NONCONTACT IN VIVO CONFOCAL LASER SCANNING MICROSCOPY OF EXFOLIATION SYNDROME

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ABSTRACT

Purpose: To visualize structural alterations of the cornea, iris, and lens in patients with exfoliation syndrome (XFS) using a noncontact in vivo laser scanning confocal microscope and to correlate these with the clinical features.

Methods: The cornea, iris, and lens of 30 eyes with XFS were imaged using the Rostock Cornea Module of Heidelberg Retina Tomograph II (50× noncontact Nikon lens, an estimated 1 to 2 µm transverse resolution, 500×500-µm field of view). Serial transverse section images, as well as anterior segment photographs, were taken and analyzed.

Results: The corneal stroma and endothelium of 19 eyes (63%) showed different amounts and sizes of scattered small hyperreflective deposits. The irides revealed hyperreflective deposits on the anterior outer surfaces and/or pupillary margin corresponding to exfoliation material (XFM) and/or pigment granules. The anterior lens capsule showed varying degrees of peripupillary fibrillar hyperreflective deposits, hyperreflective areas with apparent epithelial cells centrally, and uniform epithelial cells in the clear intermediate zone. On the anterior capsule in 4 pseudophakic eyes, XFM appeared as hyperreflective round deposits. Hyperreflective floating deposits were seen in the aqueous humor in the pupillary region of the posterior chamber of 6 eyes (20%).

Conclusions: Noncontact in vivo confocal microscopy permits visualization of XFM in the cornea, iris, and lens. This new technique may improve early detection of anterior segment abnormalities by providing information about subclinical cellular pathology, such as early pregranular XFS.

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INTRODUCTION

In vivo scanning laser confocal microscopy is a noninvasive technique that provides 1- to 2-µm resolution of anterior segment tissues. It collects serial section images within a specific thickness.[1-3] The Rostock Cornea Module (RCM) uses laser scanning technology and originally consisted of a contact lens system attachment to the Heidelberg Retina Tomograph (II). The distance from the cornea to the microscope is stabilized by a single-use contact element.[4] The contact RCM, with 800× magnification and a 250- to 400-µm field of view, can successfully visualize cellular changes in such conditions as pterygium, keratoconjunctivitis, keratitis, and corneal dystrophies.[5-21]

Exfoliation syndrome (XFS) is an age-related disease characterized by the production and progressive accumulation of an abnormal, fibrillar extracellular material in virtually all tissues of the anterior segment and in many extraocular tissues and connective tissue portions of various visceral organs.[22-28] Exfoliation material (XFM) is found by electron microscopy and indirect immunofluorescence in the stromal connective tissue of the conjunctiva, ciliary body, and iris; iris dilator muscle; and basement membrane of the corneal epithelium, pigmented ciliary epithelium, and lens capsule.[29] The presence of XFM on the anterior lens surface is the most consistent and important clinical diagnostic feature of XFS. Subclinical XFM and/or XFM-related changes in the conjunctiva, lens capsule, and zonules have been reported in the unaffected eye of patients with clinically unilateral XFS by electron microscopy and ultrasound biomicroscopy.[30-32]

The contact RCM cannot image intraocular tissues because of its limited working distance (1.5 mm). The need to examine intraocular tissue has led to the development of a novel noncontact lens prototype. The noncontact RCM, with a working distance of 13.8 mm, is less affected by anatomic lid barriers and is able to visualize ocular surface tissues as well as anterior segment structures up to the posterior lens capsule. We successfully used this lens prototype to image filtering blebs (Sbeity Z et al., Association for Research in Vision and Ophthalmology, 2008, Abstract 826) and minute anatomic changes in such anterior segment disorders as pigment dispersion syndrome and uveitis (Sbeity Z et al., World Ophthalmology Congress, 2008, Abstract).

To our knowledge, no prospective study has been done to screen the corneal layers, iris, and lens in patients with XFS using this new device. We determined the ability of this prototype to image in vivo anatomic microstructural alterations in XFS and correlated these with the clinical findings.

METHODS

The cornea, iris, and lens of eyes with XFS were imaged using the noncontact RCM of the Heidelberg Retina Tomograph II (Heidelberg Engineering Inc, Dossenheim, Germany). The study was approved by the Institutional Review Board of The New York Eye and Ear Infirmary and followed the tenets of the Declaration of Helsinki. All patients underwent a complete ophthalmic examination. The diagnosis of XFS was made by visualizing XFM on the anterior lens surface and/or pupillary margin on slit-lamp

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biomicroscopy following pupillary dilation. All phakic eyes had the classic pattern of 3 distinct zones (homogeneous central disc, intermediate clear zone, and peripheral granular zone). Pseudophakic eyes had remnants of XFM on their pupillary borders.

A 50× noncontact objective lens (Nikon, CF plan 0.45) with a working distance of 13.8 mm, an estimated 1- to 2-µm transverse resolution, and 500×500-µm field of view (Figure 1) was used.[4] Serial transverse (parallel to the cornea, iris, and anterior lens capsule) and oblique section images, in addition to slit-lamp photographs, were taken and analyzed. Patients were instructed to look straight ahead, and images of all corneal layers were taken with the focal plane parallel to the central cornea. A z-axis drive knob was used to advance the focal plane from the corneal epithelium to endothelium. The focal plane was then moved to visualize the central and midperipheral iris (pupillary margin was first imaged to confirm the location of the focal plane on the iris) and then further advanced to screen the anterior lens capsule and lens. While positioned at the anterior lens capsule, the focal plane was moved horizontally and vertically to screen for XFM in all 4 directions. For optimal visualization of the lens, patients with miotic pupils were given mydriatics before imaging. Alternatively, a darkened room was used for patients who had reactive pupils. A minimum of 3 scans consisting of 100 transverse section images were taken of the cornea, iris, and lens. All captured images were saved digitally in a video mode.

