

# Detection of Herpes Simplex Virus DNA in Pseudoexfoliation Syndrome

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## Abstract

**Background:** Pseudoexfoliation syndrome is one of the most common identifiable causes of open angle glaucoma. It has unknown etiology and pathogenesis. Infection, possibly viral, is one of the proposed pathogenic mechanisms in this condition. In the present study the presence of herpes simplex virus (HSV) in specimens of anterior lens capsule of patients with pseudoexfoliation syndrome has been assessed.

**Methods:** The presence of HSV- DNA was searched by using polymerase chain reaction method in specimens of anterior lens capsule (5 mm diameter) of 50 patients with pseudoexfoliation syndrome (study group) and 50 age-matched patients without the disease (control group) who underwent cataract or combined cataract and glaucoma surgery during a one-year (2006-2007) period in Khalili Hospital, Shiraz, Iran. The results were compared statistically with Chi-square test and independent samples *t* test using SPSS software (version 11.5).

**Results:** HSV type I DNA was detected in 18% of the patients in the study group compared with 2% in the control group (Chi square test,  $P = 0.008$ ). The difference between the ranges of intraocular pressure in the two groups was not statistically significant.

**Conclusion:** The presence of HSV type I DNA suggests the possible relationship between the virus and pseudoexfoliation syndrome. It may be a treatable etiology in this multi-factorial disorder and may help to future management of patients; especially to prevent some of the complications in this syndrome.

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**Keywords** • Cataract • glaucoma • herpes simplex virus • pseudoexfoliation syndrome • polymerase chain reaction (PCR)

## Introduction

**P**seudoexfoliation syndrome is one of the most common identifiable causes of open angle glaucoma worldwide. It was described for the first time by Lindberg in 1917.<sup>1</sup> The syndrome is characterized clinically by small whitish deposits of fibrillar-granular materials in anterior segment of the eye.<sup>2</sup> These materials can be deposited on the lens, iris, pupillary margin, cornea, zonula, ciliary body, and anterior chamber angle.<sup>3</sup> Elevated intraocular pressure with or

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without glaucomatous damage occurs in about 25% of patients with pseudoexfoliation syndrome (about 6 to 10 times more than in eyes without the syndrome).<sup>2</sup> Many case series studies have reported high rates of bilateral involvement with ratio as high as 3:1.<sup>4-8</sup>

The exact chemical composition of exfoliation materials is unknown. Histochemical and immunohistochemical methods suggest a complex glycoprotein composed of a protein core surrounded by glycoconjugates.<sup>2</sup> The protein component includes both non-collagenous basement membrane and epitopes of the elastic fiber system. Production of exfoliation materials has been suggested to be the result of disturbed basement membrane metabolism.<sup>2</sup>

It is proposed that a combination of genetic and non-genetic factors may be involved in the etiology and pathogenesis of pseudoexfoliation syndrome. Possible genetic factors including mitochondrial, autosomal, or X-linked inheritances have been suggested.<sup>9,10</sup> A number of non genetic factors have been suggested for this condition that includes ultraviolet light, autoimmunity, viral infection, and trauma.<sup>4,9,10</sup>

Significant association was proposed between this syndrome and aging.<sup>11</sup> It is more common in women than men and is a common disease in some parts of Europe such as Iceland and northern Sweden; however, the reported incidence in other parts of the world such as Ethiopia is high as well.<sup>12-14</sup> Likewise, it is claimed that hyperhomocysteinemia may be a risk factor for thromboembolic vasculopathy in patients with pseudoexfoliation syndrome and the associated glaucoma.<sup>15</sup> Dysregulation of matrix metalloproteinase and their inhibitors have been proposed to contribute to the pathogenesis of the syndrome and glaucoma.<sup>16</sup>

In one study HSV type I has been detected significantly more often in iris specimens of patients with pseudoexfoliation syndrome,<sup>17</sup> emphasizing an infectious theory for the pathogenesis of the disease. The present study was designated to evaluate the possible association between HSV infection and pseudoexfoliation syndrome by examining the presence of HSV-DNA in anterior capsule of the lens by polymerase chain reaction (PCR) method.

