

MICROSTRUCTURAL EVIDENCE OF THE ANTERIOR OCULAR SURFACE ALTERATION IN PATIENTS WITH PTERYGIUM

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ABSTRACT

PURPOSE: To study microstructural changes in cornea and conjunctiva of patients with pterygium using in vivo laser scanning confocal microscopy (LSCM).

METHODS: Seventy eyes of 64 patients with pterygium and 70 eyes of 70 healthy subjects were examined. Using LSCM in vivo images at microstructural level were captured. The density of keratocytes in the anterior and posterior stroma and basal corneal epithelial cells and the density of specific cellular structures of the pterygium were calculated. Comparative analysis of the central cornea and nasal bulbar conjunctiva of the control subjects was performed.

RESULTS: Density of basal corneal epithelial cells and anterior keratocytes in corneas with pterygium was determined at: 4678 ± 41 cells/mm² and 424 ± 17 cells/mm² respectively, which was lower than that in the controls. Dendritic cells with density 115 ± 11 cells/mm² were found in regions of pterygia. The dendritic cell density in the pterygium was found higher than the density in the nasal bulbar conjunctiva of the healthy eyes. Morphologic alterations of the sub-basal nerve plexus were observed in pterygium, as well as bright deposits at the level of the basal epithelial layer and also hyperreflective spots in the surface epithelium, corresponding to the clinically observed Fuchs' flecks.

CONCLUSIONS: In vivo LSCM is method of choice for dynamic evaluation of the morphologic alterations of pterygium, allowing precise diagnosis, prognosis and monitoring of this disease. Study highlighted microstructural alterations and increased dendritic cells presumed to be characteristic of pterygium and adjacent to it clear cornea.

Key words: *Pterygium, confocal microscopy, cornea*

INTRODUCTION

Pterygium is a pathological alteration of the conjunctiva and the cornea that is most frequently seen as a mass at the medial canthus, growing towards and invading the clear cornea (1). It is an associated with UV and blue light exposure degenerative lesion (11,14). Pterygium is common in many parts of the world and is particularly prevalent in equatorial regions and high altitudes. Anti-apoptotic and immunologic mechanisms, growth factors, cytokines, extracellular matrix modulators, genetic factors and viral infections have also been associated

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with the development of pterygia (2,3,10). Pathogenesis of pterygium remains unclear, despite the existing evidence, based primarily on histological studies.

Previous studies are performed using surgically excised tissue samples and due to tissue processing and other artefacts did not allow for analysis of the microstructural alterations of both pterygia and the adjacent clear cornea of the human eye. Moreover animal models of pterygium appear to be impossible to create.

Increasingly, *in vivo* confocal microscopy is being used to discern tissue microanatomy at a resolution previously only achieved by conventional histology. Superficial ocular tissues can be observed at cellular level by this non-invasive imaging method. Its unique physical properties enable microscopic examination of all layers of the cornea *in vivo* and have been used to investigate numerous corneal diseases. It offers a new approach to study the physiological reactions of the cornea to different stimuli and the pathophysiologic events leading to corneal dysfunction in certain diseases. A few studies are performed using LSCM to observe pterygium *in situ*, they demonstrate potential uses of LSCM in pterygium research and suggest the role of dendritic cells in pathogenesis of pterygium (1).

Currently, *in vivo* studies of morphological alterations of pterygia remain limited. In this study we sought to perform a comprehensive investigation of the morphologic characteristics of the pterygium and the adjacent cornea, allowing precise diagnosis, prognosis and monitoring of this disease. Using LSCM we also described and evaluated some specific features at microstructural level such as dendritic cells and changes in density of corneal epithelial cells and keratocytes in cornea invaded by pterygium.

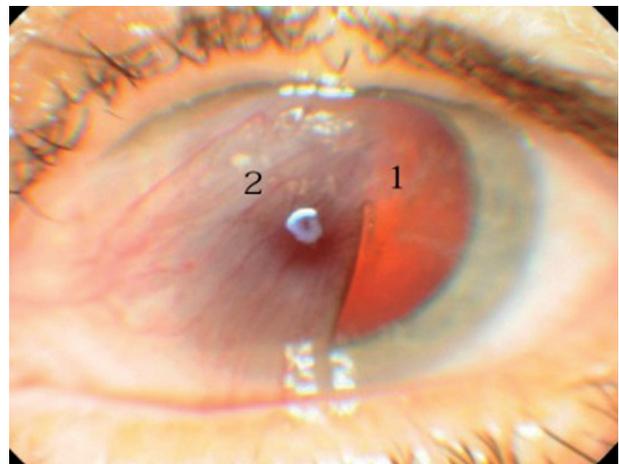
MATERIALS AND METHODS

Seventy eyes of 64 patients with pterygium were examined. As controls were included 70 eyes of 70 age-matched healthy subjects. Patients with known systemic diseases associated with ocular surface disease, a history of ocular surgery, systemic or topical drug use, or contact lens use were excluded from both groups. Comprehensive eye examination including slit-lamp photo-biomicroscopy was performed on each subject.

