

# Effect of Pseudoexfoliation Syndrome on Biochemical Characteristics of Aqueous Humour

## Aköz Humör'un Biyokimyasal Özellikleri Üzerine Psödoeksfoliasyon Sendromunun Etkisi

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### ABSTRACT

**Purpose:** The aim was to evaluate the effect of pseudoexfoliation (PEX) syndrome on the biochemical characteristics of aqueous humour.

**Materials and Methods:** This is a cross-sectional, single-center, comparative study. Aqueous humor was aspirated from consecutive senile cataract cases with and without PEX. The levels of urea, creatinine, glucose, calcium, magnesium, phosphate, chlorine, sodium and potassium were measured in the aqueous humor of both groups.

**Results:** Group-1 included 60 cases without PEX, and Group-2 included 48 cases with PEX. The calcium and chlorine levels were significantly higher in the aqueous humor of the patients with PEX (Group-2) than in the control group (Group-1), whereas the glucose levels were significantly lower in the PEX group than in the control group. The coexistence of nuclear cataracts and PEX in Group-2 was found to be statistically significant.

**Conclusion:** Between the PEX and non-PEX groups, a difference was found in the levels of the biochemical constituents in the aqueous humor. The changes in biochemical features of aqueous humor may be the cause of ocular morbidity. In the future, the ocular complications can be controlled with medicines that regulate biochemical structure of aqueous humor in patients with PEX. The quantity of aqueous humor obtained from the anterior chamber was very limited and thus, only a small number of parameters could be evaluated. In patients with PEX, further studies are needed to analyze the aqueous humor.

**Key Words:** Pseudoexfoliation, Aqueous Humor, Biochemical Characteristics.

### ÖZ

**Amaç:** Bu çalışmanın amacı, aköz humör'un biyokimyasal özellikleri üzerine psödoeksfoliasyon sendromu'nun etkisini değerlendirmektir.

**Gereç ve Yöntemler:** Bu kesitsel, tek merkezli, karşılaştırmalı bir çalışmadır. Aköz humör, psödoeksfoliasyonu olan ve olmayan arka arkaya senil katarakt vakalarından aspire edildi. Üre, kreatinin, glukoz, kalsiyum, magnezyum, fosfat, klor, sodyum ve potasyum seviyeleri, her iki grubun aköz humörlerinde ölçüldü.

**Bulgular:** Grup-1, psödoeksfoliasyonu olmayan 60 hasta içeriyordu, Grup-2, psödoeksfoliasyonlu 48 hasta içeriyordu. Kalsiyum ve klor düzeyleri psödoeksfoliasyonlu hastaların (Grup-2) aköz humörlerinde kontrol grubuna göre istatistiksel olarak anlamlı biçimde yüksek idi, buna karşın glukoz düzeyleri psödoeksfoliasyonlu grupta kontrol grubundan anlamlı biçimde düşüktü. Grup-2'de psödoeksfoliasyonla nükleer kataraktın birlikteliği istatistiksel olarak anlamlı biçimde yüksekti.

**Sonuç:** Psödoeksfoliasyonu olan ve olmayan gruplar arasında; aköz humör'un biyokimyasal bileşenlerinin düzeylerinde bir farklılık bulundu. Aköz humör'un biyokimyasal özelliklerindeki bu değişiklik oküler morbiditeden sorumlu olabilir. Gelecekte bu oküler komplikasyonlar psödoeksfoliasyonlu hastalarda aköz humörün biyokimyasal yapısını düzenleyecek ilaçlarla kontrol edilebilir. Ön kamaradan elde edilen aköz miktarı çok sınırlıydı ve bu yüzden, sadece belli sayıda parametre değerlendirilebildi. Psödoeksfoliasyonlu hastalarda aköz humörü analiz edecek başka çalışmalara ihtiyaç vardır.

