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Serum and Aqueous Humor Levels of Fetuin-A in Pseudoexfoliation Syndrome

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ABSTRACT

Purpose: To evaluate serum and aqueous humor levels of fetuin-A in patients with pseudoexfoliation syndrome (PEXS) in comparison with those of age- and sex-matched healthy subjects.

Materials and Methods: This prospective study included 25 patients with PEXS and 25 control subjects who were undergoing cataract surgery without any systemic or ocular disease. Aqueous humor and serum fetuin-A levels were measured with enzyme-linked immunosorbent assay method.

Results: The mean age of the PEXS group (14 males, 11 females, $n = 25$) was 57.7 ± 6.9 years, and the control group (13 males, 12 females, $n = 25$) was 58.1 ± 5.7 years. There was no difference between the groups in terms of age ($p = 0.77$) and sex ($p = 0.83$).

The mean serum fetuin-A level of the PEXS group did not differ from that of the control group ($p = 0.53$). The mean aqueous humor level of the PEXS group was significantly higher than that of the control group ($p = 0.032$). There were no significant correlations between aqueous humor and serum fetuin-A levels among patients with PEXS and control group ($p > 0.05$).

Conclusions: Increased levels of fetuin-A in aqueous humor of patients with PEXS may show the local effect of fetuin-A on the anterior segment. With considering the wide range of possible biological functions of fetuin-A in the pathogenesis of PEXS, further studies are needed to clarify the clinical relevance of these findings.

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Introduction

A less-understood ophthalmological disease, pseudoexfoliation syndrome (PEXS), is a common, age-related, extracellular matrix (ECM) abnormality characterized by the accumulation of fibrillar and granular material in ocular tissue, the skin, and other organs.^{1,2} Detecting accumulated pseudoexfoliative material in the vessel wall due to this ECM abnormality has thus become the goal of research seeking to clarify the relationship between PEXS and vascular diseases. Although several such studies have reported various results concerning that relationship, a recent meta-analysis additionally revealed that PEXS is associated with an increased risk of vascular disease,³ as shown by results that increased systemic arterial stiffness and decreased vascular elasticity with PEXS.^{4,5}

A well-described inhibitor of pathological vascular calcification, fetuin-A, has been suggested as an independent risk factor for increased arterial stiffness.^{6,7} At insufficiently low levels, fetuin-A is associated with cardiovascular mortality in both dialysis and type 2 diabetes patients.^{8,9}

However, though several biochemical and radiological parameters have been studied to demonstrate a relationship between PEXS and vascular diseases, serum and aqueous humor levels of fetuin-A have not yet been evaluated in patients with PEXS. Accordingly, the aim of the present study was to evaluate serum and aqueous humor levels of fetuin-A in patients with PEXS alongside age- and sex-matched healthy controls.

Materials and methods

This prospective study was conducted according to the Declaration of Helsinki and approved by the local institutional ethics committee. Written informed consent was obtained from all participants.

All participants had grade 3 cataract density according to the Lens Opacities Classification System (LOCS) III¹⁰ and were scheduled to receive cataract surgery in the Ophthalmology Department of Ankara Ataturk Training and Research Hospital. A total of 50 participants, including 25 consecutive patients with PEXS (PEXS group) and 25 age- and sex-matched control subjects (control group), were included in the study. Both prior to surgery and after pupillary dilation, each participant received a complete ocular examination for pseudoexfoliative material on the anterior lens capsule or pupillary margin in eyes with cataract, yet with a normal optic disc appearance, normal visual field findings, and intraocular pressure (IOP) of 21 mmHg or less. Control participants had no history of ocular disease except cataract.

An experienced internal medicine specialist evaluated all participants preoperatively. Patients exhibiting any systemic disease (e.g., hypertension, diabetes mellitus, cardiovascular or cerebrovascular disease, autoimmune disease, malignancy, chronic kidney failure, and chronic hepatic failure) were excluded from the study, as were all patients with a history of ocular surgery and eye trauma, chronic ocular diseases (e.g., glaucoma), ocular inflammatory disease, severe retinal disease, rheumatologic disease, or chronic topical drug usage.

Aqueous humor samples (100–200 μL) were collected at the beginning of phacoemulsification surgery by way of clear corneal paracentesis. Aqueous humor was aspirated from the central pupillary area using a 27-gauge needle on a tuberculin syringe without making vascular contact or touching the iris, lens, or corneal endothelium with the needle. Venous blood samples (5 mL) for testing for serum fetuin-A were collected after 12 h of fasting. Serum specimens were obtained after the samples were centrifuged at $2500 \times g$ for 10 min. All samples were immediately frozen to -80°C prior to analysis. Clinicians performing the biochemical analysis were blinded to diagnostic and clinical information.

