Cilt 12, Sayı 1, Sayfa 163-170, 2023 Volume 12, Issue 1, Page 163-170, 2023

Araştırma Makalesi https://doi.org/10.46810/tdfd.1250739 Research Article

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

Investigation of Some Metabolic Enzyme Activities in Samples of Serum and Humor Aqueous of Cataract Cases with Pseudoexfoliation Syndrome

Büşra ÇALIŞKAN[1*], Mine AKSOY[2], Muhammet Serhat ÖZASLAN[3], İlknur AKYOL SALMAN[1]

[1]Atatürk University, School of Medicine, Department of Ophthalmology, Erzurum, Türkiye
[2]Atatürk University, Faculty of Science, Department of Chemistry, Erzurum, Türkiye
[3]Ardahan University, Nihat Delibalta Göle Vocational High School, Department of Pharmacy Services, Ardahan, Türkiye

Büşra ÇALIŞKAN ORCID No: 0000-0002-2350-184X
Mine AKSOY ORCID No: 0000-0002-2430-8769
Muhammet Serhat ÖZASLAN ORCID No: 0000-0002-5060-2048
İlknur AKYOL SALMAN ORCID No: 0000-0003-2079-6213

**Corresponding author: drbusracaliskan@gmail.com*

(Received: 17.02.2023, Accepted: 16.03.2023, Online Publication: 27.03.2023)

Keywords
Pseudoexfoliation syndrome, Carbonic anhydrase, Aldose reductase, Glutathione reductase, Paraoxonase

Abstract: Pseudoexfoliation syndrome (PEX) is a systemic disease that occurs as a result of aggregation of extracellular matrix material in ocular tissues and visceral organs. The etiopathogenesis of the PEX is not fully known. The purpose of our study is to examine the enzyme activities in some metabolic pathways that may be associated with etiopathogenesis. Carbonic anhydrase (CA), glutathione reductase (GR), aldose reductase (AR), paraoxonase (PON) enzymes activity in aqueous and serum samples, also serum selenium levels were investigated. According to the results, CA hydratase enzyme activity levels were higher in the serum samples with PEX compared to the control samples ($p < 0.05$). Aqueous CA hydratase and aqueous/serum PON, GR and AR activity levels did not differ significantly among the study samples ($p > 0.05$). Based on the relationship of carbonic anhydrase with aqueous production and glaucoma, increased serum CA hydratase activity may help to understand the PEX etiopathogenesis and mechanism of conversion to glaucoma.

Psödoeksfoliasyon Sendromlu Katarakt Olgularının Serum ve Humor Aköz Örneklerinde Bazı Metabolik Enzim Aktivitelerinin Araştırılması

Anahtar Kelimeler
Psödoeksfoliasyon sendromu, Karbonik anhidraz, Aldoz redüktaz, Glutatyon redüktaz, Paraoksonaz

Öz: Psödoeksfoliasyon sendromu (PEX), ektraselüler matriks materyalinin oküler dokular ve visseral organlarda birikmesi sonucu ortaya çıkan sistemik bir hastalıktır. PEX'in etiyopatogenezi tam olarak bilinmemektedir. Çalışmamızın amacı, etyopatogenez ile ilişkili olabilecek bazı metabolik yolaklarda görev alan enzim aktivitelerinin değerlendirilmesidir. Bu amaçla; aköz ve serum örneklerinde karbonik anhidraz (CA), glutatyon redüktaz (GR), aldoz redüktaz (AR), paraoksonaz (PON) enzim aktiviteleri, ayrıca serum selenyum seviyeleri araştırılmıştır. Sonuçlara göre PEX'li örneklerde serum CA hidrataz enzim aktivite düzeyleri kontrol örneklerine göre daha yüksek tespit edildi ($p < 0.05$). Aköz CA hidrataz ve aköz/serum PON, GR ve AR aktivite seviyeleri, çalışma numuneleri arasında anlamlı farklılık göstermedi ($p > 0.05$). Karbonik anhidrazın aköz üretimi ve glokom ile ilişkisine dayanarak, artmış serum CA hidrataz aktivitesi, PEX'in etyopatogenezi ile glokoma dönüşüm mekanizmasının anlaşılmasına yardımcı olabilir.

1. INTRODUCTION

Pseudoexfoliation syndrome (PEX) is a systemic disease that known to occur with the aggregation of extracellular fibrillar material in visceral organs and ocular tissues. Although the cause of pathogenesis of PEX are unknown, it is thought that its formation may be relevant to environmental, genetic, viral infection, ischemia or immunological conditions [1-4]. In addition, increased oxidative damage and decreased cell protection

mechanism are thought to be one of the important pathogenetic factors [5]. It has been reported that oxidative stress is increased in eyes with PEX and decreased total antioxidant status in aqueous humor and serum. Ascorbic acid levels, an antioxidant, have been found to be decreased in aqueous samples of patients with PEX. Also, the concentration of 8-iso prostaglandin F2 alpha, which is an indicator of oxidative stress have been observed to increased in aqueous. All these studies have shown that oxidative damage due to free radicals plays an important role in the pathogenesis. [6, 7].

