

Research Paper

Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency and Late-stage Age-Related Macular Degeneration

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Abstract

Purpose: Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly in Western Countries. Evidence indicates that Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency, a common genetic abnormality, may protect against ischemic heart and cerebrovascular disease, ocular vascular disorders, and colorectal cancer. This study was undertaken to ascertain whether G6PD deficiency may protect against AMD.

Materials and Methods: 79 men with late-stage AMD and 79 male, age-matched cataract controls without AMD were recruited in March-December 2016. Smoking status, clinical history, and drug use were recorded. A blood sample was taken from each participant. Complete blood count, hemoglobin, glucose, creatinine, cholesterol, triglycerides, transaminases, bilirubin, and erythrocyte G6PD activity were measured. Stepwise logistic regression was used to investigate the association between G6PD deficiency and AMD.

Results: G6PD deficiency was found in 7 (8.9%) AMD patients and 8 (10.1%) controls, a not statistically significant difference. Stepwise logistic regression disclosed that AMD was significantly associated with increased diastolic blood pressure (OR=1.09, 95% CI=1.03-1.15, P=0.02) and LDL-cholesterol (OR=1.02, 95% CI=1.0001-1.03, P=0.049) and lower values of white blood cell (WBC) count (OR=0.71, 95% CI=0.56-0.88, P=0.02) and aspartate aminotransferase (AST) (OR=0.92, 95% CI=0.85-0.99, P=0.044).

Conclusion: Results suggest that G6PD deficiency has no protective effect on nor is a risk factor for AMD. Larger studies are necessary to confirm whether increased diastolic blood pressure and LDL-cholesterol and lower values of WBC count and AST are risk factors for AMD.

Key words: age-related macular degeneration (AMD); Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency; observational case-control study; stepwise logistic regression analysis

Introduction

Age-related macular degeneration (AMD) is a leading cause of severe, irreversible vision impairment in adults aged over 50 in the Western

Countries.¹ AMD can be classified into early and late stages. Early AMD is a clinical condition without clearly evident visual symptoms, characterized by

retinal pigment epithelium alterations and/or drusen in the macular area.² The late-stage manifestations of AMD include geographic atrophy (dry AMD) and neovascular (wet) AMD. The hallmark of wet AMD is the presence of choroidal neovascularization (CNV), defined as formation of pathological neovessels originating from the choroidal vasculature and extending through a defect in the Bruch's membrane. The pathological mechanisms underlying AMD are not clear, but clinical and experimental evidence indicates that genetic predisposition and environmental factors, such as tobacco smoking and oxidative stress, may play a major role.³⁻⁹

Glucose-6-Phosphate Dehydrogenase (G6PD) is a ubiquitous cytoplasmic enzyme converting glucose-6-phosphate into 6-phosphogluconate in the pentose phosphate pathway. G6PD is the rate-limiting enzyme of this metabolic pathway that supplies reducing energy to cells by maintaining the level of the reduced form of the extramitochondrial Nicotine-Adenosine-Dinucleotide Phosphate (NADPH) coenzyme. In red blood cells, defense against oxidative stress is strictly dependent on G6PD activity, because the G6PD/NADPH pathway is the only source of reduced glutathione (GSH).¹⁰ The gene encoding G6PD is on the distal long arm of the X chromosome (band Xq28). Hemizygous males have populations of uniformly deficient erythrocytes, whereas heterozygous females have mosaic populations of normal and G6PD-deficient red blood cells, due to random inactivation of X chromosome. G6PD deficiency is genetically heterogeneous and about 400 different variant enzymes have been described.¹¹ The G6PD-Mediterranean variant (C to T transition at nucleotide 563) is associated with undetectable levels of enzyme activity by routine methods (WHO class II). This variant is common in the island of Sardinia, Italy, where the reported prevalence of G6PD deficiency is 8-15%.¹²⁻¹⁸ G6PD deficiency represents a public health issue in Sardinia, because of the seasonal occurrence of favism, a hemolytic anemia caused by the ingestion of the broad bean (*Vicia faba*) in affected subjects.^{11,13}

Former studies have suggested that G6PD deficiency may have a protective effect on ischemic heart and cerebrovascular disease and colorectal cancer.^{14,19,20} Additionally, G6PD-deficient individuals have been reported to have a significantly decreased risk of developing retinal vein occlusion (RVO) and nonarteritic anterior ischemic optic neuropathy (NAION).^{16,21} The concept that G6PD deficiency may protect against the development of various age-related diseases is new and not yet established.

