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The Association Between the Transforming Growth Factor Beta-1 -509C>T Gene Polymorphism and Primary Open Angle Glaucoma in North Eastern Iran

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Abstract

Background: Glaucoma is a common cause of irreversible blindness. Transforming growth factor beta-1(TGF-β1) is the main isoform of TGF-β superfamily in the eye. Overexpression of TGF-β1 is shown to be related with the glaucoma. Studies have shown that the presence of mutant T allele of TGF-β1 -509C>T polymorphism (rs1800469) is associated with increased gene expression. So, in present study, association of the TGF-β1-509C>T gene polymorphism and primary open angle glaucoma (POAG) in patients from north east of Iran was investigated.

Methods: A case-control study was conducted on 112 POAG patients and 112 control participants. TGF-β1-509C>T genotyping was done by PCR-restriction fragment length polymorphism (PCR-RFLP) method using Bsu36I restriction enzyme. Moreover, cup to disk ratio(CDR), intraocular pressure (IOP) and visual acuity (VA) were measured. The obtained results were statistically analyzed.

Results: The highest frequency of genotype in the control group was related to CC genotype (44.6%), but the heterozygous CT genotype (45.6%) was observed as the highest frequency of genotypes in patient group (P value: 0.022, OR for TT genotype: 2.54 CI95% for OR: 1.22, 5.27). Also, the frequency of the T mutant allele showed a significant difference between case and control groups (P value: 0.005, OR: 1.73 CI95% for OR: 1.18, 2.53).

Conclusions: In conclusion, a significant association was seen between TGF-β1 -509C>T gene polymorphism and POAG disease and inheritance of mutant T allele is considered to be a risk factor for glaucoma in patients living in North Eastern part of Iran.

Keywords: Glaucoma, PCR, TGF-beta1, Polymorphism.

Introduction

Glaucoma is the second cause of blindness and the most common cause of irreversible blindness in the world and known as optic neuropathy. Glaucoma confirm by enlargement of the optic disc cup (cup-disc Ratio or CDR) and visual field loss based on the primary test. The main risk factors of disease are age and increased intraocular pressure (IOP). Other risk factors include decreased corneal

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thickness, race background and familial history. Primary open angle glaucoma (POAG) is the most common type of glaucoma and is referred to the form in which the anterior chamber angle and trabecular meshwork are normal. 1-2 percent of people over 40 years suffer from the PAOG, making it a major health challenge. The prevalence of glaucoma, large CDR and increased IOP are relatively higher in siblings and sons of patients with glaucoma than normal population (1-3).

Transforming growth factor beta-1(TGF-β1) is one of the immune system cytokines and a member of a large family of proteins called TGF-β superfamily. TGF-β1 has cellular immunosuppressive effects as well as pro-inflammatory effects such as monocyte and macrophage accumulation and anti-inflammatory effects, such as inhibiting lymphocyte proliferation and promoting tissue repair. Various studies showed the contribution of TGF-β1 in inflammatory autoimmune diseases disorders such as, allergy, systemic lupus erythematosus (SLE), multiple sclerosis and other diseases (4-8).

TGF-β1 is the main isoform of TGF-β superfamily in the eye and its significant level are observed in the aqueous, vitreous, and tear. Proteoglycan chondroitin sulfate is one of the main components of the extracellular matrix of the trabecular network and has effect on the outflow facility. Studies have shown that TGF-β1 plays a significant role in the production of proteoglycans. Also, its expression in the ciliary body epithelium of patients with glaucoma is higher than normal population (7, 9).

One of the known principal mechanisms in POAG is induction of proteoglycans synthesis of the extracellular matrix in the trabecular network, which reduces of the out flow facility of the anterior chamber which increase intraocular pressure. TGF-β1 increase proteoglycans synthesis, so IOP rise by reducing out flow facility. (10, 11)

To date, several polymorphic sites have been found on a variety of growth factor genes that control the growth factors production, thereby affecting the extracellular matrix of the trabecular meshwork (10-12).

TGF-β1 also has multiple polymorphic sites on chromosome 19 that affect the expression of TGF-β1 gene. Among the mentioned polymorphic sites, TGF-β1 -509C>T polymorphism on the promoter of the TGF-β1 gene plays an important role in the expression of this gene. Several studies have shown that the presence of mutant T allele is associated with increased gene expression (13-16). Therefore, due to the importance of open-angle glaucoma, in present study, the association of the TGF-β1-509C>T gene polymorphism (rs1800469) and primary open angle glaucoma (POAG) in patients from north east of Iran was investigated. Based on the high prevalence of glaucoma and the importance of its early diagnosis, finding genetic factors can help early diagnose of the disease.

