# Biochemical analysis of aqueous humor in diabetic and non-diabetic patients with cataracts

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## ABSTRACT

**BACKGROUND:** Although there are many factors stated in the etiology of cataract, the mechanisms which are formed during the formation of cataract are still not illuminated. The purpose of this study is to evaluate the biochemical analysis of aqueous humor in diabetic and non-diabetic patients with cataract in terms of the existence of pseudo-exfoliation (PSX).

**MATERIAL AND METHODS:** Seventy-six patients who presented to our ophthalmology clinic with the complaint of cataract and who were planned to undergo phacoemulsification and IOL implementation were included in the study. The patients were classified into 4 groups as Group I: Cataracts with diabetes and without PSX, Group II: Cataracts without diabetes and PSX, Group III: Cataracts with diabetes and PSX, Group IV: Cataracts without diabetes and with PSX. The groups were compared statistically in terms of biochemical analysis of aqueous humor.

**RESULTS:** The mean age of the patients was $68.0 \pm 8.5$, and 51.3% of the patients were male. In Group II, Na value was significantly higher than in Group I and Group III. In Group IV, Na value was significantly higher than in Group I and Group III. Cl value in Group IV was significantly higher than in Group I-III-III. In Group IV, Ca value was significantly higher than in Group I-III-III. In Group I, P value was significantly higher than in Group II and Group III. Glucose levels in Group I were significantly higher than in Group II-III-IV. Glucose levels in Group III were significantly higher than in Group II-IV. Na value in the PSX (+) group was significantly lower than in the PSX (–) group. In the PSX (+) group, glucose value was significantly higher than in the PSX (–) group.

**CONCLUSION:** High glucose and low Na levels in the anterior chamber may play a role in the development of PSX and PSCC. High P level in the anterior chamber may be contributed to the development of cataract in diabetic non-PSX eyes. In non-diabetic PSX (+) group, high Ca and Cl levels may be contributed to developing cataracts.

**KEY WORDS:** cataract; biochemical analysis; aqueous humor; diabetes

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## INTRODUCTION

Cataract is defined as a progressive loss of lens transparency. Some of the opacities are fixed and localized while others are progressive and widespread. Cataract is the most leading cause of curable blindness. Although there are many factors in the etiology, the exact mechanisms of cataract formation are not fully elucidated. Therefore, the prevention of cataract formation is not possible now and surgical treatment has emerged as the only option [1].

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Although there have been more than fifty years of basic and clinical research, there is no method to prevent age-related cataract and treat it without surgery. But there is a better understanding of this complexity; it is a multifactorial condition in which the occurrence and progression of this condition is modified by age, sex, radiation, oxidation, physical trauma, nutrition, diabetes, hypertension, smoking and drugs [2].

The mechanism in cataractogenesis has not been understood yet. Several risk factors have been identified in cataract development such as age, genetic predisposition, oxidative stress, and UV light exposure. It can be classified as congenital or secondary. Secondary cataract may also be due to reasons such as retinitis pigmentosa or uveitis, or systemic reasons such as diabetes or homocystinuria, or medications such as steroids [3].

The only source that meets the metabolic needs of the lens is the aqueous humor. Aqueous humor is not a simple ultrafiltrate of plasma. Changes in aqueous humor content are secondary to the active transport and dilutional changes of the vitreous due to hyaloid, iris blood vessels, lens and corneal endothelium [4–7].

Pseudoexfoliation (PSX) Syndrome is an age-related condition characterized by the production and accumulation of extracellular fibrillar materials in the anterior segment of the eye. Since the blood-aqueous barrier is affected, the protein content of the aqueous humor is changed in eyes with PSX compared to normal eyes.

The aim of this study is to evaluate the biochemical analysis of aqueous humor according to the presence of pseudoexfoliation in patients with diabetic and nondiabetic cataracts. In order to classify cataract types and to see the effect of changes in the anterior chamber, we included patients with PSX.

