

Is the GSTM1 Null Polymorphism a Risk Factor for Primary Angle-Closure Glaucoma among Iranian Population?

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Abstract- Glutathione S-transferases (GSTs) are members of multigenic family which have the essential function in cells as an antioxidant. In the present study we studied the polymorphism of GSTT1 and GSTM1 deletion genotypes in Iranian patients with primary closed angle glaucoma (PCAG) compared to healthy subjects. We conducted a study of 41 PCAG patients (24 women, 17 men) and 100 healthy participants (57 women, 43 men) to determine the prevalence of GSTT1 and GSTM1 deletion genotypes and the risk of PCAG, which were determined by multiplex polymerase chain reaction. Genotypes of GSTM1 and GSTT1 null deletions were determined in 22 (53.7%) and 7 (17.1%) patients with PCAG and 34 (34%) and 15 (15%) in healthy participants. Comparison of patients and healthy ones regarding GSTM1 and GSTT1 genotypes revealed increase of GSTM1 null deletions genotypes' in patients with PCAG ($P=0.03$). It was concluded that the increased frequencies of GSTM1 null in patients with PCAG could be associated with a risk factor for incidence of PCAG in the Iranian population.

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Introduction

Glaucoma is a complex, heterogeneous disease with a multi-factorial etiology including mechanical damage because of increased intraocular pressure (IOP), variable susceptibility of the optic nerve, mutations in specific nuclear genes, changes in the mitochondrial genome, toxic effects, and oxidative damage caused by reactive oxygen species (ROS) (1).

The prevalence of glaucoma in adults over 40 years of age has been reported from 2%- 8.8% in different parts of the world and the prevalence of glaucoma in adults 40 years of age or older in Tehran is estimated 1.44% (2).

The most common types of glaucoma include primary open-angle glaucoma (POAG) and primary closed-angle glaucoma (PCAG).

Primary angle closure is apposition or adhesion of the iris to the human trabecular meshwork (HTM). As a result of crowded anterior segment anatomy in a predisposed eye, PCAG must be differentiated from secondary forms of angle closure (3).

Oxidative free radicals and ROS can affect the cellularity of the HTM. Much evidence indicates that in this region ROS plays a fundamental pathogenic role by reducing local antioxidant activities inducing outflow resistance and exacerbating the activities of superoxide dismutase and glutathione peroxidase in glaucomatous eyes (4).

Different gene families are identified in detoxification or reduction process of the production of ROS. Glutathione S-transferases (GSTs; EC: 2.5.1.18), are members of a multi-gene family of phase II metabolic enzymes which catalyze the detoxifying reaction of a wide variety of toxic and carcinogenic compounds by conjugating them to glutathione, thus protecting cells from oxidative damage. Human cytosolic GSTs are divided into seven different classes, including I, h, and p. There are few polymorphisms in the genes of these enzymes affecting the enzyme activity. One of them is deletions in GSTM1 and GSTT1 genes which accompanied by lacking enzyme activity (5).

The blood-aqueous barrier and its scavenging system

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preserve the clarity of the lens of the eye. This barrier consists mainly of the aryl hydrocarbon hydroxylase and the glutathione S-transferase systems, and GSTs are considered to be key enzymes, protecting the eye from toxic chemicals and electrophiles (6). Therefore, the aim of this study was to determine the frequency of GST genotypes in patients with PCAG compared to controls and to find the possible relation between GSTs genotypes and PCAG in Iranian population. To our knowledge, the current study is the first investigation conducted in Iranian PCAG patients.

Materials and Methods

Patients and controls studied participants consisted of 41 patients with PCAG confirmed by clinical tests and examined by glaucoma specialist (24 male, 17 female, with mean age 55.8 ± 11.8 years), and 100 controls (43 males, 57 females with mean age 54.5 ± 7.8 years). All controls and patients were Iranian, and patients were selected from the Glaucoma Clinic at Rasoul Akram hospital and Farabi eye hospital in Tehran, Iran. Written informed consent was obtained from all participants. The research protocol was approved by the Ethics Committee of Lorestan University Medical Sciences.

Clinical evaluation

A complete medical history was taken from all participants.

Refraction, best-corrected visual acuity, slit lamp biomicroscopy, Goldmann applanation tonometry, funduscopy, and gonioscopy were performed in patients.

In patients the IOP (intraocular pressure) was higher than 21 mmHg at the time of diagnosis and Cup-to-disc ratios were between 0.6 and 0.9.

Patients with a history of eye surgery before the diagnosis of glaucoma, uveitis, and trauma or with evidence of secondary glaucoma, such as exfoliation, pigment dispersion or uveitis were excluded from the study.

Controls had no family or personal history and abnormalities suggestive of glaucoma, and their IOP were below 21 mmHg. Patients and controls were matched in terms of smoking, aging, sex, and race.

