

The cytologic analysis of aqueous humor in eyes with uveitis

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ABSTRACT

Purpose: The purpose of this study was to evaluate the cytologic analysis of aqueous humor (AH) of the eyes with Fuchs uveitis (FU) and idiopathic anterior uveitis (IAU).

Methods: AH of 25 uveitic eyes (12 FU and 13 IAU) were evaluated cytologically. The samples of the anterior chamber were taken before cataract surgery. Aqueous fluid samples were evaluated by applying a Giemsa stain.

Results: The 12 patients with FU and 13 patients with IAU were included in this study. In AH, the number of lymphocytes was similar in eyes with FU and IAU (the number of lymphocytes: min:1 and max:7). The acellular amorphous structures were detected in eyes with FU, and the same finding was not observed in the eyes with IAU. In addition, any pigmented cells or accumulation were not detected in any of the eyes.

Conclusion: The acellular amorphous structures were the remarkable finding of this study. It has been considered that amorphous structures in the AH of the eyes with FU could be related to chronic, low-grade anterior chamber inflammation and responsible for several complications of uveitis. FU is a uveitic entity with mysterious findings that await clarifications in its etiopathogenesis.

Keywords: Cytology, Fuchs uveitis, anterior uveitis.

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INTRODUCTION

The cytologic analysis of aqueous humor (AH) in uveitic eyes has numerous significances from clinical evaluation to elucidating the etiology of uveitis. In eyes with uveitis, cellular and non-cellular constituents have been detected in the AH as a reflection of the inflammatory process [1-4].

Fuchs uveitis (FU) and idiopathic anterior uveitis (IAU) are the most common causes of anterior uveitis. Although anatomically classified as anterior uveitis, they have completely different clinical findings. FU is characterized by distinctive anterior uveitis findings. It has low-grade, predominantly unilateral, and chronic inflammatory disease with widely scattered small/medium keratic precipitates (KPs), and a variable degree of iris atrophy [5]. In contrast, IAU is characterized by either acute or chronic inflammation without iris atrophy, and its KPs are spread predominantly in the inferior half of the corneal endothelium [6].

In the past years, cytocentrifuge, and flow cytometric analyses of the AH have been reported in different uveitis entities. A great number of leukocytes have been displayed in the AH during active uveitis and multiple different leukocyte forms have been described in eyes with uveitis [1-3]. FU has a different significance in all aspects from other anterior uveitic entities and several theories have been included in the etiopathogenesis of FU, recently. In light of these findings, we aimed to evaluate and compare the results of cytologic analysis of aqueous humor (AH) in eyes with FU and IAU in this study.

MATERIAL AND METHODS

This clinical prospective comparative study was performed at University of Health Science, Ulucanlar Eye Training and Research Hospital. The study protocol was approved by the Ethics Committee of Ankara Education and Research Hospital (Protocol number: 355; date:04/2018), and the study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all of the patients.

Aqueous samples were obtained from uveitic eyes that were followed up at our Uvea department and undergoing cataract surgery. The diagnostic criteria

of FU included diffusely spread medium and/or stellate KPs, chronic low-grade anterior chamber reaction, iris atrophy accompanied or not with heterochromia, varying degrees of the vitreous cell, and fibril-like vitreous appearance, absence of acute uveitis symptoms. IAU was described as acute anterior uveitis without any systemic disease, and detailed clinical and laboratory investigations were made for systemic evaluation.

In all eyes, findings consistent with the clinically inactive uveitic period were detected for 3 months before cataract surgery. A detailed medical history was obtained in all cases, including previous diagnoses, therapies, and if any surgical interventions. All patients had no systemic steroid usage. The topical dexamethasone drops (16 x 1/ day) were started one week before surgery.

Anterior chamber paracentesis was performed using a 30-gauge needle at the beginning of the surgery. AH (100-200 µl) was aspirated and immediately transferred into Eppendorf tubes. Aqueous fluid samples were centrifuged for 5 minutes at 800 rpm by cytospin and transferred into cytopsin microscope slides. Giemsa stain was applied to the prepared slides. The examination was reported by taking notes of the number of cells, and repetitive acellular amorphous structures and photographing them.