FIGURE 1

Noncontact Rostock Cornea Module: A 50× Nikon lens with 13.8-mm working distance and 500×500-µm field of view is attached to the Heidelberg Retina Tomograph (HRTII).

RESULTS

Thirty consecutive eyes of 17 patients (12 females, 5 males) with unilateral or bilateral XFS were enrolled. The mean age was 73.1 ± 10.1 years (range, 55-94 years). Twenty-one eyes (70%) had exfoliative glaucoma or ocular hypertension and were being treated with antiglaucoma medications (Table 1). Thirteen patients had bilateral XFS, and 8 eyes were pseudophakic with remnants of XFM on their pupillary borders seen biomicroscopically. All patients had clinically unremarkable corneas.

TABLE 1. DEMOGRAPHIC INFORMATION FOR PATIENTS WITH EXFOLIATION SYNDROME

Gender	
Male	5
Female	12
Race	
White	17
Black	0
Hispanic	0
Age (yr)	
Mean (±SD)	73.1 ± 10.1
Range	55-94

CORNEA

The corneal endothelium of 19 eyes (63%) revealed different degrees of round, pleomorphic, hyperreflective dots scattered throughout the endothelium (Figure 2, top). This finding was also detected in both eyes of 2 patients with clinically unilateral XFS. Two of the 19 eyes demonstrated hyperreflective fibrillar particles in the subepithelial anterior stroma (Figure 2, middle). No eyes had clinical evidence of corneal endothelial changes on slit-lamp biomicroscopy. One patient with bilateral XFS had a relatively thickened, slightly tortuous nerve plexus in the central anterior stroma bilaterally, which was more prominent in the eye with greater glaucomatous optic neuropathy (Figure 2, bottom). Few eyes demonstrated early degrees of corneal endothelial polymegathism, guttata, and/or reduced cell counts (Table 2). The noncontact RCM images of the corneal layers in 11 eyes were unremarkable.

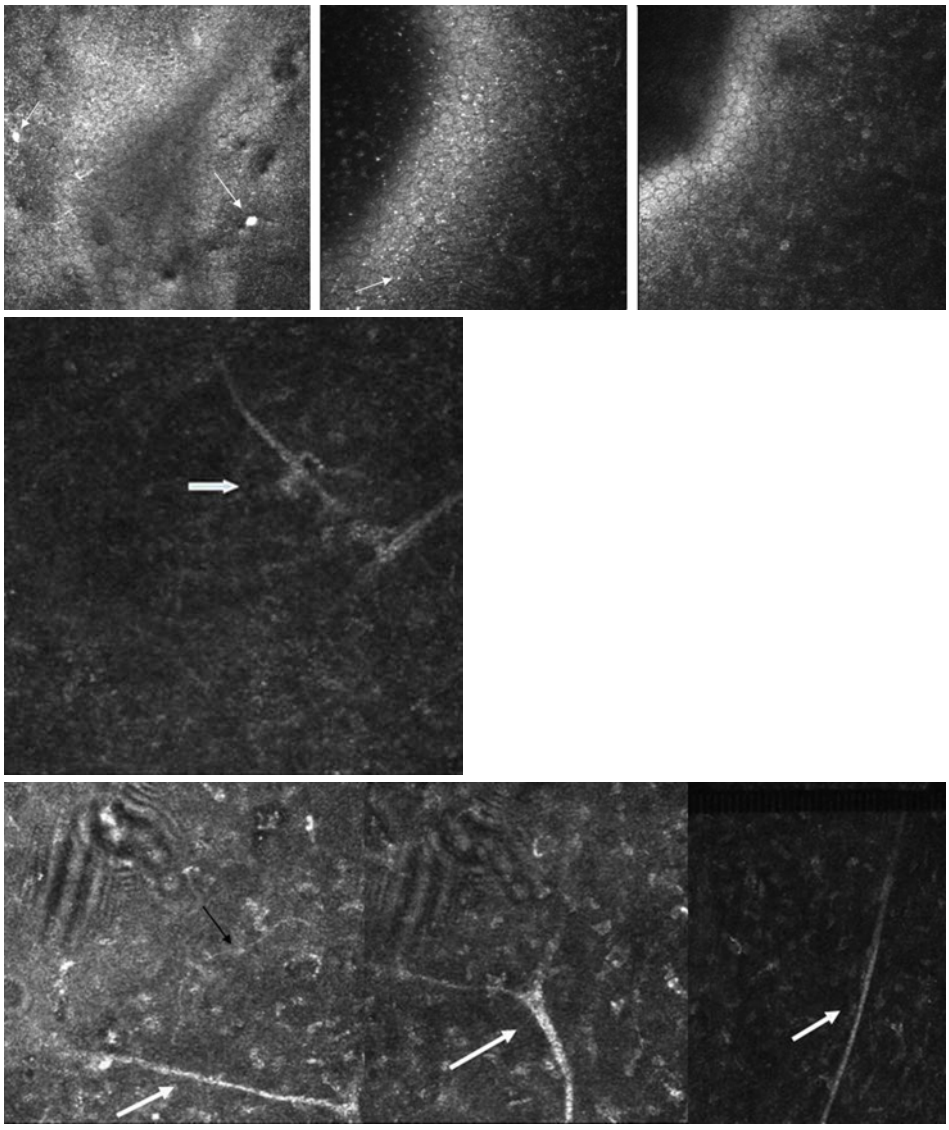

FIGURE 2

Top panel, A healthy cornea of a patient with XFS showing scattered hyperreflective deposits of different sizes (arrows) in the posterior stroma and the endothelium (left and middle images). Image of a healthy cornea in another patient with no evidence of hyperreflective deposits (right image). Middle panel, A healthy cornea of a patient with XFS revealing hyperreflective fibrillar structure in the anterior stroma (arrow) with scattered, tiny hyperreflective deposits. Bottom panel, The anterior stroma of a patient with XFS showing a bilateral thickened nerve plexus (white arrows) in all 3 images and a slightly tortuous normal-sized nerve loop (black arrow) in the anterior stroma (left image).

