Corneal and Conjunctival Manifestations in Fabry Disease: In Vivo Confocal Microscopy Study

LEONARDO MASTROPASQUA, MD, MARIO NUBILE, MD, MANUELA LANZINI, MD, PAOLO CARPINETO, MD, LISA TOTO, MD, AND MARCO CIANCAGLINI, MD

• PURPOSE: To describe the microscopic corneal and conjunctival findings in patients with Fabry disease (FD) related keratopathy by using in vivo confocal microscopy.  
• DESIGN: Prospective observational case series.  
• METHODS: Twelve eyes of six patients affected by Fabry disease, belonging to two different families, underwent in vivo confocal microscopic examination. Corneal and conjunctival morphology were assessed by means of a scanning slit corneal confocal white-light microscopy and confocal laser-scanning microscope.  
• RESULTS: Confocal microscopy examination evidenced two different types of corneal epithelial changes. The three hemizygous patients presented bright hyper-reflective intracellular inclusions located within the basal epithelial cells, while the three heterozygous patients showed fine diffusion of reflective substance at the level of superficial, basal epithelial cells and basal membrane, in all eyes. The complex basal-Bowman’s membrane appeared irregular, distorted, and nonhomogeneous in all subjects. Stromal increased reflectivity attributable to haze and epithelial ingrowth with bright intracellular inclusions was noticed in one hemizygous patient. In all patients, conjunctival epithelial involvement represented by bright roundish intracellular inclusions was evidenced, appearing more pronounced in tarsal than in bulbar conjunctiva.  
• CONCLUSIONS: Although FD-related cornea verticillata attributable to glycosphingolipids accumulation is considered to be primarily a corneal disease, in vivo confocal microscopy demonstrated structural alterations throughout the entire ocular surface epithelia. It is still unclear whether the different type of corneal epithelial lesions observed for hemizygous and heterozygous patients is related to different physiopathological mechanisms. Confocal microscopy may assist ophthalmologists in the diagnosis of FD-related ocular surface and corneal manifestations. (Am J Ophthalmol 2006;141:709–718. © 2006 by Elsevier Inc. All rights reserved.)

FABRY DISEASE IS AN X-LINKED GENETIC DISORDER with multiple organ dysfunction attributable to a congenital error in glycosphingolipid metabolism and is characterized by angiokeratoma (telangiectatic cutaneous lesions), hypohidrosis, acroparesthesia, and ocular abnormalities and vascular lesions that involves kidney, heart, and brain; this progressive vascular involvement represents the first cause of death for Fabry disease.¹

This syndrome is determined by the deficient activity of α-galactosidase A, a lysosomal enzyme, that causes an error of glycosphingolipid metabolism² and more than 200 responsible gene mutations have been reported to date.³ The consequent inability to catabolize sphingolipid globotriaosylceramide (GL-3) leads to the accumulation of this undergraded neutral glycosphingolipid in plasma and lysosomes throughout the body⁴ involving vascular endothelial cells and ultimately determines renal failure and ischemic manifestations in brain, kidneys, heart, and cochlea.¹,⁵

Ocular manifestations are also a frequent finding in Fabry disease (FD) and include conjunctival vessel microaneurysm and granular storage material within the vessel walls,⁶–⁸ cataract formation or crystalline posterior capsule linear deposits,⁶,⁸ retinal vascular tortuosity,⁶,⁸ and corneal lesions. Cornea verticillata and stromal haze are the most characteristic and frequent ocular findings.⁶–¹⁰ Nevertheless ocular manifestations are not constant in all patients with FD and, moreover, the severity of ocular and, particularly, corneal findings is highly variable among heterozygotes and hemizygotes.⁵,⁷ Cornea verticillata may occur early in life during the course of Fabry disease,⁶,¹¹ presenting as a whorl-like subepithelial opacity appearing at biomicroscopic examination as yellow or brown whirling lines, mainly involving the inferior part of the cornea.⁷

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Corneal differential diagnosis includes other diseases causing cornea verticillata, such as lipidosis, sphingolipidosis, mucopolysaccaridosis, endothelial congenital hereditary dystrophy, and amiodarone-induced keratopathy.10,11 Various histopathologic and ultrastructural changes present in the cornea of patients affected by FD have been reported in literature12–16 and include intracellular deposits within the epithelial layer12–16 and reduplication of the basal lamina of the epithelium.12,15

The purpose of this study was to evaluate micromorphological alterations using laser and white-light confocal microscopy in vivo examinations of corneal tissue and conjunctival epithelium in patients with FD.

