Mortality in a Cohort of Men Expressing the Glucose-6-Phosphate Dehydrogenase Deficiency

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The objective of this study was to test the hypothesis of a lower mortality from cancer and cardiovascular diseases among men expressing glucose-6-phosphate dehydrogenase (G6PD) deficiency. We designed a mortality study based on death certificates from January 1, 1982 through December 31, 1992 in a cohort of G6PD-deficient men. Cohort members were 1,756 men, identified as expressing the G6PD-deficient phenotype during a 1981 population screening of the G6PD polymorphism. The setting was the island of Sardinia, Italy. Outcome measures were cause-specific standardized mortality ratios (SMRs), which were computed as 100 times the observed/expected ratio, with the general Sardinian male population as the reference. Deaths from all causes were significantly less than expected due to decreased SMRs for ischemic heart disease (SMR, 28; 95% confidence interval [CI], 10 to 62), cerebrovascular disease (SMR, 22; 95% CI, 6 to 55), and liver cirrhosis (SMR, 12; 95% CI, 0 to 66), which explained 95.6% of the deficit in total mortality. All cancer mortality was close to the expectation, with a significant increase in the SMR for non-Hodgkin's lymphoma (SMR, 545; 95% CI, 147 to 1,395). A decrease in mortality from cardiovascular diseases was one of the study hypotheses, based on an earlier human report and experimental evidence. However, selection bias is also a likely explanation. Further analytic studies are warranted to confirm whether subjects expressing the G6PD-deficient phenotype are protected against ischemic heart disease and cerebrovascular disease. This cohort study is consistent with more recent case-control studies in rejecting the hypothesis of a decreased cancer risk among G6PD-deficient subjects. The observed increase in mortality from non-Hodgkin's lymphoma and decrease in mortality from liver cirrhosis were not previously reported.

GLUCOSE-6-PHOSPHATE dehydrogenase (G6PD) is a cytoplasmic enzyme that affects the production of the reduced form of the extramitochondrial nicotine-adenosine-dinucleotide phosphate coenzyme (NADPH) by controlling the step from glucose-6-phosphate to 6-phospho-gluconate in the pentose phosphate pathway.[1,2] In red blood cells, defense against oxidative damage is heavily dependent on G6PD activity, because it is the only source of NADPH, which maintains the stability of catalase and preserves and regenerates the reduced form of glutathione (GSH).[1,2] In other tissues, and particularly in liver cells, NADPH is required for various cellular functions, including fatty acid and cholesterol synthesis,[3,4] the ruling step of which is the NADPH-dependent reduction of β-hydroxy-β-methyl-glutaryl-coenzyme A (HMG-CoA) to mevalonate. Also, cytochrome P-450 isoenzymes capture electrons from the reduced coenzymes nicotinamide adenine dinucleotide (NADH) and NADPH during the cyclic redox processes activating many xenobiotics.[5,6] NADPH, and therefore G6PD activity, may be also implicated for the ready availability of GSH to support the phase two metabolism of carcinogens operated by glutathione-S-transferase isoenzymes by catalyzing the conjugation of hydrophobic electrophiles to GSH.[7]

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The gene encoding G6PD is located in the telomeric region of the long arm of the X chromosome (band Xq28).[1] More than 300 alleles have been identified that are related to point mutations in the base sequence of the G6PD gene.[1,2] One of these alleles, the Gd-Mediterranean allele, is mostly frequent among the male population in Sardinia, Italy, and it is associated with levels of enzyme activity undetectable with routine methods. This condition affects 12% to 15% of the Sardinian male population overall,[8,9] with a broad range by communes (1% to 30%) that has been related to the past incidence of malaria.[8] G6PD deficiency is a public health issue in Sardinia because of the seasonal occurrence of hemolytic crises among subjects expressing the deficient phenotype after the ingestion of fava beans (favism). In an attempt to prevent such occurrences, the Health Department of the Regional Administration of Sardinia launched in 1981 a program of free testing of the G6PD polymorphism in the general population. The data from this screening provide a unique opportunity to test early hypotheses of lower risks for cardiovascular diseases and cancer among G6PD-deficient individuals.[10-12]