Little is known about the role of G6PD deficiency in AMD. The present study was designed to assess the

frequency of G6PD deficiency in Sardinian men with AMD and ascertain whether G6PD deficiency is a risk factor for AMD or may have a protective effect against this important cause of blindness in the elderly.

Patients and Methods

The present study used a case-control design, recruiting 79 consecutive men with late-stage AMD and 79 perfectly age-matched male controls without AMD between March and December 2016. Women were excluded because of the small number of homozygote subjects with complete lack of erythrocyte G6PD activity. Sample size was computed before the survey, using a two-tailed test at 5% significance level with an 80% statistical power to detect an estimated relative risk of 0.4, assuming a G6PD prevalence rate of 8% as reported previously,^{13,17,18} and an incidence of 25,000 new AMD cases per year (data from the Istituto Nazionale di Statistica - ISTAT, 2004, Italy).

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.

The inclusion criteria for cases were male gender, Sardinian descent, and the diagnosis of late-stage AMD (neovascular AMD or geographic atrophy involving the center of the macula) in at least one eye.² All AMD patients underwent a full ophthalmic evaluation, including fluorescein angiography and OCT scans of the macula (3D OCT-1000 Mark II, Topcon Co, Tokyo, Japan).

Perfectly age-matched male controls of Sardinian ancestry were selected among patients undergoing cataract surgery. All controls underwent standard ophthalmic evaluation, including best corrected visual acuity, slit-lamp examination, applanation tonometry, and fundus examination. Patients with any clinical evidence of maculopathy (i.e. early, dry or wet AMD, cystoid macular edema, epiretinal membrane, retinal pigment epithelium changes, central serous chorioretinopathy, diabetic macular edema, etc.) and/or retinal vascular disorder (retinal vein occlusion, diabetic retinopathy, etc.) were excluded.

Medical conditions, including body mass index (BMI), systemic hypertension, hypercholesterolemia, diabetes mellitus, cardio- and cerebro-vascular status (positive history of angina, myocardial infarction, TIA, stroke), renal failure, and medication use were also recorded for both AMD patients and controls. Definitions of systemic hypertension, diabetes, and hypercholesterolemia have been reported previously.¹⁶

Smoking history was obtained by an interviewer-administered questionnaire. Current smoking status was compared with noncurrent smoking (individuals who smoked in the past or never smoked).

Sitting blood pressure was measured three times after resting for at least 5 minutes. The average of the three measurements was used for the analysis.

A blood sample was taken from each participant after an overnight fast. Standard laboratory tests, including complete blood count, hemoglobin, glucose, creatinine, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, transaminases (ALT and AST), and bilirubin were performed. Red blood cell G6PD activity was determined using a quantitative assay (G6PD/6PGD, Biomedic snc, Sassari, Italy), as described previously.¹⁶

In addition, serum vitamin B12 and folate levels were measured in all patients with AMD. Vitamin B12 and folate were measured using an IMX Analyzer (Abbott Laboratories Diagnostics Division, Abbott Park, IL). The IMX B12 assay is based on the microparticle enzyme immunoassay (MEIA) technology, while IMX folate is an ion capture assay technique. For these immunological assays, inter- and intra-assay coefficients of variation were <10%. Normal values for plasma vitamin B12 and folate are 179-1162 pg/ml and 2.7-34 ng/ml, respectively.

Descriptive analysis was performed for all variables measured. Categorical variables were compared by Z test for proportions and differences for quantitative variables were analyzed by Student's t test.

