

In vivo laser confocal microscopic findings in patients with epithelial basement membrane dystrophy

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PURPOSE. *To evaluate the corneal characteristics of patients with epithelial basement membrane dystrophy (EBMD) using an in vivo laser confocal microscope, Heidelberg Retina Tomograph II, Rostock Cornea Module (HRT II RCM).*

METHODS. *Sixteen women and 13 men who were diagnosed with or suspected to have EBMD were included in the study. The mean age of the patients was 56.4±17.2 years within a range of 25 to 81 years. Nine patients (31%) had a history of recurrent corneal erosions and 3 patients had been misdiagnosed with herpetic epithelial keratitis. The remaining 17 patients were asymptomatic and EBMD was diagnosed incidentally during routine eye examinations.*

RESULTS. *Confocal microscopy revealed highly reflective tissue in various configurations within the intermediate and basal epithelial cell layers corresponding to the abnormal basement membrane extending into the epithelium. There were thin, parallel hyperreflective lines and high contrast round lesions in sizes ranging between 10 and 250 µm within the epithelium. Basal epithelial cells around the abnormal basement membrane and cysts seemed to be highly distorted. In two subjects with bleb-like disorder, the authors observed circular or oval hyporefective areas with a diameter ranging between 40 and 100 µm at the level of basal epithelium and the Bowman layer, accompanied by hyperreflective, linear structures extending into the epithelium.*

CONCLUSIONS. *EBMD is most commonly asymptomatic and undiagnosed; however, it might be associated with recurrent corneal erosions and lead to severe complications after LASIK surgery. The confocal images are highly characteristic for EBMD; therefore, confocal microscopy seems to be a valuable tool in the diagnosis of EBMD. (Eur J Ophthalmol 2009; 19: 348-54)*

KEY WORDS. *Epithelial basement membrane dystrophy, Bleb-like dystrophy, HRT II RCM, In vivo confocal microscopy*

Accepted: September 22, 2008

INTRODUCTION

Epithelial basement membrane dystrophy (EBMD) (also known as Cogan microcystic dystrophy, map-dot-fingerprint dystrophy, or microcystic corneal dystrophy) is by far the most common anterior corneal dystrophy, with an estimated prevalence of 5% (1-3). The clinical manifestations vary considerably among patients from grayish-white intraepithelial opacities to map-like subepithelial

geographic patches or fingerprint-like lines alone or in different combinations (1-6). These findings are variable and change in number and distribution from time to time. Bleb-like disorder, which was described by Bron and Brown (7), is a less common manifestation of EBMD. It is typically observed as a symmetrical grouping of transparent, confluent, subepithelial excrescences in the central or lower two thirds of the cornea (7).

EBMD is an abnormality of epithelial turnover, maturation,

and production of basement membrane and the pathologic findings are sheetlike areas of basement membrane extending superficially into the substance of the cornea epithelium, intraepithelial cysts containing pyknotic nuclei with debris, underdeveloped hemidesmosomes, and absence of anchoring fibrils, which result in poor adherence of corneal epithelium to Bowman layer (3, 8-10). The patients are usually asymptomatic; however, EBMD may sometimes be associated with recurrent corneal erosions and irregular astigmatism (7, 11, 12). Laser in situ keratomileusis (LASIK) is not recommended in patients with EBMD, since these patients are predisposed to multiple complications after LASIK, such as epithelial sloughing/defects during the microkeratome pass in LASIK, flap microfolds, epithelial ingrowth, flap melting, and diffuse lamellar keratitis (13, 14), which emphasizes the importance of careful screening of EBMD cases undergoing LASIK surgery.