Statistical analysis

Age of the patient and the control group was compared with student's *t*-test. The *chi*-square test was applied to compare differences in sex between patients and controls. All values were represented as mean \pm S.D. Genotypes of GSTT1 and GSTM1 were grouped as

either null (homozygous deletion) or non-deleted. Odds ratio (OR) with 95% confidence limits computed by logistic regression was used to analyze the occurrence of frequencies of the GSTM1 and GSTT1 genotypes. *P*-values were two-tailed, and a value of < 0.05 was considered statistically significant. All analyses were carried out using SPSS Version 15 statistical analysis software.

Sample collection and DNA analysis

Five ml venous blood was collected in a tube containing ethylenediamine tetraacetate (EDTA) as an anticoagulant for DNA extraction. Genomic DNA was extracted from peripheral venous blood using Roche DNA extraction kit (CAT NO.117968288001).

GSTM1 and GSTT1 genetic polymorphisms were assessed using multiplex PCR technique. The PCR primers were synthesized with gene runner software and made by Cinaclon Company of Iran, Tehran. Primers for GSTM1 were 5'- GAA CTC CCT GAA AAG CTAA AGC-3' and 5' GTT GGG CTC AAA TAT ACG GTG G-3' and for GSTT1 were 5'- TTC CTT ACT GGT CCT CAC ATC TC-3' and 5'- TCA CCGGACAT GGC CAG CA-3'.

The dihydrofolate reductase (DHFR) locus was used as an internal control to avoid false-negative readings. Primers for DHFR were 5'-GGA ATG GAG AAC CAG GTC GTC TT-3' and 5' -GCA TGT CTT TGG GAT GTG GA-3'.

PCR reaction was carried out in a total volume of 25 μ l containing 10 pmol of each primer, 1.5 mmol / L of MgCl₂, 0.25 mmol/L of each deoxynucleotide triphosphate, Number of 2 unit of Taq polymerase, and 100-500 ng of genomic DNA. amplification was performed by initial denaturation at 94 0 C for 5 minutes, followed by 35 cycles at 94 0 C for 30 second, 64 0 C for 45 second and 72 0 C for 1 minute and a final extension of 72 0 C for 5 minutes. The amplified products were identified by electrophoresis in a 2% agarose gel and stained with 0.5 μ g/ml ethidium bromide. The product lengths were 215 bp, 480 bp, and 280 bp for GSTM1, GSTT1, and DHFR.

The T1M1 genotype determined by two bands of 480 bp for GSTT1 and 215 bp for GSTM1 The T1M0 genotype showed one band of 480 bp, and the T0M1 genotype showed a band of 215 bp. For the T0M0 genotype (deletion of two genotype), no bands were showed and therefor the use of DHFR as internal positive control was necessary to discerned the null genotype from aborted PCR reactions (Figure 1).



Figure 1. Polymerase chain reaction (PCR) analysis of glutathione S-transferase (GST) gene polymorphism. The line with 480 bp stands for GSTT1, 280 bp for DHFR and 215 bp for GSTM1. Columns 1 and 6 GSTT1 null/GSTM1 positive, columns 2 and 5 and 9 GSTT1 positive/GSTM1 positive, columns 3 and 7 GSTT1 positive/GSTM1 null and column 4 and 8 GSTT1 null/GSTM1 null. Columns 2 and 5 and 9 GSTT1 positive/GSTM1 positive, columns 3 and 7 GSTT1 positive/GSTM1 null and column 4 and 8 GSTT1 null/GSTM1 null.

Results

The number of patients with PCAG was 41, and the number of controls was 100. All participants were of Iranian origin.

Table 1 shows the demographic characteristics for glaucoma patients and the control group. The mean age of the control group was 54.5±9.89 years, 43 of them (43 %) were. The mean age of the PCAG group was 55.8±11.8 years, 24 of them (58.4 %) were. Patients and controls were not significantly different in terms of sex ($P=0.68$), age ($P=0.19$), and smoking status ($P=0.079$); therefore, these variables were not evaluated further.

We compared the frequencies of GSTM1 and GSTT1 null genotypes in patients with PCAG and controls. GSTM1 null was 22(53.7%) ($P=0.03$) and GSTT1 null was 7(17.1 %) ($P=0.75$) in PCAG patients.

A significant ($P=0.03$) decrease in the frequency of GSTM1 null genotypes was found between the PCAG. (Table 2)

Table 2 shows GST genotype distribution and the risk of developing primary closure-angle glaucoma. It shows that the frequency of the T1M1, T0M1 ($P=0.46$) T1M0 ($P=0.1$) and T0M0 ($P=0.08$) genotypes had no significant difference in PCAG patients than in controls.

