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Glutathione s-transferase M1 and T1 genetic polymorphisms in Iranian patients with glaucoma

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ABSTRACT

Objective(s): Glaucoma is the second leading cause of blindness and it is related to oxidative stress based on numerous studies. Glutathione S-transferases (GSTs) are members of multigenic family, which have important role in cells as an antioxidant. In the present study, we examined the polymorphism of GSTT1 and GSTM1 deletion genotypes (T0M1, T1M0, and T0M0) in 100 Glaucoma patients (41with primary open angle glaucoma (PCAG), and 59 with primary closed angle glaucoma (POAG)) compared to 100 healthy subjects.

Materials and Methods: GSTM1and GSTT1 polymorphisms were determined by multiplex polymerase chain reaction.

Results: GSTM1 and GSTT1 null deletions genotypes were determined in 22 (53.7%) and 7 (17.1%) patients with PCAG and 34 (34%) and 15 (15%) in healthy subjects. Comparison between patients and healthy subjects regarding GSTM1 and GSTT1 genotypes revealed increase of GSTM1 null deletions genotypes in patients with PCAG (P=0.03).

Conclusion: It was concluded that the increased frequencies of GSTM1 null in patients with PCAG could be a risk factor for incidence of PCAG in the Iranian population.

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Introduction

Glaucoma is a difficult disease that has multifactorial etiology consisting of mechanical damage due to increased intraocular pressure (IOP), disorders leading to progressive damage to the optic nerve, mutations in some of genes, and damage caused by oxidative stress (1).

The prevalence of glaucoma in adults above 40 years of age has been reported from 2% to 8.8% in different parts of the world (2). The prevalence of glaucoma in adults in Tehran is 1.44%, including primary open angle glaucoma (POAG) 0.46%, primary closed angle glaucoma (PCAG) 0.33%, and other types of glaucoma 0.65% (2). Oxidative stress can damage the human trabecular meshwork (HTM) by generating toxic free radicals, and increase intraocular pressure (IOP), which is described in most types of glaucoma (3).

Glutathione (GSH) is an important cellular antioxidant that defends against external toxicants such as reactive oxygen species (ROS) mediated cell injury that reduces peroxides and detoxifies multiple compounds through glutathione S-transferase (GST) conjunction (4). Glutathione S-transferases (GSTs) are members of a group of multigene and multifunctional detoxification enzymes, which protect cells against a large variety of toxic damages including chemical, metabolites, and oxidative stress (5). The glutathione S-transferaseM1 (GSTM1) (chromosome 1p13.3) encodes cytosolic enzymes, while glutathione S-transferase (GST)-μ and glutathione S-transferaseT1 (GSTT1) (22q11.2) genes encode GST-θ. These enzymes participate in phase 2 metabolism and can be deleted (6).

The most common types of glaucoma include primary open-angle glaucoma (POAG) and primary closed-angle glaucoma (PCAG). Recent studies suggest that oxidative stress affects the progressive neuronal death that is characteristic of glaucomatous optic nerve damage (14, 3). It has been shown that oxidative stress markers such as nitrotyrosine protein, carbonyls proteins, and lipid oxidation are increased in glaucoma patients (1). It is reported that oxidative stress has an important role in pathogenesis of glaucoma and deletion in GSTT1 and GSTM1 genotypes are affiliated with a variety of pathologic processes such as ophthalmologic diseases (1).

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Different studies have found GST polymorphisms as risk factors for glaucoma among different populations (1, 11-16).

To facilitate understanding the multifactorial causes of the disease, it is reasonable to study whether genetic polymorphisms of xenobiotic-metabolizing and antioxidant enzymes contribute to development of POAG and PCAG. Since GSTs have important role in the inactivation of oxidative stress products that have harmful effects leading to glaucoma, in the present study we tried to compare the polymorphisms of GSTM1 and GSTT1 in the Iranian patients with glaucoma with matching healthy controls to explore the possible association between different GST variants and the incidence of POAG and PCAG.

