pearson χ^2 test. The result was considered significant if probability value was equal to or less than 0.01. All data analyses were performed using the statistical package SPSS 8.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

The fluorescence spot test reading showed that 266 men (88%) had bright fluorescence in all the phases of the test, and according to this, their G6PD activity was classified as normal. In 36 blood samples, which showed weak fluorescence and/or no fluorescence spots, enzyme activi-

ties were determined by the spectrophotometric UV test. All blood samples with no fluorescence at 5 minutes after the beginning of the fluorescent spot test were G6PD deficient. Out of 4 samples with weak fluorescence at 5, 10, and 20 minutes, 2 were G6PD deficient. All 16 samples with weak fluorescence at 5 minutes and bright fluorescence at 10 and 20 minutes turned out to have normal G6PD activity after the spectrophotometric test (Table 1).

Glucose-6-phosphate dehydrogenase deficiency was found in 18 individuals, resulting in the prevalence rate of 6.0% (95% CI, 3.3-8.6%). Severe G6PD deficiency (lower than 10% of normal G6PD activity) was found in 14 (78%) individuals, and the rest of them were suffering from moderate deficiency (2 individuals with 18 and 22% enzyme activity and 2 more with 40 and 42% enzyme activity).

We compared our results with the rate of 0.8% (95% CI, 0.3-1.2%) which is the prevalence of G6PD deficiency among men in the Dalmatian region in the south of Croatia (12). The prevalence of G6PD deficiency in Komiža was significantly higher ($P < 0.001$, difference 5.2%, 95% CI, 3.6-6.8%).

Discussion

There are significant differences in the prevalence of G6PD deficiency among different nations, and even within the same nation, due to migrations, geographical characteristics, and various religious and cultural factors (13,14). Uneven distribution of G6PD deficiency, ranging from 1 to 30% in male infants, was found in Greece (15).

Table 1. Results of fluorescent spot test for screening of G6PD deficiency (n = 302)

	Time of test reading (minutes)			No.	
	5	10	20	total	G6PD-deficient*
No fluorescence	no fluorescence	no fluorescence	no fluorescence	14	14
No fluorescence	weak fluorescence	weak fluorescence	weak fluorescence	2	2
Weak fluorescence	weak fluorescence	weak fluorescence	weak fluorescence	4	2
Weak fluorescence	bright fluorescence	bright fluorescence	bright fluorescence	16	0
Bright fluorescence	bright fluorescence	bright fluorescence	bright fluorescence	266	0

*According to the quantitative spectrophotometric method for enzyme activity.

Population isolation as a major cause for higher prevalence of G6PD deficiency was previously demonstrated in studies conducted in some parts of Greece, Sardinia, and Israel (15-17).

We confirmed our hypothesis that the prevalence of G6PD deficiency in Komiza is significantly higher than in the entire region (5.96% vs 0.75%, in men). After obtaining screening test results, a letter was sent to all the G6PD deficient men and their general practitioners informing them about the importance of avoiding precipitating factors for clinical manifestation of G6PD deficiency, like fava beans and certain drugs. There is a relatively short list of drugs which have been shown to cause hemolytic anemia in G6PD deficient patients. The risk and severity of hemolysis are usually related to the dose and duration of therapy and the presence of additional oxidant stress such as infection (9).

An important reason for higher incidence of G6PD deficiency in Komiza is the geographical position of the island of Vis, which is an isolated island with a high degree of consanguinity, inbreeding, and founder effect in population. Also, it could be a consequence of Greek colonization in the fourth century BC. Isolate island populations are generally considered to be a suitable setting for efficient and powerful research in genetic epidemiology, and a considerable interest has recently been shown for Croatian island isolates. Some disorders that appear in Eastern Adriatic island populations were found to be related to high inbreeding levels (18). Our results suggest that rare genetic variants are more frequent in human isolate populations than in outbred general populations. Therefore, human isolates are advantageous for mapping rare genetic variants underlying human diseases, including common complex diseases. Our findings support the role of rare variants in human diseases and the value of human isolates in the identification and mapping of such variants (19,20).

