MICROSTRUCTURAL ANALYSIS OF THE CORNEAL CHANGES IN PIGMENT DISPERSION SYNDROME – CLINICAL STUDY ASSISTED BY *IN VIVO* LASER SCANNING CONFOCAL MICROSCOPY

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ABSTRACT

PURPOSE: To evaluate and define microstructural changes in the cornea of patients with pigment dispersion syndrome (PDS) using *in vivo* laser scanning confocal microscopy (LSCM). To demonstrate the option of this technology for precise diagnosis and monitoring PDS disease, including dynamic observations.

MATERIAL AND METHODS: 40 eyes of 20 patients with clinically detectable PDS were examined by *in vivo* laser confocal microscopy (HRTII Rostock corneal module). Several examinations were performed in order to facilitate quantitative and qualitative analysis of the sub-basal nerve plexus, endothelial cells and bright granules, consistent with pigment granules at the level of endothelium.

RESULTS: The most demonstrative findings were hyper-reflective, polymorphic granules on endothelial surface, measured 10-25 μm. The size and density of the particles increased in patients with higher intraocular pressure. There were a correlation between the clinical characteristic and the degree of polymegathism and pleomorphism of affected endothelial zones.

CONCLUSION: *In vivo* laser confocal microscopy demonstrates new perspectives for diagnostics and staging of the pigment dispersion syndrome. The method has wider applications for monitoring and long term prognosis.

Key words: *cornea, pigment dispersion syndrome, in vivo confocal microscopy*

INTRODUCTION

Pigment dispersion syndrome (PDS) is a clinical entity defined by excessive pigment shedding from the iris (1, 2). PDS is typically bilateral and affects young, myopic males. Classic anterior segment findings include pigment deposition on the corneal endothelium (Krukenberg spindle), dense pigmentation of the trabecular meshwork (TM), and mid-peripheral iris transillumination defects. A characteristic concave iris configuration has also been described, which is thought to facilitate pigment release through increased iridozonular rubbing (3,4). Over time, chronic pigment release can lead to elevated intraocular pressure and followed by pigmentary glaucoma. The rate of progression to pigmentary glaucoma among patients with PDS has been reported at 10%–50%, (2,5–8) with a prospective

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trial showing that the intraocular pressure (IOP) at presentation is a key risk factor for the progression (8).

In vivo confocal microscopy allows non-invasive, life imaging of the ocular surface and adnexal structures. Its unique physical properties enable microscopic examination of all cellular layers of the cornea 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. There are number of studies dedicated to corneal nerves, stromal keratocite density and endothelial characteristics of the normal human and ageing cornea (9,10,11). However, in the published literature there are only novel papers describing different pathological observations (12-14).

The purpose of this study is to describe the microstructural changes of the cornea in patients with pigment dispersion syndrome and to demonstrate *in vivo* laser confocal microscopy opportunities for precise diagnosis and long term monitoring, based on qualitative and quantitative analysis.

MATERIAL AND METHODS

Twenty patients (40 eyes) between the age 45-60 years (13 male and 7 female) with clinical signs of PDS were selected for the purpose of the study. Each eye was examined with slit-lamp biomicroscopy, fundoscopy and *in vivo* laser scanning confocal microscopy. Microstructural assessment was focused on quantity and quality of subbasal nerve plexus, keratocytes, endothelial cells and morphology and size of the pigment granules.

In this study we used Heidelberg Retina Tomograph/Rostock Cornea Module (Heidelberg Engeneering 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 sourse 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 in order to keep the objective immersed. One drop of topical anesthetic (Alcaine 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 lean against the headrests. The objective of the microscope was subsequently put in contact with the cornea, the position of the eye was monitored by a camera placed on the side of the objective. Clear images (without motion blur or compression lines) from different observation points were selected to use for cell number counting. The cells were counted using Cell Count Software (Heidelberg Engineering GmbH) in manual mode. The data are expressed as density (cells/mm^2) ±SD (standard deviation).

For each cornea three images were selected from the following levels: basal epithelium, sub basal nerve plexus, anterior stroma, mid stroma, posterior stroma and endothelium. Collected data were analyzed independently by two investigators using the same criteria and measurement tools.

RESULTS

The study highlighted significant changes at the level of epithelium, sub-basal plexus and endothelium as demonstrated on the composite figure (fig 1).