In vivo laser scanning confocal microscopy examination:

In vivo confocal microscopy enables detailed analysis of the human cornea, allowing detailed visualization of the corneal microstructure. The LSCM uses a coherent high intensity light source and the laser beam is scanned over the back of the microscope objective by a set of galvanometer scanning mirrors. In this study we used Heidelberg Retina Tomograph/Rostock Cornea Module (Heidelberg Engineering GmbH, Germany), which is an applanating device. A 63x water immersion objective lens (Zeiss) is used with a 670 nm wavelength Class I diode laser as a light source to allow a scanning area of 300x300 μm (FOV-300 μm), with a lateral resolution of 1 μm /pixel and z-resolution of 4 μm and up to 800x magnification. The objective of the microscope was covered with a disposable sterile PMMA cap previously filled with eye gel (Corneregel) in order to provide immersion. One drop of topical anesthetic (Alcain 0, 5%) was applied in the inferior cul-de-sac of the eye to be examined. The patient was positioned in front of the instrument, his/her chin and forehead adjusted against the headrests. The instrument was justified using the inbuilt software. The objective of the microscope was subsequently advanced to achieve a contact with the ocular surface, the position of the eye was monitored by a camera placed on the side of the objective.

In vivo LSCM was performed on each eye with pterygium in 2 areas (Figure 1): clear cornea adjacent



*Fig. 1. Clinical photography of pterygium, demonstrating the two areas (circles) where *in vivo* LSCM was performed: 1 adjacent clear cornea, 2 body of the pterygium*

to the pterygium (1) and pterygium body (2). Images were captured at the 2 points in the same sequence for all subjects. The central cornea and nasal bulbar conjunctiva (within 2 mm from limbus) were examined in healthy subjects. Clear images (without motion blur or compression lines) from different observation points were selected for qualitative and quantitative analysis. The cells were counted using the inbuilt Cell Count software (Heidelberg Engineering GmbH) in manual mode. The data

are expressed as density (cells/mm²) ±SD (standard deviation).

RESULTS

Microstructural characteristics of the pterygium, cornea, and the sub-basal nerve plexus were evaluated qualitatively and quantitatively. The density of basal epithelial cells and keratocytes in the anterior stroma was determined in the clear cornea adjacent to the pterygium. The density of dendritic cells in the pterygium body was also evalu-