Confocal microscopy is a noninvasive method that provides real-time corneal images with high resolution at the cellular level and allows better lateral resolution and image contrast of the corneal layers than conventional imaging devices such as the slit-lamp biomicroscopes and specular microscopes. Heidelberg Retina Tomograph II, Rostock Cornea Module (HRT II RCM) (Heidelberg Engineering GmbH, Dossenheim, Germany) is a relatively new high-resolution, high-speed, digital confocal laser-scanning microscope permitting detailed in vivo investigation of the cornea (15, 16). The morphology of EBMD was examined with various confocal microscopy devices, including HRT II RCM (17-19). In this study, we examined a relatively large group of patients with EBMD using HRT II RCM and also reported the in vivo confocal microscopic appearance of two patients with bleb-like dystrophy.

METHODS

Twenty-nine consecutive patients with clinically diagnosed or suspected EBMD were included in the study. A complete eye examination, including anterior segment slit-lamp biomicroscopy, visual acuity evaluation, fundus examination, and in vivo confocal microscopy with HRT II RCM, was performed in all patients. Patients who had typical gray geographic patches, fingerprint lines, or dot like gray-white opacities on slit-lamp examination were clinically diagnosed with EBMD, whereas patients with only a few dots or subepithelial patch like findings in a

small area of the cornea, microform epithelial erosions, or epithelial irregularity without typical map, dot, or fingerprints were suspected to have EBMD. Patients with a history of ocular surgery, trauma, or contact lens wear were excluded from the study.

HRT II is a confocal scanning laser ophthalmoscope that provides quantitative, objective, and highly reproducible three-dimensional images of the optic nerve head. With the addition of RCM, the HRT II is converted to an in vivo confocal microscope that allows the acquisition of two-dimensional images of different corneal layers. The details of the technology were explained elsewhere (Heidelberg Retina Tomograph II, Rostock Cornea Module. Operating instructions of software version 1.1. Dossenheim, Germany; 2004). HRT II RCM uses a diode laser with a wavelength of 670 nm and each optical section consists of 384x384 pixels in an area of 400x400 μ m. The study protocol was in adherence to the tenets of the World Medical Association Declaration of Helsinki and all the patients were informed of the confocal procedure and recording their data to be used in this study and their written consent was obtained. After administration of topical proparacaine hydrochloride 0.5% (Alcaine[®], Alcon Pharmaceuticals, Alcon-Couvreur, Belgium), a contact gel with high viscosity (Comfort Gel, Bausch & Lomb, GmbH, Berlin, Germany) was applied to the lower conjunctival fornix and onto the front surface of the microscope lens (Zeiss x63). The objective of the microscope is covered by a single-use polymethylmethacrylate cap (TomoCap) in sterile packaging. The subjects were positioned comfortably in the chin and forehead rest and were requested to look into the center of the objective lens. A lateral view of the eye and objective lens was obtained for each scan using a digital camera to check the position of the objective lens on the surface of the eye. The x-y position of the image and the depth of the sections are controlled manually. Several images of the cornea were taken within 5 minutes and no complications related to confocal microscopy were noted.

RESULTS

Clinical findings

There were 16 women (55%) and 13 men (45%) in the study group. The mean age of the patients was 56.4 \pm 17.2 years within a range of 25 to 81 years. Nine subjects (31%)