## Patients and Methods

Fifty consecutive patients who underwent cataract or combined cataract and glaucoma surgery by phacoemulsification, phacotrabeculectomy methods or extracapsular cataract extraction and exerted pseudoexfoliation syndrome by slit lamp examination were selected as the

study group. The study was done in Khalili hospital affiliated to Shiraz University of Medical Sciences during a one-year (2006-2007) period. The patients of the control group were selected from the same hospital and consisted of 50 patients who were considered as candidates for cataract or combined cataract and glaucoma operation but had no evidence of the syndrome. Before surgery, all of the patients underwent complete ocular examination including evaluating the intraocular pressure, examination using slit lamp before and after pupillary dilation, and funduscopy. All selected patients were more than 45 years old and from both genders (we did not match them for sex).

Exclusion criteria were history of eye infection with herpes simplex or the presence of corneal opacity in the slit lamp examination that was suspected to be caused by previous HSV infection. The diagnosis of pseudoexfoliation syndrome was based on the presence of any amount of exfoliative materials on lens capsule, pupillary border, or iris with or without transillumination defect of the iris during the examination before and after pupillary dilation with twice instillation of cyclopentolate 2%.

Written informed consent was taken from all of the enrolled patients. At the time of operation, anterior capsule of the lens was accessed after performing 5 mm capsulorrhexis using gauge 27 needle in phaco or phaco trabeculectomy method and Can Opener method with the same size in extra capsular method. Then, it held in viral transport media and stored in - 20 °C immediately for further study. The same viscoelastic material (Coatel, Bausch & Lomb, Waterford, Ireland) was used for all patients before capsulotomies. PCR was performed on all specimens as follows: viral DNA was extracted from specimens using a DNP™ DNA extraction Kit (CinnaGen Co. IRAN) according to the manufacturer's instruction. PCR was done using a CinnaGen HSV detection kit (CinnaGen Co. IRAN) according to the manufacturer's instructions. A clinical specimen which was proved to be positive for herpes and a molecular size marker used during the test. In this kit, gene of HSV1 considered as target and 256 bp fragments in comparison with positive control indicated as a positive test.

## Statistical Analysis

The results were compared with Chi-square test and independent sample *t* test using SPSS software (version 11.5, Chicago, IL, USA). *P* values less than 0.05 were considered statistically significant.

## Results

Tables 1 and 2 show the characteristics of patients and the type of operation used in the study and control groups. The age range in the study group was 46-81 years and in control group was 51-78 years. The range of intraocular pressure was 12-38 mm Hg and 12-18 mm Hg in the study and control groups respectively. Twenty percent of the patients in the study group and 9% of the patients in the control group had intraocular pressure more than 21 mmHg. However, the differences between age, sex, mean intraocular pressure and prevalence of nebular corneal opacity were not statistically significant (table 1).

**Table 1:** Characteristics of the patients in the study and control groups

Patients' Characteristics	Study group	Control group	P value
Mean age± SD (years)	65.6±1.12	63.3±0.73	0.46
M/F ratio	24/26	30/20	0.28
Mean IOP± SD (mm Hg)	17.8±0.89	15.4±0.21	0.68
Nebular corneal opacity	5/50 (10%)	8/50 (16%)	0.35

M: Male, F: Female, IOP: Intraocular pressure, SD: Standard deviation

**Table 2:** The type of operation used in the study and control groups

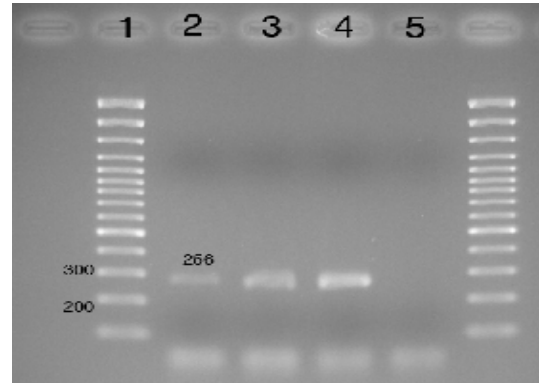
Type of operation	Study group	Control group
ECCE	14 (28%)	7 (14%)
Phaco	30 (60%)	39 (78%)
Phaco-Trab	6 (12%)	4 (8%)

ECCE: Extra capsular cataract exfoliation, Phaco: Phacoemulsification procedure, Trab: Trabeculectomy

PCR analysis on the anterior lens capsule specimens showed that 18% of specimens obtained from the study group and 2% of the specimens from the control group were positive for HSV. The difference was statistically significant (Chi-square test,  $P=0.008$ ).

