Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency in Nonarteritic Anterior Ischemic Optic Neuropathy in a Sardinian Population, Italy

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Purpose. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common human genetic abnormalities, with a high prevalence in Sardinia, Italy. Evidence indicates that G6PD-deficient patients are protected against ischemic heart and cerebrovascular disease and retinal vein occlusion. The purpose of this study was to assess the frequency of G6PD deficiency in Sardinian patients with nonarteritic anterior ischemic optic neuropathy (NAION) and ascertain whether G6PD deficiency may offer protection against NAION.

Methods. Erythrocyte G6PD activity was determined by using a quantitative assay in 140 patients with NAION and 280 age- and gender-matched comparison patients. Conditional logistic regression models were used to investigate the association between G6PD deficiency and NAION.

Results. G6PD deficiency was found in 7 (5%) patients with NAION and 34 (12.1%) control subjects. Differences between cases and controls were statistically significant ($P = 0.02$). Conditional logistic regression analysis, including as covariates G6PD deficiency, hypertension, diabetes, and hypercholesterolemia, revealed that G6PD deficiency was significantly associated with decreased risk for NAION (odds ratio [OR] = 0.4, 95% confidence interval [CI] = 0.17–0.94, $P = 0.035$). Conditional logistic regression analyses, including systolic or diastolic blood pressure and plasma glucose and cholesterol levels confirmed that G6PD deficiency was associated with a decreased risk for NAION, but the ORs were not significant at the 0.05 significance level ($P = 0.085$ and $P = 0.071$). Models including gender × G6PD deficiency interaction disclosed that gender was not an effect modifier of G6PD deficiency ($P > 0.20$).

Conclusions. The frequency of G6PD deficiency in patients with NAION was significantly lower than expected. Results suggest that G6PD-deficient patients in the Sardinian population have a significantly decreased risk of having NAION. (*Invest Ophthalmol Vis Sci.* 2008;49:1328–1332) DOI:10.1167/iovs.07-1115

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Glucose-6-phosphate dehydrogenase (G6PD) is a cytoplasmic enzyme present in all cells, where it plays the key role in regulating carbon flow through the pentose phosphate pathway. Specifically, the enzyme affects the production of the reduced form of the extramitochondrial nicotine-adenosine-dinucleotide phosphate (NADPH) coenzyme by controlling the step from glucose-6-phosphate to 6-phosphogluconate in the pentose phosphate pathway. In erythrocytes, defense against oxidative damage is heavily dependent on G6PD activity, which is the only source of NADPH.[1] The gene encoding G6PD is located in the distal long arm of the X chromosome (band Xq28). More than 300 alleles with missense point mutation in the G6PD gene sequence have been identified.[2] The G6PD Mediterranean allele, associated with levels of enzyme activity undetectable with routine methods (WHO class II), is common on the island of Sardinia, Italy, where the reported prevalence of G6PD deficiency ranges from 10% to 15%.[3–7] This condition is a public health issue in Sardinia, because of the seasonal occurrence of favism, a hemolytic anemia induced by ingestion of the broad bean (*Vicia faba*) in subjects expressing the deficient phenotype.[4,5]

Nonarteritic anterior ischemic optic neuropathy (NAION) is the most common acute optic neuropathy in patients older than 50 years, with an estimated annual incidence in the United States of 2.3 to 10.2 per 100,000 population, accounting for at least 6,000 new cases annually.[8,9] NAION is a multifactorial disease. NAION, retinal vein occlusion, cardiovascular disease, and cerebrovascular disease have several risk factors in common, including systemic hypertension, diabetes mellitus, and atherosclerosis.[10,11] Of note, a former study found supportive evidence for protection against ischemic heart and cerebrovascular disease in men with G6PD deficiency.[5] In addition, we have recently shown that both men and women with G6PD deficiency have a significantly decreased risk for retinal vein occlusion; however, this decreased risk appears to be more significant in the women.[7]

The present study was designed to assess the frequency of G6PD deficiency in Sardinian patients with NAION and ascertain whether G6PD deficiency may have a protective effect against this ischemic disorder of the optic nerve.

Methods

In the present study, we used a case–control design, recruiting 140 of 150 consecutive patients with NAION and 280 control subjects between January 1992 and December 2006. Sample size was computed before the survey with a 95% confidence level (two-tailed test) and 86% statistical power to detect an odds ratio (OR) of 0.5, assuming a G6PD prevalence rate of 12%, as reported previously.[4–7] The case–control ratio was 1:2.