Materials and Methods

The studied individuals consisted of 100 controls and 100 patients (59 with POAG and 41 with PCAG) documented by clinical tests and examined by glaucoma specialist.

All controls and patient were Iranian. Patient were selected from the Glaucoma Clinic at Rasoul Akram hospital and Eye Specialty Farabi hospital in Tehran, Iran. All subjects were given written informed consents. The research protocol was approved by the Ethics Committee of Lorestan University Medical Sciences. A complete medical history was taken from all volunteers.

Refraction, best-corrected visual acuity, slit lamp biomicroscopy, Goldmannapplanation tonometry, funduscopy, and gonioscopy were performed in patient.

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

Patients who had an eye surgery before the diagnosis of glaucoma, uveitis, trauma, or evidence of secondary glaucoma were eliminated. Controls had no family or personal history and abnormalities suggestive of glaucoma and their intraocular pressure (IOP) were below 21 mmHg. We compared age of patients and controls with student's t-test, and their genders with chi-square test. All values were represented as mean ± S.D. Genotypes were divided into two groups: present or null GSTT1 and GSTM1 gene.

GSTM1 and GSTT1 genotypes frequencies were analyzed by Odds ratio (OR) with 95% confidence intervals calculated by logistic regression. *P-values* were two-tailed and a value of < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 15 software.

Of 100 patients, 59 (59%) were patient with primary open angel and 41(41%) of them with primary close angle glaucoma.

The number of controls was 100. All patients and control subjects were of Iranian origin.

A volume of 5 ml venous blood was collected in tubes containing ethylene-diamine-tetra-acetate (EDTA) as an anticoagulant for DNA extraction. Genomic DNA was extracted from peripheral venous blood using Roche DNA extraction kit (CAT NO.117968288001).

Table 1. Primer sequences

Primers	Sequence
GSTM1forward	5' – GAA CTC CCT GAA AAGCTA AAGC-3'
GSTM1 reverse	5' GTT GGG CTC AAA TAT ACGGTG G-3'
GSTT1 forward	5' – TTC CTT ACTGGT CCT CAC ATC TC-3'
GSTT1 reverse	5' – TCA CCGGACAT GGC CAG CA-3'
DHFR forward	5' -GGA ATG GAG AAC CAG GTC GTC TT-3'
DHFR reverse	5'-GCA TGT CTT TGG GAT GTG GA-3'

The presence or absence of the GSTM1 and GSTT1 genes were determined using multiplex polymerase chain reaction (PCR) technique. The PCR primers were synthesized by gene runner software and made by cinaclon company, Tehran, Iran (Table 1).

The dihydrofolate reductase (DHFR) locus was used as internal control. PCR reaction was done in total volume of 25 µl containing 10 pmol of each primer, 1.5 mmol/l of $MgCl_2$, 0.25 mmol/l of each deoxy-nucleotide triphosphate, 2 unit of Taq polymerase and 100-500 ng of genomic DNA. Amplification was performed by initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, 64°C for 45 sec and 72°C for 1 min, and a final extension at 72°C for 5 min. The amplified products were identified by electrophoresis in a 2% Agarose gel and stained with 0.5 µg/ml ethidium bromide.

Results

Table 2 shows the demographic data 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 men, and 57 of them (57%) were women. The mean age of the glaucoma group was 57.1±12.5 years, 46 of them (46 %) were men, and 54 of them (54%) were women. Patients and controls were not significantly different with respect to sex (P=0.66), age (P=0.1), and smoking status (P=0.059); therefore, these variables were not evaluated further.

No significant difference in the frequency of GSTM1 null (P=0.061) and GSTT1 null (P=0.451) was observed between the glaucoma group and control. Moreover, it was revealed that the polymorphism of GSTM1 increased the risk of developing glaucoma in glaucoma group up to 1.72 times than the control (CI=0.97-3.045), and the polymorphism of GSTT1 increased the risk of developing glaucoma in glaucoma group up to 1.32 times than control group (CI=0.63-2.7) (Table 3).