**METHODS**

- **PATIENTS:** We examined 12 eyes of six patients (three men and three women belonging to two different families, age range 20 to 64) affected by FD (Table 1), referred to our Cornea and Ocular Surface Division of the Ophthalmic Clinic (University “G. d’Annunzio”, Chieti-Pescara, Italy). The study was approved by the University Institutional Review Board, and patients provided written informed consent before being enrolled. A detailed history was obtained for each patient.

In all cases, the diagnosis of FD had been previously determined on the basis of clinical features, biochemical evidence of decreased α-galactosidase A enzyme activity, and specific gene analysis. Plasmatic α-galactosidase A activities were also measured in patients with very low plasma enzyme activity. In all patients examined, the same α-galactosidase A gene (GLA) mutation (S78X) was detected: all patients belonged to two families that reside in the same small town. Gene analysis confirmed the

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**TABLE 1. Clinical Data on the Members of the Two Families Affected by Fabry Disease**

- **OD** = right eye; **OS** = left eye.
diagnosis of FD in heterozygous female patients. None of the patients presented a history of any other disease or drug use which could be responsible for corneal changes.

A complete ophthalmologic examination, including anterior segment slit-lamp biomicroscopy, visual acuity evaluation, applanation tonometry, corneal topography, and ophthalmoscopy, was performed in all patients.

Patient 1: a 43-year-old hemizygotes affected male (index patient A) presenting systemic manifestations including typical cutaneous angiokeratoma, proteinuria, moderate-stage heart failure, and hearing loss. Since April 2001, the patient was under treatment with fortnightly infusions of algsidase alfa recombinant enzyme (Replagal, Transkaryotic Therapies, Cambridge, Massachusetts, USA). Ophthalmologic findings included bilateral cornea verticillata, lens opacity, and retinal vessel tortuosity.

Patient 2: a 45-year-old heterozygous woman (index patient A sister) presenting clinically relevant signs of neuropathic pain and heart failure. She was under treatment with recombinant enzyme replacement therapy infusions of algsidase alfa recombinant enzyme (Replagal, Transkaryotic Therapies, Cambridge, Massachusetts, USA) since April 2004. Ocular changes were bilateral cornea verticillata and conjunctival vessel tortuosity.

Patient 3: a 20-year-old heterozygous girl (index patient A niece) presenting neuropathic pain and heart failure. She was under treatment with recombinant enzyme replacement therapy infusions of algsidase alfa recombinant enzyme (Replagal, Transkaryotic Therapies, Cambridge, Massachusetts, USA) since May 2004. Ocular findings included conjunctival vessels anomaly, cornea verticillata, lens opacity, and retinal vessel tortuosity.

Patient 4: a 37-year-old hemizygotes affected male (index patient B) presenting multiple organ involvement represented by cutaneous angiokeratoma, heart failure, cerebrovascular disease (transient ischemic attack, TIA), and auditory symptoms (hearing loss). The patient was treated with recombinant enzyme replacement therapy infusions of algsidase alfa recombinant enzyme (Replagal, Transkaryotic Therapies, Cambridge, Massachusetts, USA) since February 2004. Ocular signs were conjunctival vessels anomaly, cornea verticillata, lens opacity, and retinal vessel tortuosity.

Patient 5: a 64-year-old heterozygous woman (index patient B mother) presenting systemic clinical manifestations including heart failure and acroparesthesia. She was under treatment with animal derived algsidase beta enzyme (Fabrazyme, Genzyme, Cambridge, Massachusetts, USA) since May 2004. Ocular findings included conjunctival vessel tortuosity and cornea verticillata.