IRIS

The anterior iris surface in most eyes revealed sparse, scattered, hyperreflective deposits of different sizes, presumably corresponding to both dispersed pigment granules and XFM (Figure 3, row 1). The XFM and intact pigmentary ruff found on the pupillary margin were demonstrated as hyperreflective material (Figure 3, row 2). Regions of iris atrophy over the sphincter muscles were displayed as large hyporeflective, dark areas signifying reduced connective tissue when imaged by the noncontact RCM (Figure 3, row 3). Deep stromal iris vessels filled with fine hyperreflective dots were seen only sporadically in some XFS patients (Figure 3, row 4). Imaging of iris vessels was technically difficult because of abrupt saccadic eye movements and absence of scan depth information. Also, the increased incidence of iris small vessel obstruction in patients with XFS may play a pivotal role in the ability of RCM to detect iris blood vessels.

TABLE 2. CONFOCAL MICROSCOPY FINDINGS IN 30 EYES WITH EXFOLIATION SYNDROME

CORNEA	IRIS	POSTERIOR CHAMBER	LENS CAPSULE	LENS
Scattered pleomorphic HDs in the endothelium and posterior stroma (19/30) Thickened subepithelial nerve plexus (2/30) Pleomorphic and polymegathic endothelial cells (5/30)	HDs on the pupillary border (19/30) and iris surface area (10/30) Hyporeflective areas at the pupillary ruff	HDs in the pupillary area (6/30)	Fibrillary HDs adjacent to normal epithelial cells (22/30) Central homogenous hyperreflective material with hidden epithelial cells (22/30)	Hyporeflective spaces (10/30), hyperreflective dense fibers (4/30) Smooth IOL surface with HDs on the anterior capsule (4/6)

HD, hyperreflexive deposit; IOL, intraocular lens.

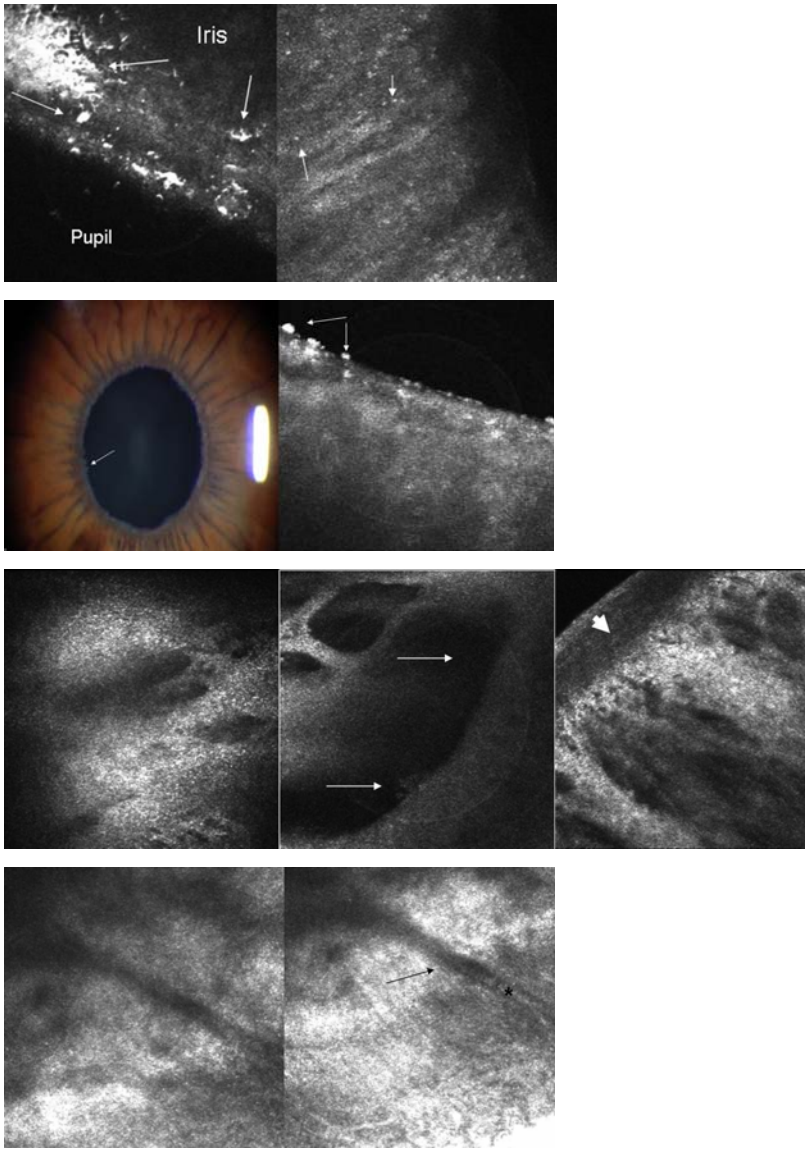

FIGURE 3

Row 1, Multiple hyperreflective deposits (arrows) on the anterior iris surface of a patient with XFS corresponding to pigment granules (right image) and XFM (left image). Row 2, The exfoliation material on the pupillary margin demonstrated hyperreflectivity (arrows) when imaged by noncontact RCM (left image). Row 3, Unlike the normal iris (left image), the iris sphincter area of a patient with XFS shows hyporeflective spaces and reduced connective tissue (arrows, middle image); the pupillary border (arrow head) is thin and without a pigment ruff (right image). Row 4, A deep stromal iris vessel (arrow) filled with tiny hyperreflective dots (*) corresponding to red blood cells seen in 2 consecutive images of the iris of a patient with XFS.