Stepwise logistic regression models were performed to calculate the estimates of odds ratios (ORs), considering AMD as dependent variable and including BMI, systolic blood pressure, diastolic blood pressure, blood glucose, creatinine, total cholesterol, LDL, HDL, triglycerides, white blood cell (WBC) count, hemoglobin, ALT and AST, bilirubin, G6PD deficiency, smoking status, concomitant cardio- and cerebro-vascular disorders (Yes/No), and use of antiplatelet and/or anticoagulant medications (Yes/No) and lipid-lowering drugs (Yes/No) as covariates (full model). Furthermore, interaction terms were added to the regression models to detect possible confounders. A significance level of 0.2 was used to remove covariates from the multivariable model. ORs and 95% confidence intervals (CIs) were obtained by a maximum likelihood estimation. P values <0.05 were considered to be statistically significant. Statistical analysis was performed with commercial software (STATA ver. 12; StataCorp, College Station, TX).

Four percent of the AMD cases and 7% 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 79 AMD men (mean age: 78±7 years, range 57-92 years). The control group included an equal number of perfectly age-matched male subjects without AMD. In both groups, all individuals were of Sardinian ancestry.

In the AMD group, 19 patients had bilateral wet AMD, 1 bilateral geographic atrophy, 3 wet AMD in one eye and geographic atrophy in the fellow one, and 56 wet AMD in one eye and early AMD in the fellow eye.

Wet AMD patients had received an average of 5 intravitreal injections of an anti-VEGF agent (bevacizumab, ranibizumab, or aflibercept) per eye.

The AMD and cataract groups had identical mean intraocular pressure values (14±2.6 mm Hg and 14±3.5 mm Hg). Four (5%) AMD and 7 (9%) cataract patients had glaucoma, a not statistically significant difference.

Demographics, medical history information, and blood test results are summarized in Table 1. All the diabetic patients had type 2 diabetes. Both AMD patients and control subjects had similar rates of diabetes, hypertension, hypercholesterolemia, cardio- and cerebro-vascular disorders, chronic renal failure, G6PD deficiency, smoking, and use of antiplatelet and/or anticoagulant medications and lipid-lowering drugs. Likewise, there were no significant differences in BMI, hemoglobin, plasma glucose, creatinine, LDL, triglycerides, ALT, and bilirubin values. On the other hand, AMD patients had significantly higher values of systolic and diastolic blood pressure, plasma cholesterol, and HDL, and significantly lower values of WBC count and AST.

Mean plasma vitamin B12 and folate values in AMD patients fell within normal ranges (299 pg/ml and 5.2 ng/ml, respectively).

Forward-stepwise selection led to a final logistic regression model with 8 variables, including diastolic blood pressure, LDL, HDL, WBC count, hemoglobin, AST, smoking status, and concomitant cardio- and cerebro-vascular disorders (Yes/No). In this model, logistic regression analysis showed that AMD was significantly associated with higher values of diastolic blood pressure (OR = 1.09, 95% CI = 1.03-1.15, P = 0.02) and LDL (OR = 1.02, 95% CI = 1.0001-1.03, P = 0.049) and lower values of WBC count (OR = 0.71, 95% CI = 0.56-0.88, P = 0.02) and AST (OR = 0.92, 95% CI = 0.85-0.99, P = 0.044). No interaction term was found to be statistically significant.

Table 1. Demographics, medical history information, and blood test results of patients with age-related macular degeneration (AMD) and cataract controls without AMD.

