Neurotropic Grades in Lattice Corneal Dystrophy Based on in Vivo Confocal Microscopy Observation

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Research article

Keywords: Lattice corneal dystrophy (LCD), in vivo confocal microscopy(IVCM), corneal neurotropic phenomenon

Posted Date: December 1st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-114232/v1

Abstract

Background: To investigate the corneal neurotropic phenomenon in patients with lattice corneal dystrophy (LCD) with in vivo laser scanning confocal microscopy (IVCM).

Methods: IVCM was performed on a total of 15 patients (28 eyes) with LCD annually at a follow-up. A collection of the data was acquired to be analyzed.

Results: As indicated by the analysis, the LCD patients' normal corneal stromal nerves (Grade 0) presented a decline with the prolongation of the follow-ups, corresponding to a gradual increase in grade I and II involving amyloid-wrapped nerve fibers, which demonstrated that the growing amount of amyloid deposit due to the corneal nerve invasion increased slowly over time.

Conclusions: The neurotropic phenomenon could increase with its severity in the corneal lesion of the patients with LCD, and also reflect the distribution of the corneal nerves, to some extent. IVCM provides a rapid, noninvasive way to observe the corneal nerves, which can be an efficient means of better understanding the development of LCD.

Background

Corneal dystrophy (CD) is a type of non-inflammatory, hereditary disease that mainly affects the central cornea. The disease progresses slowly, having little or no relationship with the systemic or environmental factors. CD has characteristic pathological changes [1.2]. Lattice corneal dystrophy (LCD) is a hereditary disease in which the amyloid deposits in the cornea, causing lattice-like opacities in the cornea with a detrimental effect on visual acuity [3]. LCD affects males and females equally, with age of onset varying as described in history and physical [4]. LCD is known to have five subtypes: LCDI, II, III, IIIA, IV; in LCDI, IIIA, and IV, mutations in the BIGH3 gene can result in amyloid deposition in the corneal stroma, but no such pathological changes are observed in other tissues [5].

In vivo corneal laser scanning confocal microscopy (IVCM) is helpful to evaluating the morphological characteristics of corneal dystrophies at the histological level and maybe helpful in diagnosis and understanding the pathophysiology of disease [6]. We observed the corneal nerves were wrapped by amyloid deposits in a gradual fashion, and the entire involvement of the corneal neural network occurred eventually; thus this condition was defined as a neurotropic phenomenon. However, little is known of the progression of corneal neurotropic phenomenon in LCD, which is essential for developing a better understanding of the disease. The aim of this study was to observe the neurotropic phenomenon in the corneas of the patients with LCD using IVCM, which could provide us with a new thinking of treatment to prevent the corneal lesions.

Methods

Patients

The study was conducted in compliance with informed consent regulations and the Declaration of Helsinki; the protocol was approved by the Internal Review Board (IRB) of Shenzhen Eye Hospital; and informed consents were obtained from the patients with LCD, who numbered 15 with eligible 28 eyes for the study in Shenzhen Eye Hospital in Shenzhen of China during the period from March 2009 to March 2018. The group of subjects was composed of 7 women and 8 men aged 35.9±4.36 y, including 2 patients monocular.

All patients underwent the conventional slit lamp examination by an ophthalmologist, the corneal stroma seen as lattice-like streaky turbidity, as indicated by the photograph of the anterior segment of the patient's eye with LCD (Figure1). A differential diagnosis was made to exclude other types of corneal dystrophy, glaucoma, uveitis, keratitis, corneal ulcer, conjunctivitis and leukoplakia. After the first examination, all the patients would undergo IVCM annually at a follow-up. Respectively, 8 patients (16 eyes) received the examination for 10 consecutive years; 5 patients (9 eyes), for 6 consecutive years; and 2 patients (3 eyes), for 4 consecutive years. The data collected covered the medical and ophthalmological history including age, age at diagnosis, gender, detailed slitlamp examination and IVCM imaging. Without exception, every individual was informed of the aim of each data recording.

IVCM

The area in contact with the cornea was examined using IVCM with magnification up to X800 (HRT II Rostock Cornea Module, diode laser 670 nm, Heidelberg Engineering GmbH, Germany), the images consisting of 384 × 384 pixels covering an area of 400 × 400 mm with a transverse optical resolution of approximately 1 mm/pixel and an acquisition time of 0.024 s (Heidelberg Engineering, Germany). After that, the patient's cornea was routinely examined. To standardize the measurements, all images were subsequently randomized and encoded by a single independent observer.