Statistical analyses

Descriptive analyses were performed using IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA). Demographic characteristics and clinical data were expressed as mean, standard deviation, frequency, or percentage.

RESULTS

This study included 25 eyes of 25 uveitic patients; 12 were FU and the remaining 13 were diagnosed as IAU. The patients with FU consisted of 5 men and 8 women with a mean age of 32.6±9.6 years old (range, 28-39), while the IAU included 3 men and 10 women with a mean age of 51.8±11.2 years old (range, 32-59).

Bilateral involvement was determined in 3 eyes (25 %) with FU. There were diffusely spread non-pigmented KPs [(medium-sized in 9 (75 %) eyes, stellate-medium in 3 (25 %) eyes)], Koeppe nodules

in 4 (33.3 %) eyes, and Busacca nodules in 2 (16.6 %) eyes with FU. The color of all eyes with FU was brown and there were varying degrees of iris atrophy in these eyes. There were no KPs and iris atrophy in the eyes with IAU. 12 (48%) eyes had posterior subcapsular cataracts, 10 (40 %) eyes had cortical cataracts, and there was a mature cataract in 3 (12 %) eyes with FU. The participants' characteristics and clinical findings are shown in Table 1.

The number of lymphocytes was similar in eyes with FU and IAU. Leukocytes were detected in the aqueous humor in a total of 4 patients; 2 with FU and 2 with IAU. 3 leukocytes and 7 leukocytes were detected in FU patients respectively. 1 leukocyte was detected in both 2 IAU patients. The striking finding in eyes with FU has detected the appearance of dense acellular sediment (uncellular amorphous structures) (Figures 1, 2, and 3). These uncellular structures were not observed in eyes with IAU. In addition, no pigment-containing cell or accumulation was observed in any eye.

DISCUSSION

The cytological analysis of AHs of eyes with uveitis has been conducted to elucidate the etiopathology of uveitis. It has been demonstrated that mixed leukocytes infiltrate composed of lymphocytes, neutrophils, and macrophages accumulated when there was an active inflammation in the AHs. The lymphocyte predominance has been reported

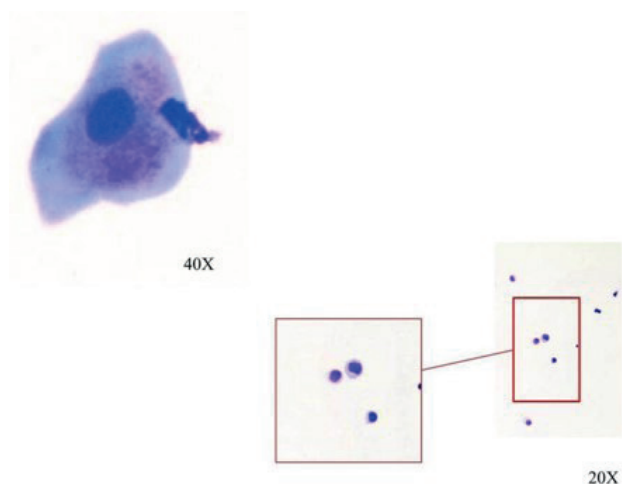


Figure 1. The lymphocytes in an aqueous humor sample of eyes with idiopathic anterior uveitis (Giemsa stain).

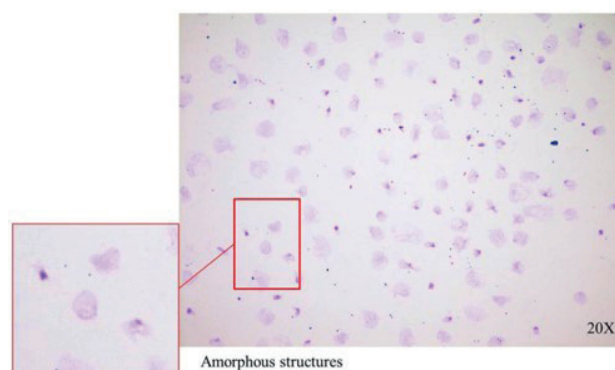


Figure 2. The amorphous structures in aqueous humor sample of eyes with Fuchs uveitis (Giemsa stain).