Although PEX occurs all over the world, its prevalence differs between countries and geographic regions [8]. PEX can present unilaterally or bilaterally. It is associated with the formation of cataracts and glaucomas. It is thought that the aqueous composition may be affected by changes in the iris vascular system and the blood-aqueous barrier, thus affecting lens metabolism and leading to earlier cataract formation [9].

Humor aqueous is bicarbonate-rich fluid that fills the space between the lens and cornea. The balance between the release and outflow of aqueous humor is closely related to intraocular pressure [10]. Decreased aqueous flow was observed in PEX positive eyes and it was concluded that aqueous humor production and composition were affected in PEX. Various studies have shown that the total aqueous protein quantity in the eyes is increased as a result of influencing the blood-aqueous barrier in PEX [11, 12]. Similarly, changes in the aqueous level of acid phosphatase [13], α_1-lipoprotein and ceruloplasmin [14], α_1-antitrypsin [15], transferrin [16], cellular⁄plasma fibronectin [17] and growth factors [18-20] have been reported.

CA is a metalloenzyme that contains Zn^{2+} ions in its active site. CA is an important enzyme that reversibly catalyzes the reactions of hydration of carbon dioxide and dehydration of bicarbonate ion in the living. CA enzyme functions as a pH regulating enzyme in many tissues including erythrocytes [21, 22].

CA carries out bicarbonate transport through a rapid conversion pathway between HCO_3^- and CO_2 across the ciliary epithelium. It affects liquid transport through optimum active transport of Na^+ by regulating pH with bicarbonate formation. Thus, it is directly related to aqueous production [23].

PON is a calcium-mediated esterase with antioxidant properties. It has been reported that there is a relationship between decreased activity of PON and increased oxidative stress in serum [24] [8] [9].

GR is a dimeric disulfide oxidoreductase that catalyzes the reduction of one molar equivalent of glutathione disulfide (GSSG) to two molar equivalents of reduced glutathione (GSH). It is known that GSH plays a critical role in maintaining the reducing environment of the cell and resisting oxidative stress by detoxifying some xenobiotics and heavy metals [25]. GR uses a FAD prosthetic assembly and NADPH to reduce GSSG to GSH [26]. Therefore, GR is essential in maintaining the GSH/GSSG intracellular ratio in the cell [27].

AR reduces various aldehydes using NADPH as the reductant. It is responsible for catalyzing the conversion of glucose to sorbitol in metabolism [28].

Selenium is an essential element that participates in the structure of many enzymes and acts as a cofactor. It is involved in many metabolic events such as antioxidant enzyme system, regulation of immune system and thyroid hormone system. Selenoproteins are proteins containing selenium in the form of selenocysteine in their active sites and play an important role in antioxidant defence [29].

In this study, aqueous and serum samples were examined from the cases cataract with PEX and the control group was selected from the cataract group without PEX. The aim of this study is to investigate the activities of CA, GR, AR, PON enzyme in patients and control groups. In addition, it was aimed to investigate the selenium levels in serum samples and to compare them with the control group.

2. MATERIAL AND METHOD

2.1. Materials

Paraoxon (diethyl p-nitrophenyl phosphate), NADPH (β-Nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate), D,L-glyceraldehyde, 4-Nitrophenyl acetate, L-Glutathione oxidized and other chemicals were obtained from Sigma-Aldrich (Taufkirchen, Germany).

2.2. Sample Collection

Ethics committee report numbered B.30.2.ATA.0.01.00/176 was received for the conduct of the study. Patients who applied to Atatürk University Faculty of Medicine, Department of Ophthalmology with the complaint of low vision and were diagnosed with cataract were included in the study. The study population comprised 66 individuals, including 33 patients with cataract with PEX, 33 patients with age-related cataracts without PEX. Each participant underwent a full ophthalmologic examination. Individuals with ocular diseases such as glaucoma, corneal trauma, uveitis, diabetic retinopathy were excluded from the study. Also systemic, cronic and inflammatory diseases such as diabetes mellitus, liver-kidney failure, rheumatological diseases were excluded from the study.

Aqueous samples were obtained at the beginning of cataract surgery. About 100–150 µl of the aqueous humor was aspirated by utilizing a 30- gauge needle attached to a micro syringe under an operating microscope. Particular attention was paid to avoid touching the iris, lens, and corneal endothelium and to prevent the contamination of the aqueous sample by the irrigation fluid and blood from the conjunctiva. Venous blood samples (5 mL) were drawn from the participants. The blood samples were centrifuged for 15 min. The centrifuged serum samples

were distributed to Eppendorf tubes. The Eppendorf tubes containing both serum and aqueous samples were placed at 4 °C for 1 h. Then all samples were stored in the freezer at − 80 °C until the enzyme activity measurement [30].

