The Risk of Primary Open Angle Glaucoma and Glutathione S-Transferase M1 and T1 Polymorphism among Egyptians

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Abstract: Purpose: Glaucoma, the second leading cause of blindness, is characterized by changes in the optic disc and visual field defects. The elevated intraocular pressure was considered the prime factor responsible for the glaucomatous optic neuropathy involving death of retinal ganglion cells and their axons. Extensive investigations into the pathophysiology of glaucoma now reveal the role of multiple factors in the development of retinal ganglion cell death. Genetic factors and oxidative damage have been shown to have a role in the development of primary open angle glaucoma (POAG). Glutathione S-transferases (GSTs) are a family of enzymes that inactivate xenobiotics and endogenous end products formed as secondary metabolites during oxidative stress. In humans, GSTT1 and GSTM1 deletion genotypes are associated with a variety of pathologic processes including certain ophthalmologic diseases. The aim of this study was to determine the effects of genetic polymorphisms of glutathione S-transferase GSTM1 and GSTT1 on the risk of POAG in an Egyptian population. Methods: We compared the prevalence of GSTT1 and GSTM1 deletion genotypes, which were determined by multiplex polymerase chain reaction, in 32 patients with primary open angle glaucoma to 16 age, sex, and ethnically matched controls. Results: The GSTM1 positive genotype had an increased risk of developing POAG (p< 0.05, OR 4.681, 95% CI 1.190 – 18.412). The risk of glaucoma also increased significantly in subjects with a combination of GSTM1 positive and GSTT1 null genotypes (p< 0.05, OR 4.700, 95% CI 0.959 – 23.033). Conclusion: The GSTM1 positive genotype or the combination of both GSTM1 positive and GSTT1 null genotypes may be associated with the increased risk of development of POAG in the Egyptian population. The overall results indicate a possible variable association between various GSTT1 and GSTM1 genotypes and primary open angle glaucoma. Decreased GST function might interfere with the metabolism of oxidative intermediates and exacerbate the direct or indirect damaging effects of oxidative stress on the optic nerve. It is possible that these GST polymorphisms may be risk factors for primary open angle glaucoma [Journal of American Science 2010;6(12):375-381]. (ISSN: 1545-1003).

Keywords: Glaucoma; optic disc and visual field defects; primary open angle glaucoma (POAG); Glutathione S-transferases (GSTs)

1. Introduction

Glaucoma is the most common optic neuropathic process affecting human and the second most common cause of blindness worldwide (1). It is a disease in which progressive loss of retinal ganglion cells is characterized by a recognizable pattern of both visual function loss and optic nerve head pallor and excavation. If, untreated, the natural course is towards blindness, or at least significant visual loss disability (2).

Primary open angle glaucoma (POAG), which affects almost 2% of the world population, accounts for most of the glaucoma cases. Although the pathophysiology of POAG is not precisely known, its causes are clearly multifactorial. It is a result of multiple interactive genetic and environmental effects. Although the most prominent known risk factor for developing POAG is elevated intraocular pressure, there are also other suspected risk factors such as positive family history, age, hypertension and diabetes. Its prevalence increases with age. It is well known that POAG is an age-related disorder. There is a general consensus that cumulative oxidative damage is responsible for aging, and may, therefore, play an important role in the pathogenesis of an age-related disorder such as glaucoma. Oxidant stress and antioxidant systems are potentially important for ocular tissues. Exposure to light by photosensitizing mechanisms may lead to the formation of reactive oxygen species. Many of the ocular tissues regenerate slowly, causing an increase in the risk for an accumulation of oxidant-inflicted damage in the tissue components. The damage caused by xenobiotics and oxidants can result in a number of molecular changes that contribute to the development of glaucoma, cataract, and other age related diseases. Therefore the eye must posses efficient reducing systems, as well as, detoxification enzymes such as
catalase, superoxide dismutase, glutathione peroxidase and glutathione S-transferase (GST) for protection from oxidative damage. The ocular ciliary epithelium expresses genes coding for GST and other enzymes involved in the glutathione cycle, such as glutathione peroxidase. Several epidemiological studies suggested that individual susceptibility to several disorders, including eye diseases might be connected with the GST system (2).