Patient 6: a 42-year-old hemizygotes affected male (index patient B cousin) presenting multi-systemic symptoms including proteinuria and neuropathic pain. He was treated since September 2002 with recombinant enzyme replacement therapy infusions of algsidase alfa recombinant enzyme (Replagal, Transkaryotic Therapies, Cambridge, Massachusetts, USA). Ocular changes included typical bilateral whorl-like corneal opacity and conjunctival vessel tortuosity.

Each patient was examined using both a scanning slit corneal confocal white-light microscopy (Confoscan 3, Nidek Technologies, Padova, Italy) and a recently introduced digital corneal confocal laser-scanning microscope (LSM) (HRT II Rostock Cornea Module, Heidelberg Engineering GmbH, Heidelberg, Germany). Confoscan 3 was used to study the central corneal morphology and to assess layer reflectivity and cellular density while the HRT II LSM was used to evaluate central and peripheral corneal tissue and conjunctival epithelium morphology.

The technical characteristics and examination methods for the Confoscan 3 were previously described. The center of the cornea was studied in all examinations and two complete confocal analyses of the entire central cornea were performed for each eye. The main parameters for the acquisition sequence of each complete corneal examination were set using three passes, and a z-axis range of movement of 1000 µm, thus giving a theoretical z-axis distance between images in the scans of 10 µm. Each frame is approximately 400 × 300 µm (area of 0.12 mm²). The position on the z-axis of the corneal thickness of each image was obtained using the Z-Scan function of the instrument. The Z-Scan is a graph showing the depth (expressed in microns) on the x-axis and the level of reflectivity (expressed in arbitrary numerical units called light reflectance units, LRU) on the y-axis, of each corneal image included in the scan. A reflectivity index was calculated for superficial and basal epithelial layers and for anterior and posterior stroma (depth range from 50 to 100 µm and from 300 to 400 µm from the basal lamina of the epithelium, respectively). The reflectivity values obtained using the Z-Scan function were calculated by averaging five values obtained from reliable Z-Scan curves during which the illumination lamp source was kept.
constant to obtain comparable reflectivity values between different patients. Mean keratocyte density of anterior and posterior stroma was calculated using the analysis software provided with the instrument, which allows a manual cell count within a selected region of interest (ROI) of standard dimensions (0.1 mm²). The keratocyte cell density values of the anterior (depth range 50 to 100 μm from the basal lamina of epithelium) and rear stroma (depth range 300 to 400 μm from the basal lamina of epithelium) were obtained by averaging five analyses for each eye. Mean endothelial cell density was calculated as the mean of three manual cell counts within a 0.1 mm² standardized ROI in the central cornea obtained by using the Confoscan 3 dedicated analysis software.

HRT II Rostock Cornea Module LSM is a confocal laser scanning device equipped with a water immersion objective (Zeiss, Oberkochen, Germany 63×/N.A. 0.95 W). The wavelength of the beam emitted from a diode-laser is 670 nm. HRT II LSM technical characteristics and examination methods were previously described.18 The theoretical confocal section thickness of the LSM is approximately 10 μm and images are 300×300 μm in size. The eyes were properly aligned for the HRT II examinations by using a dedicated target mobile red light provided with the instrument that the patient used for fixation with the contralateral eye, permitting the acquisition of tangential optical sections of the center and the periphery of the cornea and bulbar conjunctiva. 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. Sequential images derived from automatic scans and manual frame acquisitions throughout the central and peripheral cornea (superior, inferior, nasal, and temporal quadrants, approximately 2 mm internal to the limbal edge), and bulbar conjunctiva at approximately 2 mm from superior, inferior, nasal, and temporal limbus were obtained for each examined eye. All patients also underwent HRT II LSM examination of the tarsal conjunctiva. After eversion of the upper eyelid, the objective lens was properly aligned to maintain a correct contact with the tarsal conjunctiva, and manual image acquisitions of different areas of conjunctival epithelium were performed.