LENS

Imaging of the anterior lens capsule with the classic 3-zone pattern of XFM distribution revealed (1) different extents of hyperreflective, fibrillar, peripupillary deposits (granular peripheral zone); (2) homogenous, hyperreflective areas and hidden (covered) epithelial cells centrally (central disc); and (3) uniform epithelial cells in the intermediate zone (clear zone; Figure 4, top). The unaffected eye of patients with clinically unilateral XFS showed either a normal lens capsule or signs of possible early pregranular changes (Figure 4, bottom). Remnants of XFM deposits on the peripupillary anterior capsule in a pseudophakic eye revealed hyperreflective round deposits (Figure 5). While imaging the anterior capsule in 6 eyes (5 phakic and 1 pseudophakic), we noted multiple floating hyperreflective dots of different sizes in the pupillary area of the posterior chamber corresponding to either liberated XFM or pigment granules (Figure 5). All 6 eyes had glaucoma and were being treated with hypotensive medication with no signs of pigment cells in their anterior and posterior chambers on slit-lamp biomicroscopy. Unfortunately, there were no images taken both before and after pupillary dilation to study the effect of this on these floating particles. Pupillary dilation was thought not to be a factor in inducing these floating deposits, as 3 of these eyes were not dilated when imaged. Imaging the cataractous lenses demonstrated hyperreflective, dense cortical fibers with multiple large hyporeflective cavitations. This corresponded to the vacuoles seen clinically (Figure 6). The noncontact RCM could also visualize intraocular lenses. In all pseudophakic eyes the central optic of the IOL showed a smooth surface with few scratches and no evidence of hyperreflective deposits (Figure 6).

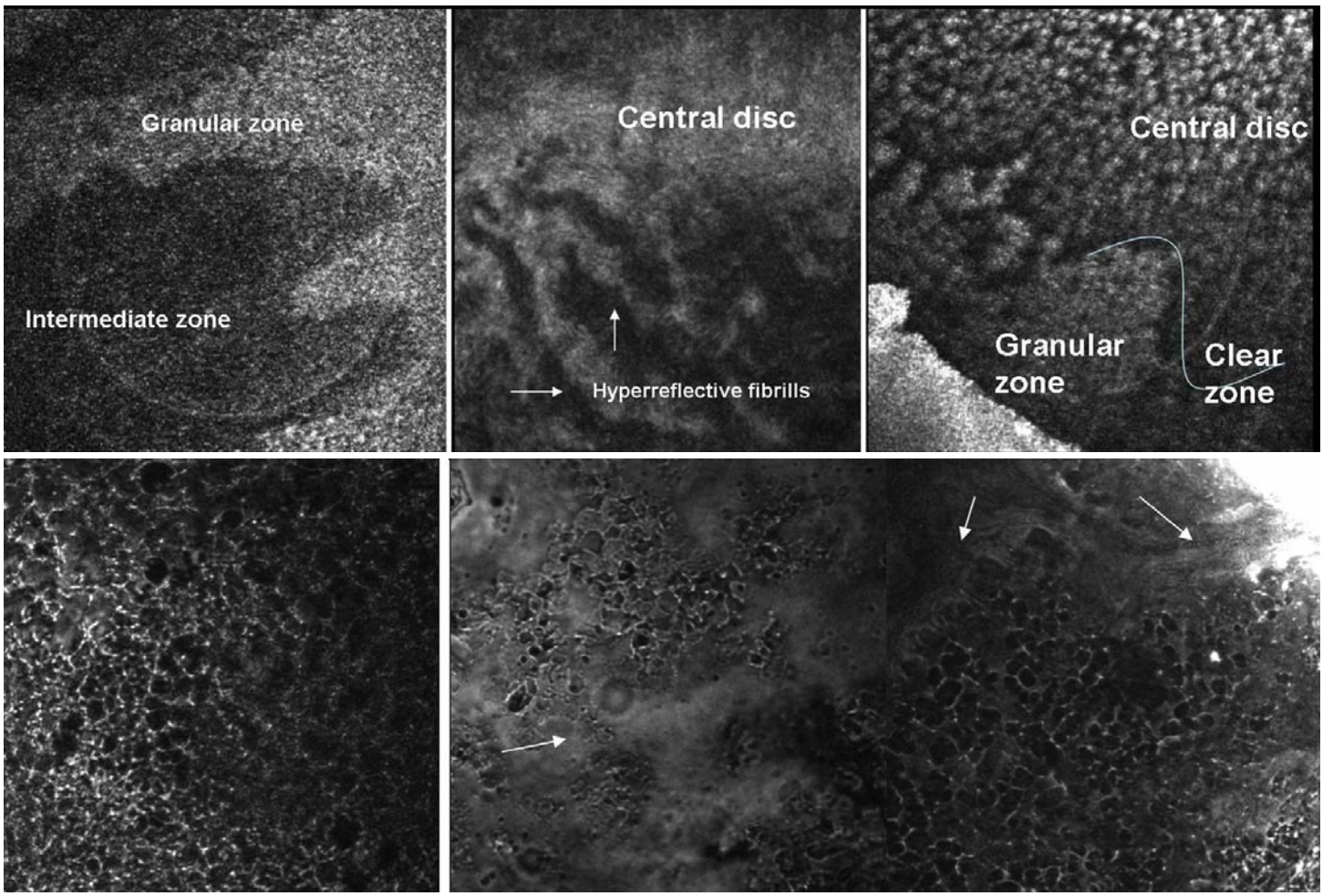

FIGURE 4

Top, Images of the 3 zones of exfoliation material accumulation on the anterior lens capsule; unlike the intermediate zone, the granular zones and the central disc are hyperreflective. Bottom, Image of anterior lens capsule epithelial cells in the unaffected eye of a patient with clinically unilateral XFS (left). The right image shows presumably early pregranular XFM (arrows).