The inclusion criterion for the case group was the diagnosis of NAION, established by a history of sudden visual loss accompanied by findings of optic nerve edema (or pallor with a known history of disc edema) and characteristic retinal nerve fiber layer defects revealed by conventional automated visual field examination. All patients with

NAION underwent a full ophthalmic evaluation, including best corrected visual acuity (BCVA), slit lamp examination, applanation tonometry, fundus biomicroscopy, fluorescein angiography, and automated visual field examination (Humphrey Field Analyzer 30-2 test; Carl Zeiss Meditec, Oberkochen, Germany). Visual field types were determined by a team of two readers (AP, AC). Medical conditions, including systemic hypertension, diabetes mellitus, hypercholesterolemia, and cardio- and cerebrovascular status (presence of angina, myocardial infarction, transient ischemic attacks, and stroke) were also recorded. Exclusion criteria included age <18 years, non-Sardinian ancestry, and evidence of any other neurologic, systemic, or ocular disorder that could be responsible for optic disc edema and visual impairment.

Two age- and gender-matched control subjects per case were randomly selected from the cataract register. Exclusion criteria included age <18 years, non-Sardinian ancestry, and previous history of AION, retinal vein occlusion, or retinal artery occlusion. All control subjects underwent standard ophthalmic evaluation, including BCVA, slit lamp examination, applanation tonometry, and fundus examination. Medical conditions, including systemic hypertension, diabetes mellitus, hypercholesterolemia, and cardio- and cerebrovascular status (presence of angina, myocardial infarction, transient ischemic attacks, and stroke) were also recorded. Control subjects were recruited concurrently during the patients' recruitment period.

Definitions used for systemic hypertension, diabetes, and hypercholesterolemia are described elsewhere.[7]

Institutional ethics review board approval was obtained, and the study was conducted in full accord with the tenets of the Declaration of Helsinki. Each participant received detailed information and provided informed consent before inclusion.

Red blood cell G6PD activity was determined with a quantitative assay (G6PD/6PGD; Biomedic snc, Sassari, Italy), as described previously.[7] Quantitative testing for G6PD deficiency is routinely performed in all patients admitted to our hospital.

Categorical values were compared by χ^2 test. The differences between cases and controls for quantitative variables were analyzed by Student's *t*-test. Conditional logistic regression analysis, including as covariates known risk factors for NAION, such as systemic hypertension, diabetes mellitus, and hypercholesterolemia, was used to determine the significance of the association between G6PD deficiency and NAION.[12] Conditional logistic regression models, including as covariates systolic or diastolic blood pressure, plasma glucose level, and cholesterol level, were also performed. Models including the gender × G6PD-deficiency interaction were fitted to test whether gender is an effect-modifier of G6PD deficiency. OR and 95% CI were calculated. $P \leq 0.05$ were considered to be statistically significant. Statistical analysis was performed with commercial software (Stata ver. 9.0; StataCorp, College Station, TX).

Seven percent of the NAION cases and 4% of the control subjects who were eligible for the study declined to participate. The major reason was "not interested."

Results

The study group consisted of 140 patients with NAION (68 men, 72 women; mean age: 63.6 ± 11.2 years). The right and left eyes were affected approximately equally. Mean visual acuity was 0.44 ± 0.38 (range: 0.001–1) in the affected eye and 0.85 ± 0.29 (range: 0–1) in the fellow eye. The affected and the fellow eyes had almost identical mean intraocular pressure values (14.6 ± 2.1 and 14.7 ± 2.1 mm Hg). All the NAION patients had no history of glaucoma. No patient complained of pain with eye movements before visual loss. All the affected eyes showed no recovery of vision over time. Automated visual field examination disclosed altitudinal field defect in 81 (57.9%) eyes, generalized depression in 24 (17.1%), cecocentral defect in 15 (10.7%), broad arcuate scotomas in 11 (7.9%), and nasal depression in 9 (6.4%). Second-eye involvement was documented in 14 (10%) of the 140 patients.

The patients' and control subjects' systemic characteristics are reported in Table 1. Both patients and control subjects had similar levels of plasma glucose and similar rates of diabetes and angina–myocardial infarction ($P > 0.30$). On the other hand, NAION patients had significantly higher levels of systolic and diastolic blood pressure and plasma cholesterol, a significantly higher frequency hypercholesterolemia, and a significantly lower frequency of G6PD deficiency than the control subjects. For systemic hypertension and transient ischemic attacks/stroke, the data suggested a higher prevalence in NAION compared to control, but it was not significant at the 0.05 significance level ($P = 0.07$).