RESULTS

● CENTRAL CORNEA: The central corneal findings of in vivo confocal microscopy are summarized in Table 2. An abnormal morphology of the corneal epithelial layer was observed using in vivo confocal microscopy (IVCM) in all patients. Two different types of epithelial presentation, which distinguished males from females, were seen in this study. A bright hyper-reflective intracellular inclusion located within the basal epithelial cells was the main epithelial pathologic feature in males (Figure
This characteristic was observed in all eyes of patients 1, 4, and 6, and appeared to homogeneously involve the basal epithelium throughout the examined areas. In all eyes, superficial epithelial cells appeared irregular, highly reflective with hardly discernible borders, and large bright desquamating cells were also observed. Additionally, some intracellular hyper-reflective inclusions, presenting as granular cytoplasmatic deposits, were evidenced within superficial cells (Figure 2). In all eyes of male subjects, the basal-Bowman’s membrane complex appeared irregularly distorted characterized by hypo-reflective striae and nonhomogeneous structure inducing folding of the basal epithelial layer and basal membrane (Figure 2).

A different pattern of epithelial involvement was observed in all eyes of female patients. Patients 2, 3, and 5 showed the presence of a fine diffusion of a reflective substance at the level of superficial, basal epithelial cells and basal membrane (Figure 3), which presented nonhomogeneous distribution of substance accumulation in the central cornea, resulting in areas of intense reflectivity interspersed between hyporeflective normal epithelial zones. The mean basal epithelial reflectivity was similar to the superficial epithelial reflectivity, though always lower than the values recorded for male subjects (Table 2). Basal and Bowman’s membrane appeared distorted with irregular foldings (Figure 3).

Subepithelial nerve fibers presenting as thin reflective fibers, but with normal appearing nerve fiber density were seen in all patients. Anterior stromal reflectivity and anterior stromal keratocyte morphology and density appeared normal in all but one male patient (patient 6) who presented evident bilateral pathologic involvement of the anterior stroma with diffuse accumulation of hyper-reflective extracellular substance of a nonhomogeneous density within the extracellular matrix and presence of needle-shaped bright inclusions (Figure 4). Moreover, this patient bilaterally presented well defined epithelial ingrowths within the most anterior stroma associated with Bowman’s membrane disruption at the same site (Figure 4). Morphologically, the areas of epithelial protrusions involving the Bowman’s membrane and the stroma presented the same features of bright intra-cytoplasmatic inclusions within basal epithelial cells (Figure 4). In this patient, anterior stromal reflectivity was markedly increased and keratocyte density lower compared with the other patients (Table 2).

Posterior stromal and endothelial layers in all examined eyes, as reported in Table 2, showed normal morphology, cellular density, and reflectivity.

**Peripheral Cornea:** Pathologic microscopic findings observed for the peripheral cornea in the examined patients were limited to epithelial layers. The different patterns of epithelial involvement between male and female patients observed in the central cornea were maintained for peripheral corneal epithelium. The inferior quadrant showed a greater presence of epithelial inclusions with respect to nasal and temporal areas, while a lower epithelial involvement was observed in the superior quadrant in all examined eyes (Figure 5).

![Figure 2](image-url)
CONJUNCTIVA: Conjunctival microstructural observations are reported in Table 2. Bulbar conjunctival epithelium was imaged in all cases. The same features of conjunctival epithelial involvement were observed for both male and female patients. Epithelial morphology appeared irregular, with poorly distinguishable cell borders and presence of reflective nonhomogeneous material, widely distributed or interspersed between areas of normal cellular architecture presenting less reflective epithelium (Figure 6).

Tarsal conjunctival epithelium was visualized in all patients and presented the most evident pathologic findings. The presence of roundish hyper-reflective intracellular structures involving most of the cells was the main observed feature (Figure 6). The pattern of tarsal conjunctival epithelial lesions between female and male subjects did not differ. The analysis of different areas of tarsal conjunctiva typically showed two different types of epithelial distribution of the reflective inclusions: (1) papillary distribution and (2) columnar distribution following the conjunctival epithelial folding (Figure 6).