**Anahtar Sözcükler:** Psödoeksfoliasyon, aköz humor, biyokimyasal özellikler.

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## INTRODUCTION

Pseudoexfoliation (PEX) is a generalized syndrome associated with aging which affects the extracellular matrix of the eye. This condition develops when glycoproteins and cross-linked amyloid-like fibrils are secreted and form extracellular fibrillar aggregates which subsequently accumulate in the anterior region of the eye.<sup>1,2</sup> Moreover, this PEX material can also occur in elastic connective tissue of other organs.<sup>3</sup>

Patients with PEX are predisposed to develop ophthalmic pathologies including glaucoma and intra-operative and post-operative complications of cataract surgery.<sup>3,4</sup> In addition, PEX patients are more commonly affected by late intraocular lens dislocation.<sup>5,6</sup>

The multifactorial, genetic nature of the PEX syndrome is recognized, while the pathogenesis of the disorder is not completely understood.<sup>7</sup> The suggestion that proteins involved in inflammation, oxidative stress and coagulation may contribute to PEX pathogenesis is based on the higher levels of these proteins found in the eyes of PEX patients.<sup>7</sup>

In order to determine the important effective pathogenetic factors of PEX, an examination of the components of the aqueous humor of patients suffering from this disorder is called for.<sup>8</sup>

Consequently, this study aimed to compare the biochemical characteristics of the aqueous humor in patients with and without PEX.

## MATERIAL AND METHODS

This cross-sectional, single-center, comparative study was conducted in the Departments of Ophthalmology and Biochemistry at the Numune Training and Research Hospital in Adana, Turkey. The study was approved by the Ethics Committee of the hospital and was in compliance with the tenets of the Declaration of Helsinki. Informed consent was obtained from patients participating in the study.

Consecutive patients with senile cataract were evaluated for the study between January and November 2015. Detailed demographic and medical information was collected from each patient. Patients with systemic disease and/or ocular disorders and those with history of intraocular or laser surgery were excluded from the study.

Best-corrected visual acuity (BCVA) was measured with Snellen charts. The anterior segment was evaluated through biomicroscopic examination, and intraocular pressure was measured by using a Goldman applanation tonometer. After pupil dilation, a fundus examination of each patient was performed. In those with PEX syndrome, PEX material deposits were observed on the anterior segment during the examination. The patients were divided into two groups. Group-1 included 60 control patients with cataract only, and Group-2 included 48 patients diagnosed with both cataract and PEX.

## Sample collection and analysis

The operation was conducted in an operation room under sterile conditions, and the patients vital signs were monitored throughout the operation. The eyes were dilated with 1% tropicamide (Tropamide; Bilim İlaç, Istanbul, Turkey) and 2.5% phenylephrine (Mydfrin; Alcon, Fort Worth, Tex., USA) drops. Following topical anesthesia through proparacaine HCl 0.5%, 10% betadine was used to wipe the periocular area. Before the operation the inside of the eye was cleaned with 5% betadine, and the surgeon waited for 3 min.

Upon commencement of the cataract surgery, aqueous humor specimens (~0.1–0.2 cc) were collected from the anterior chamber through 30 gauge needle and each specimen was taken to the laboratory.

The urea, creatinine, glucose, calcium, magnesium, sodium, potassium, chlorine and phosphate levels in the samples were determined using an analyzer (Cobas 6000 c-501, Roche Diagnostics GmbH, Germany; Hitachi High-Technologies Corporation, Japan).

The hexokinase method was used to determine the glucose levels, and the kinetic urease-glutamate dehydrogenase method was employed for the urea measurements. Creatinine levels were assessed via the kinetic alkaline picrate (Jaffe) method. Other measurements were made using the NM-BAPTA method for calcium, ammonium phosphomolybdate for phosphate, the photometric method for magnesium, and an ion-selective electrode measuring system (ISE) for sodium, potassium and chloride.

## Statistical analysis

The Statistical Package for Social Sciences for Windows software (SPSS version 16.0, SPSS Inc., Chicago, USA) was used for data analysis, the Kolmogorov–Smirnov test for the normality distribution of the variables, the Student's t-test for comparison of the variables with normal distribution, and the Mann–Whitney U test for comparison of variables with abnormal distribution. The Chi-square and Fisher's exact tests were employed for the comparison of categorical variables presented as frequency (%). The descriptive statistics of normally distributed continuous variables were expressed as mean  $\pm$  standard deviation. Differences with a  $p$  value of  $< 0.05$  were considered statistically significant.