## MATERIAL AND METHODS

The patients who were admitted to Adana Numune Training and Research Hospital, Department of Ophthalmology between May 2014 and November 2015 underwent phacoemulsification and IOL implantation for prospective investigation. The study was approved by the Ethics Committee of Adana Numune Training and Research Hospital in Adana in Turkey.

Seventy-six patients who underwent lensectomy and intraocular IOL implantation with phacoemulsification technique were included to the study. The same phacoemulsification equipment was used in all operations. These operations were made by the same two specialists.

Patients with glaucoma, glaucoma surgery, history of previous vitrectomy, corneal transplantation, and those with intravitreal injection history were excluded from the study. Those with systemic diseases other than diabetes and those with systemic steroid treatment for any reason were excluded.

The patients were grouped as followings:
— Group I: Cataracts with diabetes and without PSX;
— Group II: Cataracts without diabetes and PSX;
— Group III: Cataracts with diabetes and PSX;
— Group IV: Cataracts without diabetes and with PSX.

In all patients, the best corrected visual acuity (BCVA), eye pressure (To), biomicroscopic examination of anterior segment, and detailed fundus examination were performed preoperatively. BCVA was evaluated with Snellen chart. To was measured by an applanation tonometer. Cataract type was determined according to anatomic location. Fundus examination was performed with the 90D lens.

Approximately 10–15 IU of aqueous humor from the anterior chamber was taken with a 27-gauge insulin needle before the operation. Then, intraocular pressure was normalized by intraocular irrigation. Capsulorhexis + hydrodissection were performed. The nucleus was phacoemulsified. Cortex residues were aspirated by bimanual irrigation and aspiration (I/A). The collapsible acrylic hydrophilic intraocular lens (IOL) was implanted into the capsule bag. The anterior chamber was purged with I/A. 1 mg/0.01 ml cefuroxime axetil was given to the anterior chamber.

The samples were delivered to the biochemistry laboratory. Glucose, urea, creatinine (Cr), calcium (Ca), phosphate (P), magnesium (Mg) sodium (Na), potassium (K) were studied in the cobas 600 c-501 (Roche Diagnostics GmbH, Germany; Hitachi High-Technologies Corporation, Japan) autoanalyzer. Glucose was measured by hexokinase method, urea by urease-glutamate dehydrogenase enzymes by kinetic method, creatinine by Jaffe alkaline picrate method, calcium by NM-BAPTA, phosphate by ammonium molybdate, sodium and potassium by magnesium xylidil blue by photometric method.

## STATISTICAL METHOD

In the descriptive statistics of the data, mean, standard deviation, median, lowest, highest, fre-

quency and ratio values were used. The distribution of the variables was measured by Kolmogorov-Smirnov test. ANOVA (Tukey test), Kruskal-Wallis, Mann-Whitney U test, and Independent Sample t-test were used to analyze the quantitative data. Chi-square test was used for the analysis of qualitative data and Fischer test was used since the Chi-square test conditions were not met. SPSS 22.0 program was used in the analysis.

## RESULTS

The average age of the patients who were included into this study was 68.0 ± 8.5. 48.7% of the patients were female and 51.3% of them were male. The percentage of the patients who were smoking was 35.5% and the percentage of the patients who were not smoking was 64.5%. The mean examination findings were Vo: 0.11 ± 0.12 and To: 12.5 ± 2.4. The percentage of the posterior subcapsular cataract was 21.1%, nuclear cataract was 56.6%, and nuclear cataract + posterior subcapsular cataract was 22.4%. The mean values in the laboratory results were found as Na: 147.5 ± 3.8, K: 4.1 ± 0.2, Cl: 120.8 ± 4.2, Ca: 5.2 ± 1.2, P: 2.1 ± 0.5, Mg: 1.6 ± 0.1, Glucose: 102.0 ± 57.9, Urea: 34.4 ± 14.0, Cr: 0.2 ± 0.1 (Tab. 1)

The age of the patients in Group I was significantly lower than in Group II, Group III and Group IV ($p < 0.05$). In Group II, PSCC was significantly higher than in Group III and Group IV ($p < 0.05$) (Tab. 2).