The glutathione S-transferases (GSTs) are a family of enzymes consisting of numerous cytosolic, mitochondrial, and microsomal proteins capable of multiple reactions with endogenous and xenobiotic substrates. They catalyze the conjugation of reduced glutathione to electrophilic centers via the sulfhydryl group on a wide variety of substrates. GSTs bind toxins, function as transport proteins, detoxify endogenous compounds such as peroxidized lipids, and inactivate endogenous end products formed as secondary metabolites during oxidative stress (1).

The GST isoenzymes expressed in human tissues comprise the alpha, mu, pi, theta, kappa, sigma, zeta and omega gene families. As many GST genes are polymorphic, there has been considerable interest in determining whether particular allelic variants are associated with altered risk (or outcome) of a variety of pathologies including cancers, cardiovascular diseases and respiratory diseases. Of these classes of GSTs, five (GSTM1, GSTM3, GSTT1, GSTP1 and GSTZ1) have been shown to be polymorphically distributed. Five mu-class genes (GSTM1–GSTM5) are situated on chromosome 1.11 Polymorphisms identified in GSTM1 are GSTM1*0, GSTM1*A and GSTM1*B. GSTM1*0 is deleted, and homozygotes (GSTM1 null genotype) express no protein. GSTM1*A and GSTM1*B differ by a single base, and thecatalytic effectiveness of the enzymes encoded by these alleles is similar. There are two theta-class genes, GSTT1 and GSTT2, located on chromosome 22.6 GSTT1 is represented by two alleles: a functional or wild allele (GSTT1*1), and a nonfunctional or null allele (GSTT1*0). Studies have shown that the GSTT1*0 allele corresponds to a total or partial deletion of the gene, causing a deficiency in enzymatic activity (3).

Because of the role of GSTs in inactivating endogenous end products formed as secondary metabolites during oxidative stress, we decided to compare the distribution of GSTM1 and GSTT1 polymorphisms in Egyptian patients with POAG, compared to the distribution in matching healthy controls so as to explore the possible association between different GST variants and the incidence of POAG.

2. Subjects and Methods

Patient and Control Selection:

This case–control study was comprised of 48 subjects; thirty two patients with POAG and sixteen disease-free controls. The studied subjects were recruited from the Research Institute of Ophthalmology and Fayoum University Teaching Hospital in the period from January 2009 to January 2010. A complete examination was done to detect other abnormalities, a full medical history was taken and a thorough pedigree analysis was conducted to determine consanguinity and other affected family members. An informed consent was obtained from all subjects after explanation of the nature of the study.

The sixteen age-matched healthy volunteers were selected as control group; they were non smokers and had neither diabetes nor any systemic illness. They had no family or personal history of glaucoma. They had clinical healthy appearing optic discs as demonstrated by indirect ophthalmoscope with a cup-to-disc ratio of 0.3 or lower, and glaucoma hemifield test (GHT) within normal limits. Mean intraocular pressure (IOP) level of the controls was 13.1 ± 3.0 mmHg (range 10 and 21 mmHg).

Diagnosis of POAG required all of the following: open angle: intraocular pressure higher than 21 mmHg ; characteristic optic changes (e.g., vertical cup -to-disc ratio higher than 0.6); thin or notched neuroretinal rim or disc hemorrhage; and characteristic visual field changes. The mean IOP level was 24.2 ± 2.1 mm Hg (range 22 –28 mm Hg) at the time of diagnosis. Cup-to-disc ratios were between 0.6 and 0.9. Patients with a history of eye surgery before the diagnosis of glaucoma, or with evidence of secondary glaucoma, such as exfoliation, pigment dispersion or uveitis, were excluded. The patients with POAG who met the inclusion criteria were selected consecutively.

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 gender between patients and controls. All values were represented as mean ± S.D. GSTT1 and GSTM1 genotypes were classified as either null (homozygous deletion) or non-deleted. Odds ratio (OR) with 95% confidence limits calculated 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 performed using SPSS v. 11.5 statistical analysis software.
Specimen Collection:
Two ml venous blood was collected by venapuncture in a tube containing ethylenediamine tetraacetate (EDTA) as an anticoagulant for DNA extraction.