2.3. Biochemical Analyses

For biochemical analysis, both hydratase and esterase activity of CA enzyme were investigated in serum and aqueous. Other enzyme activities were also examined in serum and aqueous.

2.3.1. Principle of CA hydratase activity measurement method

Hydratase activity of CA was determined with the Wilbur-Anderson method [31]. In the method; the time taken for the pH to decrease from 8.2 to 6.3 due to the H^+ released as a result of the hydration of CO_2 is calculated using the bromine thymol blue indicator.

According to this method, an enzyme unit (EU) for CA activity is calculated by determining the CO_2 hydration time that occurs without the enzyme and the CO_2 hydration time with the enzyme. The formula EU= $((t_0-t_c)/t_c)$ was used to calculate the enzyme unit.

2.3.2. Principle of CA esterase activity measurement method

It is the method that shows that it also has CA esterase activity. In principle, CA hydrolyzes p-nitro phenyl acetate, which is used as a substrate, to p-nitro phenol or p-nitro phenolate, which gives absorption at 348 nm [32]. The esterase activity of the CA was calculated in EU mL^{-1} by applying the following formula:

$$EU/mL = \frac{\Delta OD}{5} \times \frac{V_T}{V_E} \times D_f$$

EU mL^{-1}: enzyme unit in 1 mL, ΔOD: absorbance change per minute, extinction coefficient ($M^{-1}.cm^{-1}$): 5, V_T= total cuvette volume, V_E = the volume of enzyme sample D_f=dilution factor.

2.3.3. Principle of PON enzyme activity measurement method

The activity measurement of PON is determined by measuring the absorbance change at 412 nm caused by p nitrophenol formed as a result of hydrolysis of paraoxon [33]. The activities of PON in EU mL^{-1} were calculated by applying the following formula:

$$EU/mL = \frac{\Delta OD}{18.29} \times \frac{V_T}{V_E} \times 1000$$

EU mL^{-1}: Enzyme unit in 1 mL, ΔOD: absorbance change per minute, extinction coefficient ($M^{-1}.cm^{-1}$): 18.290, V_T: total cuvette volume, V_E: the volume of the enzyme sample, 1000: Enzyme unit conversion factor.

2.3.4. Principle of AR enzyme activity measurement method

The AR enzyme activities were determined with a modified procedure used by Cerelli et al [28]. Activity measurement was carried out by considering the decrease in absorbance at 340 nm of the amount of NADPH spent in the reaction medium where DL-glyceraldehyde was used as the substrate. The activity of the enzyme was calculated in EU mL^{-1} by applying the following formula:

$$EU/mL = \frac{\Delta OD}{6.22} \times \frac{V_T}{V_E} \times D_f$$

EU mL^{-1}: enzyme unit in 1 mL, ΔOD: absorbance change per minute, extinction coefficient ($M^{-1}.cm^{-1}$): 6.22, V_T= total cuvette volume, V_E = volume of enzyme sample D_f=dilution factor.

2.3.5. Principle of GR enzyme activity measurement method

Spectrophotometric method was used to measure the activity of GR enzyme. The method described by Carlberg and Mannervik is based on the absorbance of NADPH, which decreases due to the oxidation of NADPH at 340 nm in the presence of GSSG [26]. Absorbance decrease was recorded at 1-minute time intervals. The GR activities were calculated in EU mL^{-1} by applying the following formula:

$$EU/mL = \frac{\Delta OD}{6.22} \times \frac{V_T}{V_E} \times D_f$$

EU mL^{-1}: enzyme unit in 1 mL, ΔOD: absorbance change per minute, extinction coefficient ($M^{-1}.cm^{-1}$): 6.22, V_T= total cuvette volume, V_E = volume of enzyme sample D_f=dilution factor.

2.4. Determination of Selenium in Serum

Selenium levels analyzes of serum samples taken from the control group and patients with PEX were studied in the infrastructure of the Research Hospital Biochemistry laboratory.

2.5. Statistical Analysis

SPSS 20.0 software was used to analyze the data (SPSS Inc., Chicago, Illinois, USA). Each group was tested for normality. According to normality tests, only the groups of CA esterase aqueous sample, selenium serum sample are normally distributed ($p>0.05$, Table 1). Independent Samples t Test was used for group comparison of these two variables. Mann-Whitney U test was used for all other variables.