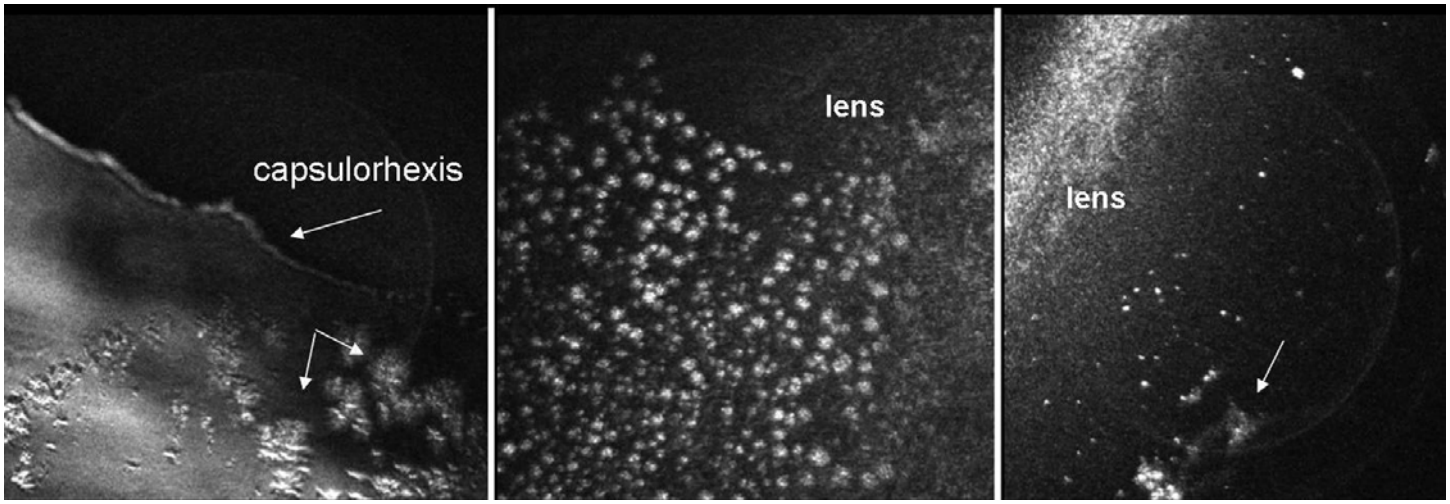

FIGURE 5

Hyperreflective round deposits (arrows) shown on the anterior lens capsule of a pseudophakic eye (left image), and on the lens and in the posterior chamber (middle and right images). A large dendritic-like hyperreflective structure in the posterior chamber is presumably an inflammatory cell or a large melanocyte (arrow, right image).

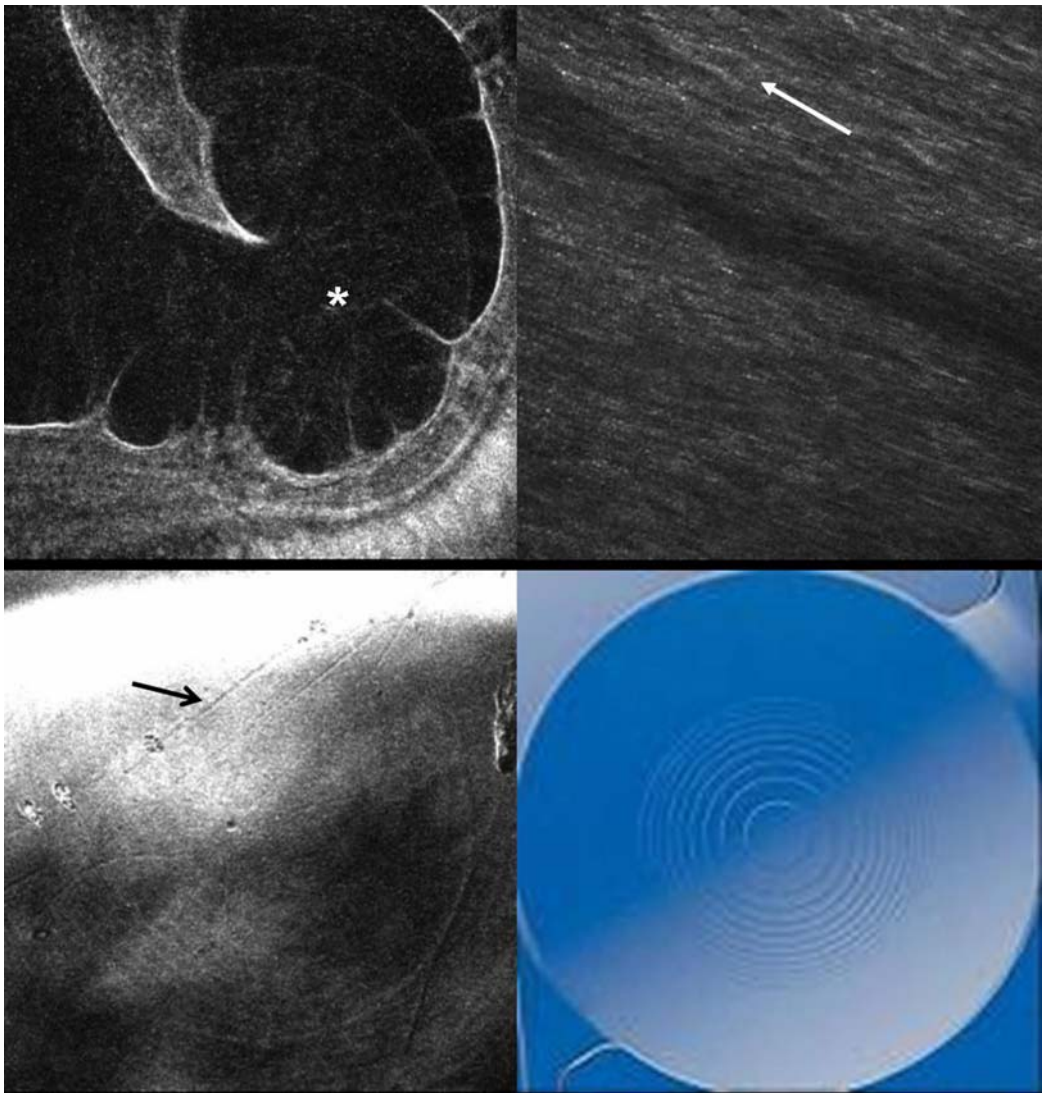

FIGURE 6

Cataractous lens showing large hyporeflective spaces (vacuoles; asterisk upper left image) and dense hyperreflective cortical fibers (arrow, upper right image). Image of the optic of a lens implant (lower left) shows a smooth surface with few superficial scratches (arrow). Note the circular lines in the confocal image which are characteristic of a multifocal diffractive IOL (lower right).