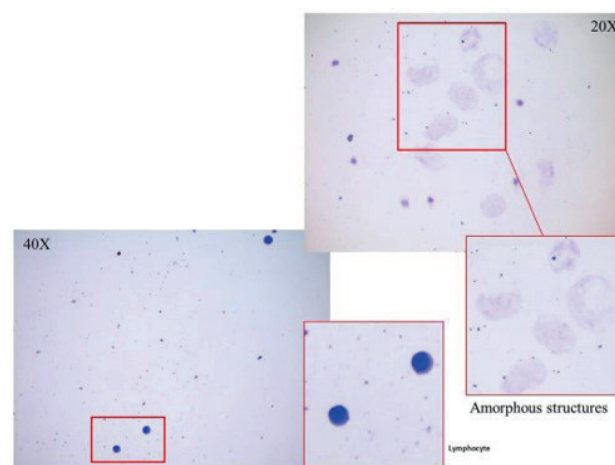


Figure 3. The lymphocytes and amorphous structures in aqueous humor sample of eyes with Fuchs uveitis (Giemsa stain).

Table 1. Demographic and clinical characteristics of the participants

	Age (years) Mean±SD (min-max)	Sex, n (%)	Keratic Precipitates n (%)	Koepe Nodules n (%)	Busacca Nodules n (%)	Cataract Type n (%)		
						Posterior Subcapsular	Cortical	Mature
Fuchs uveitis (n=12)	32.6±9.6 (28-39)	10 M (83.3) 2 F (16.7)	Medium-sized 9 (75) Stellate-medium 3 (25)	4 (33.3)	2 (16.6)	9 (75)	0	3 (25)
Idiopathic Anterior Uveitis (n=13)	51.8±11.2 (32-59)	5 M (38.5) 8 F (61.5)	No	No	No	3 (23.1)	10 (76.9)	0

in AH of the majority of idiopathic or HLA-B27 anterior uveitis. Examinations of herpes-viral uveitis or FU, which do not have any neutrophils, have shown the most severe cases of a deviation in distributions of leukocytes [1-3]. The comparison of the cellular phenotypes and the cytokine profile between patients with FU and IAU has been done by the research of Muhaya et al. [7]. According to this, while CD4+ T cells were more in IAU, CD8 + T cells were more dominant in FU [7].

FU is mainly unilateral, low-grade, chronic anterior uveitis, bilateral involvement has been reported in 5–10% of patients with FU [5,8]. Approximately 2-11% of all uveitis cases include patients with FU and the rate of FU in Turkish patients with uveitis has been reported as 6.3% [5,9,10]. Although the etiology of FU is not completely known, immunologic and infectious etiologies are among the most important reasons in recent studies. In addition, ocular fluid analysis findings in patients with FU were reported to indicate a predominance of CD8 + T cells supporting chronic low-grade inflammation [11].

FU and IAU have differences from each other in both clinical appearance and the steroid response. Thus, we evaluated the cytological analysis of the AHs of two different uveitis entities. The number of lymphocytes was similar in eyes with FU and IAU and these findings were consistent with the clinically inactive uveitic period before cataract surgery. The most remarkable feature of this study was the uncellular amorphous structures in eyes with FU. These structures were not detected in eyes with IAU.

The proteins of human AH are low in concentration (0.1–0.5 mg/ml) [12]. Richardson et al. [13] utilized an enhanced proteomic viewpoint to identify the AH proteome by depleting abundant (albumin) proteins and employing multidimensional protein identification technology. To the best of our knowledge, uncellular amorphous structures were detected in eyes with FU which were not reported before. They are not artifacts since the samples were collected and stained at different times. We are considering that these accumulations can be “proteinous amorphous structures”. It can be interpreted that these accumulations can develop as a result of low-degree and chronic anterior

chamber inflammations. It has been known that the low degree of inflammation of the eyes with FU was not regressed in response to steroids, even though the anterior chamber inflammations in other uveitis entities mostly can be managed with local and/or systemic steroids. On the other hand, patients with FU have frequently and unnecessarily used these steroids. Based on this, we can assume that these accumulations’ “proteinous amorphous structures” may be a revealing cause of steroid nonresponsiveness in eyes with FU.