G6PD deficiency was found in 7 (5%) out of 140 patients with NAION and 34 (12.1%) of 280 control subjects. Enzyme deficiency was total in men (hemizygotes) and partial in women (homozygotes). None of the patients with G6PD deficiency had shown clinical manifestations of favism or drug-induced hemolysis in the 2 years before their enrollment in the study.

Conditional logistic regression results are reported in Tables 2 to 4. In the model including G6PD deficiency, systemic hypertension, diabetes, and hypercholesterolemia, the ORs for NAION revealed that G6PD deficiency was significantly associated with decreased risk for the vascular disorder (Table 2). In this model, hypercholesterolemia, but not hypertension or

Table 1. Systemic Characteristics of Patients with NAION and Control Subjects

	NAION ($n = 140$)	Control ($n = 280$)	*P* Total vs. Control
Systolic blood pressure, mm Hg (mean) ± SD	142.0 ± 19.6	134.1 ± 13.7	<0.0001
Diastolic blood pressure, mm Hg (mean) ± SD	83.1 ± 10.2	80.8 ± 7.8	0.01
Plasma glucose, mg/dL (mean) ± SD	105.5 ± 38.2	105.5 ± 30.0	0.99
Plasma cholesterol, mg/dL (mean) ± SD	207.6 ± 41.5	186.9 ± 28.1	<0.0001
G6PD deficiency, *n* (%)	7 (5.0)	34 (12.1)	0.02
Systemic hypertension, *n* (%)*	77 (55.0)	128 (45.7)	0.07
Diabetes mellitus, *n* (%)†	17 (12.2)	45 (16.1)	0.30
Hypercholesterolemia, *n* (%)‡	48 (34.3)	51 (18.2)	0.0001
Cardiovascular history, *n* (%) (angina/myocardial infarction)	16 (11.4)	24 (8.6)	0.35
Cerebrovascular history, *n* (%) (transient ischemic episodes/stroke)	10 (7.1)	9 (3.2)	0.07

* Blood pressure >140 mm Hg systolic or >90 mm Hg diastolic or taking antihypertensive medication.

† Fasting plasma glucose ≥126 mg/dL and/or plasma glucose ≥200 mg/dL 2 hours after a 75-g oral glucose load or taking insulin or oral hypoglycemics.

‡ Fasting plasma cholesterol >220 mg/dL or taking lipid-lowering drugs.

Table 2. Conditional Logistic Regression Analysis, Including G6PD Deficiency, Hypertension, Diabetes, and Hypercholesterolemia

Factor	OR	NAION 95% CI	*P*
G6PD deficiency (yes/no)	0.40	0.17–0.94	0.035
Hypertension (yes/no)	1.50	0.95–2.36	0.084
Diabetes (yes/no)	0.64	0.34–1.20	0.163
Hypercholesterolemia (yes/no)	2.28	1.40–3.72	0.001

Data show ORs for NAION ($n = 140$). Controls, $n = 280$.

Table 3. Conditional Logistic Regression Analysis, Including G6PD Deficiency, Systolic Blood Pressure, Plasma Glucose, and Cholesterol

Factor	OR	NAION 95% CI	*P*
G6PD deficiency (yes/no)	0.46	0.19–1.11	0.085
Systolic blood pressure (mm Hg)	1.03	1.02–1.05	0.001
Plasma glucose (mg/dL)	1	0.99–1	0.96
Plasma cholesterol (mg/dL)	1.02	1.01–1.02	0.001

Data are as described in Table 2.

Table 4. Conditional Logistic Regression Analysis, Including G6PD Deficiency, Diastolic Blood Pressure, Plasma Glucose, and Cholesterol

Factor	OR	NAION 95% CI	*P*
G6PD deficiency (yes/no)	0.46	0.19–1.07	0.071
Diastolic blood pressure (mm Hg)	1.02	0.99–1.05	0.12
Plasma glucose (mg/dL)	1	0.99–1	0.98
Plasma cholesterol (mg/dL)	1.02	1.01–1.02	0.001

Data are as described in Table 2.

diabetes, was significantly associated with increased risk for NAION.