Table 2. Demographic data of the study groups

Study Groups	Control group	Glaucoma group	*P*-value
Number of Subjects	100	100	-
Sex			
Men, n (%)	43(43%)	46(46%)	0.66
Women, n (%)	57(57%)	54(54%)	
Age (years) Mean ± SD	54.5 ±9.89	57.1 ± 12.5	0.1
Smoker, n (%)	6%	14%	0.059

Table 3. Glutathione s-transferase genotypes and the risk of developing glaucoma

Genotype	Control group (n =100)	Glaucoma group (n=100)	OR	$CI_{95\%}$	*P*-value
GSTM1					
Present n (%)	66 (66%)	53 (53%)	1.72	0.97-3.045	0.061
Null n (%)	34 (34%)	47 (47%)			
GSTT1					
Present n (%)	85 (85%)	81 (81%)	1.32	0.63-2.7	0.451
Null n (%)	15 (15%)	19 (19%)			

Table 4. Glutathione s-transferase genotypes and the risk of developing primary open angle glaucoma

Genotype	Control group (n =100)	POAG (n=41)	OR	$CI_{95\%}$	*P*-value
GSTM1					
Present n(%)	66 (66%)	34 (57.6%)	1.4	0.73-2.7	0.29
Null n (%)	34 (34%)	25 (42.4%)			
GSTT1					
Present n (%)	66 (66%)	47 (79.7%)	1.4	0.62-3.3	0.38
Null n (%)	34 (34%)	12 (20.3%)			

We compared the frequency of GSTM1 null and GSTT1 null within POAG with normal group.

The frequency of the GSTM1 null genotype in POAG patients (42.4%) was higher than in controls (34%). It showed no significant difference between POAG patients and controls. (*P*=0.29). Moreover, the frequency of GSTT1 null genotypes among patient with POAG were 20.3 % (*P*=0.38) that shows no significantly difference between POAG patients and controls (Table 4).

We also compared the frequency 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 decrease in the frequency of GSTM1 genotypes was found between the PCAG and control group. It means the increased risk of PCAG is associated with GSTM1 null genotype (OR: 2.2, 95% CI=1.3-4.7, *P*=0.03) (Table 5).

The product lengths were 215 bp, 480 bp, and 280 bp for GSTM1, GSTT1 and DHFR (Figure1).

Table 5. Glutathione s-transferase genotypes and the risk of developing primary closed angle glaucoma

Genotype	Control group (n =100)	PCAG (n=41)	OR	$CI_{95\%}$	*P*-value
GSTM1					
Present n (%)	66 (66%)	19 (46.3%)	2.2	1.3-4.7	0.03
Null n (%)	34 (34%)	22 (53.7%)			
GSTT1					
Present n (%)	66 (66%)	34 (83.9%)	1.1	0.43-3.1	0.75
Null n (%)	34 (34%)	7 (17.1%)			

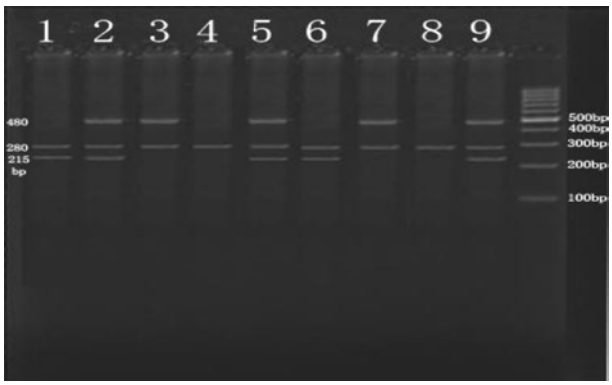

Figure 1. Amplified PCR products of GSTT1, GSTM1 and DHFR gene polymorphism. Lines (2, 5 and 9) heterozygous for GSTT1 and GSTM1. Lines (1and 6) were homozygous deletion for GSTT1. Lines (3 and 7) were homozygous deletion for GSTM1, and lines (4 and 8) were deletion for both GSTM1 and GSTT1

Discussion

Glaucoma is a complex disease that has important variants such as POAG and PCAG. These variants have different damage to optic nerve (7).