In Group II, Na value was significantly higher than in Group I and Group III ($p < 0.05$). In Group IV, Na value was significantly higher than in Group I and Group III ($p < 0.05$). There was no significant difference ($p > 0.05$) between Group I and Group III for Na value. Cl value in Group IV was significantly higher than in Group I-III-III ($p < 0.05$). In Group I-II-III, there was no significant difference ($p > 0.05$) between Cl values. In Group IV, Ca value was significantly higher than in Group I-III-III ($p < 0.05$). In Group I-II-III, there was no significant difference for Ca value ($p > 0.05$). In Group I, P value was significantly higher than in Group II and Group III ($p < 0.05$). In Group I-IV,

| Table 1. The demographic characteristics and examination findings of the patients | | Min–Max | Median | Mean ± SD/N | % |
|---|---|---|---|---|---|
| Age | | 51.0–86.0 | 65.5 | 68.0 ± 8.5 | |
| Gender | Female | | | 37 | 48.7% |
| | Male | | | 39 | 51.3% |
| Smoking | Positive | | | 27 | 35.5% |
| | Negative | | | 49 | 64.5% |
| Va | | 0.01–0.50 | 0.05 | 0.11 ± 0.12 | |
| Ip | | 2.0–21.0 | 12.5 | 12.5 ± 2.4 | |
| Biomicroscope | PSCC | | | 16 | 21.1% |
| | NC | | | 43 | 56.6% |
| | NC + PSCC | | | 17 | 22.4% |
| Fundus | Normal | | | 19 | 25.0% |
| | Abnormal | | | 57 | 75.0% |
| Na | | 141.0–162.0 | 147.5 | 147.5 ± 3.8 | |
| K | | 3.5–4.6 | 4.1 | 4.1 ± 0.2 | |
| Cl | | 114.2–136.0 | 120.0 | 120.8 ± 4.2 | |
| Ca | | 2.3–7.1 | 5.6 | 5.2 ± 1.2 | |
| P | | 1.4–5.3 | 2.0 | 2.1 ± 0.5 | |
| Mg | | 1.2–2.9 | 1.6 | 1.6 ± 0.1 | |
| Glucose | | 40.0–367.5 | 85.1 | 102.0 ± 57.9 | |
| Urea | | 16.1–127.0 | 32.1 | 34.4 ± 14.0 | |
| Cr | | 0.0–0.7 | 0.2 | 0.2 ± 0.1 | |

PSCC — posterior subcapsular cataract; NC — nuclear cataract; Va — vision acuity; Ip — intraocular pressure; Na — sodium; K — potassium; Cl — chlorine; Ca — calcium; P — phosphate; Mg — magnesium; Cr — creatine; SD — standard deviation