The atrophy of iris stroma was classically the first pathologic finding of the eyes with FU and it can be clinically observed as varying degrees of iris depigmentation and/or heterochromia. Atrophy of the iris stroma and ciliary body has not occurred equally in each eye with FU [5,14,15]. Hence, it indicates that the pathologic involvement of uveal pigment is at different levels in these eyes. Even though pigmented cells have been observed in AHs or on corneal endothelium in various uveitic entities such as herpetic uveitis or chronic uveitis [6], pigmented KPs are occasionally observed in patients with FU [15], and also, our electron microscopic investigation showed no pigment accumulation on the anterior capsule of the lens [16]. In our study, we did not identify any pigmented cells in AH. The absence of any pigment formation in eyes with FU was another striking result of this study.

In studies, the cataract development rate has been reported as 50%-70% in many uveitis entities [17-19]. Posterior subcapsular cataract (PSCC) is the most common cataract type in uveitic eyes [20,21]. Tugal-Tutkun et al. [22] reported that 8-year the risk of cataract development was 56% in 181 eyes with FU without using steroids. The development of mature cataracts has not frequently occurred in eyes with other uveitis entities except in the end-stage phase of uveitis complication, but the mature cataract is a clinical finding encountered in eyes with FU [17,18,22-24]. In the study of Tugal-Tutkun et al. [22] mature cataract was reported in 4 of 181 eyes (2.2%) and PSCC in 108 of 181 eyes (9.7 %) with FU. Also, Zareiet al. [23] reported that the rates of mature cataracts and PSCC were 7% and 80% in 89 eyes with FU, respectively. In our previous study, mature cataracts and PSCC were shown in 8 of 281 eyes (2.8%) and 121 of 281 eyes (43.1 %) with FU,

respectively [24]. It can be interpreted that this proteinous material, identified in this study, can be accumulated in the lens tissue and can be one of the causes of cataract development. Therefore, we think that this finding should be supported by a more detailed evaluation of lens contents in eyes with uveitis cataracts. It has been demonstrated that proteinous amorph materials have been detected in the human body and have a role in the etiopathology of some diseases. Accumulated proteinous material is named amyloid protein. Research about this accumulation has been ongoing in diseases such as multiple sclerosis, Alzheimer and Parkinson [25,26]. The blood-aqueous barrier of the eye provides a limited selective diffusion of paracellular transport. Dysfunction of the barrier contributes to the pathophysiology of ocular inflammations through the vascular leakage of inflammatory cells and blood-borne molecules into the anterior chamber [27,28]. In the literature, a mild alteration of the blood-aqueous barrier has been detected in Fuchs uveitis [29]. Therefore, there could be a relationship between the number of lymphocytes in the aqueous and the amorphous material due to the breakdown of this barrier and the ease of passage. Also, it can be argued that the amorph materials in the eyes with FU could accumulate in the bodies of patients. Thus, there is a need for more comprehensive studies on this subject.

The intraocular pressure (IOP) elevation in FU patients is generally intermittent. Elevated IOP is typically not seen at admission, it has been estimated approximately 50% of the patients during the follow-up period. Many reasons have been proposed as the cause of IOP elevations such as the use of topical corticosteroids, recurrent hypema, angle neovascularization, and trabeculitis [5,30,31]. As far as we know, there is no study in the literature on the accumulation of amorphous material at the anterior chamber angle. The presented study may lead to future studies on amorphous material accumulation that may cause increased intraocular pressure in Fuchs patients. Besides, we showed hyperreflective spots in the vitreolenticular interface in Fuchs patients in our last study [32]. The hyperreflective points we visualized in Berger's space may be the amorphous materials we detected in this histological study. This material may be accumulating both in the angle and the Berger area and it could be one of the reasons triggering the development of glaucoma.

The present study showed that clinically inactive uveitic periods were confirmed with AH analysis in eyes with uveitis. The acellular amorphous structures and no pigment formation in eyes with FU were the remarkable findings of this study. The limitation of our study is the limited number of patients. Furthermore, other staining or examination techniques were not used. More comprehensive studies and examinations are needed.