Table 1. Test of normality and homogeneity of variances

			Shapiro-Wilk testi		F-test	
Variant	**Samples**	**Groups**	**W**	**p-value**	**F**	**p-value**
CA Esterase	Serum	SC	0.9202	0.01856	1.3587	0.3906
		SP	0.95958	0.2515		
	Aqueous	AC	0.94583	0.1005	0.89875	0.7646
		AP	0.94421	0.0901		
CA Hydratase	Serum	SC	0.90526	0.007312	0.36245	0.005258
		SP	0.88312	0.001999		
	Aqueous	AC	0.9565	0.2054	0.51173	0.06244
		AP	0.92964	0.03418		
PON	Serum	SC	0.90494	0.007173	0.99841	0.9964
		SP	0.97432	0.608		
	Aqueous	AC	0.65821	1.61e-07	1.4146	0.3316
		AP	0.79135	2.217e-05		
AR	Serum	SC	0.92692	0.02861	0.61508	0.1747
		SP	0.89593	0.004185		
GR	Serum	SC	0.95785	0.2245	0.76814	0.4598
		SP	0.92223	0.02114		
	Aqueous	AC	0.898 01	0.004732	1.0491	0.893
		AP	0.9664	0.3876		
		AP	0.30499	2.254e-11		
Selenium	Serum	SC	0.95593	0.1978	1.0013	0.9971
		SP	0.95156	0.1478		

SC: serum control group, SP: serum patient group, AC: aqueous control group, AP: aqueous patient group

3. RESULTS

Enzyme activities were studied in aqueous and serum samples of 66 individuals. The demographic data of the our study were previously given by Çalışkan *et al.* in the study that included same sample [30].

The test results of the group comparisons for each variant of the serum samples are presented in Table 2. When the table is examined, there is a statistically significant difference between PEX group and control patients regarding only the variables named CA hydratase ($p<0.05$).

The test results of group comparisons for each variable of aqueous samples are presented in Table 3. No statistically significant difference was observed between the groups in the test results ($p>0.05$).

Table 2. Enzyme activities comparisons in serum of patients with cataract with PEX and control subjects (cataracts without PEX).

			Group Comparison	
Variant	**Groups**	**Median**	**Test statistics (u)**	**p-value**
CA Esterase	SC SP	0.72 0.77	631*	0.267
CA Hydratase	SC SP	20.39 41.61	815*	0.0003967
PON	SC SP	30.23 40.69	630.5*	0.270
AR	SC SP	0.14 0.13	512.5*	0.680
GR	SC SP	0.27 0.45	587*	0.584
Selenium	SC SP	77 75	-0.033443**	0.9734

*: Mann-Whitney U test, **: Independent Samples t-test
SC: serum control group, SP: serum patient group, AC: aqueous control group, AP: aqueous patient group

Table 3. Enzyme activities comparisons in aqueous samples of patients with cataract with PEX and control subjects (cataracts without PEX).

			Group Comparison	
Variant	**Groups**	**Median**	**Test statistics (u)**	**p-value**
CA Esterase	AC AP	0.06 0.05	0.80197	0.4255
CA Hydratase	AC AP	29.04 34.60	650	0.176
PON	AC AP	11.37 11.41	833.5	0.272
GR	AC AP	0.45 0.46	587.5	0.580

SC: serum control group, SP: serum patient group, AC: aqueous control group, AP: aqueous patient group

Hydratase activity of CA was found to be 46.5 EU mL^{-1} in the PEX group and 27.3 EU mL^{-1} in the control group in serum samples. According to the results, serum CA hydratase enzyme activity levels were significantly higher in the PEX group compared to the control group. ($p=0.013$).

Hydratase activity of CA was found to be 34.8 EU mL^{-1} in the group of cataract cases with PEX and 29.1 EU mL^{-1} in the group of cataract cases without PEX in aqueous samples.

Despite an increase in aqueous CA hydratase levels of the PEX group compared to control group, this increase did not create a statistically significant difference. ($p=0.176$). (Fig.1).

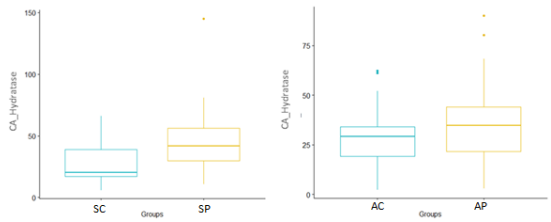

Figure 1. Carbonic anhydrase enzyme hydratase activities in serum and aqueous humor of patients with cataract with PEX and control subjects (cataracts without PEX). # Significantly elevated carbonic anhydrase enzyme hydratase activities in the serum of patients (Mann-Whitney U; p=0.013) compared with control subjects. (SC: Serum Control, SP: Serum PEX, AC: Aqueous Control, AP: Aqueous PEX)

AR activities were not detected in the study performed in aqueous samples of both groups.

4. DISCUSSION AND CONCLUSION

PEX, a systemic disease, occurs as a result of the aggregation of extracellular matrix material in the ocular and extraocular tissues in the body. Many ocular problems (corneal endotheliopathy, glaucoma, cataract, zonular instability, central retinal vein occlusion, decreased dilatation, and increased cataract surgery complication risk) are associated with PEX [34].