The most prominent observation at superficial level was the change in morphology of the basal epithelial cells. This is presumed to correlate to the clinical stage of the disease. In advanced cases epithelial mosaic was irregular with signs of oedema (fig 1, first row).

Consistent pathological changes were found at the level of sub-basal nerve plexus. With advancement of pigmentation, the nerves appeared to decrease in density and increase in diameter. Another change in morphology was looping and beading of the nerve branches (fig 1, second row).

Corneal stroma had less prominent changes but generally with advancement of disease decreased number of keratocytes was highlighted (fig. 1, third row).

Study also observed a correlation between clinical characteristics and degree of polymegathism and pleomorphism of affected endothelial zones. Quantitative analysis found a mean density of the pleomorphic endothelium to be 713±63 cells/ mm^2. However the peripheral endothelium appeared to be normal with density 2302±56 cells/ mm^2. This

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observation is not clinically detectable, which is one of the main disadvantages of the wider used technology in the everyday practice.

At the level of endothelium the most significant findings were hyper-echogenic, polymorphic granules on the back side of endothelial cells, measured 20 μm ±15. The size and density of the granules in patients with high intraocular pressure was significantly enlarged (fig 1, D).

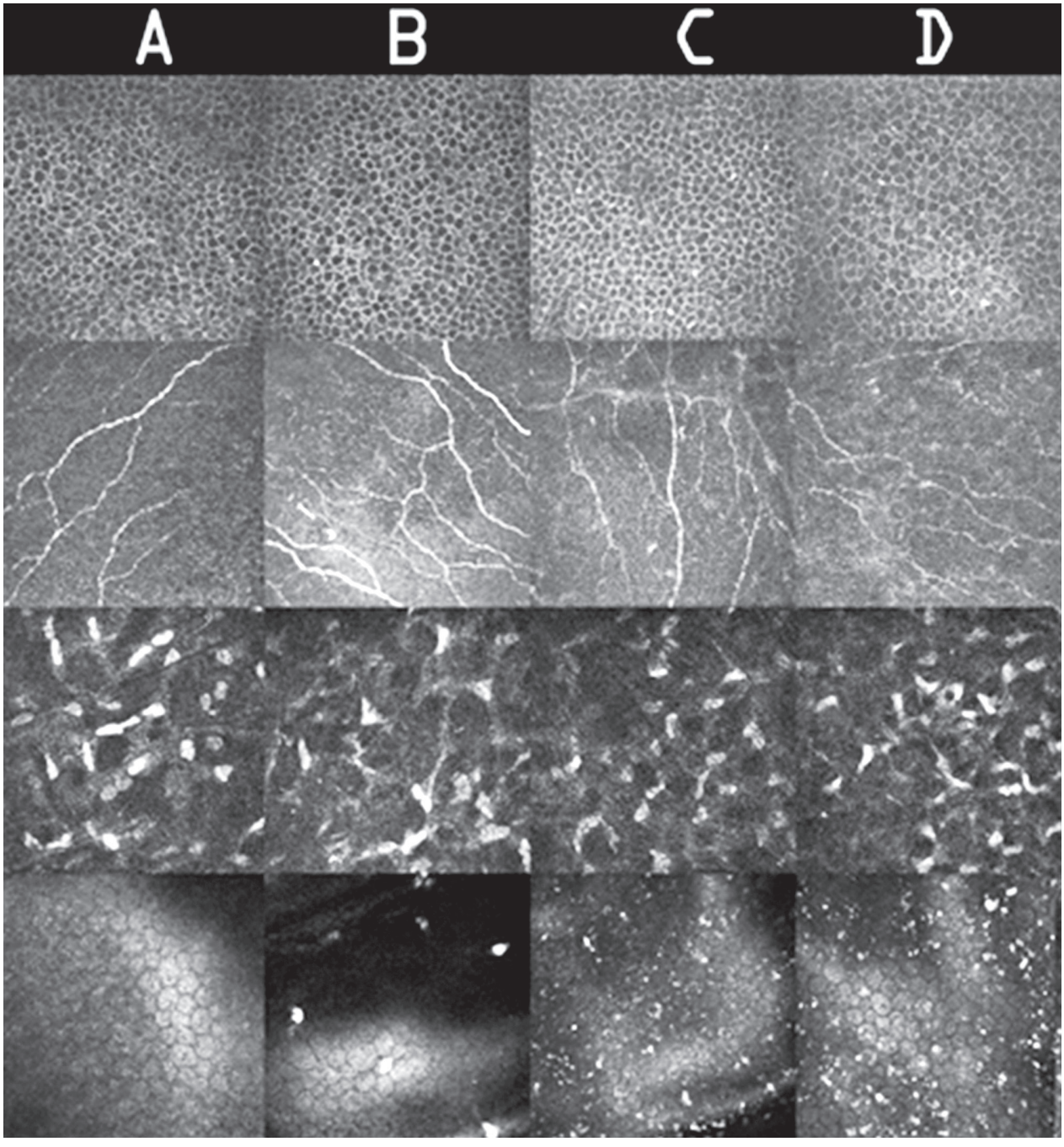

Fig.1. In vivo laser scanning confocal microscopy at different levels of the cornea affected by PDS; Rows from up to bottom: epithelium, sub-basal nerves, stroma and endothelium. The columns are demonstrating the specified levels for normal cornea (A) and corneas with mild (B), moderate (C) and advance development of the PDS (D).

DISCUSSION

In the literature there are limited number of published case reports describing PDS using *in vivo* confocal microscopy for morphometric analysis (15). Our study demonstrated new perspectives for diagnostic and staging the pigment dispersion syndrome. The observations would have a monitoring and prognostic advantage, as well as differential diagnostic value regarding conditions associated with clinically similar pigment deposits on the back surface of the endothelium (inflammation, post-surgical and post-traumatic pigment dispersion, Fuch's endothelial dystrophy).

In our study we described interesting changes in nerve morphology, which differs from other observations. The most prominent feature was thickening of the sub-basal corneal nerves in advancement of disease. We believe that this is related to the PDS, as the patients had no history of any other ocular or systemic disease, nor prior cataract surgery. Whether this findings is isolated and nonspecific or thickened sub-basal nerves are more frequent in PDS patients requires further observations.