MATERIALS AND METHODS

The population screening of the G6PD polymorphism covered all of 1981. Blood withdrawals (1 to 2 mL) were performed for free in numerous public health services dispersed throughout the region. The G6PD polymorphism was assayed in erythrocytes with the Beutler's fluorescent spot test[13] in four central laboratories. About 2% of the resident population participated in the screening. Participation was voluntary and covered the whole region, although the sample size ranged from 1% to 42% of the resident population by commune. The lowest participation rates were in the central western area, where G6PD deficiency is mostly frequent (~30% of the male residents).[9] Among the participants, 15,964 were men, 1,905 (11.9%) of whom expressed a complete enzymatic deficiency in erythrocytes. Records were kept only for G6PD-deficient subjects (ie, subjects showing no enzyme activity at the test) most likely related to G6PD alleles associated with very severe reduction in enzyme activity and for men with partial enzyme activity related to other G6PD alleles relatively less common.[1,2] These subjects were not considered as G6PD-deficient for the purposes of this study. Women were also excluded because of the small number of homozygote subjects with complete lack of erythrocyte G6PD activity.

The Computer Center of the Regional Administration of Sardinia provided a complete list of the 1,905 men diagnosed as G6PD-deficient during the 1981 screening. Available information included name, date of birth, and complete address. Tracing was extended to all Italian territory. Vital status was successfully determined as of December 31, 1992 for 96.8% of the subjects at the municipalities of residence. Table 1 shows the number of subjects excluded from study by criteria for exclusion and the number of subjects who entered the cohort by vital status at the end of follow-up. Forty-three subjects who could not be identified (missing or wrong identification data) were excluded from study. Eighteen subjects identified and subsequently lost to follow-up contributed to person-years up to date of last known vital status. For subjects found to be deceased, the Public Health Departments of the Local Health Units covering the area where death occurred provided the death certificate upon request. Thirteen men who died in 1981 did not enter the cohort. Subjects less than 15 years of age were also excluded because regional mortality rates were not available in computerized form for the younger ages. Thus, an additional 93 subjects, including 91 subjects who reached age 15 after the end of follow-up and 2 subjects who died before age 15, were excluded from the cohort. Therefore, the cohort comprised all men identified as G6PD-deficient during the 1981 survey, identified as alive on January, 1 1982, and 15 years of age or more (N = 1,756). Each subject entered the cohort on January, 1 1982 or thereafter at age 15.

One-hundred twenty-one deaths were identified, with death certificates available for 117 (97%). Primary causes of death were coded following the International Classification of Diseases (9th revision).[14] Expected deaths were calculated by applying the 5-year age group and 5-year period of follow-up specific mortality rates in the Sardinian general male population to the person-years of follow-up in the correspondent strata of the study population. The Italian National Institute of Health (Istituto Superiore di Sanità) made reference rates available in computerized form. To preserve the same diagnostic level as in the reference population, we did not make attempts to confirm the accuracy of the causes of death reported on the death certificates. The measure of association between the G6PD-deficient phenotype and cause-specific mortality was the standardized mortality ratio (SMR) computed as 100 times the ratio of observed versus expected deaths. Ninety-five percent confidence intervals (CIs) of SMRs were calculated according to Liddell.[15] The results were considered statistically significant when the 95% CI did not include 100.

RESULTS

The average age at entry into follow-up was 41.6 years (median, 40 years; standard deviation [SD], 18.4 years). The 1,756 cohort members accumulated a total of 15,164.7 person-years. Average age at death was 67.9 years (median, 72 years; SD, 15.0 years). Only 2 deaths, 1 from bronchopneumonia and another from accidental death, occurred among the 93 subjects excluded from follow-up because of the age criterion.

Table 1. Definition of the Cohort and Number of Subjects by Vital Status at the End of Follow-Up

Total subjects	1,905
Subjects not identified	43
Died before entering follow-up*	15
Subjects born after December 31, 1977†	91
Entered the cohort	1,756
Lost to follow-up	18
Alive	1,617
Dead	121
With death certificate	117
Without death certificate	4

*Includes 13 subjects who died before start of follow-up and 2 subjects who died before attaining age 15.

†Alive but less than 15 years of age at the end of follow-up.