	AMD patients (n = 79)	Controls (n = 79)	P values (Cases vs Controls)
Systemic hypertension, n (%) [*]	55 (69.6)	51 (64.6)	0.5
Diabetes mellitus, n (%) [†]	14 (17.7)	20 (25.3)	0.25
Hypercholesterolemia, n (%) [‡]	37 (46.8)	42 (53.2)	0.43
Body mass index (kg/cm ²), mean ± SD	27.2 ± 5.5	27.1 ± 3.7	0.84
Cardiovascular and Cerebrovascular disease, n (%)	11 (13.9)	10 (12.7)	0.82
Chronic renal failure	3 (3.8)	2 (2.5)	0.65
G6PD deficiency, n (%)	7 (8.9)	8 (10.1)	0.79
Smoking, n (%)	11 (8.7)	11 (8.7)	1
Systolic blood pressure (mm Hg), mean ± SD	141.2 ± 18.4	134.1 ± 14.4	0.0075
Diastolic blood pressure (mm Hg), mean ± SD	80.6 ± 9.3	76.1 ± 9.9	0.0039
White blood cell count (x10 ³ /μL)	7.0 ± 1.9	7.99 ± 2.4	0.0073
Hemoglobin (g/dL), mean ± SD	14.0 ± 1.62	14.0 ± 2.2	0.99
Plasma glucose (mg/dL), mean ± SD	107.8 ± 23.4	121.1 ± 57.4	0.06
Total cholesterol (mg/dL), mean ± SD	193.3 ± 35.6	178.6 ± 42.5	0.02
HDL (mg/dL), mean ± SD	54.7 ± 12.1	49.3 ± 12.3	0.0055
LDL (mg/dL), mean ± SD	116 ± 35.2	105.0 ± 38.4	0.0643
Triglycerides (mg/dL), mean ± SD	103.5 ± 53.3	117.6 ± 58.4	0.11
Creatinine (mg/dL), mean ± SD	0.9 ± 0.4	1.07 ± 0.6	0.06
ALT (U/L), mean ± SD	16.6 ± 7.7	19.8 ± 14.2	0.08
AST (U/L), mean ± SD	18.3 ± 6.2	22.4 ± 12.2	0.0075
Bilirubin (mg/dL), mean ± SD	0.8 ± 0.4	0.9 ± 0.5	0.09
Antiplatelet and/or anticoagulant drug use, n (%)	28 (35.4)	28 (35.4)	1
Use of lipid-lowering drugs, n (%)	24 (30.4)	27 (34.2)	0.61

* 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.

Discussion

G6PD deficiency is the most common enzyme deficiency in humans, with an estimated 400 million people affected worldwide.¹¹ It has a high prevalence (5-30%) in the tropical and sub-tropical regions of the world, including Africa, Asia, the Middle East, the Mediterranean, and Papua New Guinea.^{22,23} In the U.S., Afro-American males are commonly affected, with a prevalence of 10%.²³ The island of Sardinia is one of the areas with the highest prevalence, with rates of between 8 and 15%.¹²⁻¹⁸

The geographic distribution of G6PD deficiency is highly correlated with the distribution of current or past malaria endemicity. This finding might be the result of a balanced polymorphism conferring resistance to infection with *falciparum* malaria.¹¹ Furthermore, recent research has found supportive evidence for protection against ischemic heart and cerebrovascular disease, RVO, NAION, and colorectal cancer in G6PD deficient individuals.^{14,16,19-21} Overall, these data seem to suggest that G6PD deficiency may have a protective effect against the development of various age-related diseases.

The main risk factors for the development of late-stage AMD include ageing, genetics, ethnicity, and oxidative stress.³⁻⁹ Tobacco smoking is the main modifiable risk factor consistently identified in many studies;^{6,24} thus, stopping smoking is strongly recommended in patients at risk for AMD. A number of population-based and case-control studies have investigated the correlation between AMD, systemic hypertension, and other cardiovascular diseases.²⁵⁻³² These studies have reported conflicting results.

Little is known about the role of G6PD deficiency in the pathogenesis of AMD. To the best of our knowledge, we are unaware of any previous investigation exploring a possible relationship between late-stage AMD and G6PD deficiency. In theory, a decrease in NADPH production might paradoxically result in protection against oxidative stress, as reported by a recent survey associating high G6PD activity and elevated NDAPH levels with endothelial and vascular dysfunction.³³ In fact, G6PD-derived NADPH, a cofactor for NADPH oxidase, enhances superoxide anion generation, thus increasing oxidative stress.³³ G6PD deficiency might also offer protection against choroidal