**Table 2. The comparison of demographic characteristics and examination findings in groups I–IV**

| | | Group I | | Group II | | Group III | | Group IV | | p |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | Mean ± SD | 62.8 ± 5,8 | | 67.9 ± 9.0 | | 71.7 ± 7.6 | | 71.9 ± 8.2 | | **0.006** |
| | Median | 62.0 | | 65.0 | | 70.5 | | 72.0 | | |
| | | N | % | N | % | N | % | N | % | |
| Gender | Female | 12 | 70.6 | 16 | 45.7 | 4 | 33.3 | 5 | 41.7 | 0.190 |
| | Male | 5 | 29.4 | 19 | 54.3 | 8 | 66.7 | 7 | 58.3 | |
| Smoking | Negative | 10 | 58.8 | 23 | 65.7 | 8 | 66.7 | 8 | 66.7 | 0.958 |
| | Positive | 7 | 41.2 | 12 | 34.3 | 4 | 33.3 | 4 | 33.3 | |
| Biomicroscope | PSCC | 3 | 17.6 | 12 | 34.3 | 1 | 8.3 | 0 | 0.0 | **0.043** |
| | NC | 9 | 52.9 | 14 | 40.0 | 10 | 83.3 | 10 | 83.3 | |
| | NC + PSCC | 5 | 29.4 | 9 | 25.7 | 1 | 8.3 | 2 | 16.7 | |
| Fundus | Normal | 6 | 35.3 | 6 | 17.1 | 3 | 25.0 | 4 | 33.3 | 0.465 |
| | Abnormal | 11 | 64.7 | 29 | 82.9 | 9 | 75.0 | 8 | 66.7 | |
| Va | Mean ± SD | 0.11 + 0.11 | | 0.11 + 0.12 | | 0.11 + 0.11 | | 0.13 + 0.13 | | 0.718 |
| | Median | 0.10 | | 0.05 | | 0.05 | | 0.08 | | |
| Ip | Mean ± SD | 11.7 + 3.0 | | 12.5 + 2.3 | | 12.8 + 1.9 | | 13.2 + 2.4 | | 0.691 |
| | Median | 13.0 | | 12.0 | | 12.5 | | 13.0 | | |

PSCC — posterior subcapsular cataract; NC — nuclear cataract; Va — vision acuity; Ip — intraocular pressure; SD — standard deviation

P value was not significant ($p > 0.05$). There was no significant difference ($p > 0.05$) between Group II and Group III. Glucose levels in Group I were significantly higher than in Group II-III-IV ($p < 0.05$). Glucose levels in Group III were significantly higher than in group II-IV ($p < 0.05$). Glucose levels in Group II were significantly higher than in Group IV ($p < 0.05$). In Group I-II-III-IV, there was no significant difference ($p > 0.05$) for K, Mg, Urea and Cr (Tab. 3).

Patients with PSX (+) and PSX (–) were not significantly different in terms of age, sex distribution, and smoking rate ($p > 0.05$). Visual acuity, intraocular pressure, cataract distribution, and fun-

**Table 3. The biochemical findings of aqueous humor in groups I–IV**

| Mineral | Mean/Median | Group I | Group II | Group III | Group IV | p |
|---|---|---|---|---|---|---|
| Na | Mean ± SD | 146.2 ± 2.4 | 148.1 ± 3.7 | 145.1 ± 3.2 | 149.8 ± 4.8 | 0.007 |
| | Median | 145.0 | 148.0 | 144.0 | 150.0 | |
| K | Mean ± SD | 4.2 ± 0.2 | 4.1 ± 0.2 | 4.0 ± 0.2 | 4.1 ± 0.2 | 0.068 |
| | Median | 4.1 | 4.0 | 4.0 | 4.0 | |
| Cl | Mean ± SD | 119.2 ± 3.6 | 120.8 ± 3.9 | 119.9 ± 2.6 | 124.2 ± 5.6 | 0.011 |
| | Median | 118.2 | 120.3 | 120.0 | 124.4 | |
| Ca | Mean ± SD | 5.2 ± 0.9 | 4.8 ± 1.3 | 5.6 ± 0.7 | 6.0 ± 0.6 | 0.024 |
| | Median | 5.6 | 5.4 | 5.7 | 5.9 | |
| P | Mean ± SD | 2.2 ± 0.4 | 2.0 ± 0.3 | 1.8 ± 0.2 | 2.4 ± 1.0 | 0.018 |
| | Median | 2.2 | 2.0 | 1.8 | 2.1 | |
| Mg | Mean ± SD | 1.6 ± 0.2 | 1.6 ± 0.1 | 1.6 ± 0.1 | 1.6 ± 0.2 | 0.075 |
| | Median | 1.5 | 1.6 | 1.6 | 1.7 | |
| Glucose | Mean ± SD | 148.5 ± 6.95 | 92.7 ± 56.0 | 105.5 ± 21.5 | 60.0 ± 11.4 | 0.032 |
| | Median | 139.3 | 77.5 | 102.4 | 60.8 | |
| Urea | Mean ± SD | 33.0 ± 7.6 | 31.5 ± 8.7 | 38.5 ± 9.0 | 40.7 ± 29.2 | 0.084 |
| | Median | 33.1 | 30.8 | 34.2 | 31.9 | |
| Cr | Mean ± SD | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.2 | 0.079 |
| | Median | 0.1 | 0.2 | 0.2 | 0.2 | |