The differences between age, gender, the range of intraocular pressure, and corneal opacity between the PCR positive and PCR

negative patients in the study group were not statistically significant ( $P=0.95$ ,  $P=0.82$ ,  $P=0.45$ ,  $P=0.56$  respectively). Table 3 shows the characteristics of patients with positive PCR in both the study and control groups. Figure 1 shows the PCR results in two positive and one negative specimens in comparison with the positive control.



**Figure 1:** (1): DNA ladder from 80-1031bp; (2): 256 bp for positive control; (3,4): patients with positive results for HSV PCR in anterior lens capsule. (5): patient with negative result for HSV in anterior lens capsule.

## Discussion

Ocular manifestations of pseudoexfoliation syndrome involve all of the anterior segment structures. It has been recognized as a common identifiable cause of secondary glaucoma,<sup>4</sup> or progression of cataract.<sup>18</sup> Even in apparently unilateral cases, the syndrome may produce zonular weakness in clinically unaffected fellow eye and leads to a complicated cataract surgery.<sup>19</sup> The exact etiology of pseudoexfoliation syndrome remained obscure. Many theories for the pathogenesis of this disease were described; one of them is evidence of an infectious origin.<sup>4</sup> Even though many genetic causes were investigated and proved to be related to the occurrence of this syndrome, environmental factors may also influence the phenotypic expression of the syndrome.<sup>20</sup>

**Table 3:** Characteristics of patients with positive PCR in the study and control groups

Patients	Study group/control group	Gender	Age	Method of Surgery	Intraocular pressure (mm Hg)
1	SG	M	63	ECCE	18
2	SG	M	68	Phaco	16
3	SG	M	58	Phaco	14
4	SG	F	63	Phaco	18
5	SG	F	61	Phaco	14
6	SG	M	71	Phaco-Trab	36
7	SG	F	59	ECCE	16
8	SG	M	64	Phaco-Trab	34
9	SG	F	61	Phaco	15
10	CG	M	68	Phaco	18

SG: study group, CG: control group, ECCE: Extra capsular cataract exfoliation, Phaco: Phacoemulsification procedure, Trab: Trabeculectomy, PCR: Polymerase chain reaction

In Norway eye screening study,<sup>21</sup> the prevalence of pseudoexfoliation syndrome in both partners of 343 married couples (3.2%) was significantly higher than expected. Ringvold noted a striking morphology of fibrillar materials in pseudoexfoliation syndrome, suggesting the possibility of a viral disorder.<sup>22</sup> Further evidence for the possibility of an infectious agent came from observation of younger patients developing the syndrome after intraocular surgeries,<sup>23-26</sup> including one patient with keratoconus who developed the disease after penetrating keratoplasty.<sup>27</sup>

Detection of DNA of HSV type 1 in pseudoexfoliation syndrome and exfoliation glaucoma in the specimens obtained from the iris of patients with this disorder has been reported.<sup>17</sup> Likewise, long-term presence of HSV in the lens after the resolution of an acute retinitis, has been observed.<sup>28</sup> In addition, the role of HSV infection in conditions such as iridocorneal endothelial syndrome has been suggested.<sup>29</sup>

In our study the presence of HSV-DNA in specimens obtained from the anterior capsule of patients with pseudoexfoliation syndrome was significantly higher than the patients in the control group while both groups had no statistically significant differences regarding age, sex, intraocular pressure, presence of nebular corneal opacity or type of surgery. Meanwhile, presence of virus in lens capsule is in contrast to what observed by Detorakis and co-workers who concluded that the anterior capsule was not a favorable site for interaction between HSV and pseudoexfoliation syndrome.<sup>17</sup>

## Conclusion

Presence of HSV type I DNA in lens capsule specimens of patients with pseudoexfoliation syndrome suggests the role of sub clinical HSV infection in eyes with this syndrome and may help to future therapeutic interventions; at least to prevent some of the associated complications. However, the syndrome is a multi factorial disorder and infectious causes may potentiate phenotypic expression of corresponding genes or have effects on other environmental causes.

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**Conflict of Interest:** None declared

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