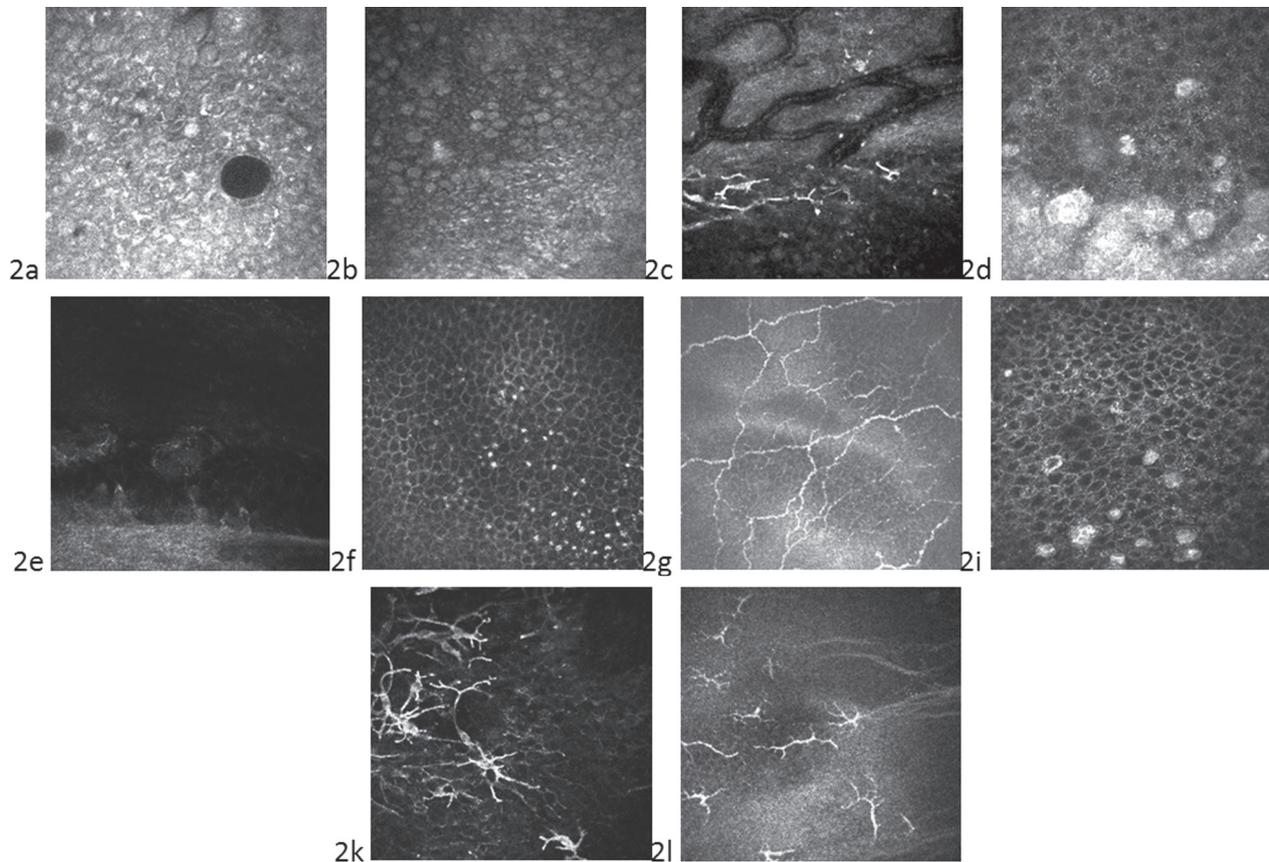


Fig. 2. In vivo confocal microscopy demonstrating microstructural characteristics of pterygium altered tissues.

Microcysts with hypo-reflective core and hyper-reflective round borders in pterygium surface. (2a)

Pterygium surface featuring many Goblet cells. (2b)

Rich blood vessels network and dendritic cells with branching phenotype in pterygium stroma. (2c)

Pterygium head showing infiltration of hyper reflective spots in the corneal epithelium in front of the pterygium, corresponding to clinically seen Fuchs' flecks. (2d)

Columnar appearance of basal epithelial cells at the border of pterygium head and clear cornea. (2e)

Bright deposits in basal corneal epithelium, presumed to be melanin deposits. (2f)

Irregular sub-basal nerve plexus - tortuosity, localized bulge and looping formations. (2g)

Goblet cells in epithelial layer of adjacent clear cornea. (2i)

Dendritic cells with wire-net appearance in the epithelium of pterygium surface. (2k)

Dendritic cells with obvious dendritic appearance in sub-epithelial layers. (2l)

ated. In control subjects, the density of basal corneal epithelial cells and keratocytes in the anterior stroma in the central cornea was determined. The density of dendritic cells was calculated in the nasal bulbar conjunctiva.

In vivo laser scanning confocal microscopy images of pterygium:

The *in vivo* LSCM images of the pterygium epithelium showed tightly packed cells with light-gray bodies, dark borders, and punctiform hyper-reflective nuclei. Inflammatory cells, primarily dendritic cells, were distributed throughout the epithelium of the pterygium. Microcysts with hypo-reflective contents and hyper-reflective even borders were found in the sub-epithelium (Figure 2a). Goblet cells, appearing as large hyper-reflective oval-shaped cells, were also observed in the pterygium's epithelium (Figure 2b). The stroma of the pterygium had a dense fibrovascular structure with rich network of blood vessels filled with hyper-reflective blood cells and minimal infiltration of inflammatory cells in the surrounding tissue (Figure 2c).

In vivo laser scanning confocal microscopy images of the clear cornea adjacent to the pterygium:

Basal corneal epithelial cells appeared as tightly packed cells with bright borders and dark core. They were found with density of 4678 ± 41 cells/mm² in the clear central cornea adjacent to the pterygium and 6850 ± 38 cells/mm² in the central cornea of healthy controls. The basal epithelial cell density in the control group was higher than that in the pterygium group.

At the pterygium-cornea junction, hyper-reflective pterygium epithelial cell clusters were found scattered within the basal corneal cell layer (Figure 2d), presumed to correspond to the clinically seen Fuchs' flecks (6). The corneal basal cells located at the border were in some cases arranged in a columnar fashion (Figure 2 e).

Bright deposits, presumed to be melanin deposits, histologically proven by previous investigators (7), were sporadically found at the level of the basal epithelium (Figure 2 f).

Morphologic irregularities, including breakage, tortuosity, and localized

looping and bulge formation, were commonly seen in the sub-basal nerve plexus of pterygium subjects (Figure 2 g).

In the surface epithelial layer of the clear cornea were observed Goblet cells (Figure 2 i).

The keratocyte density in the anterior stroma was 424 ± 17 cells/mm² in the pterygium patients, which was lower than the density of 770 ± 20 cells/mm² in the central cornea of the control group.

Distribution and density of dendritic cells:

Dendritic cells were found in the nasal bulbar conjunctiva of the control group with a density of 25 ± 12 cells/mm². In the pterygium group, dendritic cells were found in the the pterygium body with a density of 115 ± 11 cells/mm².