The pathogenesis and etiology of PEX are not yet fully known. It is argued that various factors have played a role in the etiology and pathogenesis of PEX, and it has been demonstrated by many studies that the aqueous content of patients with PEX is altered [35, 36]. A total of 269 protein groups were defined in the proteomic study conducted in patients with and without PEX, among patients with eye diseases such as cataracts and glaucoma. As a result of label-free protein quantification, no significant difference was observed between the aqueous humor proteomes of glaucoma and cataract. In the study, protein amounts were determined as 4.6±1.7 µg/mL in cases with cataract, 2.5±1.6 µg/mL in cases with pseudoexfoliative cataract, 4.3±1.8 µg/mL in cases with primary open angle glaucoma, and 2.7±0.7 µg/mL in cases with pseudoexfoliative glaucoma [37].

Oxidative stress (OS) results from excessive ROS formation or insufficient antioxidant defense. ROS production allows the presence of sufficient ROS to perform cellular signaling tasks. "Redox control" maintains the delicate balance between the advantageous and harmful effects of ROS, which is essential for the survival of living things. Redox control maintains homeostasis and protects against OS in living things. Overproduction of ROS damages cells by causing OS in them. As a result, cells have antioxidant defense systems to counteract excess ROS and maintain redox balance, both of which are essential for cell survival [38-40]. Several active oxidative agents are known to exist in aqueous humor, such as superoxide anions and hydrogen peroxide [41]. Therefore, oxidative markers in aqueous samples were frequently investigated in cases with PEX and/or glaucoma [42-44]. Until now, enzyme activities such as superoxide dismutase [44, 45], catalase [5, 43, 46], glutathione peroxidase [43, 46] and biomarkers such as malondialdehyde [44], glutathione [47, 48], nitric oxide [44] have been examined as oxidative stress markers. In addition, arylesterase and PON activities were measured in the study, which aimed to investigate the oxidative stress status of aqueous humor and serum in patients with PEX and pseudoexfoliative glaucoma (PEG). It was determined that total oxidative stress values were higher (< 0.05) in patients with PEX and PEG, and total antioxidant capacity, PON and ARE aqueous humor levels did not differ significantly between PEX and PEG groups [49]. In our study, the activities of PON and GR as oxidative markers were determined in serum and aqueous samples. The activities of PON and GR aqueous and serum samples did not differ significantly between cataract cases with PEX and control groups. In addition, in our study, AR levels were investigated in serum and aqueous samples, since both AR enzyme and oxidant-antioxidant pathways work in a NADPH-dependent manner [50]. There was no statistically significant difference between the two groups in serum AR levels (p=0.5235). AR enzyme activity was not detected in the study performed in aqueous samples of both groups. Aldose reductase (AR) is catalyzing the conversion of glucose to sorbitol in metabolism. During hyperglycemia the flux of glucose the polyol pathway increases significantly, leading to excessive formation of sorbitol. [51]. Since diabetic patients were excluded from the study population, aldose reductase enzyme activity may not have been detected under normoglycemic conditions in aqueous.

Serum and aqueous levels of trace elements have been evaluated by various studies in cases of cataract and PEX [52-55]. Selenium; It is an essential element that plays the role of a cofactor by participating in the structure of many enzymes. Along with various enzyme groups, it is also an engagement element for antioxidant enzymes [56]. In one study, selenium was investigated in the aqueous humor and serum of control groups and patients with PEX. The mean selenium levels in the serum of patients with PEX syndrome (115.25±25.20 µg/L) were found to be lower than the control group (124.25±14.40 µg/L), but no statistically significant result was obtained (P <.325) [57]. In our study, serum selenium levels for each group were found to be 80.2 µg/L in the control group and 80.5 µg/L in the PEX group. There was no statistically significant difference between the two groups in serum selenium levels (p: 0.9734).

Human CAs are metalloenzymes known for their ability to catalyze the hydration of CO_2 to bicarbonate anion and proton, and an association between glaucoma and the CA enzyme has been demonstrated [22, 58]. Inhibition of human CA isozymes such as hCA-I, II, IV, and XII, which reduces aqueous humor secretion and elevated intraocular pressure, is the mechanism of action of well-known anti-glaucoma agents [59]. Based on the relationship of carbonic anhydrase with aqueous production and glaucoma, in our study investigated the both activities of esterase and hydratase of CA in aqueous and serum

samples. According to the our results, a statistically significant difference was found in terms of CA hydratase activities in the serum samples of the patient group. Although there is no statistically significant difference, an increase in aqueous CA hydratase levels was also observed in patients with PEX.