This is the first study to measure the occurrence of these polymorphisms among Iranian patients with these two types of glaucoma. Glaucoma patients in this study had POAG and PCAG. Controls were well matched to the patients regarding age, sex, smoking, diabetic or non-diabetic state, and ethnicity.

In this study, we found no significant relation-ship between the GSTM1 genotype and the incidence of glaucoma overall (*P*=0.061). Separately in this study, we found no significant relationship between

the GSTM1 genotype and the incidence of POAG (*P*=0.29). This result is supported by a previous study by Jansson *et al* in Swedish population (8) who found no significant difference between cases and controls of GSTM1 genotype among patient with POAG.

Our results are on the contrary to that of Juronen *et al* in Estonian population (9), Khaled *et al* in Arab population (1), Unal *et al* in Turkish population (10), and Hoiyda *et al* in Egyptian population (11), who found that the frequency of GSTM1-positive individuals was significantly higher in the glaucoma group compared with the control group. They proposed that GSTM1 might be a risk factor for the expansion of POAG.

Nevertheless, Izotti *et al* in an Italian population (12), Yildirim *et al in* Turkish population (13) and Auta *et al* in Brazilian population (14), reported that POAG was associated with the GSTM1 null genotype. In our 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 (*P*=0.03). Our results are supported by a previous study in the Arab population who showed that GSTM1 null genotype is a risk factor for developing PCAG (*P*=0.001). In our study the frequency of the GSTT1 null genotype was

not statistically different between the glaucoma group and the controls (*P*=0.451). We found no statistical difference in GSTT1 polymorphism between POAG group and the controls (*P*=0.38). The results of our study are supported by the study of Yildrim *et al* (14) and the study by Izzotti *et al* (13). Our results are contrary to those of Unal *et al* (11) and Khaled *et al* (1) where they reported that GSTT1 null genotype was significantly associated with POAG. We also found that there is no relationship between GSTT1 and PCAG (*P*=0.75). This result is supported by the study of Khaled *et al* (*P*=0.38) (1).

Apparently, there is only one previous case study (in Arab population) about the relationship between GST and PCAG and we are confined to compare our results with other populations.

Several factors might affect the different results between similar studies such as ethnic, genetic, and environmental background of the populations studied, the number of subjects studied, and method of the experiments. For example, Jansson *et al* (8), who reported that there was no association between GSTM1 and glaucoma in the Swedish population, used two methods for genotyping: multiplex PCR and pyrosequencing. In contrast, Juronen *et al* (9) performed their analysis using ELISA only.

The GST genes are located in complex genomic regions that could be affected by a number variation and rearrangements, so different genotyping methods could give different results. PCAG has been shown primarily to increase IOP caused by anatomic changes in the anterior (15) and posterior (16) globe. Nevertheless, ocular phenomena must play a role in the posterior globe to cause optic nerve damage (16-18), and recent studies confirm the hypothesis that oxidative stress may affect glaucomatous optic nerve injury at several levels (12). Oxidative stress may lead to optic nerve neuronal cell death (19).

Although we could not find any significant difference between GSTM1 and T1 polymorphism in glaucoma group overall, our results revealed that polymorphisms of GSTT1 and M1 null increase the risk of developing glaucoma up to 2.7 times than that in controls. It may support the idea that GSTs, which have important role in decreasing oxidative stress, are fewer in glaucoma group overall than controls.

Conclusion

This study indicated no association between GSTT1 and GSTM1 polymorphisms to glaucoma overall and POAG, although it showed that GSTM1 null in patients with PCAG is associated with a risk factor for the incidence of PCAG in the Iranian population. These results can help us to realize the pathogenesis of glaucoma and may be helpful in the prevention and treatment of this disease.

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Conflict of interest

The authors declare no conflict of interest for the present research outcome.

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