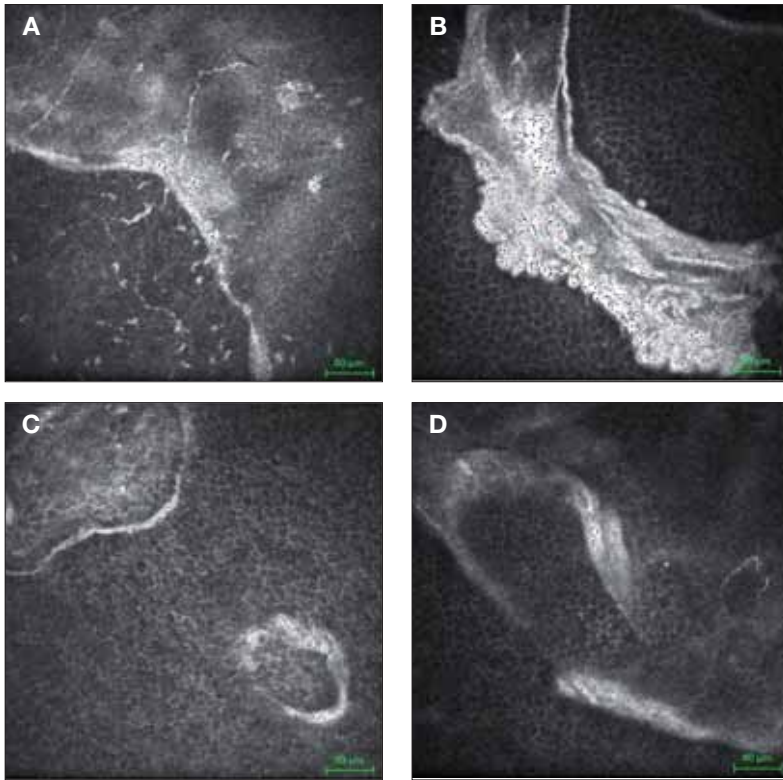


Fig. 1 - (A) A sheet of condensed, hyperreflective layer, lying over the basal epithelial layer. **(B)** Hyperreflective tissue within the basal epithelial cells. **(C)** Hyperreflective ring-like structures within the basal epithelial cells. **(D)** Hyperreflective tissue seems to roll over itself, forming a spiral pattern.

were diagnosed with EBMD previously and had history of recurrent corneal erosions. Three patients had been misdiagnosed with recurrent herpetic epithelial keratitis. Two subjects were using topical acyclovir ointment and antibiotic drops and 8 subjects were using tear substitutes only. The remaining 17 patients were asymptomatic and the disorder was identified or suspected incidentally by slit-lamp examination during routine eye examinations. Twenty-four subjects were clinically diagnosed with EBMD, whereas 5 subjects were suspected to have EBMD. Best-corrected visual acuities ranged between 20/60 and 20/20. Seven patients had typical map-dot-fingerprint pattern, 7 patients had map-dot pattern, 5 patients had dot-fingerprint pattern, 3 had only dot like findings, 3 had map pattern, and 2 had map-fingerprint pattern. A female patient (44 years of age), who presented with the complaint of recurrent corneal erosions for the last few years, and a male patient (61 years of age), who was referred to us with a diagnosis of recurrent herpetic epithelial keratitis, had bleb-like corneal dystrophy with remarkable fingerprint lines and faint map-like geographical patches. None of the patients had been treated with phototherapeutic keratectomy or anterior stromal puncture.

In vivo confocal microscopy

The superficial epithelial cell layer was considered morphologically normal in all patients. Within the intermediate and basal epithelium, a highly reflective tissue corresponding to an aberrant thickened basement membrane invaginating into the epithelium was observed in most of the eyes. The abnormal tissue represented in various configurations, such as a sheet of condensed layer lying over the basal epithelial layer (Fig. 1A), odd forms (Fig. 1B), hyperreflective ring-like structure (Fig. 1C), or in a spiral-shape pattern (Fig. 1D). There were thin, parallel hyperreflective linear structures within the epithelium (Fig. 2, A and B), which were clearly shown to extend from the basement membrane into the epithelium in tangential sections (Fig. 2C). In four patients, less hyperreflective streaks were observed and in two of them, these streaks were separating the basal epithelial cells and creating empty spaces among them (Fig. 2D). A high-contrast tissue area which might correspond to a scar tissue was also noted in this area. There were multiple high contrast round lesions in different sizes (Fig. 2, E and F) ranging between 10 and 250 μm within the epithelium. Basal ep-