PSCC — posterior subcapsular cataract; NC — nuclear cataract; Na — sodium; K — potassium; Cl — chlorine; Ca — calcium; P — phosphate; Mg — magnesium; Cr — creatine; SD — standard deviation

| Table 4. The demographic characteristics and biochemical findings of PSX (+) and PSX (–) groups | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | PSX (+) | | | PSX (–) | | | p |
| | | Mean ± SD | | Median | Mean ± SD | | Median | |
| Age | | 66.5 ± 7.8 | | 65.0 | 69.9 ± 8.9 | | 68.0 | 0.214 |
| | | N | % | | N | % | | |
| Gender | Female | 16 | 55.2 | | 21 | 44.7 | | 0.374 |
| | Male | 13 | 44.8 | | 26 | 55.3 | | |
| Smoking | Negative | 18 | 62.1 | | 31 | 66.0 | | 0.731 |
| | Positive | 11 | 37.9 | | 16 | 34.0 | | |
| Va | | 0.1 ± 0.1 | | 0.1 | 0.1 ± 0.1 | | 0.1 | 0.948 |
| Ip | | 12.2 ± 2.6 | | 13.0 | 12.7 ± 2.4 | | 12.0 | 0.672 |
| Biomicroscope | PSCC | 4 | 13.8 | | 12 | 25.5 | | 0.388 |
| | NC | 19 | 65.5 | | 24 | 51.1 | | |
| | NC + PSCC | 6 | 20.7 | | 11 | 23.4 | | |
| Fundus | Normal | 9 | 31.0 | | 10 | 21.3 | | 0.340 |
| | Abnormal | 20 | 69.0 | | 37 | 78.7 | | |
| Na | | 145.7 ± 2.8 | | 145.0 | 148.6 ± 4.0 | | 149.0 | 0.001 |
| K | | 4.1 ± 0.2 | | 4.1 | 4.1 ± 0.2 | | 4.0 | 0.477 |
| Cl | | 119.5 ± 3.2 | | 119.0 | 121.6 ± 4.6 | | 120.8 | 0.053 |
| Ca | | 5.3 ± 0.8 | | 5.6 | 5.1 ± 1.3 | | 5.6 | 0.638 |
| P | | 2.1 ± 0.4 | | 1.9 | 2.1 ± 0.6 | | 2.0 | 0.894 |
| Mg | | 1.6 ± 0.1 | | 1.6 | 1.6 ± 0.1 | | 1.6 | 0.392 |
| Glucose | | 130.7 ± 58.4 | | 114.8 | 84.4 ± 50.5 | | 71.7 | 0.000 |
| Urea | | 35.2 ± 8.5 | | 33.1 | 33.8 ± 16.6 | | 30.8 | 0.113 |
| Cr | | 0.2 ± 0.1 | | 0.2 | 0.2 ± 0.1 | | 0.2 | 0.600 |

PSCC — posterior subcapsular cataract; NC — nuclear cataract; Va — vision acuity; Ip — intraocular pressure; Na — sodium; K — potassium; Cl — chlorine, Ca — calcium; P — phosphate; Mg — magnesium; Cr — creatine; PSX — pseudoexfoliation; SD — standard deviation

dus status did not differ significantly (p > 0.05) in patients with PSX (+) and PSX (-). Patients with PSX (+) and PSX (–) were not significantly different (p > 0.05) in terms of K, Cl, Ca, P, Mg, urea and Cr.