DISCUSSION

OCULAR FINDINGS ARE CONSIDERED A HALLMARK OF FD, and in some cases, result in diagnosis. Cornea verticillata, defined as a brown epithelial whorl-like opacity, is the most typical and frequent ocular manifestation in FD.

FIGURE 3. In vivo confocal microscopic findings of central corneal epithelial pathologic features in patient 5, a 64-year-old heterozygotes female patient affected by Fabry disease. White light microscopy of superficial (Top left) and basal (Top right) epithelial layers in central cornea showed a fine diffusion of reflective substance. The same micro-morphologic features were evident in laser scanning confocal microscopy examination: superficial epithelial cells appeared irregular and highly reflective (arrows) (Middle left) and basal epithelial and layer showed a diffuse accumulation of hyper-reflective substance (arrows) (Middle right) which presented an irregular distribution leading to areas of intense reflectivity (arrow) near areas of normal epithelium (arrowhead) (Bottom left). Basal and Bowman’s membrane layers evidenced focal alterations and areas of distortion (arrow), through which portions of subepithelial nerve fibers were visible (arrowheads) (Bottom right). Bar represents 100 µm.

FIGURE 4. In vivo confocal microscopic findings of stromal involvement observed in patient 6, a 42-year-old hemizygotes male affected by Fabry disease. Laser scanning confocal microscopy images showed areas of Bowman’s membrane disruption; stromal keratocytes were visible in the upper part of the image where Bowman’s membrane with nerve fibers was interrupted (arrow), adjacent to the curve basal epithelial edge (arrowheads) (Top left), associated with well defined epithelial ingrowth in the anterior stroma (arrowheads) (Top right to Bottom left), in which the same roundish intracellular accumulation of hyper-reflective substance observed in basal epithelial layer was visible. White light confocal microscopy showed pathologic stromal increased reflectivity attributable to extracellular haze (Bottom right). Bar represents 100 µm.
The whorl-like lines are related to deposition and accumulation of glycosphingolipids in the epithelial and subepithelial layer of the cornea near or at the level of Bowman's membrane.\(^{8,10,12}\) The whorl-like opacities are typically cream-colored, but they can range from white to brown, or appear very faint, presenting various shapes and distribution patterns,\(^{10}\) and generally do not impair visual function. This corneal feature is also considered a marker of carrier status in heterozygous females.\(^{9,10}\) Nevertheless, cornea verticillata may vary considerably in frequency and severity among female carriers and affected male patients.

In the series published by Sher and associates,\(^{10}\) cornea verticillata was present in 94% of the eyes of hemizygous and in 88% of heterozygous patients. Nguyen and associates\(^{8}\) reported similar data observing and incidence of 94% and 72% of cornea verticillata, in hemizygous and heterozygous, respectively. Conversely, in the series published recently by Orssaud and associates,\(^{6}\) cornea verticillata was observed in only 43.7% of the eyes of hemizygous patients and was always associated with a subepithelial corneal haze, which appeared as the most frequent corneal finding (84.3% of eyes). Furthermore, the same authors observed a different type of corneal lesion in FD, described as isolated brown lines in the subepithelial layers (occasionally reported\(^{19}\)), in 28% of the eyes in their series.

In this study, we evaluated six patients belonging to two different families affected by FD in whom the same \(\alpha\)-galactosidase A gene (GLA) mutation had been detected. Three hemizygous patients presented cornea verticillata bilaterally and one (patient 6) had concomitant
bilateral subepithelial haze. Two female heterozygous patients presented cornea verticillata, while the third female patient (the youngest) presented a normal cornea at biomicroscopic examination (patient 3).

Both white-light and laser scanning in vivo confocal microscopy examinations were performed in these patients to assess corneal and conjunctival tissues morphology. In fact, in vivo white-light confocal microscopy has been widely used in the recent years as a valid diagnostic technique for the “in vivo” microscopic imaging of corneal structure, both in healthy and pathologic corneas.20,21 Recently, laser confocal microscopy was employed to assess conjunctival epithelial morphology with a greater precision and reliability than white-light microscopy.22

In our study, central corneal microscopic morphology was analyzed and quantitative data (that is, cell density and sublayers reflectivity) were obtained using white-light confocal microscopy, while qualitative micro-morphologic evaluation of central and peripheral cornea, bulbar, and tarsal conjunctiva was performed using laser scanning confocal microscopy.

