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Original Article

Investigation of eNOS G894T Gene Polymorphism in Patients with Pseudoexfoliation Syndrome: A Preliminary Study

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Abstract

Objectives: The aim of this study is to investigate the relationship between pseudoexfoliation syndrome (XFS) and pseudoexfoliative glaucoma (XFG) and endothelial nitric oxide synthase (eNOS) G894T polymorphism.

Methods: Seventy-eight eyes of 78 patients who had undergone uncomplicated cataract surgeries for senile cataract were included in this study. Forty patients with XFS were included in the study group, and 38 patients without XFS constituted the control group. Patients with XFS were divided into two subgroups according to their XFG development, and subgroup analysis was performed. Venous blood samples were taken from all patients before surgery and 894 G>T (rs1799983) polymorphism on the eNOS gene was evaluated by RT-PCR.

Results: While the mean age in the control group was 65.97±10.64 years (23 males and 15 females), the mean age in the study group was 73.05±6.79 years (30 males and 10 females), ($p<0.001$). Regression analysis of the risks caused by the genotype and alleles between the control and study groups revealed that the homozygous alleles were more common in the study group, and heterozygous or mutant alleles have reduced the development of XFS approximately 2-folds. However, this was not statistically significant ($p=0.11$). Similarly, when subgroup analysis was performed, it was found that there was no significant relationship between XFG in patients with XFS and gene polymorphism.

Conclusion: In this study, it was observed that there was no relationship between the G894T polymorphism in the eNOS gene and the development of XFS/XFG.

Keywords: Endothelial nitric oxide synthase, G894T polymorphism, nitric oxide, pseudoexfoliation syndrome, pseudoexfoliative glaucoma

Introduction

Pseudoexfoliation syndrome (XFS) is a systemic disease characterized by the accumulation of fibrillar extracellular pseudoexfoliation material (XFM) in ocular tissues and organs (1). Vascular structures are the most important areas of involvement in this disease, and shown that associated with many systemic events such as systemic hypertension, angina, myocardial infarction, abdominal aortic aneurysm, transient ischemic attack, and Alzheimer's Disease (2-5). Apart from the accumulation of XFM in all these systemic organs, it is known that this material can accumulate densely in intraocular tissues. It has been demonstrated that especially non-pigmented ciliary epithelial cells, trabecular endothelial cells, and anterior equatorial lens epithelial cells may be involved densely in this accumulation process (6). Due to the accumulation of XFM in the iridocorneal angle region, resistance to

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aqueous humor outflow occurs and pseudoexfoliative glaucoma (XFG), which is the most common form of secondary open angle glaucoma, may develop (7).

Although the etiology of XFS development is not precisely known, the important role of oxidative stress in this situation is the generally accepted opinion (8-10). Oxidative stress is a status where the balance between oxidants and antioxidants in the cell is disturbed in favor of oxidants and reactive oxygen radicals as well as reactive nitrogen species play an important role in this imbalance. Hydrogen peroxide, superoxide, hydroxyl, nitric oxide (NO), and their toxic metabolites are the main oxidant radicals and cause DNA damage by joining the structure of the basic components in the cell (11). Under normal conditions, NO has many physiological roles in the body, especially related to the vascular system (12). Three types of synthase enzyme isoforms that mediate NO synthesis have been identified (neuronal, inducible, and endothelial) and endothelial NO synthase (eNOS) is the isoform responsible for the synthesis in the vascular endothelium (12). Due to the different functions of NOS, disorders that occur in the activation of this enzyme may play a role in the development of different diseases by causing nitrergic dysregulation (13). The most studied gene polymorphisms, that are thought to cause disorders in the NOS gene, are 786 T>C (rs2070744) polymorphism in the promoter region and 894 G>T (rs1799983) in exon 8 and 27 bp VNTR (rs61722009) in the intron (14,15). These polymorphisms in the NOS gene have been analyzed in many studies and suggested that they might be risk factors for different diseases. It has also been reported that polymorphisms in this gene might be associated with the development of glaucoma (16).

It is known that oxidative stress may have a role in the etiology of XFS and XFG. The G894T polymorphism of the eNOS gene, which is responsible for the synthesis of NO (an important mediator in oxidative stress), may be a factor that may play a role in this etiology. To the best of our knowledge, until now, there has been no study evaluating the relationship between XFS/XFG and eNOS G894T gene polymorphism, and the aim of this study is to investigate this possible relationship.