DISCUSSION

In a single patient with bilateral XFS and clinically healthy corneas, Martone and associates[33] found small hyperreflective corneal endothelial cell deposits, polymegathism, and pleomorphism using the contact RCM. They concluded that these deposits corresponded to subclinical XFM. In the study reported here, biomicroscopic XFM and pigment granules on the lens and iris were visualized as hyperreflective deposits when imaged by the noncontact RCM of 30 eyes with XFS and clinically unremarkable corneas. Nineteen (63%) had variable degrees of hyperreflective deposits in either the anterior corneal stroma and/or the endothelium. We presume that these deposits correspond to subclinical XFM, as they were very tiny (smaller than an endothelial cell) and irregular in shape. The incidence of subclinical corneal XFM in our patients (63%) is similar to that of microscopic XFM in the anterior lens capsule of the unaffected eye of patients with clinically unilateral XFS.[34] The conjunctiva and iris of eyes of patients suspected of having XFS or of the unaffected eyes of patients with unilateral XFS have evidenced a similar or somewhat greater incidence of subclinical XFM by electron microscopy.[31,35,36] It would be interesting to know the incidence of hyperreflective corneal deposits in controls, in patients suspected of having XFS, and in the companion eye of a larger group of patients with unilateral XFS. Although thickened nerve plexuses are not typically seen in XFS, reduced corneal sensitivity has been reported as a sign of exfoliative keratopathy.[37-39] Thickened corneal nerves are usually present in isolated ocular conditions (keratoconus, Fuchs dystrophy, herpes keratitis) as well as in systemic disorders such as congenital ichthyosis, Refsum disease, multiple endocrine neoplasia, and leprosy.[40] Contact RCM imaging of stromal and subepithelial nerves in leprosy reveals both thickened stromal nerves and thin, tortuous, and beaded basal nerves.[41] In our patient, the RCM images of the thickened nerves were bilateral but asymmetric. The patient had no history of any other ocular or systemic disease and had cataract surgery in one eye. Whether this is an isolated nonspecific finding or whether thickened nerve plexuses may be more frequent in patients with XFS requires further study.

Two patients had large, hyperreflective cellular structures (dendritic-like cells) in the aqueous of the posterior chamber, presumably inflammatory cells or large melanocytes (Figure 5, right image), consisent with a suggested role of inflammation in the pathogenesis of XFS.[42] Liberation of pigment granules and XFM in the posterior chamber is an important risk for intraocular pressure spikes in patients with XFS. Slit-lamp biomicroscopy is sufficient to detect pigment liberation in the anterior chamber of eyes with XFS after pupillary dilation. The noncontact RCM may help detect liberated pigment or XFM in the posterior chamber, where these particles are almost impossible to see by slit-lamp biomicroscopy. We detected these in 6 eyes. Noncontact RCM easily detected XFM on the lens and possible lens epithelial changes related to early pregranular changes. This should allow earlier diagnosis of XFS. Scheimpflug photographic imaging of the anterior lens capsule of patients with XFS has also been considered diagnostically useful.[43]

The quality of corneal images obtained using the noncontact lens was satisfactory and comparable to that of images taken using the contact lens. The use of artificial tears or lubricant gels can reduce strong surface reflections and optimize image quality by balancing refractive index differences. Technical issues such as gain, time lag, and a minimally oblique scan may still contribute to histologic-microscopic discrepancies. The inability to determine the approximate depth of the focal plane adds another limitation to the noncontact RCM. We used common ocular structures such as the central cornea and pupillary borders to assist in image localization. The noncontact RCM provides a novel method of examining cellular alterations in XFS between the cornea and the lens, as well as identifying other anterior segment ocular pathology. To date, ex vivo histology remains the gold standard and only reliable method of diagnosing and differentiating microstructural abnormalities and in detecting subclinical disorders. This new in vivo technology may hopefully now be used to screen for cellular abnormalities.

In conclusion, the noncontact RCM permits visualization of microstructural abnormalities within the cornea, iris, and lens. This promising, rapid, noncontact imaging technique could improve early detection of anterior segment pathology by delivering information about the cellular and subcellular tissues in subclinical abnormalities, such as pregranular exfoliation syndrome. This approach needs to be further studied and optimized for imaging intraocular tissues and determining the depth of the focal plane.

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Conformity With Author Information: The study was approved by the Institutional Review Board of The New York Eye and Ear Infirmary and followed the tenets of the Declaration of Helsinki.

REFERENCES

1. Cavanagh HD, Jester JV, Essepian J, Shields W, Lemp MA. Confocal microscopy of the living eye. *CLAO J* 1990;16:65-73.
2. Cavanagh HD, Petroll WM, Jester JV. The application of confocal microscopy to the study of living systems. *Neurosci Biobehav Rev* 1993;17:483-498.
3. Stave J, Zinser G, Grümmer G, Guthoff R. Modified Heidelberg Retinal Tomograph HRT. Initial results of in vivo presentation of corneal structures. *Ophthalmologe* 2002;99:276-280.