Materials and methods

The present study was a case-control study in which 112 patients with primary open-angle glaucoma (POAG) with age over 40 years who referred to the Glaucoma Clinic of Khatam-Al-Anbia eye Hospital, Mashhad, Iran were enrolled. POAG diagnosis was performed by a glaucoma fellowship. The control group was included 112 participants over 40 years of age who were referred to other clinics of Khatam-Al-Anbia eye Hospital, with negative diagnosis of glaucoma and without family history of disease**.** The diagnosis of primary open angle glaucoma was according to increased intra ocular pressure (IOP), abnormal perimetry changes and an increased cup to disc ratio (CDR) with a normal gonioscopy. The exclusion criteria were the history of ocular trauma, uveitis, and abnormal gonioscopy, any history of eye surgery or laser iridectomy. Slit lamp and fundus examination were performed in all participants and visual acuity, cup to disc ratio and Intra ocular pressure (by Goldman tonometry) were measured. After obtaining the consent letter from all participants in the case and control groups, personal data including age, sex, and mentioned clinical data were recorded.

To determine the association between TGF-β1 gene polymorphism at C-509T position(rs1800469) and primary open-angle glaucoma (POAG), five ml of whole blood sample was taken from each participant in an anticoagulant tube containing EDTA. The DNA from blood samples was done by routine salting out method (17). The TGF-β1-509C>T genotyping was done by PCR-restriction

fragment length polymorphism (PCR-RFLP) method using *Bsu36I* restriction enzyme. the primers sequence and the restriction enzyme is shown in Table 1. PCR amplification was done according to the following condition: initial denaturation step at 95 °C for 4 minutes followed by 35 cycles of denaturation step at 94 °C for 45 seconds, annealing step at 56 °C for 30 seconds, extension step at 72 °C for 45 seconds and 1cycle of ending extension step at 72 °C for 5 minutes.

Table 1. Primer sequences and restriction enzyme for TGF-1 -509 C>T polymorphism genotyping

Primers	Restriction enzymes (Restriction site)	PCR product	Genotype
Forward: GTCGCAGGGTGTTGAGTGACAG Reverse: AGGGGGCAACAGGACACCTTA	*Bsu36I* (5-'CCTNAGG-3')	808bp	CC: 808 bp CT: 808 bp+ 617 bp+ 191 bp TT: 617 bp+ 191 bp

In order to determination of the TGF-β1-509 C>T gene polymorphism**,** the amplified sequences were digested with *Bsu36I* restriction enzyme. Briefly, ten microliters of PCR products were digested by 0.5- 0.25 units of *Bsu36I* restriction enzyme for 24 hours at 37 °C. As shown in table 2, the digested PCR products were separated based on their lengths using 2% agarose gel electrophoresis followed by a UV light visualization (Fig. 1).

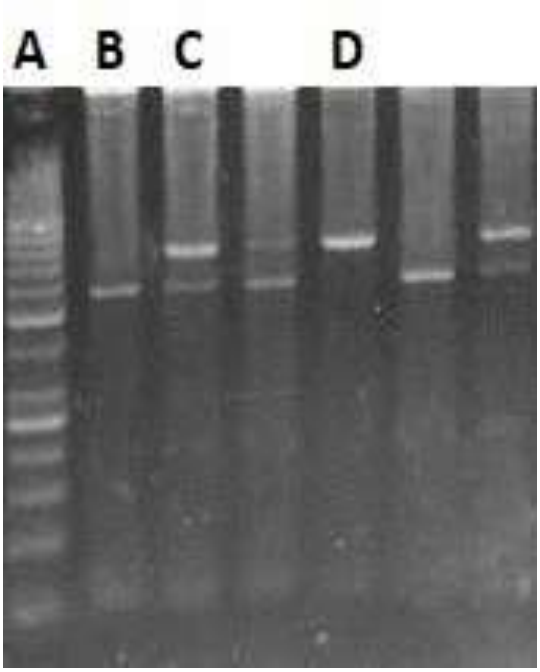

Fig. 1. to genotyping of TGF-β1 -509 C>T polymorphism, the amplified sequences were digested with Bsu36I restriction enzyme. Then, the digested PCR products were separated and visualized based on the lengths of the DNA fragments using 2% agarose gel electrophoresis followed by a UV light. A: 50 base pairs(bp) DNA molecular weight marker, B: TT genotype with 617bp+191bp fragments, C: CT genotype with 808 bp+ 617 bp+ 191 bp fragments, D: CC genotype with 808bp fragment.