PEX is known to be one of the most common causes of glaucoma. Progressive degeneration and obstruction occur with the accumulation of PEM in trabecular meshwork in PEX patients. Glaucoma can be seen in both eyes in cases with unilateral PEX or no glaucoma can be seen in all eyes with PEM suggests that the underlying cause may be due to different reasons. One of these reasons may be the increased activity of CA hydratase and the fact that this triggers the production of more aqueous in patients with PEX.

In conclusion, some oxidative markers and carbonic anhydrase activities were investigated in aqueous humor and serum samples of cataracts with PEX, in the control group without PEX. In addition, serum selenium levels were studied and compared with the control group. It is thought that the parameters obtained in our study may be related to the etiopathogenesis of pseudoexfoliation csyndrome and contain important findings in this respect. Especially, in the literature, we did not find a study on CA enzyme activity in serum and aqueous samples in patients with PEX. Therefore, we believe that this study can be a reference.

Acknowledgement

The authors thank Mr. Erkan OKTAY for their support in the statistical analysis of the article. This research did not receive any specific grant or project support. There is no statement of interest.

REFERENCES

[1] Damji KF, Bains HS, Amjadi K, Dohadwala AA, Valberg JD, Chevrier R, et al. Familial occurrence of pseudoexfoliation in Canada. Can J Ophthalmol. 1999;34(5),257-265.

[2] Konstas AG, Williamson TH. Coexistence of exfoliation syndrome, previous iris surgery and heterochromia. Acta Ophthalmol. 1993; 71(6),850-852.

[3] Orr AC, Robitaille JM, Price PA, Hamilton JR, Falvey DM, De Saint-Sardos AG, et al. Exfoliation syndrome: clinical and genetic features. Ophthalmic Genet. 2001;22(3),171-185.

[4] Ringvold A, Blika S, Elsas T, Guldahl J, Brevik T, Hesstvedt P, et al. The Middle-Norway eye-screening study. I. Epidemiology of the pseudo-exfoliation syndrome. Acta Ophthalmol. 1988;66(6):652-8.

[5] Koliakos GG, Befani CD, Mikropoulos D, Ziakas NG, Konstas. AGP. Prooxidant–antioxidant balance, peroxide and catalase activity in the aqueous humour and serum of patients with exfoliation syndrome or exfoliative glaucoma. Graefes Arch Clin Exp Ophthalmol. 2008;246:1477–1483.

[6] Koliakos GG, Konstas AGP, Schlötzer-Schrehardt U, et al. 8 isoprostaglandin F2α and ascorbic acid concentration in the aqueous humour of patients with exfoliaition syndrome. Br J Ophthalmol. 2003;87:353-6.

[7] Koliakos GG, Konstas GP, Schlötzer-Schrehardt U, Bufidis T et al. Ascorbic acid concentration is reduced in the aqueous humor of patients with exfoliation syndrome. American J Oph. 2002; 134;879-83.

[8] Ringvold A. Epidemiology of the pseudo-exfoliation syndrome. Acta Ophthalmol Scand. 1999;77(4),371-375.

[9] Sangal N, Chen TC. Cataract surgery in pseudoexfoliation syndrome. Semin Ophthalmol. 2014;29(5-6), 403-408.

[10] Goel M, Picciani RG, Lee RK, Bhattacharya SK. Aqueous humor dynamics: a review. Open Ophthalmol J. 2010;4,52-59.

[11] Kuchle M, Nguyen NX, Hannappel E, Naumann GO. The blood-aqueous barrier in eyes with pseudoexfoliation syndrome. Ophthalmic Res. 1995;27Suppl 1,136-142.

[12] Nguyen NX, Kuchle M, Martus P, Naumann GO. Quantification of blood-aqueous barrier breakdown after trabeculectomy: pseudoexfoliation versus primary open-angle glaucoma. J Glaucoma. 1999;8(1),18.

[13] Mizuno K, Hara S, Ishiguro S, Takei Y. Acid phosphatase in eyes with pseudoexfoliation. Am J Ophthalmol. 1980;89(4),482-489.

[14] Baba H. Investigation of the pathogenesis of glaucoma capsulare with special discussion of alpha-lp and cp in aqueous-humor. Graefes Archive for Clinical and Experimental Ophthalmology. 1982;218(6), 283-286.

[15] Smolenska-Janicowa D, Bernacka K. Relationship between exfoliation syndrome and autoimmunity. Results of the analysis of aqueous humor. Klin Oczna. 1982;84(4),99-100.

[16] Koliakos GG, Konstas AGP, Dimitrakoulias N, Triantos A, Kardasopoulos A, Dimitriadou A, et al. Possible role of transferrin in exfoliation syndrome. Acta Ophthalmol Scan. 1996;74(2), 155-159.