The Committee on Medical and Public Health Research of the European Economic

Community decided in 1983, at a workshop for screening and management of potentially treatable genetic metabolic disorders, that screening for G6PD deficiency should be performed in high-risk populations, ie, Greeks and Sardinians.

In high-risk populations, it is very important to accurately and rapidly diagnose G6PD deficiency early in the neonatal period, before the onset of jaundice, and before discharging the neonate from hospital (21). Neonates with a severe G6PD deficiency may develop hyperbilirubinemia sufficiently severe to cause kernicterus and death. Therefore, neonatal jaundice resulting from G6PD deficiency requires careful monitoring and earlier beginning of therapy than jaundice resulting from other causes (9). International Committee for Standardization in Hematology recommended in 1979 the fluorescent spot test as the most acceptable and most reliable screening test for G6PD deficiency (11). In comparison with other commercially available screening tests for G6PD deficiency, it was found to have 100% sensitivity and 98% specificity (21). However, an abnormal screening test should be confirmed with a definitive, quantitative test. In this study, absence of fluorescence at 5 minutes of incubation definitely confirmed G6PD deficiency.

Screening is recommended for infants of both sexes (22). In Sardinia, screening for G6PD deficiency has been obligatory since 1971, whereas in Greece, it was incorporated into the existing national phenylketonuria screening program in 1977. Reported prevalence of G6PD deficiency among men was 7.5% in Sardinia and 4.5% in Greece. A marked decline in favism was noticed after the beginning of population screening, which showed that the decision was justified (15,16). Screening is not only useful in preventing favism, but also in the prevention of severe neonatal hyperbilirubinemia (23).

With the prevalence of G6PD deficiency in Dalmatian region in the south of Croatia being only 0.75% among men and 0.14% among

women, there is no need for a continuous screening program (12). However, on the basis of our findings, we believe that neonatal screening for G6PD deficiency, together with a comprehensive education program, should be performed for newborns of both sexes on the island of Vis, in order to predict those at risk for developing favism, jaundice, and severe acute hemolytic attacks. Identification of G6PD-deficient neonates, together with the awareness of the condition and its danger, will allow a selective approach to their hospital discharge and follow-up surveillance. In addition, our results indicate that determination of G6PD deficiency is necessary for all the island isolates in Mediterranean basin and warrants further studies.