Image Analysis

For the convenience of description, the corneal stromal nerve fibers that were not affected by LCD lesions were divided into three categories according to the pathway and thickness of the nerve in the corneal stroma by IVCM: The first type was composed of the straight nerve fibers (Fig 2A); the second type, of the curved, thinner nerve fibers (Fig 3A); and the third type, of the branching, thicker nerve fibers (Fig 4A).

Neurotropic Grades

1. Grade 0: As indicated by the IVCM, the corneal stromal nerve fibers, which were not affected by the lesion, had a highly reflective strip-like structure running continuously, with the boundary being smooth and the structure being clear. This was defined as Grade 0 of the neurotropic phenomenon (Figure 2A, 3A & 4A). As shown in. Figure 2A, the corneal stroma nerves were not involved in LCD lesions, showing a clear structure, smooth border and highly reflective strip-like structure running straight. As shown in Figure 3A, the corneal stroma nerves of LCD were not involved, relatively curved and slender, the structure being highly reflective and its boundary being clear. Moreover, the corneal

stroma nerves of LCD were not involved, showing an obvious branching structure, whereas the nerve fibers were relatively coarse (Figure 4A).

2. Grade I: Part of the agglomerate and high-reflective structure wrapped the corneal nerve, the corneal nerve slightly thickened, beaded or segmental, which was defined as Grade I of the neurotropic phenomenon (Figure 2B, 3B & 4B). Some of the amyloid-coated nerve fibers of the corneal stroma were thickened by the affected nerve fibers, while the unwrapped nerves were thinner, thus resulting in beaded nerve fibers (Figure 2B). The bent and curved nerve fibers were partially thickened by the LCD lesions, presenting uneven thicknesses (Figure 3B). There were curved nerve fibers that are not affected in the lower left. Some of the amyloid-coated nerve fibers were bifurcated in the corneal stroma, the nerve fibers being segmental (Figure 4B).
3. Grade II: A large number of agglomerate and highly reflective structures wrapped the corneal nerve, with the corneal nerve significantly thickened. This neurotropic phenomenon of LCD was defined as Grade II (Figure 2C, 3C & 4C). In the nerve fibers a large number of amyloids enveloped the corneal stroma, the affected nerve fibers significantly thickened, which was defined as Grade II of the neurotropic phenomenon of LCD (Figure 2C). When wrapped by LCD lesions, the nerve fibers of the corneal stroma became significantly thickened, which was defined as Grade II of neurotropic phenomenon of LCD (Figure 3C). Additionally, when a large number of amyloids wrapped the nerve fibers in the corneal stroma, the nerve fibers were all significantly thickened without segmentation, which was also defined as Grade II of the neurotropic phenomenon of LCD (Figure 4C).

Statistical methods

The data were analyzed using IBM SPSS Statistics for Windows, Version 19.0(IBM Corp, Armonk, NY, USA) and reported as mean±SD.

Results

At each follow-up examination with IVCM, the cornea was divided into 4 quadrants, each taken once every 100um depth. According to the criteria, the nerve fibers were graded, and each nerve structure found was counted 1 as follows:

As shown in Table 1, 8 patients (16 eyes) underwent a 10-year observation, Out of the corneal neuropathic data averaged came a trend chart, as shown in Figure 5A. With the prolongation of the observation period, the nerves of Grade 0 as normal presented a gradual decrease, while Grade I and II nerves affected, the amyloid-encapsulated nerve fibers, did a gradual increase.

As indicated in Table 2, the data of the neurotropic phenomenon of LCD were acquired from the remaining patients, 9 undergoing 6-year follow-ups and 2 (3 eyes) from 2 having 4-year follow-ups. The trend chart generated as shown in Figure 5B, although the observation period was shorter than that as indicated in Figure 5A, the nerves of Grade 0 as normal presented a gradual decrease, while those of

Grade I and II, the amyloid-encapsulated nerve fibers, did a gradual increase in the trend; However, the slope was smaller than that as indicated in Figure 5A, considering the shorter period of observation.

Discussion

LCD, a clinically common hereditary disease, can lead to severe visual impairment and significant heritability. The condition is characterized by the deposition of amyloid in the cornea leading to the appearance of corneal stroma. These deposits create linear, lattice-like opacities arising primarily in the central cornea, while the peripheral cornea is often spared [7]. When illuminated by post-illumination, the lattice lines and nodules are visible double-profiles with an optically transparent core that can be directed to the periphery (Generally, it does not reach the limbus of the cornea) and deep expansion of the matrix; it can also stretch the epithelial layer to make the surface of the corneal epithelium irregular [8] .