Results of the mortality analysis are reported in Table 2. Mortality from all causes was significantly reduced among cohort members (SMR, 76). Mortality from cardiovascular diseases was about half the expectation (SMR, 46), mostly due to a decrease in deaths from ischemic heart disease (SMR, 28) and cerebrovascular disease (SMR, 22). Digestive diseases also showed a significant decrease in risk (SMR, 24), which was mostly due to a significant deficit in mortality from liver cirrhosis (SMR, 12). Nonmalignant respiratory diseases were also less than expected, but the SMR was not statistically significant. The deficit in mortality from ischemic heart disease, cerebrovascular disease, and liver cirrhosis (15.1 + 14.6 + 7.4 = 37.1) accounted for 95.6% of the deficit in total mortality (37.1/38.8).

Mortality from all cancers combined was nearly identical to the expectation, with a nonsignificant reduction in lung cancer. Nonsignificant excess risks were observed for oral and pharyngeal cancer, prostate cancer, and cancer of the lymphatic and hematopoietic system. The excess of lymphatic and hematopoietic cancer was entirely due to a significant increase in mortality from non-Hodgkin's lymphoma (SMR, 545; based on 4 deaths).

DISCUSSION

In this mortality follow-up study of G6PD-deficient individuals, we found a decrease in deaths from ischemic heart disease, cerebrovascular disease, and liver cirrhosis and a significant 5.4-fold increase in mortality from non-Hodgkin's lymphoma.

In an early cross-sectional study, 7.2% of patients affected by coronary artery disease carried the A-G6PD phenotype (associated with partially deficient enzyme activity), whereas they accounted for 14.1% of all other patients, although the frequency of hypertensive disease did not vary by G6PD phenotype.[10] On the other hand, higher blood pressure levels were reported among African-American men with the G6PD A-allele in another study.[16] To the best of our knowledge, no other studies have explored the hypothesis of a decreased risk for cardiovascular diseases among subjects expressing G6PD deficiency. A large body of experimental evidence linking G6PD activity, cholesterol synthesis, and cell growth has accumulated in recent years.[17] However, despite their genetic condition, G6PD-deficient individuals grow normally.[18] It is plausible that alternative sources of NADPH, such as the extramitochondrial isocitrate dehydrogenase enzyme and the malic enzyme, provide enough NADPH to support the endogenous cholesterol synthesis required for normal cell replication.[17] Data on the consistency in deficient enzyme activity across different tissues from the same individual expressing erythrocyte G6PD deficiency have been published,[19-22] but a Medline search from 1966 onwards did not list any study providing data specifically for endothelial cells. Therefore, the hypothesis that G6PD-deficient individuals might be less susceptible to ischemic heart diseases and cerebrovascular diseases, because of difficulties in providing enough NADPH for the intima cell proliferation during the formation of the atheroma,[23] is only speculative.

Balance between nitric oxide (NO) synthase activity, which is NADPH-dependent,[24] and levels of GSH, a physiological

Table 2. Cause-Specific SMRs Among a Cohort of G6PD-Deficient Subjects: 1982-1992

ICD-9	Cause of Death	Observed	Expected	SMR 95% CI
001-999.9	All causes	121	159.8	76 (63-91)
140-208.9	All malignant neoplasms	44	43.1	102 (74-137)
140-149.9	Lip, oral cavity, and pharynx	5	1.9	258 (83-601)
151-151.9	Stomach	5	3.4	148 (48-346)
155-156.9	Liver and biliary tract	3	3.4	88 (18-258)
162-162.9	Trachea, bronchus, and lung	7	12.6	56 (22-115)
185	Prostate	6	3.0	199 (73-433)
200-208.9	Lymphatic and hematopoietic tissue	6	3.1	195 (71-424)
200-200.8, 202-202.9	Non-Hodgkin's lymphoma	4	0.7	545 (147-1,395)
250-250.9	Diabetes	3	4.0	75 (15-219)
390-459.9	Cardiovascular diseases	29	62.6	46 (31-67)
410-414.9	Ischemic heart disease	6	21.1	28 (10-62)
430-438.9	Cerebrovascular disease	4	18.6	22 (6-55)
460-519.9	Nonmalignant respiratory diseases	10	14.0	71 (34-131)
520-579.9	Digestive diseases	3	12.6	24 (5-69)
571-571.9	Liver cirrhosis	1	8.4	12 (0-66)
580-608.9	Genitourinary diseases	4	2.1	193 (52-495)
780-799.9	Ill defined conditions	13	15.4	85 (45-145)
800-999.9	Accidental deaths	11	11.7	94 (47-169)