4. Guthoff, RF, Baudouin C, Stave J. Principles of confocal in vivo microscopy. In: Philipp M, ed. *Atlas of Confocal Laser Scanning In-vivo Microscopy in Ophthalmology*. Berlin Heidelberg: Springer; 2006:3-15.
5. Tervo T, Moilanen J. In vivo confocal microscopy for evaluation of wound healing following corneal refractive surgery. *Prog Retin Eye Res* 2003;22:339-358.
6. Zhivov A, Stachs O, Kraak R, Stave J, Guthoff RF. In vivo confocal microscopy of the ocular surface. *Ocul Surf* 2006;4:81-93.
7. Messmer EM, Mackert MJ, Zapp DM, Kampik A. In vivo confocal microscopy of normal conjunctiva and conjunctivitis. *Cornea* 2006;25:781-788.
8. Bailly N, Sherif ZA, Pleyer U, Rieck P. Confocal microscopy in corneal dystrophies: a comparison between confocal slit scanning (ConfoScan P2) and laser scanning microscopy (Rostock Cornea Module-HRT II). *Klin Monatsbl Augenheilkd* 2006;223:735-742.
9. Gheck L, Dupas B, Denion E, Amar N, Baudouin C. Advantages of in vivo confocal microscopy for investigation of the pterygium. *J Fr Ophtalmol* 2007;30:703-710.
10. Lim LL, Hoang L, Wong T, et al. Intravital microscopy of leukocyte-endothelial dynamics using the Heidelberg confocal laser microscope in scleritis and allergic conjunctivitis. *Mol Vis* 2006;12:1302-1305.
11. Hu Y, Adan ES, Matsumoto Y, et al. Conjunctival in vivo confocal scanning laser microscopy in patients with atopic keratoconjunctivitis. *Mol Vis* 2007;13:1379-1389.
12. Sheppard JD Jr, Lattanzio FA Jr, Williams PB, Mitrev PV, Allen RC. Confocal microscopy used as the definitive, early diagnostic method in Chandler syndrome. *Cornea* 2005;24:227-229.
13. Garibaldi DC, Schein OD, Jun A. Features of the iridocorneal endothelial syndrome on confocal microscopy. *Cornea* 2005;24:349-351.
14. Messmer EM, Zapp DM, Mackert MJ, Thiel M, Kampik A. In vivo confocal microscopy of filtering blebs after trabeculectomy. *Arch Ophthalmol* 2006;124:1095-1103.
15. Guthoff R, Klink T, Schlunck G, Grehn F. In vivo confocal microscopy of failing and functioning filtering blebs: results and clinical correlations. *J Glaucoma* 2006;15:552-558.
16. Labbé A, Dupas B, Hamard P, Baudouin C. In vivo confocal microscopy study of blebs after filtering surgery. *Ophthalmology* 2005;112:1979-1986.
17. Matsumoto Y, Dogru M, Sato EA, et al. The application of in vivo confocal scanning laser microscopy in the management of acanthamoeba keratitis. *Mol Vis* 2007;13:1319-1326.
18. Iordanidou V, Sultan G, Boileau C, Raphael M, Baudouin C. In vivo corneal confocal microscopy in Marfan syndrome. *Cornea* 2007;26:787-792.
19. Zhivov A, Stave J, Vollmar B, Guthoff R. In vivo confocal microscopic evaluation of langerhans cell density and distribution in the corneal epithelium of healthy volunteers and contact lens wearers. *Cornea* 2007;26:47-54.
20. Brasnu E, Bourcier T, Dupas B, et al. In vivo confocal microscopy in fungal keratitis. *Br J Ophthalmol* 2007;91:588-591.
21. Labbé A, Nicola RD, Dupas B, Auclin F, Baudouin C. Epithelial basement membrane dystrophy: evaluation with the HRT II Rostock Cornea Module. *Ophthalmology* 2006;113:1301-1308.
22. Ritch R, Schlötzer-Schrehardt U. Exfoliation syndrome. *Surv Ophthalmol* 2001;45:265-315.
23. Schlötzer-Schrehardt U, Koca MR, Naumann GOH, Volkholz H. Pseudoexfoliation syndrome. Ocular manifestation of a systemic disorder? *Arch Ophthalmol* 1992;110:1752-1756.
24. Streeten BW, Li ZY, Wallace RN, Eagle RCJ, Keshgegian AA. Pseudoexfoliative fibrillopathy in visceral organs of a patient with pseudoexfoliation syndrome. *Arch Ophthalmol* 1992;110:1757-1762.
25. Dark AJ, Streeten BW, Conward CC. Pseudoexfoliative disease of the lens: a study in electron microscopy and histochemistry. *Br J Ophthalmol* 1977;61:462-472.
26. Asano N, Schlötzer-Schrehardt U, Naumann GO. A histopathologic study of iris changes in pseudoexfoliation syndrome. *Ophthalmology* 1995;102:1279-1290.
27. Takei Y, Mizuno K. Electron-microscopic study of pseudoexfoliation of the lens capsule. *Albrecht von Graefes Arch Klin Exp Ophthalmol* 1978;205:213-220.
28. Naumann GO, Schlötzer-Schrehardt U, Küchle M. Pseudoexfoliation syndrome for the comprehensive ophthalmologist. Intraocular and systemic manifestations. *Ophthalmology* 1998;105:951-968.
29. Schlötzer-Schrehardt U, von der Mark K, Sakai LY, Naumann GO. Increased extracellular deposition of fibrillin-containing fibrils in pseudoexfoliation syndrome. *Invest Ophthalmol Vis Sci* 1997;38:970-984.
30. Oliveira C, Schlötzer-Schrehardt U, Vieira G, Liebmann J, Ritch R. Early diagnosis of exfoliation syndrome in the offspring of affected patients. *Acta Ophthalmol Scand* 2006;84:512-515.
31. Hammer T, Schlötzer-Schrehardt U, Naumann GO. Unilateral or asymmetric pseudoexfoliation syndrome? An ultrastructural study. *Arch Ophthalmol* 2001;119:1023-1031.
32. Sbeity Z, Dorairaj SD, Reddy S, Tello C, Liebmann JL, Ritch R. Ultrasound biomicroscopy of zonular anatomy in clinically unilateral exfoliation syndrome [published online ahead of print Feb 8, 2008]. *Acta Ophthalmol* doi:10.1111/j.1600-0420.2007.01105.x.
33. Martone G, Casprini F, Traversi C, et al. Pseudoexfoliation syndrome: in vivo confocal microscopy analysis. *Clin Experiment Ophthalmol* 2007;35:582-585.