Methods

Before this study, necessary ethical permissions were obtained from Mersin University Clinical Research Ethics Committee and the study was conducted in accordance with the Declaration of Helsinki. Informed consent forms were obtained from all patients included in the study. Seventy-eight eyes of 78 patients who applied to Mersin University Faculty of Medicine, Department of Ophthalmology and had undergone uncomplicated cataract surgeries due to senile cataract were included in the study. Forty patients with XFS were included in the study group and 38 patients without XFS constituted the control group. In addition, patients with XFS were divided into two subgroups according to their XFG development, and subgroup analyses were performed. Patients with pseudoexfoliation material on the lens and iris formed the pseudoexfoliation group. In this group, the patients diagnosed with glaucoma and using glaucoma drugs formed the XFG and the remaining patients formed the XFS group.

Patients aged 50–90 years with senile cataract were included in the study. Patients were not excluded according to the stage and type of cataract. Patients with previous eye surgery or trauma were not included in the study. Routine ophthalmologic examinations of all patients included in the study were performed before cataract surgeries, and patients with ocular pathologies other than cataract, XFS, and XFG were excluded from the study. In addition, patients with a history of vascular diseases such as coronary disease, myocardial infarction, and cerebral ischemia as well as erectile dysfunctions and malignancies; the ones using chronic medications other than antihypertensives were excluded from the study. The history of hypertension was questioned in detail in all patients and attention was paid to its similar distribution in both groups.

Genotyping

Venous blood samples were taken from all patients before surgeries and stored at + 4°C in sterile EDTA tubes until the planned analyses were performed. DNA samples were isolated from blood samples using DNA isolation kit (Cat No: 1796828, Roche Diagnostics, GmbH, Mannheim, Germany) in accordance with the procedures. To evaluate the 894 G>T (rs1799983) polymorphism on the eNOS gene, the TaqMan® SNP Genotyping Assay (Cat No: 4351379, NOS3 rs1799983, Thermo Fisher Scientific, USA) kit was used in accordance with the procedures recommended by the company. 15 µL of reaction mixture (Master Mix, Primer-probe Assay, H2O) and 5 µL of DNA were added to the multiwell plate for each sample. Analysis was performed on RT-PCR (LC480, Roche) (Table 1).

Table 1. PCR procedure for NOS3 rs1799983 polymorphism analysis

PCR Steps	Target temperature (°C)	Standby time (sec)
Denaturation	95	600
Amplification (40 Cycle)		
Denaturation	95	15
Anneal/Extend	60	60
Cooling	40	30

PCR: Polymerase chain reaction, Sec: Second.

Statistical Analysis

All statistical analyses were performed using SPSS software package program, version 17 for Windows (SPSS, Chicago, IL). Chi-square (X^2) test was used to evaluate the distribution of the eNOS G894T genotype/alleles among patients and control subjects. The odds ratio with a 95% confidence interval of the possible risk factors for XFS/XFG was evaluated by binary logistic regression analysis.

Results

While the mean age in the control group was 65.97±10.64 years (23 males and 15 females), the mean age in the study (XFS) group was 73.05±6.79 years (30 males and 10 females), and the difference in age distribution was statistically significant among groups (p<0.001). Gender distribution and presence of hypertension were similar in both groups (p=0.12, p>0.05, respectively). In the subgroup analyses performed in the study group, patients with XFS and XFG were found to have a similar distribution in terms of age and gender (p=0.89, p=0.09, respectively).

Regression analysis of the risks caused by the genotype and alleles between the control and study groups revealed that the homozygous allele was more common in the study group, and heterozygous or mutant alleles reduced the development of XFS approximately 2-folds. However, this was not statistically significant; there was no statistical difference between pseudoexfoliation and control groups (p=0.11) (Table 2). Similarly, when subgroup analyses were performed, it was found that there was no significant relationship statistical difference between XFG development in patients with XFS and gene polymorphism (Table 3).

Discussion

In this study, eNOS G894T gene polymorphism was evaluated in patients with XFS and XFG, at the first time in the literature. In the previous studies, it was shown that G894T gene polymorphism was associated with vascular diseases such as hypertension, atherosclerosis, coronary spasm, myocardial infarction, and cerebral aneurysm (17-19). It has also been suggested that this gene polymorphism might be associated

Table 2. Distribution of eNOS genotypes in control and study groups and their risks

G894T Genotype	Control n=38 (%)	Pseudoexfoliation n=40 (%)	Relative Risk (Odd's Ratio)	Confidence Interval (%95)	p
GG	17 (44.7)	25 (62.5)	-----		
GT	18 (47.4)	14 (35)	0.529	0208–1.342	0.18
TT	3 (7.9)	1 (2.5)	0.227	0.022–2.366	0.215
GT+TT	21 (55.3)	15 (37.5)	----		
GG	17 (44.7)	25 (62.5)	2.059	0.833–5.088	0.118
Allele frequency					
T	24 (31.6)	16 (20)	-----		
G	52 (68.4)	64 (80)	1.846	0.889–3.834	0.1

G: Guanine, T: Thymine.