Our results indicate that two different (apparently, gender-dependent) microscopic patterns of epithelial changes can be observed in FD-related cornea verticillata. Hemizygous affected males disclosed the presence of highly reflective intracellular roundish and well defined inclusions distributed almost homogeneously within the basal epithelial cell layer. Superficial epithelial cells also appeared to be affected showing an increased reflectivity of cytoplasm and undefined cell borders, but without the features of distinct intracellular bright inclusions. Conversely, all heterozygous carrier females showed epithelial lesions characterized by a discontinuous reflective diffusion (which we called “fine diffusion”) of material within all epithelial layers, clearly different from the hemizygous typical pattern. These differences were not dependent in any way on the family relationship and on age. Additionally, the 20-year-old female heterozygous patient (patient 3), in which corneal examination at slit-lamp appeared normal, showed at confocal microscopy examination the same pattern of epithelial changes, although less pronounced, observed for the other heterozygous. It is possible that no signs of cornea verticillata were visible at slit-lamp biomicroscopy while confocal microscopy, because of its greater magnification and resolution, was capable of showing early preclinical changes in the epithelium of this patient. Because of the fact that α-galactosidase A activity is variably reduced but not absent in heterozygous patients23 and that onset of symptoms is usually delayed, it has been proposed that corneal manifestations are limited to the first stage of involvement (cornea verticillata) in female carriers with reduced but residual enzyme activity and the progression to the stage of haze is rarer.6 Our results however indicate that heterozygous-related corneal involvement may not be an earlier stage than that seen in hemizygous. In fact, although the 20-year old female patient presented less microscopic signs of epithelial involvement with respect to the other older females (one belonging to the same family), the pattern of changes was clearly similar and, moreover, the 64- and 45-year-old heterozygous (patients 5 and 2, respectively) maintained the same type of corneal changes, different from the hemizygous features, although advanced-stage cornea verticillata was evident at biomicroscopy.

The analysis of average epithelial sublayer reflectivity also showed differences in epithelial changes between hemizygous and heterozygous, indicating that basal epithelial reflectivity in hemizygous was similar (in one patient) or greater (in both eyes of two patients) than the superficial epithelial reflectivity. While in all eyes of heterozygous patients, sublayer epithelial reflectivity levels were similar; and in every case, they were lower than the values found for males. These observations, especially for the basal epithelium, were correlated with the microscopic morphology of the two different kinds of lesion (highly reflective granules for males and fine dusty reflectivity for females).

Confocal microscopic analysis indicated that although the inferior part of the cornea showed the more evident pathologic findings of cornea verticillata at slit-lamp examination, the other quadrants of peripheral cornea presented the same type, but with a lower degree, of epithelial involvement. Previous clinical studies reported that the corneal accumulation of glycosphingolipids leading to whorl-like opacities in FD may also occur at the level of the Bowman’s membrane.5,10,19 Histopathological analysis of corneal tissue in FD-related cornea verticillata reported granular cytoplasmic inclusion bodies, mainly located in the apical part of basal epithelial cells12,16 similar to those observed in chloroquine keratopathy and amiodarone keratopathy,13 and regional reduplication of the basal lamina associated with differences in thickness of the subepithelial area.12,15 In the cases presented in our study, the basal-Bowman’s membrane complex appeared irregularly distorted with increased reflectivity, inducing folding of the overlying basal epithelium, evidencing strie containing nerve fibers bundles. These findings were consistent with thickening or regional reduplication of basal-Bowman’s membrane, potentially associated with accumulation of material within the extracellular tissue. Although basal epithelial changes observed in our series of patients affected by FD presented characteristics similar to those observed with in vivo confocal microscopy for other pathologies inducing cornea verticillata, such as amiodarone-induced keratopathy,24 the alterations seen in the subepithelial layer in FD seem to be typical.