The Na value in the PSX (+) group was significantly lower than in the PSX (–) group (p < 0.05). In the PSX (+) group, glucose value was significantly higher than in the PSX (–) group (p < 0.05) (Tab. 4).

## DISCUSSION

The incidence of cataract in patients with diabetes mellitus increases and cataract is considered to be a major cause of progression of visual impairment in diabetic patients. Many clinical studies have shown that cataract occurs more frequently and earlier in diabetic patients than in non-diabetic patients [8–10].

In our study, while other demographic factors showed similar characteristics in the diabetic group, it was found to cause cataract at the level that required surgery in younger patients. This finding supports that the presence of diabetes accelerates cataract formation in accordance with the literature. Increased glucose levels in the aqueous humor may induce glycation of lens proteins, may result in superoxide radicals ($O_2$) production, and may lead to a process leading to the formation of advanced glycation end products.

Glucose is taken from the aqueous humor by simple diffusion and facilitated diffusion. When the glucose increases in the body, the glycolysis is stopped by anaerobic glycolysis with the end products, glucose enters the sorbitol pathway and sorbitol is formed. Because the permeability of the lens to the sorbitol is high, sorbitol accumulates in the lens, water enters and opacity in the lens occurs [7]. In this study, in the aqueous humor analysis of the diabetic groups, glucose was found to be significantly higher. This finding supports the increasing effect of glucose on cataract formation in diabetic patients.

Studies that shed light on the pathophysiology of diabetic cataract have led to the development of anticataract therapies in diabetic patients. Two studies are presented below for this purpose:

— numerous experimental studies of anticataract therapy in diabetic patients support the role of ARI (Aldose-Reductase Inhibitors) in preventing diabetic cataract formation and progression. In an experimental diabetic rat model, animals were treated with AR inhibitor Renirestate [8];

— pyruvate, an endogenous antioxidant, has recently shown interest in the preventive effects of diabetic cataract formation on sorbitol formation and lipid peroxidation [9].

In epidemiological cross-sectional studies, the relationship between PSX and cataract has been established. Australian Blue Mountains Eye Study showed similar findings with our study. The PSX was associated with a significant nuclear cataract in accordance with the literature. The relationship between nuclear cataract and PSX has been established. Although the pathogenesis of PSX is not yet fully understood, it is probably a multifactorial condition associated with factors such as genetic and aging. In our study, biochemical analysis of the aqueous humor showed that in the presence of PSX a statistically significant level of glucose was found to be high and sodium was found to be low.

A significantly higher incidence of posterior subcapsular cataract in eyes with nondiabetic and nonexfoliative cataracts and a significantly higher Na concentration in this group suggest that there may be a relationship between aspirated cataract and aqueous humor Na concentration. Na is introduced into the lens according to the chemical concentration from the posterior capsule and then actively pumped from the epithelium with Na-K ATPase. According to this model, the K is found on the front of the lens, while the Na is more intense at the posterior of the lens [10–13].

Studies with calcium showed that calcium plays a special role in the development of cataract in humans. Calcium is associated with cataract level. It has been found that elevated calcium levels in human lenses play an important role in cortical cataracts.

In the present study, we performed biochemical analysis of aqueous humor in the presence of two diseases with the most common association with cataract. Diabetic patients had significantly higher amounts of glucose and lower calcium in the aqueous humor. Posterior subcapsular cataract was more frequent in diabetic patients. In diabetic non-PSX eyes, P value was significantly higher.

In PSX patients, glucose was found to be higher and sodium was lower than in the non-PSX group. This group was more frequently associated with cortical cataract. Ca and Cl ratio were significantly higher in nondiabetic PSX group.

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### STATEMENT OF COMPETING INTERESTS

The authors report no competing interests.

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