microangiopathy because of the decreased cholesterol synthesis.¹⁵

In our study, we found that late-stage AMD patients and cataract controls without AMD had similar values of G6PD prevalence (8.9% and 10.1%, respectively). Stepwise logistic regression analysis showed that G6PD deficiency has no significant protective effect against AMD, nor is a risk factor for this degenerative macular disorder. Even though there does not appear to be a substantial protective or enhancing effect of G6PD deficiency on the development of AMD, there is still the possibility of a less pronounced effect that is missed because of a type II error due to the relatively small sample size, largely dependent on the incidence of AMD. Indeed, if we, hypothetically, increased by 60 times the number of cases and controls (4,740 instead of 79) and maintained the same prevalence rates of G6PD deficiency as above, we would find a statistically significant protective effect of G6PD deficiency on late-stage AMD (OR = 0.86; 95% CI = 0.75-0.99; P = 0.036).

Our investigation also shows that late-stage AMD is significantly associated with higher values of diastolic blood pressure and LDL, even though, particularly for LDL, the OR is pretty small. Our results are consistent with former studies identifying systemic hypertension and serum cholesterol levels as risk factors for AMD,^{25,28,32,34-37} but conflicting with others.^{26,37,38} Lipid metabolism has long been hypothesized to be important in the pathogenesis of AMD.³⁹ Indeed, lipid deposits in Bruch's membrane represent at least 40% of the volume of drusen.⁴⁰

A correlation between systemic hypertension, cholesterol, and AMD would suggest the presence of shared risk factors for cardiovascular disease and AMD, and may support the hypothesis of a similar pathogenesis. However, in the present survey, AMD patients and control subjects without AMD had similar rates of cardio- and cerebro-vascular disorders, a result contrasting with the former hypothesis.

In our study, we found that patients with AMD had a significantly lower WBC count; furthermore, AMD risk was significantly associated with lower values of WBC count. Previous epidemiological studies have shown conflicting results regarding the association between WBC count and AMD. Whereas multiple studies have reported correlations between higher WBC counts and an increased risk of AMD,^{36,41,42} other studies have failed to find such an association.⁴³⁻⁴⁵ This evidence is further complicated by our finding, showing an inverse correlation, i.e. that a higher WBC count is associated with a lower risk of AMD. Overall, these results suggest that the

role of leukocyte count in AMD is far from clear.

In our survey, patients with AMD had significantly lower values of AST, and AMD risk was significantly associated with lower AST values. We are unaware of any former study exploring a possible relationship between AMD and transaminases. The significance of our finding, implying that higher AST values may have a protective effect against AMD, remains obscure and needs further investigation.

It is well known that smoking significantly increases the risk of AMD. Nevertheless, in our study, AMD patients and cataract controls without AMD had similar rates of smoking. This result does not come as a surprise, because smoking is a modifiable risk factor not only for AMD, but also for senile cataract.⁴⁶⁻⁴⁸

In our investigation, the controls were selected among patients undergoing cataract surgery. We feel strongly that this strategy did not lead to a selection bias.⁴⁹ Actually, the prevalence rate (10.1%) of G6PD deficiency in the control group was the same as in the male Sardinian population,^{13,17,18} a finding consistent with former large studies showing that G6PD deficient patients do not have a higher risk of developing cataract.^{17,50}

Our study has several important limitations, including its cross-sectional design and the relatively small sample size. Furthermore, it was restricted to a limited, genetically homogeneous group of patients (i.e. those of Sardinian descent). Therefore, our findings may not be applicable to AMD patients of non-Sardinian ancestry. Last, but not least, even though both wet and dry forms of AMD have common underlying pathological features and causes, we analyzed a small number of patients with geographic atrophy.

In conclusion, our results suggest that G6PD deficiency has no protective effect against AMD, nor is a risk factor for this degenerative macular disorder. On the other hand, AMD was significantly associated with higher values of diastolic blood pressure and LDL and lower values of WBC count and AST. These findings need to be confirmed by larger scale studies, also involving patients of non-Sardinian ancestry.

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Competing Interests

The authors have declared that no competing interest exists.

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