34. Parekh P, Green R, Stark W, et al. Electron microscopic investigation of the lens capsule and conjunctival tissues in individuals with clinically unilateral pseudoexfoliation syndrome. *Ophthalmology* 2008;115:614-619.
35. Speakman JS, Ghosh M.The conjunctiva in senile lens exfoliation. *Arch Ophthalmol* 1976;94:1757-1759.
36. Prince AM, Streeten BW, Ritch R, Dark AJ, Sperling M. Preclinical diagnosis of pseudoexfoliation syndrome. *Arch Ophthalmol* 1987;105:1076-1082.
37. Naumann GO, Schlötzer-Schrehardt U. Keratopathy in pseudoexfoliation syndrome as a cause of corneal endothelial decompensation: a clinicopathologic study. *Ophthalmology* 2000;107:1111-1124.
38. Seitz B, Müller EE, Langenbucher A, Kus MM, Naumann GO. Endothelial keratopathy in pseudoexfoliation syndrome: quantitative and qualitative morphometry using automated video image analysis. *Klin Monatsbl Augenheilkd* 1995;207:167-175.
39. Stefaniotou M, Kalogeropoulos C, Razis N, Psilas K. The cornea in exfoliation syndrome. *Doc Ophthalmol* 1992;80:329-333.
40. Sethi HS, Pal N, Dada T. Bilateral juvenile glaucoma with iridotrabecular dysgenesis, congenital ectropion uveae, and thickened corneal nerves. *Eye* 2005;19:1347-1349.
41. Zhao C, Lu S, Tajouri N, Dosso A, Safran AB. In vivo confocal laser scanning microscopy of corneal nerves in leprosy. *Arch Ophthalmol* 2008;126:282-284.
42. Ovodenko B, Rostagno A, Neubert TA, et al. Proteomic analysis of exfoliation deposits. *Invest Ophthalmol Vis Sci* 2007;48:1447-1457.
43. Goder GJ, Rechlin RG. The exfoliation in the Scheimpflug photography. *Acta Ophthalmol* 1998;66 (Suppl):44-47.

PEER DISCUSSION

DR. DON MINCKLER: It has been a pleasure and a privilege to review and discuss this paper describing the first clinical application of non-contact in vivo laser scanning microscopy in exfoliation syndrome (XFS) patients, extending the depth of tissue analysis possible with contact confocal imaging. The authors modified the optical system of a corneal module (Heidelberg Retina Tomograph II) to allow non-contact imaging of the anterior segment with estimated transverse resolution of 1-2 microns. Their images extend through the iris and anterior lens structure. This report includes images at various depths from 30 subjects including many with exfoliation syndrome and makes clinical correlations with their findings.

As with any new clinical tool, its proper place in our armamentarium will take some time to establish. I found the endothelial images (Fig 2a) impressive, and presume this device could be applied to many corneal disorders, including those with various degrees of edema or even hypotony precluding contact confocal studies. The clinical correlation with exfoliative deposits around the pupil and on the lens are dramatic and reinforce the authors' suggestion that earlier stages of exfoliation might be detectable with this non-contact technique than by currently available methods including clinical examination or contact confocal imaging.

Interpretation of some image details, particularly specific cell types in the aqueous and vascular structures in iris is problematic without morphologic correlation. Sequential non-contact confocal imaging and histology at both light and electron microscopic levels of autopsy eyes from patients with exfoliation syndrome and other ocular disorders seem highly desirable. Clinical studies of numerous entities besides exfoliation will hopefully follow, if working distance and tissue penetration permit, including angle structures, aqueous veins and collector channels in normals and other forms of chronic open-angle glaucoma, in addition to XFS. Any disease entity involving structural alterations or cell accumulations of material in anterior segment tissues including epithelial or fibrous ingrowths, ICE, corneal dystrophies, congenital anomalies, tumors, uveitis, and neovascularization would seem amenable to study with this technology.

My only somewhat substantive criticism of this initial paper is the lack of companion normal anterior segment structures for comparison by the reader. I look forward to extensive further use of this methodology including perhaps an atlas of confocal images of normal and pathologic anterior segment structures.

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DR. ROBERT RITCH: I have no conflict of interest. We did get a free instrument on loan to use for the study. I would like to thank Dr. Minckler for his kind review. I would like to ask just one question. Don, you have been using two photon microscopy, which is really cutting edge technology. In addition to looking at the posterior segment, have you had a chance to look at anterior segment structures, and, if you have, how does that compare with confocal technology? To answer your other comment, at this point we need more correlation and more normal tissues. We are just at the stage of figuring out exactly what we are looking at and how to appraise it. I think over a period of time, we are going to get more information from this device and I think it is very going to be very useful.

DR. EDWARD L. RAAB: I have no conflict of interest. Since all of your patients were identified by presently existing criteria as having pseudo-exfoliation syndrome, I think it is entirely possible that the findings you described could be just corroborative evidence and not really early findings by which you could anticipate the development of clinical signs. I was trying to think of a way around that problem because I do not see how you could really do that practically as a prospective matter. I wonder if you have any ideas for how you are going to try to attack that question.

DR. ZAHER SBEITY: Thank you for asking this question. Actually this was an initial exploratory study in which we wished to differentiate exfoliative material from other cellular pathologies. We started a prospective comparative study of patients with frank exfoliation syndrome, exfoliation syndrome suspects, and normal controls. I believe that is the only way we can identify early changes of preclinical exfoliation syndrome. I would like to thank the AOS committee for their invitation and Dr. Ritch for his great support, especially during the last two days when he had his birthday and I was still rehearsing for him. Thank you very much.