One patient presented bilateral epithelial ingrowth in the anterior stroma and the epithelial protrusions showed the same characteristic basal epithelial changes, with roundish reflective intracellular inclusions, found in hemizygous patients. In this case, the anterior stroma appeared hazy, with increased stromal reflectivity. It is possible to hypothesize that considerable alteration of the Bowman’s
membrane may lead to epithelial ingrowth into the stroma, inducing a more complex system of anterior corneal changes attributable to glycosphingolipids accumulation in FD. However, all the other examined eyes of hemizygous and heterozygous patients yielded normal reflectivity and keratocyte density of both the anterior and rear stroma, and the endothelial layer did not appear to be involved in any of the subjects.

To date, conjunctival manifestations described in FD are represented by the clinical finding of vascular abnormalities presenting as vascular aneurismal dilation and increased vessel tortuosity. Histopathological studies reported that the presence of intracytoplasmic deposits may be observed not only in the corneal epithelium but also in the epithelium, goblet cells, endothelium, and mural cells of blood vessels in the conjunctiva. In this study, the conjunctival epithelium analyzed in vivo by means of laser scanning confocal microscopy disclosed evident alterations represented by the presence of reflective inclusions apparently with intracellular location. In the bulbar conjunctiva, these features, although ranging from minor to marked, were present in all subjects without differences in pattern morphology between hemizygous and heterozygous patients. Interestingly, the same type of epithelial lesion was found in the tarsal conjunctiva, and intracellular epithelial reflective inclusions were distributed diffusely following the columnar pattern of conjunctival crypts and folding or the papillae. Tarsal conjunctival involvement appeared greater in severity than bulbar conjunctival manifestation. Conjunctival changes appeared less pronounced in heterozygous patients. These bulbar and tarsal conjunctival epithelial alterations were similar in appearance to the changes observed for corneal epithelium, and were consistent with intracellular deposits of glycosphingolipids, which represent a novel finding in FD. Whether conjunctival and corneal epithelial substance accumulation are of the same nature must still be determined, considering that the mechanism of formation of cornea verticillata in FD, especially since light and electron microscopy histopathologic findings appeared variable in different reports, is still unknown.

The hypothesis which best describes the reported results is that lipids, such as sphingolipids, are deposited in the epithelial and subepithelial area of the cornea in FD, and that variation of density of these accumulations leads to the typical whorl-like superficial opacity. However, the physiopathological meaning of the differences in microscopic confocal morphology of corneal epithelial lesions observed between hemizygous and heterozygous subjects in our study is still unclear.

In conclusion, our study documented for the first time in vivo confocal microscopic alterations of the cornea and conjunctiva in FD, evidencing that the entire ocular surface epithelial structures appeared to be involved in intracellular deposition processes (cornea verticillata presumably being the “tip of the iceberg”) and that basal and Bowman’s membrane were altered, possibly leading to the formation of pathologic epithelial ingrowth and stromal haze. These observations suggested that white-light and laser confocal microscopy are valuable diagnostic procedures for the microstructural assessment of the living cornea and conjunctival epithelium, and could be useful to ophthalmologists in facilitating the diagnosis of FD-related ocular surface manifestation. Moreover, it is possible to presume that in vivo confocal microscopy may be an important method to perform quantitative follow-up of ocular surface lesions in FD, and to detect variations while monitoring the effect of enzyme replacement therapy. However, further studies on a larger sample of patients are needed.

REFERENCES


Biosketch

Leonardo Mastropasqua, MD, born in Barletta, Italy in 1954. He was a unconfirmed University Researcher for the Ophthalmology Clinic from 1983–1986. He then became a confirmed University Researcher in 1992. In 1996, Dr Mastropasqua became an Associate Professor. Currently, Dr Mastropasqua is a Full Professor, Chair of Diseases of the Visual Apparatus, and Faculty of Medicine and Surgery. He has performed more than 10,000 operations on the globe. His fields of clinical and research interests are refractive surgery, cataract, glaucoma, corneal diseases, and surgery.