Method:
Genomic DNA was extracted from peripheral venous blood using a salting out protocol, as described by Miller et al., 1988 (4). GSTM1 and GSTT1 genetic polymorphisms were evaluated using multiplex polymerase chain reaction (PCR) technique. The PCR primers were synthesized according to Arand et al., 1996, (5). Primers for GSTM1 were 5' – GAA CTC CCT GAA AAG CTAA AGC and 5' GTT GGG CTC AAA TAT ACG GTG G and for GSTT1 were 5' – TTC CTT ACT GGT CCT CAC ATC TC and 5' – TCA CCG GACAT GGC CAG CA. The β – globin locus was used as an internal control to avoid false-negative readings. Primers for β – globin were 5' – CAA CTT CAT CCA CGT TCA CC and 5' – GAA GAG CCA AGG ACA GTG AC. PCR reaction was carried out in a total volume of 25 ul containing 10 pmol of each primer, 2.5 mmol / L of MgCl2, 0.2 mmol/L of each deoxynucleotide triphosphate, 1 unit of Taq polymerase. And 100 ng of genomic DNA. Amplification was performed by initial denaturation at 94 °C for 5 minutes, followed by 30 cycles at 94 °C for 1 minute, 64 °C for 1 minute and 72 °C for 1 minute and a final extension of 72 °C for 7 minutes. The amplified products were identified by electrophoresis in a 1.5% agarose gel and stained with 0.5 ug/ml ethidium bromide. The product lengths were 215 bp, 480 bp, and 268 bp for GSTM1, GSTT1 and β – globin, respectively. Absence of PCR product for GSTM1 or GSTT1 in the presence of the β – globin band was indicative of a null genotype for GSTM1 or GSTT1. Individuals with one or two copies of the relevant gene were classified as a positive genotype and individuals with homozygous deletions as a null genotype.

3. Results:
Table (1) shows the demographic data for POAG patients and the control group. The mean age of the control group was 47.30 ± 11.60 years, 7 of them (43.5 %) were males and 9 of them (56.5 %) were females. The mean age of the POAG group was 51.03 ± 14.68 years, 15 of them (47.5 %) were males and 17 of them (53.5 %) were females. The groups were not statistically different with respect to age and gender (p > 0.05).

Table (2) shows the GST genotype distribution among all POAG patients and the control group. The frequencies of GSTT1 and GSTM1 – null genotypes were 25 % and 31.5 % respectively in the POAG patients. The proportion of GSTT1 null genotypes was higher in the POAG patients as compared to controls but with no significant difference (25% versus 6.25%) (OR: 0.183, 95% CI: 0.02–1.683). The proportion of GSTM1 null genotypes was higher in the control group as compared to the POAG group (62.5% versus 31.5%), p<0.05. The GSTM1 present genotype had an increased risk of developing POAG (OR: 4.681, 95% CI: 1.190-18.412).

Table 3 shows the association between GST genotype profile and the development of POAG. The data suggested a trend of decreasing risk of POAG with the combination of GSTM1 null genotype and GSTT1 positive genotype. (p: <0.05, OR: 4.700, 95% CI: 0.959-23.033).

Table (1) Demographic Data of the Study Groups

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Control Group</th>
<th>POAG Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Subjects</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>7/16 (43.5%)</td>
<td>15/32 (47.5%)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>9/16 (56.5%)</td>
<td>17/32 (53.5%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>47.30 ± 11.60</td>
<td>51.03 ± 14.68</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>----</td>
<td>5/32 (17.5%)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>----</td>
<td>11/32 (34.5%)</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>----</td>
<td>10/32 (31.5%)</td>
</tr>
<tr>
<td>Consanguinity, n (%)</td>
<td>----</td>
<td>11/32 (34.5%)</td>
</tr>
<tr>
<td>Family history, n (%)</td>
<td>----</td>
<td>5/32 (17.5%)</td>
</tr>
</tbody>
</table>