Finally, the Genotype distributions in case and control groups were evaluated by Hardy Weinberg equilibrium. Moreover, the obtained results were statistically analyzed using Descriptive statistics, Independent T-Test, One-way ANOVA, Chi-Square and Fisher's exact test, Logistic regression test (and alternative nonparametric tests of the mentioned statistical tests if applicable). The mean is shown as the mean± the standard deviation(SD). *P* value <0.05 was considered as significant.

Results

In present study, the mean age of the patient and the healthy group were 58.8±12 and 58.6±12.2 years, respectively which were no statistically significant differences (P value: 0.856). In addition, in both groups, the sex frequencies did not statistically different (P value: 0.784). Table 2 shows the demographic data of the participants in the case and control groups.

As shown in table 3, the genotype distribution of TGFβ-1 -509 C >T were in Hardy Weinberg (HW) equilibrium in both case (HW P value: 0.07) and control (HW P value: 0.34) groups. The frequency of TGFβ-1 -509 C >T genotypes in the control and patient groups, in the codominant state, the highest frequency of genotype in the control group was related to CC genotype (44.6%), but the heterozygous CT genotype (45.6%) was observed as the highest frequency of genotypes in patient group. There was a significant difference in the frequency of TGFβ-1 -509 C >T genotypes between patients and control groups (P value: 0.022, OR for TT genotype: 2.54 CI 95% for OR: 1.22, 5.27). Also, the frequency of the T mutant

allele in the patient and control groups were 49.5% and 36.2%, respectively, which showed a significant difference between case and control groups (P value: 0.005, OR: 1.73 CI 95% for OR: 1.18, 2.53). Moreover, in the dominant state, there was a significant difference between the frequency of genotypes between the patient and the control group (Table 3).

Table 2. Demographic characteristics of the Glaucoma and control groups

	Number of participants	Sex	Age(mean±SD)
Glaucoma	112	69 (61.6%) Male 43 (38.4%) Female	58.8±12 years
Control	112	71 (63.4%) Male 41 (36.6%) Female	58.6±12.2 years
***P* value**	–	0.784	0.856

Table 3. Genotype and allele frequencies TGF-β1 -509 C >T gene polymorphism in glaucoma and control groups

TGFβ-1 -509 C >T (rs1800469)		Glaucoma patients n=112	Controls n = 112	P value	Odds ratio (95% CI)
Codominant	**C/C**	50 (44.6%)	31 (27.7%)	0.022	1.00 (Reference)
	C/T	43 (38.4%)	51 (45.5%)		1.93(1.04, 3.5)
	T/T	19 (17%)	30 (26.8%)		2.54 (1.22, 5.27)
Hardy Weinberg P value		0.07	0.34		
Dominant	**C/C**	50 (44.6%)	31 (27.7%)	0.008	1.00 (Reference)
	C/T-T/T	62 (55.4%)	81 (72.3%)		2.1 (1.2, 3.67)
Recessive	**C/C-C/T**	93 (83%)	82 (73.2%)	0.074	1.00 (Reference)
	T/T	19 (17%)	30 (26.8%)		1.79 (0.93, 3.42)
Overdominant	**C/C-T/T**	69 (61.6%)	61 (54.5%)	0.28	1.00 (Reference)
	C/T	43 (38.4%)	51 (45.5%)		1.34 (0.78, 2.28)
Alleles					
	C	113 (50.5%)	143 (63.8%)	0.005	1.00 (Reference)
	T	111 (49.5%)	81 (36.2%)		1.73 (1.18, 2.53)

Table 4. Association of TGF-β1 -509 C >T gene polymorphism with clinical characteristics in glaucoma patients

		TGFβ-1 -509 C >T (rs1800469)			P value	total
		CC	CT	TT		
cup-to-disc ratio (CDR±SD)	**Right eye**	0.68±0.26	0.75±0.22	0.70±0.26	0.585	0.72±0.22
	Left eye	0.64±0.22	0.77±0.22	0.65±0.24	0.06	0.70±0.24
Intraocular pressure (IOP mmHg ± SD)	**Right eye**	18.04±12.14	20.17±10.4	18.85±8.83	0.634	19.19±10.76
	Left eye	17.08±10.9	17.81±6.88	17.52±5.84	0.913	17.5±8.33
Visual acuity (VA Log MAR ± SD)	**Right eye**	0.42±0.37	0.37±0.34	0.34±0.31	0.533	0.38±0.35
	Left eye	0.41±0.38	0.32±0.32	0.45±0.30	0.145	0.37±0.34