[17] Vesaluoma M, Mertaniemi P, Mannonen S, Lehto I, Uusitalo R, Sarna S,et al. Cellular and plasma fibronectin in the aqueous humour of primary open-angle glaucoma, exfoliative glaucoma and cataract patients. Eye(Lond). 1998;12,886-890.

[18] Ho SL, Dogar GF, Wang J, Crean J, Wu QD, Oliver N, et al. Elevated aqueous humour tissue inhibitor of matrix metalloproteinase-1 and connective tissue growth factor in pseudoexfoliation syndrome. Br J Ophthalmol. 2005;89(2),169-173.

[19] Koliakos GG, Schlotzer-Schrehardt U, Konstas AG, Bufidis T, Georgiadis N, Dimitriadou A. Transforming and insulin-like growth factors in the aqueous humour of patients with exfoliation syndrome. Graefes Arch Clin Exp Ophthalmol. 2001;239(7),482-487.

[20] Schlotzer-Schrehardt U, Zenkel M, Kuchle M, Sakai LY, Naumann GO. Role of transforming growth factor-beta1 and its latent form binding protein in pseudoexfoliation syndrome. Exp Eye Res. 2001;73(6),765-780.
[21] Aggarwal M, Boone CD, Kondeti B, McKenna R. Structural annotation of human carbonic anhydrases. J Enzyme Inhib Med Chem. 2013;28(2),267-277.
[22] Supuran CT, Scozzafava A. Carbonic anhydrase inhibitors and their therapeutic potential. Expert Opinion on Therapeutic Patents. 2000;10(5), 575-600.
[23] Dobbs PC, Epstein DL, Anderson PJ. Identification of isoenzyme C as the principal carbonic anhydrase in human ciliary processes. Invest Ophthalmol Vis Sci. 1979;18(8),867-870.
[24] Li HL, Liu DP, Liang CC. Paraoxonase gene polymorphisms, oxidative stress, and diseases. J Mol Med (Berl). 2003;81(12),766-779.
[25] Circu ML and Aw TY: Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med.2010;48(6):749-762.
[26] Carlberg I, Mannervik B. Purification and characterization of glutathione reductase from calf liver.An improved procedure for affinity chromatography on 2',5'-ADP-Sepharose-4B. Anal Biochem. 1981;116(2),531-536.
[27] Circu ML and Aw TY. Glutathione and modulation of cell apoptosis. Biochim Biophys Acta. 2012;1823(10): 1767-1777.
[28] Cerelli MJ, Curtis DL, Dunn JP, Nelson PH, Peak TM, Waterbury LD. Antiinflammatory and aldose reductase inhibitory activity of some tricyclic arylacetic acids. J Med Chem. 1986;29(11),2347-2351.
[29] Duntas LH, Benvenga S. Selenium: an element for life. Endocrine. 2015;48(3),756-775.
[30] Caliskan B, Ozaslan MS, Aksoy M, Salman IA. Prolidase activity in aqueous and serum samples of cataract cases with pseudoexfoliation syndrome. Exp Eye Res. 2022;214,108880.
[31] Wilbur KM, Anderson NG. Electrometric and colorimetric determination of carbonic anhydrase. J Biol Chem. 1948;176(1),147-154.
[32] Verpoorte JA, Mehta S, Edsall JT. Esterase activities of human carbonic anhydrases B and C. J Biol Chem. 1967;242(18), 4221-4229.
[33] Eckerson HW, Romson J, Wyte C, La Du BN. The human serum paraoxonase polymorphism: identification of phenotypes by their response to salts. Am J Hum Genet. 1983;35(2),214.
[34] Naumann GO, Schlotzer-Schrehardt U, Kuchle M. Pseudoexfoliation syndrome for the comprehensive ophthalmologist. Intraocular and systemic manifestations. Ophthalmology. 1998;105(6),951-968.
[35] Berlau J, Lorenz P, Beck R, Makovitzky J, Schlotzer-Schrehardt U, Thiesen HJ, et al. Analysis of aqueous humour proteins of eyes with and without pseudoexfoliation syndrome. Graefes Arch Clin Exp Ophthalmol. 2001;239(10),743-746.
[36] Schumacher S, Nguyen NX, Kuchle M, Naumann GO. Quantification of aqueous flare after phacoemulsification with intraocular lens implantation in eyes with pseudoexfoliation syndrome. Arch Ophthalmol. 1999;117(6),733-735.
[37] Kliuchnikova AA, Samokhina NI, Ilina IY, Karpov DS, Pyatnitskiy MA, Kuznetsova KG, et al. Human aqueous humor proteome in cataract, glaucoma, and pseudoexfoliation syndrome. Proteomics. 2016;16(13),1938-1946.
[38] Varışlı B, Caglayan C, Kandemir FM, Gür C, Ayna A, Genç A, et al. Chrysin mitigates diclofenac-induced hepatotoxicity by modulating oxidative stress, apoptosis, autophagy and endoplasmic reticulum stress in rats. Molecular Biology Reports. 2023;50(1), 433-442.
[39] Kucukler S, Benzer F, Yildirim S, Gur C, Kandemir FM., Bengu AS, et al. Protective effects of chrysin against oxidative stress and inflammation induced by lead acetate in rat kidneys: a biochemical and histopathological approach. Biological Trace Element Research. 2021;199(4),1501-1514.
[40] Caglayan, C., Kandemir, F. M., Darendelioğlu, E., Küçükler, S., & Ayna, A. Hesperidin protects liver and kidney against sodium fluoride-induced toxicity through anti-apoptotic and anti-autophagic mechanisms. Life Sciences. 2021;281,119730.
[41] Spector A, Garner WH. Hydrogen peroxide and human cataract. Exp Eye Res. 1981;33(6),673-681.
[42] Goyal A, Srivastava A, Sihota R, Kaur J. Evaluation of oxidative stress markers in aqueous humor of primary open angle glaucoma and primary angle closure glaucoma patients. Curr Eye Res. 2014;39(8),823-829.
[43] Uzun L, Duzguncinar O, Evren O, Demirpence E, Uzun A, Gürsel E. Antioxidant status in the aqueous humor of patients with exfoliation syndrome. MN Ophtalmol. 2006;13(4),273-278.
[44] Yagci R, Gurel A, Ersoz I, Keskin UC, Hepsen IF, Duman S, et al. Oxidative stress and protein oxidation in pseudoexfoliation syndrome. Curr Eye Res. 2006;31(12),1029-1032.
[45] DelaPaz MA, Epstein DL. Effect of age on superoxide dismutase activity of human trabecular meshwork. Invest Ophthalmol Vis Sci. 1996;37(9),1849-1853.
[46] Ferreira SM, Lerner SF, Brunzini R, Evelson PA, Llesuy SF. Antioxidant status in the aqueous humour of patients with glaucoma associated with exfoliation syndrome. Eye. 2009;23(8), 1691-1697.
[47] Costagliola C, Iuliano G, Menzione M, Rinaldi E, Vito P, Auricchio G. Effect of vitamin E on glutathione content in red blood cells, aqueous humor and lens of humans and other species. Exp Eye Res. 1986;43(6),905-914.
[48] Veach J. Functional dichotomy: glutathione and vitamin E in homeostasis relevant to primary open-angle glaucoma. Br J Nutr. 2004;91(6), 809-829.
[49] Dursun F, Vural Ozec A, Aydin H, Topalkara A, Dursun A, Toker MI, et al. Total oxidative stress, paraoxonase and arylesterase levels at patients with pseudoexfoliation syndrome and pseudoexfoliative glaucoma. Int J Ophthalmol. 2015;8(5),985-990.
[50] Srivastava SK, Ramana KV, Bhatnagar A. Role of aldose reductase and oxidative damage in diabetes