To conclude, the cytological analysis will lead to the understanding of complex mechanisms in eyes with uveitis. FU is a uveitic entity with mysterious findings that await clarifications in its etiopathogenesis.

Author contribution

Study conception and design: YÖE, BK, BS, RK; data collection: YÖE, BK, PK, BS, GB, RK, PÖ; analysis and interpretation of results: YÖE, BK, BS, RK, PÖ; draft manuscript preparation: YÖE, BK, PK, BS, GB, RK, PÖ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Ethics Committee of Ankara Education and Research Hospital (Protocol no. 355).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

- [1] Denniston A, Jurnow SC. What Can the Aqueous Humour Tell Us About Uveitis?. Pleyer U, Foster JV. Uveitis and Immunological Disorders Progress III (Essentials in Ophthalmology), Germany, Springer, 19-27. 2008.
- [2] Deschênes J, Char DH, Kaleta S. Activated T lymphocytes in uveitis. *Br J Ophthalmol* 1988; 72:83-7.
- [3] Wang XC, Norose K, Yano A, Ohta K, Segawa K. Two-colour flow cytometric analysis of activated T lymphocytes in aqueous humor of patients with endogenous vs. exogenous uveitis. *Curr Eye Res* 1995; 14:425-33.
- [4] Kalogeropoulos CD, Malamou-Mitsi VD, Asproudis I, Psilas K. The contribution of aqueous humor cytology in the differential diagnosis of anterior uvea inflammations. *Ocul Immunol Inflamm*. 2004;12(3):215-25.
- [5] Sun Y, Ji Y. A literature review on Fuchs uveitis syndrome: An update. *Surv Ophthalmol*. 2020;65(2):133-143.
- [6] Michel SS, Foster CS. Definition, Classification, Etiology, And Epidemiology. In *Diagnosis and Treatment of Uveitis* [1 ed.] (Ed) Foster CS, Vitale AT. USA, Saunders, 17-26, 2002.
- [7] Muhaya M, Calder V, Towler HM, Shaer B, McLauchlan M, Lightman S. Characterization of T cells and cytokines in the aqueous humor in patients with Fuchs' heterochromic cyclitis and idiopathic anterior uveitis. *Clin Exp Immunol* 1998;111:123-128.
- [8] Tandon M, Malhotra PP, Gupta V, Gupta A, Sharma A. Spectrum of Fuchs uveitic syndrome in a North Indian population. *Ocular Immunol Inflammation*. 2012;20:429-433.
- [9] Hovakimyan A, Baglivo E, Cunningham ET Jr. Fuchs Uveitis Syndrome in the developing world. *Int Ophthalmol Clin*. 2010 ;50(2):145-53.
- [10] Yalçındağ FN, Özdal PC, Özyazgan Y, Batioğlu F, Tugal-Tutkun I; BUST Study Group. Demographic and Clinical Characteristics of Uveitis in Turkey: The First National Registry Report. *Ocul Immunol Inflamm*. 2018;26(1):17-26.
- [11] Kreps EO, Derveaux T, De Keyser F, Kestelyn P. Fuchs' Uveitis Syndrome: No Longer a Syndrome? *Ocul Immunol Inflamm*. 2016 ;24(3):348-57.
- [12] Bansal R, Gupta A. Protein Biomarkers in Uveitis. *Front Immunol*. 2020, 3;11:610428
- [13] Richardson MR, Price MO, Price FW, Pardo JC, Grandin JC, You J, et al. Proteomic analysis of human aqueous humor using multidimensional protein identification technology. *Mol Vision*. 2009,15:2740-50.
- [14] Rutzen AR, Raizman MB. Fuchs' Heterochromic Iridocyclitis. In: *Albert & Jakobiec's principles and practice of ophthalmology*. (Eds.) Albert DM, Jakobiec FA, Miller JW. Philadelphia, PA; Edinburgh. pg: 1231-1243, Saunders/ Elsevier.
- [15] Peizeng Yang. *Atlas of Uveitis Diagnosis and Treatment*, Singapore, Springer, 2020.