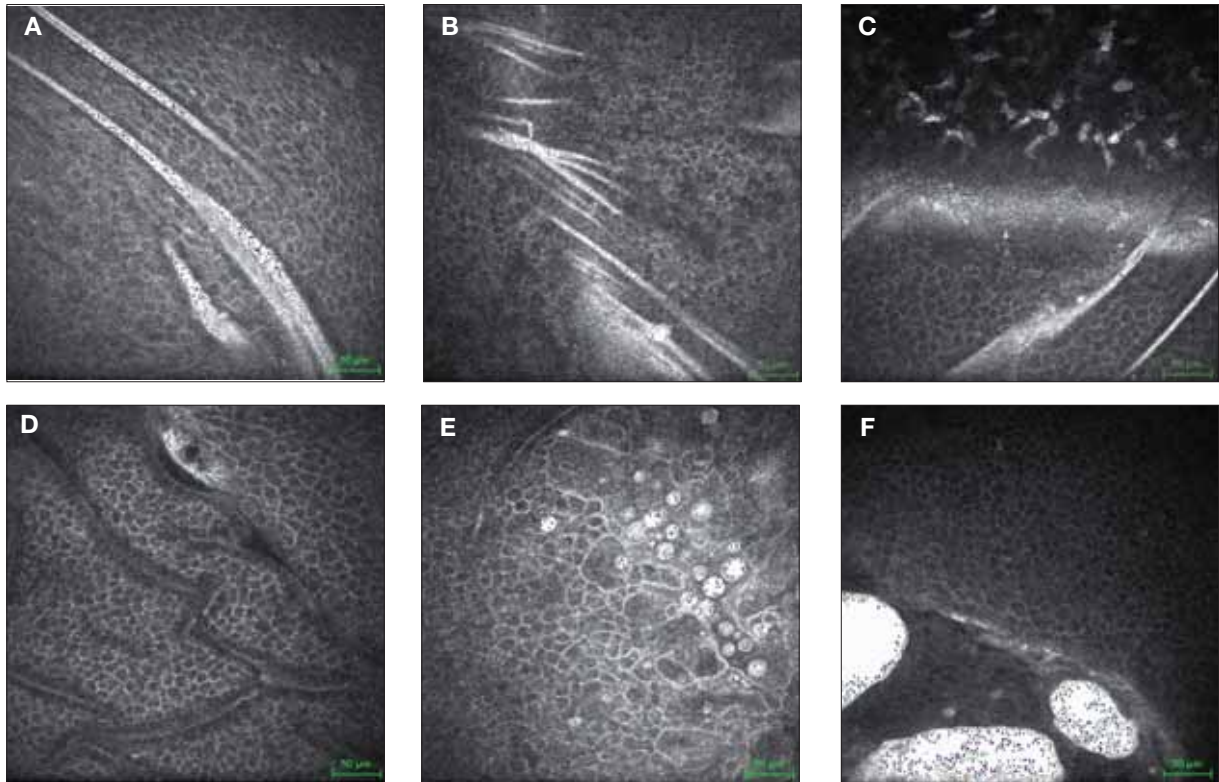


Fig. 2 - (A, B) Thin, hyperreflective parallel lines in the basal epithelium. **(C)** A tangential section, showing thin, hyperreflective linear structures extending from the basement membrane into the epithelium. **(D)** Less hyperreflective streaks with dark spaces among basal epithelial cells and hyperdense scar tissue were noted. **(E, F)** Multiple hyperreflective cystic lesions in different sizes ranging between 10 and 250 μm within the basal epithelial cells, usually located near the abnormal basement membrane. Some of the basal epithelial cells around the microcysts were highly distorted **(E)**.

ithelial cells distant from the abnormal basement membrane were normal, whereas especially in sections with microcysts, the epithelial cells seemed to be highly distorted (Fig. 2E). In two subjects with bleb-like disorder, we observed circular or oval shaped hyporeflexive areas with a diameter ranging between 40 and 100 μm at the level of basal epithelium in a depth of 50 μm from the corneal surface (Fig. 3A) and the Bowman layer (Fig. 3, B and C), accompanied by hyperreflective, parallel, linear structures extending into the epithelium (Fig. 3D), consistent with fingerprint dystrophy. In five patients, basal epithelial layer had islets of highly reflective cells, perhaps caused by intracellular deposits, and only one of the subjects was on amiodarone treatment. Only in one patient with recurrent cornea erosions, we showed highly reflective deposits intermixed with activated keratocytes in the anterior stroma. Otherwise, there were no abnormal findings in the stroma and the endothelium associated with the dystrophy.