## RESULTS

The study included a total of 108 patients, 60 of whom were in Group-1 and 48 in Group-2. The two groups were similar in terms of demographic characteristics and baseline visual acuity.

The demographic features of the patients with and without PEX are presented in Table 1.

**Table 1.** Demographic data of patients with and without PEX.

	Group I, n=60	Group II, n=48	p values
Age	67.9±9.0	71.9±8.2	0.465
Gender: Female	45.7 %	41.7 %	0.190
Male	54.3 %	58.3 %	
Smoking: Yes	34.3 %	33.3 %	0.958
No	65.7 %	66.7 %	
VA (Snellen)	0.11±0.12	0.13±0.13	0.718
IOP (mmHg)	12.5±2.3	13.2±2.4	0.691

VA: Visual acuity, IOP: Intraocular pressure

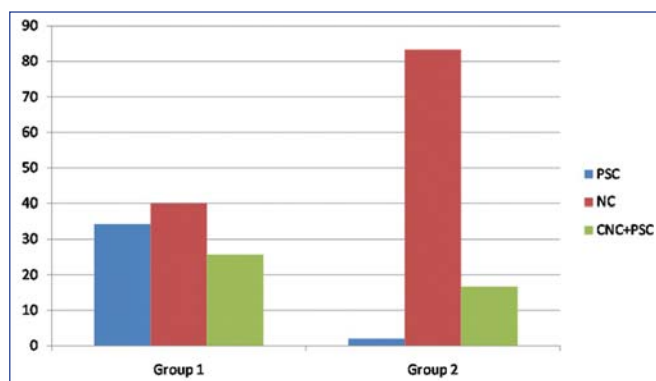
The mean values of calcium and chlorine in the aqueous humor were higher in the PEX group than in the control group, while the mean glucose levels were significantly lower in the PEX group compared to the control group ( $p<0.05$ ).

The urea, creatinine, phosphate, magnesium, sodium and potassium levels were similar in both groups. The results of the biochemical analysis of the aqueous humor samples in Group-1 and Group-2 are presented in Table 2.

In addition, the co-existence of nuclear cataract and PEX in Group-2 was found to be statistically significant (Figure 1).

**Table 2.** Results of biochemical analysis of aqueous humor in both groups.

	Group I	Group II	P values
Sodium	148,1±3,7	149,8±4,8	0,186
Potassium	4,1±0,2	4,1±0,2	0,240
Chlorine	120,8±3,9	124,2±5,6	<b>0,029</b>
Calcium	4,8±1,3	6,0±0,6	<b>0,007</b>
Phosphorus	2,0±0,3	2,4±1,0	0,162
Magnesium	1,6±0,1	1,6±0,2	0,320
Glucose	92,7±56,0	60,0±11,4	<b>0,000</b>
Urea	31,5±8,7	40,7±29,2	0,169
Creatinine	0,2±0,1	0,3±0,2	0,168

**Figure 1.** Distribution of cataract types in both groups.

PSC: Posterior subcapsular cataract, NC: Nuclear cataract, CNC+PSC: Corticonuclear and posterior subcapsular cataract.

## DISCUSSION

Pseudoexfoliation syndrome is a complex disorder involving many factors, both genetic and non-genetic.<sup>9</sup> The pathogenesis of PEX along with the precise makeup of the associated PEX material have yet to be determined. The secretion of PEX material is strongly associated with the circulation of the aqueous humor and consequently, is affected by its composition.<sup>10</sup>

The analysis of the aqueous humor in PEX syndrome has been reported in studies around the world and possible causes for PEX have been suggested, with some indicating that oxidative stress mechanisms may be important contributing factors to PEX pathogenesis.<sup>11-13</sup>

Tissue damage is caused by oxidative stress, as the ocular tissues are affected by oxygen radicals and lipid peroxidation.<sup>10</sup> Moreover, oxidative damage has been found to be a contributing factor in cataract development.<sup>14,15</sup> Nuclear cataract, in particular, was associated with PEX in the Australian Blue Mountains eye study.<sup>16</sup> Consistent with the literature, this study demonstrated the relationship between nuclear cataract and PEX syndrome.