Table 3. Distribution of eNOS genotypes in pseudoexfoliation and XFG groups and their risks

G894T Genotype	Pseudoexfoliation n=21 (%)	XFG n=19 (%)	Relative Risk (Odd's Ratio)	Confidence Interval (%95)	p
GG	13 (61.9)	12 (63.2)	-----		
GT	7 (33.3)	7 (36.8)	1.083	0.293–4.011	0.959
TT	1 (4.8)	0 (0)			
GT+TT	13 (61.9)	12 (63.2)	----		
GG	8 (38.1)	7 (36.8)	1.055	0.263–3.418	0.935
Allele frequency					
T 9 (21.4)	7 (18.4)	-----			
G 33 (78.6)	31 (81.6)	1.208	0.401–3.363	0.737	

G: Guanine, T: Thymine, XFG: Exfoliation glaucoma.

with oncological diseases (20,21). Therefore, thinking that a similar situation might be valid for patients with XFS, we have established a hypothesis about the association of eNOS G894T gene polymorphism and developing XFS. However, in this study, no relationship was found between the development of XFS and eNOS G894T gene polymorphism, and it was observed that the homozygous GG allele might pose a risk for the development of XFS, although it was not statistically significant. In addition, attention was paid to the similar distribution of patients with a history of hypertension in both the study and control groups, and patients with vascular problems were excluded from the study.

Today, it is known that the most important modifiable risk factor for glaucoma development and progression is intraocular pressure. However, the increase in intraocular pressure alone is insufficient to explain the development and progression of glaucoma, and it is suggested that vasospasm and vascular dysregulation are effective in the development of glaucoma (16). Many endothelial factors, notably NO, endothelin, and angiotensin, are effective on ocular vascular regulation. NO is synthesized by eNOS in the aqueous humor outflow pathway, and recent studies suggest that abnormalities in NOS expression and control may be an important factor underlying the development of glaucomatous optic neuropathy (16,22). It has been shown that, NO can play a role not only in glaucomatous damage but also in pathological processes such as uveitis, retinitis, and retinal degeneration through apoptosis (23). However, the results obtained from these studies evaluating the NO level in patients with XFS and XFG are controversial. Borazan et al. found that aqueous humor NO levels were high in XFG, while Aydın et al. showed that serum NO levels in patients with XFG were decreased (24,25). Apart from all these studies, it was known that the G894T polymorphism in the eNOS gene have caused a decrease in basal NO production and thus functional abnormalities have occurred (19,26). It might be thought that these functional abnormalities might contribute to the development of XFS and XFG due to the underlying mechanisms. However, in this study, no significant relationship was found between the polymorphism presented in XFS and XFG patients and the development of glaucoma. In different studies evaluating this polymorphism of eNOS, it was not found risky in both primary open-angle glaucoma and normotensive glaucoma patients (16,27,28). The result obtained from our study seems to be compatible with the literature. However, this polymorphism has been studied in different ocular pathologies and has been shown to cause a serious increase in the risk of developing diabetic retinopathy, while it is not associated with retinopathy of prematurity (29,30).

The limitation of this study is the relatively small number of patients included in the study; in these small and selected groups of patients, we may fail to show some correlations. Further studies in larger XFS and XFG groups are needed for evaluating this eNOS polymorphism and its possible relation for developing of XFS and XFG. However, the biggest challenge for researchers here will be to rule out vascular problems other than XFS in study group of patients. It is known that the development of XFS increases with age and that the prevalence of chronic diseases such as vascular problems is higher in this group, as well. In this study, all patients with a history of vascular disease other than systemic hypertension were excluded from the study, and therefore, the number of patients in study group was limited. In addition, care was taken to ensure that patients with a history of systemic hypertension to be similar in the two groups.

Conclusion

It was found that there was no significant relationship statistical difference between G894T polymorphism in eNOS gene and development of XFS and XFG. This possible relationship was first evaluated in this study and it would be appropriate to conduct new studies in larger groups.

Disclosures

Ethics Committee Approval: Mersin University Clinical Research Ethics Committee, 17-388, 18/09/2019.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

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