In the models including systolic or diastolic blood pressure, plasma glucose level, and cholesterol level (Tables 3, 4), G6PD deficiency was associated with decreased risk for NAION, but the ORs were not significant at the 0.05 significance level ($P = 0.085$ and $P = 0.071$). In both models, plasma cholesterol, but not glucose, was found to be significantly associated with increased risk for NAION. Systolic blood pressure, but not diastolic blood pressure, was significantly associated with an increase in the risk for NAION.

Models including gender × G6PD-deficiency interaction disclosed that gender was not an effect modifier of G6PD deficiency ($P > 0.20$).

Discussion

This study demonstrates that G6PD-deficient patients in the Sardinian population are at a significantly decreased risk for NAION. Our results also corroborate earlier reports associating hypercholesterolemia with NAION.[11,13,14]

G6PD deficiency is the most common enzyme deficiency in humans, affecting an estimated 400 million people worldwide.[2] The disorder is found mainly in the tropical and subtropical regions of the world, with the highest rates, usually 5% to 30%, in Africa, Asia, the Middle East, the Mediterranean, and Papua, New Guinea.[15,16] In the United States, black males are commonly affected, with a prevalence of approximately 10%.[16] Sardinia is one of the areas with the highest prevalence, with reported rates ranging from 10% to 15%.[4–7] Several studies have suggested that the geographic distribution of G6PD deficiency, which correlates highly with the distribution of current or past malaria endemicity, is the result of a balanced polymorphism that confers resistance to infection with falciparum malaria.[17,18]

NAION, a common cause of sudden visual loss in patients between the ages of 55 and 70 years, is presumed to result from circulatory insufficiency within the optic nerve head. Many systemic conditions, which may decrease optic nerve head perfusion via either microvascular occlusion or reduced perfusion pressure, have been reported as risk factors. These include systemic hypertension,[10,11,19,20] diabetes mellitus,[10,11,13,19–21] hypercholesterolemia,[11,13,14] ischemic heart disease,[10,13,20] and acute blood loss.[22] Hayreh et al.[23] have suggested that marked nocturnal arterial hypotension, particularly in patients taking oral antihypertension drugs, may play a major role in the development of NAION.[23–25] In addition, a small anomalous optic disc with little or no physiologic cup ("crowded disc") may predispose to NAION, either by creating relative mechanical obstruction to axoplasmic flow or by amplifying the microvascular compression produced by optic disc edema of any origin.[26–28]

Unlike arteritic AION, where giant cell arteritis causes infiltration of the short posterior ciliary arteries and ischemic necrosis of the prelaminar, laminar, and retrolaminar portions of the optic nerve, the exact pathophysiology of NAION remains unproven. The rapid onset, stable course with generally poor recovery and association with vascular risk factors have implied a vascular cause of NAION. According to the most commonly proposed pathogenetic theory, impairment of the optic disc circulation, exacerbated by structural "crowding" of the nerve and its supporting structures at the nerve head, eventually reaches a point at which inadequate oxygenation produces ischemia and swelling of the disc.[29] In some cases, a cycle of ischemia, axonal swelling, microvascular compression, and further ischemia may lead to progressive nerve damage. Nocturnal systemic hypotension may contribute to the development of ischemia.[23–25]

In this case-control study, patients with NAION had significantly higher levels of plasma cholesterol and a significantly higher frequency of hypercholesterolemia. Furthermore, conditional logistic regression analysis revealed that hypercholesterolemia was significantly associated with increased risk for NAION. Our results are consistent with earlier studies, suggesting that hypercholesterolemia, a well-known risk factor for coronary heart disease, may also be an important risk factor for NAION.[11,13,14] Hypercholesterolemia is known to damage small blood vessels by impairing endothelial vasodilator function, probably by interfering with nitric oxide (NO), a potent vasodilator with antiatherogenic effects.[29]

Even though patients with NAION had significantly higher levels of systolic and diastolic blood pressure than did control subjects, both had similar rates of systemic hypertension. Likewise, conditional logistic regression analysis failed to find an association between systemic hypertension and NAION, but it also disclosed a significant association between systolic blood pressure and NAION ($P = 0.001$). Our results are in agreement with former studies,[11,13] but in disagreement with others.[10,11,19,20]

Unlike other reports,[13,19–21] we found that both patients and control subjects had similar rates of diabetes and similar levels of plasma glucose. This can be explained by several factors, including the high incidence of diabetes in Sardinia and the increased susceptibility to cataract in diabetic patients.[30,31]