scavenger of NO,[25] might also be an important factor in preventing the occurrence of cardiovascular diseases. It is unknown whether the two factors balance out in G6PD-deficient individuals. NO itself and/or its S-nitrosocysteine adduct are powerful vasodilators,[26] and its other properties that are relevant for the cardiovascular homeostasis include acting as a scavenger of superoxide radicals abrogating their toxicity[27] and preventing the oxidation of low-density lipoproteins (LDL)[28] and inhibiting platelet aggregation, leukocyte adhesion, and vascular smooth muscle proliferation.[29]

Early suggestions of a decrease in cancer risk among G6PD-deficient individuals[11,12] were not supported by more recent case-control studies.[30,31] The present cohort study confirms that G6PD-deficient subjects do not differ from the general population in terms of mortality from all cancers combined. Among single cancer sites, non-Hodgkin's lymphoma showed a 5.4-fold increase. However, this was generated by 4 deaths only, and previous studies did not find a higher proportion of G6PD-deficient subjects among patients with non-Hodgkin's lymphoma.[32,33] Most of the decrease in mortality from all digestive diseases observed in the present study was due to a deficit in deaths from liver cirrhosis, which was not previously described.

There are limitations that must be considered. The selection of the surveyed sample (~2% of the total Sardinian male population) was not random. Subjects who volunteered for the test were presumably unaware of their G6PD status. However, because the proportion of G6PD-deficient subjects in the screened population (11.9%) was smaller than previously reported for the Sardinian general male population,[8] it does not seem likely that a positive family history of favism was an important factor in the decision of volunteering for the test. It is plausible that individuals who participated were more concerned about their health status than were nonparticipants. Unfortunately, no information was collected at the time of the survey on lifestyle habits, including diet and smoking, of the individuals who were screened, and no records were kept of the individuals who were found to carry the wild-type G6PD phenotype. As it may be derived from the mean age at entry in the follow-up among cohort members, a large proportion of the cohort consisted of men who reached adulthood or even became elderly without awareness of their G6PD phenotype. This implies that they did not suffer negative health effects from their genetic condition. On the other hand, one cannot exclude a priori that the genetic condition of G6PD deficiency itself contributed to a hypothetical healthier condition among these subjects.

Smoking-related deaths, such as lung cancer and nonmalignant respiratory diseases, were below the expectation in this cohort, although the respective SMRs were not statistically significant. Indeed, in a case-control study of cancer risk by G6PD phenotype, G6PD-deficient individuals were found to smoke less frequently than subjects with the wild-type phenotype (53.8% *v* 70.7%, respectively).[30] However, observed and expected deaths from smoking-related cancer sites other than the lung (oral cavity, pancreas, larynx, bladder, and kidney) combined were similar (8 observed deaths *v* 8.4 expected). Also, we estimated the number of deaths from cardiovascular diseases, which would have been expected if the proportion of smokers in the general population (~70%) were the same as among G6PD-deficient subjects (53.8%)[33] and obtained a corrected SMR of 49 (95% CI, 33 to 71), which is still significant. This finding suggests that smoking was unlikely to greatly bias our results. No information is available on consumption of alcoholic beverages among G6PD-deficient individuals to assess the proportion of decrease in mortality from liver cirrhosis that could be explained by an alcohol consumption lower than the average in the Sardinian general male population. Also, whether alcohol metabolism is affected by the G6PD-deficient condition is unclear, because NAD+ and not NADP+ binds the alcohol dehydrogenase isoenzymes receiving the hydrogen ion from alcohol.[34]

In conclusion, selection bias is a likely explanation for our finding of a decrease in deaths from ischemic heart disease and

cerebrovascular disease among G6PD-deficient individuals. However, these results are partially consistent with an early report and are corroborated by experimental studies. Future studies of long-term health outcomes associated with the G6PD polymorphism should include information on diet, smoking, and health history to evaluate the impact of the informational programs and any subsequent lifestyle changes on disease risk.

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