POAG = primary open angle glaucoma
Table (2) Glutathione S Transferase (GST) Genotypes and the Risk of Developing Primary Open Angle Glaucoma (POAG).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control Group (N = 16)</th>
<th>POAG (N= 32)</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1&lt;sup&gt;b&lt;/sup&gt; Present, n (%)</td>
<td>6 (37.5%)</td>
<td>22 (67%)</td>
<td>1.0</td>
<td>Reference 1.190 – 18.412</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Null, n (%)</td>
<td>10 (62.5%)</td>
<td>10 (31.5%)</td>
<td>4.681</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTT1&lt;sup&gt;b&lt;/sup&gt; Present, n (%)</td>
<td>15 (93.75%)</td>
<td>24 (75%)</td>
<td>1.0</td>
<td>Reference 0.02 – 1.683</td>
<td>NS</td>
</tr>
<tr>
<td>Null, n (%)</td>
<td>1 (6.25%)</td>
<td>8 (25%)</td>
<td>0.183</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR: odds ratio  
CI: Confidence interval from binary logistic regression  
<sup>b</sup>: Carriers of at least one intact allele are used as reference  
POAG: primary open angle glaucoma

Table (3) Association between GST Genotype Profile and the Development of POAG

<table>
<thead>
<tr>
<th>Genotype Combination</th>
<th>GSTM1</th>
<th>GSTT1</th>
<th>Control Group (N =16)</th>
<th>POAG Group (N = 32)</th>
<th>OR</th>
<th>95 %CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present Present</td>
<td>5 (31.25%)</td>
<td>14 (43.5%)</td>
<td>1</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present Null</td>
<td>1 (6.25%)</td>
<td>8 (25%)</td>
<td>0.014</td>
<td>0.00 – 986.944</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null Present</td>
<td>10 (62.5%)</td>
<td>10 (31.5%)</td>
<td>4.700</td>
<td>0.959 – 23.033</td>
<td>&lt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR: odds ratio  
CI: Confidence interval from binary logistic regression  
POAG: primary open angle glaucoma

Figure (1): Amplified PCR products of the GSTT1 and GSTM1 gene polymorphism in the patients with primary open angle glaucoma (POAG). The product lengths were 215bp for GSTM1, 480bp for GSTT1 and 268bp for Beta globin. Lanes (1,2,4,5&7) heterozygous for GSTT1and GSTM1. Lanes (3&8) were homozygous deletion for GSTT1. Lane 6 was homozygous deletion for GSTM1 and then lane M was ØX marker.
4. Discussion:

The basic cause of glaucoma is largely unknown. First degree relatives of glaucoma cases have 8–10 times increased risk of developing the disease, making genetic predisposition a strong risk factor (2). Most genetic polymorphisms do not cause a recognizable change in the organism in which they occur. However, some either cause a disease or alter disease susceptibility. A large number of studies had attempted to show links between disease susceptibility and GST polymorphic variants. In addition, some studies have focused on the risk of association between the GST polymorphisms and ocular diseases including cataract, senile macular degeneration and glaucoma. In this study, we aimed to determine the effects of genetic polymorphisms of GSTM1 and GSTT1 on the risk of POAG in an Egyptian population.

As the pathogenic role of ROS in glaucoma has been suggested by many studies, cellular defense mechanisms alleviating the toxic manifestations of oxidative insult must have an important role in 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 (3).

The results presented in this study imply that defects in GST activity may well be risk factors for developing POAG. The exact mechanisms by which this occurs are not clear, which is not surprising given that the exact mechanisms of GST activity have yet to be elucidated.

Glaucoma patients in this study met strict criteria for POAG. Controls in this study were well matched to patients for age, sex and ethnicity.