Electron microscopy studies have shown that PEX fibrils accumulate when the levels of calcium are high.<sup>17</sup> The present study found significantly higher calcium levels in the aqueous humor of the Group-2 eyes (with PEX) compared to those of Group-1 (control). The explanation for the high concentration of calcium in the aqueous humor maybe found in the results of several studies. In one study, for example, a new genetic locus associated with calcium metabolism (CACN1A1) was proposed as a risk factor associated with the PEX syndrome.<sup>18</sup> Additionally, oxidation is a major factor in pseudoexfoliation development. The oxidation may lead to impairment in the calcium metabolism of the lens, as the  $\text{Ca}^{2+}$ -ATPase pumps of the lens are highly sensitive to oxidation.<sup>19-21</sup> Elevated  $\text{Ca}^{2+}$  levels contribute significantly to cortical cataract development.<sup>22-24</sup> In this study, cortical cataracts are the second most frequently occurring type in eyes with PEX. Consequently, the cortical cataract seen in PEX syndrome may be due to the disrupted calcium metabolism.

The study show that the eyes with PEX had lower glucose concentrations in the aqueous humor compared to the controls. It is a challenge to explain this result because there is no information about such a finding in the literature. Previous studies have indicated that both oxidative stress and pro inflammatory cytokine levels are higher in the anterior chamber in PEX.<sup>7</sup> In the cerebrospinal fluid of patients with cerebral infarction, the glucose levels decrease as the glucose is consumed due to peroxidation and inflammation.<sup>25</sup> A similar mechanism may be responsible for the aforementioned finding in PEX.

The secretion of chlorine ( $\text{Cl}^-$ ) may inhibit the rate at which the aqueous humor is produced, thus making  $\text{Cl}^-$  a crucially

important anion.<sup>26</sup> Electroneutral transporters transfer the  $\text{Cl}^-$  from the stroma to the pigment epithelium (PE) cells, where, via the gap junctions, it is disseminated to the non-pigmented epithelium (NPE) cells. The  $\text{Cl}^-$  is then released, chiefly through the  $\text{Cl}^-$  channels of the  $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$  co-transporter and the  $\text{Cl}^-/\text{HCO}_3^-$  and  $\text{Na}^+/\text{H}^+$  exchangers.<sup>26</sup>

The present study revealed higher  $\text{Cl}^-$  levels in the aqueous humor of Group-2 (with PEX) than in Group-1 (control), while the  $\text{K}^+$  and  $\text{Na}^+$  concentrations were similar in both groups.

It is possible that none of the mentioned mechanisms are the reason for the increased  $\text{Cl}^-$  levels. It has been suggested that A3 adenosine receptors (A3ARs) may contribute to the defense mechanisms or the pathogenesis of PEX.<sup>26</sup> These receptors may be one of the chief causes of open-angle glaucoma because they are overexpressed by the NPE in certain types of glaucoma and by outflow pathway cells in PEX syndrome.<sup>26</sup> As expected, intraocular pressure (IOP) was seen to be increased by A3AR-selective agonists and decreased by A3AR-selective antagonists, in addition to their significantly inhibiting the resultant reaction to adenosine.<sup>27</sup> Some studies have reported that whole-cell  $\text{Cl}^-$  currents of cultured human NPE cells are triggered by the A3AR-selective agonists.<sup>28</sup> Thus, there may be a relationship between A3ARs and the increased  $\text{Cl}^-$  levels in PEX.

## CONCLUSION

Different biochemical characteristics were found in the aqueous humor of the PEX and non-PEX groups. The increased levels of calcium and chlorine or the decreased levels of glucose may be responsible for the ocular morbidity of PEX syndrome. Changes in biochemical features of aqueous humor may be the cause of this damage. In the future, the ocular complications can be controlled with medicines that regulate biochemical structure of aqueous humor in patients with PEX. However, in the present study, the quantity of aqueous humor obtained from the anterior chamber was very small and only a limited number of parameters could be evaluated. Further studies are warranted for the analysis of the aqueous humor in PEX patients.

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