DISCUSSION AND CONCLUSIONS

In EBMD, abnormally thickened basement membrane originating from the basal epithelial cells of the cornea was found to extend superficially into the substance of the epithelium (8, 9). Maturing epithelial cells migrating from the deeper to the superficial layers of the epithelium become entrapped beneath the sheets of basement membrane and are prevented from surfacing and discharging from the corneal surface (3). They become vacuolated and liquefied and form cysts posterior to an intraepithelial extension of the basement membrane. Maps histologically represent areas of aberrant multilaminar basement membrane within the epithelium; gray-white dots correspond to the intraepithelial microcysts, which form at various depths in the corneal epithelium and contain degenerated and necrotic cellular debris and lipid; fingerprints are curvilinear clusters of reduplicated and

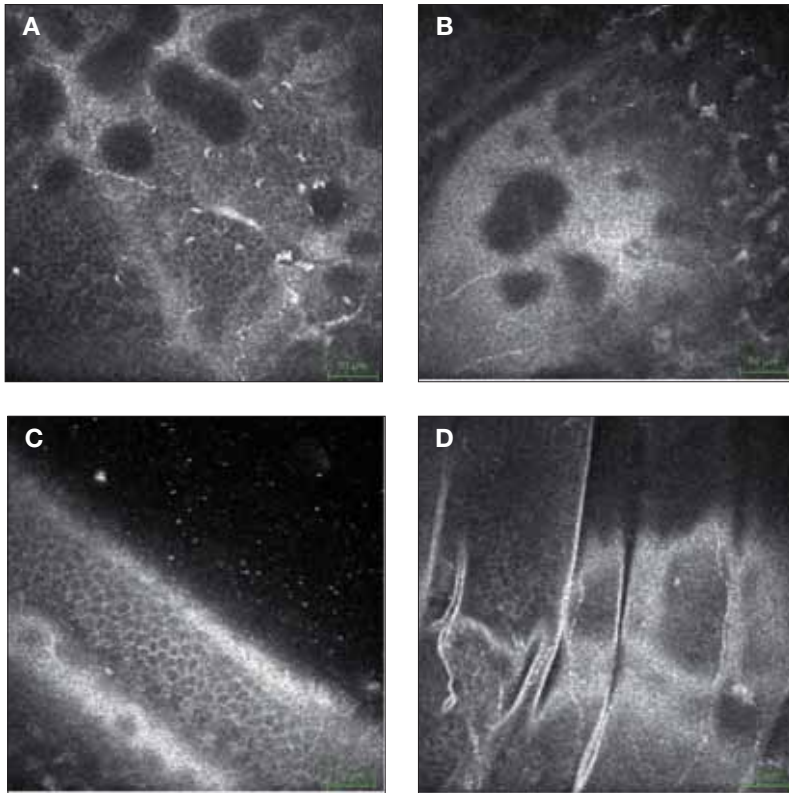


Fig. 3 - Circular or oval hyporeflective areas with a diameter ranging between 40 and 100 μm at the level of basal epithelium in a depth of 50 μm from the corneal surface (**A**) and the Bowman layer (**B, C**), accompanied by hyperreflective, parallel, linear structures extending into the epithelium (**D**).