In our study the GSTM1 positive genotype was significantly more common in the POAG group compared to the control group which shows a correlation between the GSTM1 positive genotype and the incidence of POAG. The results of our study are in concordance with a previous study by Unal et al, 2007 (3) in the Turkish population who found that GSTM1 positive genotype was a risk factor for developing POAG. Juronen et al, 2000 (6), who were the first to examine the possible association between the polymorphic GST genotypes and adult-onset POAG in an Estonian population, also found a similar relationship between the GSTM1 genotype and the incidence of POAG. They found that the
frequency of GSTM1 positive individuals was significantly higher in the glaucoma group compared with the control group. They suggested that the GSTM1 positive phenotype might be a genetic risk factor for the development of POAG. The same results were reported in the study by Khaled et al., 2008 (1) among an Arab population. We believe that several factors might explain the association between the GSTM1 positive genotype and POAG. Although GST enzymes catalyse detoxification reactions, they also take part in reactions that result in toxic products, which may cause structural changes in the proteins present in the trabecular meshwork and aqueous humor. This can lead to aggregation or modification of the proteins in the trabecular meshwork and promote the development of POAG (6). In addition, subjects with the GSTM1 null genotype have been shown to express fewer GST mu-class enzymes than subjects with the GSTM1 positive genotype (7 & 8). This may selectively cause stimulation of other non-toxic end products producing biotransformation enzyme systems to detoxify the substrates that were originally detoxified by the GST enzymes. Further evidence for involvement of GSTM in glaucoma comes from studies on autoimmunity. Yang et al., 2001 (9) showed that GST antigen was found in 52% of cases with glaucoma and in 20% of controls. The patients had significantly higher titres of anti-GST antibody compared with controls. Furthermore, the related retinal antigen belonged to the GST mu class (9). Thus, it may be hypothesized that people who express GSTM1 are at increased risk of developing autoantibodies against this protein, which is connected to an increased risk of developing glaucoma.

Contrary to our results Izzotti et al, 2004 (10) reported that POAG was associated with the GSTM1 null genotype in an Italian population. In addition, in another study by Yildirim et al., 2005 (2), the GSTM1 null genotype has been found to be associated with an increased incidence of POAG in a Turkish population. Another study by Jansson et al., 2003 (11) reported that there was no evidence of association between GSTM1 and glaucoma in the Swedish population. Further evidence for involvement of GSTM in glaucoma comes from studies on autoimmunity. Yang et al., 2001 (9) showed that GST antigen was found in 52% of cases with glaucoma and in 20% of controls. The patients had significantly higher titres of anti-GST antibody compared with controls. Furthermore, the related retinal antigen belonged to the GST mu class (9). Thus, it may be hypothesized that people who express GSTM1 are at increased risk of developing autoantibodies against this protein, which is connected to an increased risk of developing glaucoma.

In our study the frequency of the GSTT1 null genotype was not statistically different between the POAG cases and the controls. The results of our study supports the study of Yildirim et al., 2005 (2) and the study by Izzotti et al., 2003 (10). Our results are contrary to Unal et al., 2007 (3) and Khaled et al., 2008 (1) where they reported that GSTT1 null genotype was significantly associated with POAG. The combination of GSTM1 null genotype and GSTT1 positive genotype in our study showed a 4.7 fold decreased risk of glaucoma. It has been already suggested that combination of the GST polymorphisms rather than individual polymorphism make humans more susceptible to genotoxic insults (12).

Many factors might account for the difference in results between similar studies. Firstly, it may reflect the differences in the ethnic, genetic and environmental background of the populations studied. For instance, GSTT1 deficiency is less frequent than GSTM1 deficiency, but in both cases the frequency in the population varies between different ethnic groups (13). There may be differences even in the same population because of genetic and environmental factors. Secondly, the differences in the number of subjects studied in genetic researches may also lead to different outcomes. Thirdly, methodological issues should also be considered. For example, Jansson et al, 2003 (11), who reported that there was no evidence of association between GSTM1 and glaucoma in the Swedish population, used two methods for genotyping: multiplex PCR and pyrosequencing. In contrast, Juronen et al., 2000 (6), performed their analysis using only ELISA. The GST genotypes are located in complex genomic regions that could be affected by copy number variation and rearrangements, so different genotyping methods could give different results.

The present study suggests that the GSTM1 positive genotype may be a genetic risk factor for the development of POAG. The combination of GSTM1 null genotype and GSTT1 positive genotype decreased the risk of POAG. It has already been suggested that the combination of the GST polymorphisms rather than individual polymorphisms makes humans more susceptible to genotoxic insults.

In conclusion, this study is only one in a series of case-control studies of the possible association between glaucoma and GST. Some find evidence of GST positive genotypes being predisposing to glaucoma and others that GST positive genotypes being protective from glaucoma. These results imply that further studies of the precise mechanisms by which genetic polymorphism of metabolizing enzymes influences the nature history of glaucoma development are merited.

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6/1/2010