- [16] Tekin K, Erol YO, Sargon MF, Inanc M, Ozdal PC, Berker N. Effects of Fuchs uveitis syndrome on the ultrastructure of the anterior lens epithelium: A transmission electron microscopic study. *Indian J Ophthalmol*. 2017;65(12):1459-1464.
- [17] Foster CS, Rashid S. Management of coincident cataract and uveitis. *Curr Opin Ophthalmol*. 2003;14;1:1-6.
- [18] Okhravi N, Lightman SL, Towler HM. Assessment of visual outcome after cataract surgery in patients with uveitis. *Ophthalmology* 1999;106:710-22.
- [19] Kump LI, Cervantes-Castañeda RA, Androudi SN, Foster CS. Analysis of pediatric uveitis cases at a tertiary referral center. *Ophthalmology* 2005;112:1287-92.
- [20] Zigler JS Jr, Gery I, Kessler D, Kinoshita JH. Macrophage mediated damage to rat lenses in culture: a possible model for uveitis-associated cataract. *Invest Ophthalmol Vis Sci* 1983;24:5:651-4.
- [21] Secchi AG. Cataracts in Uveitis. *Trans Ophthalmol Soc U K*. 1982;102 Pt3:390-4.
- [22] Tugal-Tutkun I, Güney-Tefekli E, Kamaci-Duman F, Corum I. A cross-sectional and longitudinal study of Fuchs uveitis syndrome in Turkish patients. *Am J Ophthalmol*. 2009;148(4):510-515.
- [23] Zarei M, Darabeigi S, Mehrpour M, Roohipour R, Ghassemi H, Ebrahimiadib N. Fuchs' Uveitis in Iranian Patients: A Review of 89 Eyes. *Ocul Immunol Inflamm*. 2019;27(7):1077-1085.
- [24] Özdamar Erol Y, İnanç M, Özdal P. Fuchs' Uveitis: Is It Different from What We Know? *Ocul Immunol Inflamm*. 2020;24:1-6.
- [25] Ivanova MI, Lin Y, Lee YH, Zheng J, Ramamoorthy A. Biophysical processes underlying cross-seeding in amyloid aggregation and implications in amyloid pathology. *Biophys Chem*. 2020, 19;269:106507.
- [26] Matías-Guiu JA, Oreja-Guevara C, Cabrera-Martín MN, Moreno-Ramos T, Carreras JL, Matías-Guiu J. Amyloid Proteins and Their Role in Multiple Sclerosis. Considerations in the Use of Amyloid-PET Imaging. *Front Neurol*. 2016;31;7:53.
- [27] Coca-Prados M. The blood-aqueous barrier in health and disease. *J Glaucoma*. 2014;23(8 Suppl 1):S36-S38.
- [28] Braunger BM, Gießl A, Schlötzer-Schrehardt U. The Blood-ocular Barriers and their Dysfunction: Anatomy, Physiology, Pathology. *Die Blut-Augen-Schranken und ihre Störungen: Anatomie, Physiologie, Pathologie*. *Klin Monbl Augenheilkd*. 2023;240(5):650-661.
- [29] Küchle M, Nguyen NX. Lasertyndallometrische Analyse der Blut-Kammerwasser-Schranke bei 31 Augen mit Heterochromiezyklitis Fuchs mit und ohne sekundäres Offenwinkelglaukom [Analysis of the blood aqueous barrier by measurement of aqueous flare in 31 eyes with Fuchs' heterochromic uveitis with and without secondary open-angle glaucoma]. *Klin Monbl Augenheilkd*. 2000;217(3):159-162.

- [30] Nilforushan N, Yadgari M, Alemzadeh SA. Surgical management of glaucoma in Fuchs uveitis syndrome: Trabeculectomy or Ahmed glaucoma valve. *J Curr Ophthalmol*. 2018;31(1):24-30. Published 2018 Dec 28.
- [31] Standardization of Uveitis Nomenclature (SUN) Working Group. Classification Criteria for Fuchs Uveitis Syndrome. *Am J Ophthalmol*. 2021;228:262-267.
- [32] Özdamar Erol Y, Kaya P. Evaluation of Vitreolenticular Interface in Eyes with Fuchs Uveitis [published online ahead of print, 2023 Jan 26]. *Ocul Immunol Inflamm*. 2023;1-6.