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References

- Hundsdoerfer P, Vetter B, Kulozik AE. Chronic haemolytic anaemia and glucose-6-phosphate dehydrogenase deficiency. Case report and review of the literature. *Acta Haematol.* 2002;108:102-5. [Medline:12187030](#)
- Beutler E. Glucose-6-phosphate dehydrogenase deficiency. *N Engl J Med.* 1991;324:169-74. [Medline:1984194](#)
- Beutler E, Vulliamy TJ. Hematologically important mutations: Glucose-6-phosphate dehydrogenase. *Blood Cells Mol Dis.* 2002;28:93-103. [Medline:12064901](#)
- Barisic M, Korac J, Pavlinac I, Krzelj V, Marusic E, Vulliamy T, et al. Characterization of G6PD deficiency in southern Croatia: description of a new variant, G6PD Split. *J Hum Genet.* 2005;50:547-9. [Medline:16143877](#)
- Terzic J, Krzelj V, Drmic I, Andelinovic S, Primorac D, Mestrovic J, et al. Genetic analysis of the glucose-6-phosphate dehydrogenase deficiency in a southern Croatia. *Coll Antropol.* 1998;22:485-9. [Medline:9887604](#)
- Cikes V, Abaza I, Krzelj V, Terzic IM, Tafra R, Trlaja A, et al. Prevalence of Factor V Leiden and G6PD 1311 silent mutations in Dalmatian population. *Arch Med Res.* 2004;35:546-8. [Medline:15631882](#)
- Beutler E. G6PD deficiency. *Blood.* 1994;84:3613-36. [Medline:7949118](#)
- Luzzatto L, Mehta A. Glucose-6-phosphate dehydrogenase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. *The metabolic and molecular bases of inherited disease.* 7th ed. New York (NY): McGraw-Hill; 1995. p. 3367-98.
- Mehta A, Mason PJ, Vulliamy TJ. Glucose-6-phosphate dehydrogenase deficiency. *Baillieres Best Pract Res Clin Haematol.* 2000;13:21-38. [Medline:10916676](#)
- Sheba C, Szeinberg A, Ramot B, Adam A, Ashkenazi I. Epidemiologic surveys of deleterious genes in different population groups in Israel. *Am J Public Health.* 1962;52:1101-5. [Medline:13911534](#)
- Beutler E, Blume KG, Kaplan JC, Lohr GW, Ramot B, Valentine WN. International Committee for Standardization in Haematology: recommended screening test for glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. *Br J Haematol.* 1979;43:465-7. [Medline:497122](#)
- Krzelj V, Zlodre S, Terzic J, Mestrovic M, Jaksic J, Pavlov N. Prevalence of G-6-PD deficiency in the Croatian Adriatic coast population. *Arch Med Res.* 2001;32:454-7. [Medline:11578763](#)
- Rodrigues MO, Freire AP, Martins G, Pereira J, Martins MD, Monteiro C. Glucose-6-phosphate dehydrogenase deficiency in Portugal: biochemical and mutational profiles, heterogeneity, and haplotype association. *Blood Cells Mol Dis.* 2002;28:249-59. [Medline:12064920](#)
- Sukumar S, Mukherjee MB, Colah RB, Mohanty D. Molecular basis of G6PD deficiency in India. *Blood Cells Mol Dis.* 2004;33:141-5. [Medline:15315792](#)
- Missiou-Tsagaraki S. Screening for glucose-6-phosphate dehydrogenase deficiency as a preventive measure: prevalence among 1,286,000 Greek newborn infants. *J Pediatr.* 1991;119:293-9. [Medline:1824550](#)
- Meloni T, Forteleoni G, Meloni GF. Marked decline of favism after neonatal glucose-6-phosphate dehydrogenase screening and health education: the northern Sardinian experience. *Acta Haematol.* 1992;87:29-31. [Medline:1585769](#)
- Cohen T. Genetic markers in migrants to Israel. *Isr J Med Sci.* 1971;7:1509-14. [Medline:5144594](#)
- Bakija-Konsuo A, Basta-Juzbasic A, Rudan I, Situm M, Nardelli-Kovacic M, Levanat S, et al. Mal de Meleda: genetic haplotype analysis and clinicopathological findings in cases originating from the island of Mljet (Meleda), Croatia. *Dermatology.* 2002;205:32-9. [Medline:12145432](#)
- Wright A, Charlesworth B, Rudan I, Carothers A, Campbell H. A polygenic basis for late-onset disease. *Trends Genet.* 2003;19:97-106. [Medline:12547519](#)
- Peltonen L, Palotie A, Lange K. Use of population isolates for mapping complex traits. *Nat Rev Genet.* 2000;1:182-90. [Medline:11252747](#)
- Kaplan M, Leiter C, Hammerman C, Rudensky B. Comparison of commercial screening tests for glucose-6-phosphate dehydrogenase deficiency in the neonatal period. *Clin Chem.* 1997;43:1236-7. [Medline:9216462](#)
- Kaplan M, Hammerman C, Kvit R, Rudensky B, Abramov A. Neonatal screening for glucose-6-phosphate dehydrogenase deficiency: sex distribution. *Arch Dis Child Fetal Neonatal Ed.* 1994;71:F59-60. [Medline:8092877](#)
- Kaplan M, Hammerman C. Severe neonatal hyperbilirubinemia. A potential complication of glucose-6-phosphate dehydrogenase deficiency. *Clin Perinatol.* 1998;25:575-90. [Medline:9779335](#)