parallel rows of thickened basement membrane; and blebs are localized deposits of a fibrogranular material between the epithelium and Bowman layer. The morphology of EBMD was examined by confocal microscopy (17-19). Hernandez-Quintela et al (17) examined five patients with EBMD using Confoscan Model P2 (Tomey, Erlangen-Temmenlohe, Germany) and found a thickened basement membrane, extending into the epithelium as a dense reflective linear structure, highly reflective irregular material in the posterior epithelium and the anterior stroma, abnormal basal epithelial cells with distended cytoplasm and very reflective nuclei, epithelial cystic lesions with sizes ranging between 50 and 400 μm , and parallel linear structures below the epithelium. Rosenberg et al (18) examined eight patients with EBMD with tandem scanning confocal microscope. They found islets of highly reflective cells with presumed intracellular deposits, drop-shaped configurations, or streaks in the basal epithelium, folding of the Bowman layer, activation of anterior keratocytes, and various pathologic findings in subbasal nerve plexus; however, they could not observe epithelial cystic lesions described by Hernandez-Quintela et al (17). Labbé et al (19) evaluated 22 patients

with EBMD with HRT II RCM and showed abnormal epithelial basement membrane protruding into the corneal epithelium, epithelial cell abnormalities, and microcysts in all of the eyes. No abnormalities could be found in superficial epithelial cells or the stroma. They could not show any activation in keratocytes or deposits in the anterior stroma and concluded that they might miss the abnormal areas, since an optical section has an area of only 400x400 μm (19).

In all of our patients, we observed an abnormal basement membrane showing extension into the epithelium as a highly reflective layer ending in strange configurations forming the map component of the disease. There were round, high contrast lesions in different depths of epithelium with sizes ranging between 10 and 250 μm corresponding to microcysts in histology and gray-white dots in clinical examination. We also noted thin, parallel hyperreflective lines, which presumably correspond to fingerprint pattern in EBMD. Basal epithelial cells around abnormal basement membrane and microcystic lesions seemed to be highly distorted and enlarged with dark, empty spaces among them. In all of the subjects, we showed the typical confocal findings which further con-

firmed the diagnosis. In subjects with suspected epithelial basement dystrophy, the abnormal basement membrane and cysts were found in a limited area; therefore, we had to take pictures from various areas to catch those figures. To our knowledge, there are no published reports about the in vivo confocal microscopic appearance of bleb-like corneal disorder. Bleb-like corneal disorder is a lesser-known entity among the corneal dystrophies and should be considered in the differential diagnosis of microcystic corneal diseases, such as Meesmann epithelial cornea dystrophy. In bleb-like dystrophy, localized deposits of a fibrogranular material between the epithelium and Bowman layer and intrusion of this low refractive material into the epithelial layer produce an appearance on retroillumination identical to that of intraepithelial cysts (20). Using in vivo confocal microscopy, we showed hyporeflexive, circular, or oval structures at the level of basal epithelium and the Bowman layer (Fig. 3, A-C). Bleb-like degeneration is a subepithelial condition and hyporeflexive features in the epithelium (Fig. 3A) might be explained by the circular structures within the thickened basement membrane and the Bowman layer (Fig. 3C), which push the basal epithelial cells forward past the plane of focus of confocal microscopy. In both subjects, a concomitant fingerprint dystrophy was observed by slit-lamp biomicroscopy and confocal microscopy. EBMD may sometimes be associated with recurrent corneal erosions. Faulty adhesion of the epithelium to the basement membrane might predispose to recurrent cornea erosions, which might be due to the abnormal

basement membrane and hemidesmosomes. Unfortunately, in vivo confocal microscopy cannot provide visualization of subcellular structures such as hemidesmosomes due to limited resolution. In two patients with recurrent cornea erosions, we were able to demonstrate less hyperreflective streaks separating the basal epithelial cells and creating empty spaces among them, which might be the weak area of the cornea leading to erosion and hyperreflective tissue might be the fibrotic scar area after the healing process.

EBMD is most commonly asymptomatic and undiagnosed; however, it might be associated with recurrent corneal erosions and lead to severe complications after LASIK surgery. The images obtained with HRT II RCM are highly characteristic for EBMD and consistent with the histologic features of the disease; therefore, confocal microscopy seems to be a valuable tool in diagnosis of EBMD.

The authors have no proprietary interests or research funding and the work has not previously been presented at a meeting.

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