We are unaware of any previously reported study that explored the hypothesis of a decreased risk for NAION in subjects with G6PD deficiency. In our study, the frequency of G6PD deficiency in patients with NAION was lower than expected. Conditional logistic regression analysis, including as

covariates G6PD deficiency, systemic hypertension, diabetes, and hypercholesterolemia, revealed that G6PD deficiency was significantly associated with decreased risk for NAION. Two other models, including systolic or diastolic blood pressure and plasma glucose and cholesterol levels, confirmed that G6PD deficiency was associated with a decrease in the risk for NAION, but the ORs fell just short of significance ($P = 0.085$ and $P = 0.071$). Models including gender × G6PD-deficiency interaction disclosed that there was not a significant difference in the effect of G6PD deficiency on NAION between the men and women.

Overall, our data suggest that G6PD deficiency may have a protective effect against NAION. This result, along with the previous finding of a significantly decreased risk for retinal vein occlusion in G6PD-deficient patients,[7] seems to suggest that individuals with G6PD deficiency may be less susceptible to ocular vascular disorders.

The reason that a partial deficiency, found in heterozygous females, may offer similar protection against NAION and retinal vein occlusion as a total deficiency, found in hemizygous males, is unclear and needs further research. Other studies examining a possible protective effect of G6PD deficiency found a similar paradoxical gender difference. Indeed, heterozygous females with mosaic populations of normal and G6PD-deficient erythrocytes, due to random X chromosome inactivation, have been shown to have malaria resistance similar to or greater than hemizygous males with populations of uniformly deficient erythrocytes.[32,33]

Similar to other studies analyzing the relationship between certain risk factors of vascular disease and NAION, the control group was selected from patients undergoing cataract surgery.[14,15] It is highly unlikely that this strategy introduced a selection bias, because the frequency (12.1%) of G6PD deficiency found in the control group was the same as in the general Sardinian population.[4–7] This result rules out the hypothesis that G6PD deficiency might result in increased susceptibility to cataract, in full agreement with former studies showing that G6PD-deficient patients do not have a higher risk for cataract.[7,34]

A clear limitation of this study is that it was restricted to a limited, genetically homogeneous group of patients (i.e., those of Sardinian ancestry). As a result, our findings may not be applicable to patients with NAION of non-Sardinian ancestry. Another potential limitation is that we did not assess the impact of tobacco smoking. However, in a recent large-cohort study, Hayreh et al.[20] found no association between NAION and tobacco smoking.

A large body of experimental evidence linking G6PD activity, cholesterol synthesis, and cell growth has accumulated in recent years.[6,35] Of note, Batetta et al.[6] have recently shown that the Mediterranean variant of G6PD deficiency is characterized by peculiar alterations in plasma and intracellular cholesterol metabolism, such as reduced synthesis and esterification. As a consequence, in G6PD-deficient subjects the reduced ability to esterify and accumulate cholesterol in the arteries may account for a lower risk for atherosclerotic disease. These findings may explain our results, calling into question a mechanistic connection between G6PD deficiency and NAION. Theoretically, the slower progression of the atherosclerotic process involving the short posterior ciliary arteries may reduce the degree of impairment in the normal autoregulatory mechanisms of the optic nerve head vasculature at the level of the prelaminar region and therefore may reduce the risk for NAION.

Balance between the levels of NO and glutathione, a physiological scavenger of NO,[36] may also be an important factor in preventing the occurrence of vascular diseases. It is unknown whether the two factors are out of balance in G6PD-deficient individuals. Both NO and its *S*-nitrosocysteine adduct are powerful vasodilators and act as scavengers of superoxide radicals, thus abrogating their toxicity and preventing the oxidation of low-density lipoproteins.[37–41] They also inhibit platelet aggregation, leukocyte adhesion, and vascular smooth muscle proliferation.[42,43] In particular, NO-related inhibition of platelet aggregation may in turn inhibit the release of platelet serotonin, which is thought to play a role in the development of ischemic optic nerve damage by causing vasoconstriction and impairment of autoregulation.[44,45]

Overall, our study suggests that patients with G6PD deficiency in the Sardinian population may have a significantly lower risk for NAION. However, further studies are necessary for a better understanding of the mechanism by which G6PD deficiency may offer protection against vascular disorders and to establish whether G6PD-deficient patients of non-Sardinian ancestry show an equally reduced susceptibility to NAION.

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