Biochemical analysis

Fetuin-A levels in serum and aqueous humor were determined using the quantitative sandwich enzyme-linked immunosorbent assay in accordance with the manufacturer's instructions (Aviscera Bioscience Inc., Santa Clara, CA, USA). The plate was pre-coated with an antibody specific for human fetuin-A. The antibody can bind to the human fetuin-A in the standard and samples. Serum samples were diluted as 200,000-fold while aqueous humor samples were diluted as 10,000-fold with dilution buffer. Samples, standard dilutions, and blank were added into the wells of plate and incubated for two hours. After washing the plate of any unbound substances, a biotinylated antibody against human fetuin-A was added to the wells and incubated for two hours. After a second washing of the plate, streptavidin horseradish peroxidase conjugate was added and incubated for one hour in the dark room. After the last wash to remove any unbound enzyme, a substrate solution was added to the wells and incubated for 30 minutes. Then, stop solution was added to the wells. Optical density of each well was measured with microplate plate reader at 450 nm absorbance (Biorad Model 680 microplate reader). The sensitivity of the assay was 0.15 $\mu\text{g/mL}$, and the linear range of the standard was 31.25–2000 $\mu\text{g/mL}$. The intra-assay and inter-assay coefficients of variation were 6–8% and 8–10%, respectively. Fetuin-A levels of serum and aqueous humor were represented as $\mu\text{g/mL}$ and g/L , respectively.

Statistical analysis

All statistical tests were performed using SPSS, version 20 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA). The normality of the data was confirmed using the Shappiro–Wilk test. Chi-square test was used for the comparison of categorical variables. Normally distributed variables were presented as mean \pm standard deviation, and differences in measured parameters between two groups were analyzed by an independent samples t-test. Abnormally distributed variables were presented as mean (minimum–maximum), and differences between two groups were analyzed by Mann–Whitney U test. The correlation between variables was evaluated by using Pearson correlation analysis. The differences were considered significant when the probability was <0.05 .

Results

In the PEXS group ($n = 25$), there were 14 male and 11 female patients. In the control group ($n = 25$), there were 13 male and 12 female patients. The mean age was 57.7 ± 6.9 years (range: 55–67) in the PEXS group and 58.1 ± 5.7 years (range: 54–63) in the control group. There was no significant difference between the PEXS and control groups in terms of age ($p = 0.77$) and sex ($p = 0.83$). Table 1 summarizes the demographic properties of the study participants.

The mean serum fetuin-A level of the PEXS group did not differ from that of the control group ($p = 0.53$) (Table 2).

The mean aqueous humor fetuin-A level of the PEXS group was significantly higher than that of the control group ($p = 0.032$) (Table 2).

There were no significant correlations between aqueous humor and serum fetuin-A levels among patients with PEXS and control group ($p > 0.05$) (Table 3).

Discussion

Fetuin-A is a multifunctional, circulating glycoprotein primarily secreted by the liver that plays a major role in inhibiting vascular and ectopic calcification linked to inflammation by binding calcium and phosphate.^{6,11} Several studies have shown that an insufficiently low level of fetuin-A is

Table 1. Demographic characteristics of the patients in study groups.

	PEXS ($n = 25$)	Controls ($n = 25$)	p
Age (years) mean \pm SD Range	57.7 ± 6.9 (55–67)	58.1 ± 5.7 (54–63)	0.77
Gender			
Female (n)	11	12	0.83
Male (n)	14	13	

Table 2. Aqueous humor and serum fetuin-A levels among study groups.

	PEXS ($n = 25$)	Controls ($n = 25$)	p
Fetuin-A levels			
Serum (g/L)	0.150 ± 0.037	0.143 ± 0.034	0.53
Aqueous Humor ($\mu\text{g/mL}$)	0.83^* (0.097–1.414)	0.32 (0.092–4.197)	0.032

Serum Fetuin-A levels are presented as means \pm SD, Aqueous Humor Fetuin-A levels are presented as median (minimum–maximum). PEXS indicates pseudoexfoliation syndrome. $p < 0.05$ was accepted as statistically significant. * p was significant compared to control patients.

Table 3. Correlation of aqueous humor and serum fetuin-A levels among study groups.

Correlation	<i>p</i>	<i>r</i>
Pseudoexfoliation Syndrome		
Aqueous humor—serum fetuin-A	0.50	1.36
Control		
Aqueous humor—serum fetuin-A	0.23	0.25

Pearson correlation test.

associated with cardiovascular mortality in both dialysis and type 2 diabetes patients.^{8,9} In men with normal kidney function, Roos et al. found a negative correlation between fetuin-A and aortic pulse wave velocity, which reflects arterial stiffness.¹² By contrast, Mori et al. reported a positive correlation between carotid arterial stiffness and fetuin-A, which they attributed to the biphasic effect of fetuin-A.⁶ The close relationship between fetuin-A and vascular diseases has thus raised questions about the relationship between fetuin-A and PEXS, particularly because systemic arterial stiffness and decreased aortic root elasticity have also been found in patients with PEXS.^{4,5}