The dendritic cell density in the body of the pterygium was higher than the density in the nasal bulbar conjunctiva of healthy controls.

The majority of the dendritic cells in the pterygium displayed the branching dendritic shape. In some cases, the dendritic cells were so numerous that they took on a wire net appearance (Figure 2 k). Interestingly the dendritic cells were also observed in deeper layers and demonstrated same characteristic appearance (Figure 2 l).

DISCUSSION

Pterygium is a vision-impairing wing-shaped fibrovascular lesion that grows across the corneal surface and is associated with sunlight exposure (11). Previous histopathologic studies based on surgically excised tissue have shown an invasion of fibroblastic tissue separating the basal corneal epithelial cell layer from the Bowman layer, with destruction of the underlying superficial corneal stroma (2-6). In our current study, we performed an *in vivo* dynamic examination of the structure of corneas with pterygium *in situ* using *in vivo* LSCM. Our findings were in accordance with those of other studies that used similar devices to report histopathologic structure alterations of the clear cornea adjacent to the pterygium (2-6).

We found changes in keratocyte volume and arrangements, using *in vivo* LSCM. At the pterygium-cornea junction we observed a columnar appearance of the basal corneal epithelium, that was noted by previous investigators (2). Our study however, led to

the conclusion that this may be due to the mechanical traction on the basal cells, during the invasion by the pterygium head between the epithelium and the Bowman membrane (2).

Different *ex vivo* studies have found increased numbers of various immunologic cells, such as mast cells, lymphocytes, plasma cells, CD4+ and CD8+T cells and dendritic cells in pterygium tissue samples(8). We calculated higher number of bright round and dendritic cells in pterygia than in healthy conjunctiva. This is suggestive of a chronic inflammation of the pterygium.

The wide distribution of dendritic cells throughout the pterygium suggests involvement of an immunologic mechanism in the formation of pterygia. Dendritic cells are specialized antigen-presenting cells, part of immune responses of the ocular surface. Langerhans cells are a type of dendritic cell found in the corneal and conjunctival epithelium (9). Mutation and migration of Langerhans cells in detail are still to be evaluated. Pro-inflammatory chemokines and cytokines such as IL-1, TNF- α , and CCR5 can mediate the recruitment of Langerhans cells into the cornea. Mediators of inflammation play an important role in the pterygium development (7, 8) and may also be involved in dendritic cell migration. However, dendritic cells were distributed along the sub-basal nerve plexus in corneas with pterygium, suggesting possible "via the nerve" route to the cornea. We found bright deposits in basal epithelium of clear cornea, presumed to be melanin deposits, histologically proven by previous investigators (7). They found positive correlation between mast cell count and presence of melanin in pterygium. In the surface epithelial layer of the clear cornea we observed Goblet cells, which is a sign of stem cells deficiency, according to some investigators (12).

Morphological irregularities in the sub-basal nerve plexus and decreased corneal epithelial cell and keratocyte density were other interesting findings of the current study. Corneal innervation has important trophic function to the cornea (16, 17), so irregularities in sub-basal nerve morphology may play a role in reducing corneal epithelial cell and keratocyte densities in corneas with pterygium. The average keratocyte density in the anterior stroma of healthy human central cornea was reported to

be much higher than that in the posterior stroma (15). In our study, we found an obvious decrease of the keratocyte density in anterior stroma. Increased levels of IL-1 are thought to upregulate other matrix metalloproteinases and mediators of inflammation, potentially contributing to pterygium development (10). One could consider that inflammatory mediators such as IL-1 may also contribute to the decreased keratocyte density in the anterior stroma by triggering keratocyte apoptosis.

In this study, *in vivo* LSCM clearly demonstrated characteristic alterations of the pterygium and adjacent cornea at micro structural level. Other studies have also shown *in vivo* LSCM to be an effective way to observe the morphologic characteristics of the limbus *in vivo*(13). Because of the focusing effect of the cornea, UV light is concentrated onto the limbus and this is the Coroneo popular theory for limbal stem cell damage from excessive UV exposure (14). Future studies using *in vivo* LSCM to investigate the relationship between limbal changes and pterygium would be beneficial for proper management.

In conclusion, our study utilized *in vivo* LSCM to demonstrate *in situ* morphological alterations in corneas with pterygium. The use of this new modality of imaging may help to decrease the current recurrence rates after pterygium, providing detailed microstructural characteristics before and after surgery.

Microstructural observations of the cellular elements in pterygium and adjacent structure provide a new insight for future pterygium research. *In vivo* LSCM could be used in studies to observe histopathologic alterations throughout the progression of pterygium, to study the characteristics of recurrent pterygium, and to monitor the effects of different treatment strategies for pterygium. Such studies would also improve the current understanding of the pterygium pathogenesis.

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