As depicted in table 4, the mean cup to disc ratio (CDR) in patients carrying CT genotype were 0.75 ± 0.22 in the right eye and 0.77 ± 0.21 in the left eye which were higher than CC and TT

genotypes. However, the mean differences of cup to disc ratio (CDR) of patient in TGFβ-1 -509 C >T genotypes were not significant in the left eye (P value: 0.06) and the right eye (value: 0.58). The Intraocular pressure (IOP) of the right and left eyes in CT carrying genotype patients with were 20.17 ± 10.40 mmHg and 17.81 ± 6.88 mmHg in right and left eyes, respectively, which were higher than IOP of TT and CC genotypes. However, there were no significant mean differences of IOP of the right eye (P value: 0.527) and left eye (P value: 0.946) between genotypes. Finally, there were no statistically significant mean difference of the mean visual acuity (VA) of right eye (P value: 0.533) and left eye (P value: 0.145) of patients between TGF-β1 -509C>T genotypes.

Usually patient with severe and advanced disease are resistant to medical treatment and glaucoma surgery have to be considered. In this study there was no significant correlation between TGF-β1 -509C>T genotypes and necessity to glaucoma surgery (P=0.983).

Discussion

In present study, the association between TGF-β1 -509C>T gene polymorphism and POAG and its related symptoms was studied. Based on the obtained results of the study, there was a significant association between TGF-β1 -509C>T gene polymorphism and POAG. As seen in result section, inheritance of the T mutant allele of TGF-β1 -509C>T gene polymorphism is considered as a risk factor for the POAG. The presence of CT and TT genotypes increases the risk POAG by 1.913 and 2.547, respectively.

As Yang et al. study about the functional characteristics of TGF-β1 -509C>T gene polymorphism, they stated that the inheritance of the T mutant allele induced the TGF- β 1 gene expression (18). Moreover, in a study by Gayathri et al. In 2016, which assess the level of TGF-β1 in the urea fluid of glaucoma patients, they reported that TGF-β1 level in POAG patients were significantly higher than The control group (19). Also, based on Kuchtey et al. study, the mean serum level of TGFβ-1 in POAG patients was significantly higher than control subjects (20). These results are consistent with the results of the present study, which indicates that TGF-1 levels can contribute to the development of POAG. In a study by Krumbiegel et al. With the aim of investigating the association of the FBN1, LTBP2, MFAP2, TGM2, TGF-1 and CLU polymorphisms in European patients with pseudoexfoliation syndrome and related glaucoma, they reported a significant association between a variety of mentioned polymorphisms and the risk of glaucoma associated with pseudoexfoliation (21).

In a study by Sripriya et al. to investigate the role of TGF-β1 -509C>T gene polymorphism in 106 primary open-angle glaucoma patients and 104 controls in the Indian population, they concluded that there was no statistically significant association between TGF-β1 -509C>T gene polymorphism and POAG. Moreover, IOP and CDR had no significant association with TGF-β1 -509C>T genotype (22). In a study by Hui-Ju Lin et al.in China to investigate the association of TGF-β1 -509C>T gene polymorphism and myopia, they reported that the frequency of CC genotype in the case group was higher than that of the control group and C wild allele increased the risk of myopia (23). However, no significant association was seen between the TGF-β1 -509C>T gene polymorphism and myopia according to Hayashi et al study in Japan (24).

In conclusion, the results of present study showed that the TGF-β1 -509C>T gene polymorphism could play a role in the development of primary open-angle glaucoma (POAG). As shown, a significant association was seen between TGF-β1 -509C>T gene polymorphism and glaucoma disease and inheritance of mutant T allele of TGF-β1 -509C>T gene polymorphism is considered to be a risk factor for glaucoma in patients living in North Eastern part of Iran. However, no significant association between visual acuity(VA), Intraocular pressure (IOP) and cup to disc ratio (CDR) with the TGF-β1 -509C>T gene polymorphism were seen. Evaluation of the TGF-β1 expression level in POAG patients carrying mutant alleles of TGF-β1 -509C>T are recommended for further studies.

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