Table 1. Demographic characteristics of the study groups

Study Groups	Control Group	PCAG Group	P. Value
Number of Subjects	100	100	-
Sex	Male, n (%)	43(43%)	24 (58.4%)
	Female, n (%)	57(57%)	17 (41.6%)
Age (years) Mean ± SD	54.5 ±9.89	55.8±11.8	0.19
Smoker, n (%)	6%	11%	0.079

Table 2. Glutathione S transferase genotypes and the risk of developing PCAG

Genotype	Control Group (N=100)	PCAG (N=41)	OR	CI95%	P. Value
GSTM1	Present n (%)	66(66%)	19(46.3%)	2.2	1.3-4.7
	Null n (%)	34(34%)	22(53.7%)		
GSTT1	Present n (%)	66(66%)	34(83.9%)	1.1	0.43-3.1
	Null n (%)	34(34%)	7(17.1%)		

Table 3. Glutathione S-transferase genotypes and the risk of developing PCAG

Genotype	Control Group	PCAG group	OR	CI95%	P. Value
T1M1	56(56%)	17(41.5%)	Reference	-	-
T1M0	29(29%)	17(41.5%)	1.9	0.86-4.3	0.1
T0M1	10(10%)	2(4.9%)	0.65	0.31-3.3	0.46
T0M0	5(5%)	5(12.2%)	3.2	0.85-8.7	0.08
Total	100(100%)	100(100%)	-	-	-

Discussion

Glaucoma, the second leading cause of blindness, is characterized by changes in the optic disc and defects of the visual field. The basic cause of glaucoma is largely unknown. First-degree relatives of glaucoma patients have 8–10 times increased risk of developing the disease, making genetic predisposition a strong risk factor (7).

As the pathogenic role of ROS in glaucoma has been indicated by many reseaches, cellular defense mechanisms alleviating the toxic manifestations of oxidative insult must have an important role in the protection against the development of glaucoma. As GST enzymes are one of the important families of enzymes against oxidative stress, their genetic polymorphisms may alter the critical function of the enzymes in protecting against electrophiles and the products of oxidative stress in glaucoma (8). Glaucoma patients in this study had PCAG. Controls were well matched to patients for age, sex, smoking, and ethnicity.

PCAG has been attributed primarily to elevated IOP caused by anatomic changes in the anterior and posterior globe (9,10). However, in each variant, ocular phenomena must interact with the posterior globe to reason optic nerve injury (10-12), and recent evidence supports the hypothesis that ROS and oxidative stress may play a chipping in task in glaucomatous optic nerve injury at several levels (13). Oxidative stress may be directly involved in optic nerve neuronal cell death (14). In addition, human trabecular meshwork (TM) possesses abundant antioxidant activity, and ROS compromises TM integrity (4,15).

In current study, the GSTM1 Null genotype was significantly more common in the PCAG group compared to the control group which shows a correlation between the GSTM1 Null genotype and the incidence of PCAG. Present results are supported by previous ones in the Arab population in which GSTM1 Null genotype is a risk factor for developing PCAG ($P=0.001$).

Also, we found that there is no association between GSTT1 and PCAG ($P=0.75$). This result is consistent with the study by Khaled *et al.*, 2008 (1). Apparently there is only one previous case study (in Arab population) about association between GST and PCAG, therefore we compare present results with other populations.

However, we know that the oxidative stress is the result of increased production of reactive oxygen and nitrogen species that has been implicated retinal

ganglion cell (RCG) death. Recent in vitro studies using primary culture of RCG have also provided evidence that different glaucomatous stimuli involve increased generation of reactive oxygen species, and antioxidant treatment provides additional protection. However, although the association of free radicals in the pathophysiology of PCAG has not been approved yet, we suggest their possible role in this disease.

However oxidative stress primary might acceleratethe risk of optic nerve damage in glaucoma. This may be correct even in glaucoma variants that seem primarily anatomic in the mechanism of optic nerve injury such as PCAG. For example a study by Izotti *et al.*, suggests that oxidative DNA damage is significantly increased in the trabecular meshwork of glaucoma patients and GSTM1 gene deletion predisposed to more severe oxidative DNA damage in glaucoma patients (15). Ferreira *et al.*, in Argentine suggests that free radicals action play a role in the worse damage observed in glaucoma eyes (16). Yang *et al.*, 2001 showed that GST antigen was found in 52% of patients with glaucoma and in 20% of controls (17). The patients had significantly higher titers of anti-GST antibody compared with controls. Furthermore, the related retinal antigen belonged to the GST mu class (17).

Thus, it can be inferred that people who have GSTM1 genotype are at increased risk of developing autoantibodies against this protein, which is connected to an increased risk of glaucoma.

Also many studies have suggested that GSTM1 null is increased in patients with POAG that it is another type of glaucoma. For example Izzotti *et al.*, in an Italian population 2004 (13) and Yildirim *et al.*, in Turkish population 2005 (18) and Rossini *et al.*, in Brazilian population 2000 (19), reported that POAG is associated with the GSTM1 null genotype.

In conclusion, increased frequencies of GSTM1 null in patients with PCAG could be associated with a risk factor for incidence of PCAG in the Iranian population. These findings may contribute to understanding the pathogenesis of glaucoma and may be useful in the prevention and treatment of this disease. Further investigations are warranted into the precise mechanism by which these genetic polymorphisms may influence the development of PCAG.

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