and the consequent potential for therapeutic options. Endocr Rev. 2005;26(3), 380-392.
[51] Singh M, Kapoor A, Bhatnagar A. Physiological and Pathological Roles of Aldose Reductase. Metabolites. 2021;27;11(10):655.
[52] Aydin E, Cumurcu T, Ozugurlu F, Ozyurt H, Sahinoglu S, Mendil D, Hasdemir E. Levels of iron, zinc, and copper in aqueous humor, lens, and serum in nondiabetic and diabetic patients: their relation to cataract. Biol Trace Elem Res. 2005;108(1-3),33-41.
[53] Dawczynski J, Blum M, Winnefeld K, Strobel J. Increased content of zinc and iron in human cataractous lenses. Biol Trace Elem Res. 2002;90(1-3),15-23.
[54] Gunduz G, Gunduz F, Yucel I, Senturk UK. Levels of zinc and magnesium in senile and diabetic senile cataractous lenses. Biol Trace Elem Res. 2003;95(2),107-112.
[55] Lakomaa EL, Eklund P. Trace element analysis of human cataractous lenses by neutron-activation analysis and atomic-absorption spectrometry with special reference to pseudoexfoliation of the lens capsule. Ophthalmic Res. 1978;10(5-6),302-306.
[56] Roman M, Jitaru P, Barbante C . Selenium biochemistry and its role for human health. Metallomics. 2014;6(1), 25-54.
[57] Yilmaz A, Ayaz L, Tamer L. Selenium and pseudoexfoliation syndrome. Am J Ophthalmol. 2011;151(2),272-276 e271.
[58] Aggarwal M, McKenna R. Update on carbonic anhydrase inhibitors: a patent review (2008-2011). Expert Opin Ther Pat. 2012;22(8),903-915.
[59] Carta F, Supuran CT, Scozzafava A. Novel therapies for glaucoma: a patent review 2007-2011. Expert Opin Ther Pat. 2012;22(1),79-88.