In some cases, uveitis can cause pigment epitelitis of the iris with release of pigment, inflammatory cells and debris behind endothelium. After resolution of the inflammation clinical observations might be mistaken for PDS, as other authors had demonstrated (16). Cataract surgery also can cause features similar to PDS, with iris trans-illumination defect, pigment behind endotelium and increase IOP (17-23). In our study however, we utilized *in vivo* confocal microscopy to rule out prior incidents by observing morphology of endothelial cells and characteristics of the granules. We believe that polymegathism and pleomorphism in PDS are localized in the area of Kruckenberg spindle and the rest of endothelium is relatively normal. Our comparative observations demonstrated in cases of inflammation the condition is very asymmetric and corneal periphery is equaly involved. In post-cataract cases the endothelium is also diffusely damaged. That could be an important diagnostic characteristic for differentiation of those other conditions.

The largest study in the literature by Harold and al. reported 409 patients with PDS, clinically observed over a period of 30 years in population suspected for glaucoma. PDS comprised 4.4% of the entire "glaucoma in suspect" group. An average frequency real pigmentary glaucoma was 13.1 per year among patients with PDS (24). Regardless this small incidence, PDS is an important often underdiagnosed condition because it affects young people with potential risk of irreversible blindness. Laser scanning *in vivo* confocal microscopy may not only detect pigment particles in early stages when impossible to be observed by slit-lamp biomicroscopy, but also precisely measure the size and record changes in morphology. Furthermore this technique allows observation at microstructural level of all corneal levels and precise follow up of the dynamic changes in epithelium, sub-basal plexus and endothelium. Therefore, eye specialist may rely not only on precisely diagnose but may utilize prognostic value and utilize those those advantages for follow up.

CONCLUSIONS

In vivo laser scanning confocal microscopy provides a novel method for examining micro-structural alterations in PDS in the cornea, as well as other corneal pathology and might be useful for diagnosis and differentiating number of subclinical disorders. This new *in vivo* technology may hopefully now be used to screen for abnormalities at cellular level. Laser scanning *in vivo* confocal microscopy is an instantaneous, noninvasive imaging technique with strong perspective to improve early detection of sub-clinical anterior segment pathology. The study demonstrated its ability for diagnostics of the pigment dispersion syndrome, including staging. The method has wider applications that are still to be developed and utilised for monitoring and long term prognosis.

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