At the same time, fetuin-A is known as a negative acute phase reactant that decreases in response to inflammation.¹³ The literature contains several studies in which various molecules, including omentin,¹⁴ YKL-40,¹⁵ high-sensitivity C-reactive protein,¹⁶ anti-phospholipid antibody,¹⁷ and α 1-antitrypsin¹⁸ related to inflammatory processes, have been studied in the serum of patients with PEXS. Based on the results of these studies, inflammation clearly characterizes the pathogenesis of PEXS. Furthermore, proinflammatory cytokines such as IL-6 and tumor necrosis factor (TNF) increased in the serum of patients with PEXS.^{19,20} It has been shown that the hepatic expression of fetuin-A is inversely regulated by proinflammatory cytokines such as IL-6 and TNF.²¹ However, we did not find any differences in the serum fetuin-A levels of PEXS and healthy participants, perhaps due to the small sample size or the stage of PEXS.

In our study, we detected significantly increased fetuin-A levels in the aqueous humor of patients with PEXS, yet no correlation between the aqueous humor and serum levels of fetuin-A between the groups. Although the serum levels of fetuin-A were similar between the groups, the increased levels of fetuin-A in the aqueous humor could be explained by blood aqueous barrier defects associated with PEXS.²² Wang et al. have shown elevated fetuin-A levels in a time-dependent manner, levels that nevertheless returned to baseline at 72 h in an animal model of focal cerebral ischemia. This finding was associated with the transient elevation of blood–brain barrier permeability following cerebral ischemia.²³ We thus suggest that the disruption of the blood–aqueous barrier due to the well-known hypoperfusion and anterior chamber hypoxia in PEXS could increase fetuin-A levels in the aqueous humor.²⁴ We could make clearer comments if we could quantify the blood–aqueous barrier disruption with a method such as laser flare-cell meter and correlate with aqueous humor levels of fetuin-A. We believe that fetuin-A has different functions in the eye's anterior segment. PEXS is known as a generalized

disorder of the ECM characterized by intraocular and systemic production and the accumulation of abnormal fibrillar extracellular material. During abnormal matrix processing, transforming growth factor β 1 (TGF- β 1) in the aqueous humor has been suggested to mediate matrix formation in eyes with PEXS.²⁵ TGF- β 1 has also been shown to stimulate the expression and accumulation of fibrillin-1, fibronectin, elastin, latent transforming growth factor binding proteins 1 and 2, and proteoglycans which are confirmed components of pseudoexfoliative material.^{25–27} Additionally, imbalances between matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) caused by TGF- β 1 were deemed responsible for the accumulation of abnormal fibrillar extracellular material.^{28,29} Fetuin-A exhibits amino acid sequence homology to type II TGF- β receptors and acts as a natural antagonist of TGF- β cytokine.³⁰ In our study, increased fetuin-A levels in the aqueous humor of patients with PEXS may have been a compensatory mechanism to antagonize the effect of TGF- β . If so, then fetuin-A may be an option for future therapeutic strategies in treating PEXS. TGF- β 1 was also demonstrated to be an important cytokine in conjunctival wound healing after glaucoma filtration surgery by playing a role in scarring and tissue fibrosis.^{31,32} In future studies, fetuin-A should be investigated in terms of whether it reduces the excessive scar formation of the filtering bleb by antagonizing TGF- β 1.

In evaluating the elemental composition of pseudoexfoliative material by using energy-filtering transmission electron microscopy, Schlötzer-Schrehardt et al. found that calcium signals related directly to microfibrils and aggregating fibers, thus suggesting that calcium is essential for aggregating microfibrillar subunits.³³ That study's finding implied fetuin-A's inhibitory role in ectopic calcification, whereas in aqueous humor, it could increase as a compensatory mechanism to protect the eye's anterior segment from aggregating pseudoexfoliative material.

A chief limitation of our study was its small sample size. However, because fetuin-A levels may be affected by systemic hypertension, cardiovascular diseases, and diabetes mellitus—all common during ages that overlap with PEXS—we could include only a limited number of patients. Moreover, patients with pseudoexfoliation glaucoma could have been evaluated as a third group since fetuin-A levels could be affected by the stage of pseudoexfoliation. As another limitation, we did not know whether vascular calcification was present or absent either histopathologically or radiologically in the two study groups.

In conclusion, similar levels of fetuin-A in serum and increased levels in aqueous humor in patients with PEXS may be associated with a wide range of fetuin-A's biological functions, including the inhibition of vascular or ectopic calcification and the interaction of biogenic molecules that function in ECM synthesis or inflammation. Since all of these functions may play a role in the pathogenesis of PEXS, if we assume that PEXS is a complex puzzle, then we do not yet know the exact status of fetuin-A therein. As such, further studies should seek to clarify and evaluate the role of fetuin-A

in PEXS, all toward improving the treatment of a poorly understood disorder.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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