We performed IVCM on the cornea of LCD patients at each follow-up. As suggested by Figure 2A-C, the amyloid deposit could have an increasing effect on the coarser nerve fibers running straight through the corneal stroma; the straight nerve fibers could not be affected by the lesions (Figure 2A); as the condition progressed, some of amyloid-like nerve fibers could have a tendency to be wrapped, with the nerves becoming beaded (Figure 2B); and at the late stage of the lesion, a large amount of amyloid could encapsulate the nerve fibers, with the nerves becoming significantly thickened (Figure 2C).

As suggested by the series of Figure 3A-C, the amyloid deposit, could affect the finer nerve fibers that were bent in the corneal stroma; the nerve fibers that were bent finely could not be affected by the lesions (Figure 3A); as the condition progressed, a small amount of amyloid-like nerve fibers, with time, could be wrapped, with the nerves irregularly ringed (Figure 3B); and at the late stage of the lesion, a large amount of amyloid could encapsulate the nerve fibers, with the nerves becoming significantly thicker and curved, and with multiple flower rings (Figure 3C) which could be connected into a mass, if they continued to develop.

As suggested by the series of Figure 4A-C, the amyloid deposit could have a tendency to affect the large corneal nerve branches; the lesion was unlikely to affect the branching of the corneal stroma, and the coarser nerve fibers (Figure 4A); as the condition progressed, some of amyloid-like nerve fibers could grow to be wrapped, the nerves becoming segmental (Figure 4B); and at the late stage of the lesion, a large amount of amyloid was likely to encapsulate the nerve fibers, the nerves becoming significantly thickened under the confocal microscope (Figure 4C).

We defined this phenomenon as neurotropic phenomenon that amyloid deposits could wrap along with the corneal nerve in LCD patients, eventually involving the entire corneal neural network. By using IVCM to observe the cornea of LCD patients at follow-ups for years, we could better understand the development of the lesion. While conventional light microscopes are limited by light scatter from structures outside of the focal plane, IVCM creates a point source of light by a pinhole aperture, focused by an objective lens on the tissue, which can well facilitate the observation of the structure of living lesions in LCD patients [9]. Since the slit lamp examination shows no pathologic changes or mild lattice-like corneal turbidity at the

early stage, corneal lesions and neurotropic phenomenon with mild degree could be observed by using IVCM, which will be better for early diagnosis [10]. As previously reported, the progresses of corneal lesions in LCD patients could actually reflect the distribution of nerve fibers in the corneal stroma to some extent [11].

According to the trend charts (Figure 5A & Figure 5B), the normal corneal stromal nerve, defined as Grade 0, could have a tendency to deteriorate during the long-term observation. Moreover, the affected nerve wrapped in amyloid, defined as Grade I and II, could present a gradual increase. The statistical results also suggested that the corneal nerve invagination due to amyloid deposits in LCD patients tends to aggravate over time. In view of the findings, it can be hypothesized that the mechanism of corneal lesion in LCD refers to the nerve fibers in the corneal stroma which are wrapped and thickened, with their density increased over time.

As shown in Figure 5A, the observation time of 4.5 year is the break point whereas the time is 3.5 year in Figure 5B. The Grade 0 did a faster decrease and Grade I and II showed a faster increase after the break point time. This trend remind us to inform patients that the cornea lesions will aggravates after 3–4 years, which need a shorter regular visits to hospital, and corneal transplantation will be needed in severe cases.

Limitations of this study included the small sample size and inconsistency of observation time, which resulted in wide 95% CIs that could be reduced with a larger number of patients. Therefore, more LCD patients are needed to observe for a longer period of time. The extraction and identification of neurotropic substances can be of great benefit to the design of anti-neuronal drugs for clinical treatment of LCD patients, which may prevent the corneal lesions.

Conclusions

IVCM provides a rapid, noninvasive way to observe the corneal nerves, which can be an efficient means of better understanding the development of LCD. The neurotropic phenomenon could increase with its severity in the corneal lesion of the patients with LCD, and also reflect the distribution of the corneal nerves, to some extent. Whether the neurotropic phenomenon of LCDs with different gene phenotypes are identical, etc., further research is merited. Ideally, the current study can help us conduct in-depth researches in this direction.

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Tables

Due to technical limitations, table 1-2 is only available as a download in the Supplemental Files section.

Figures

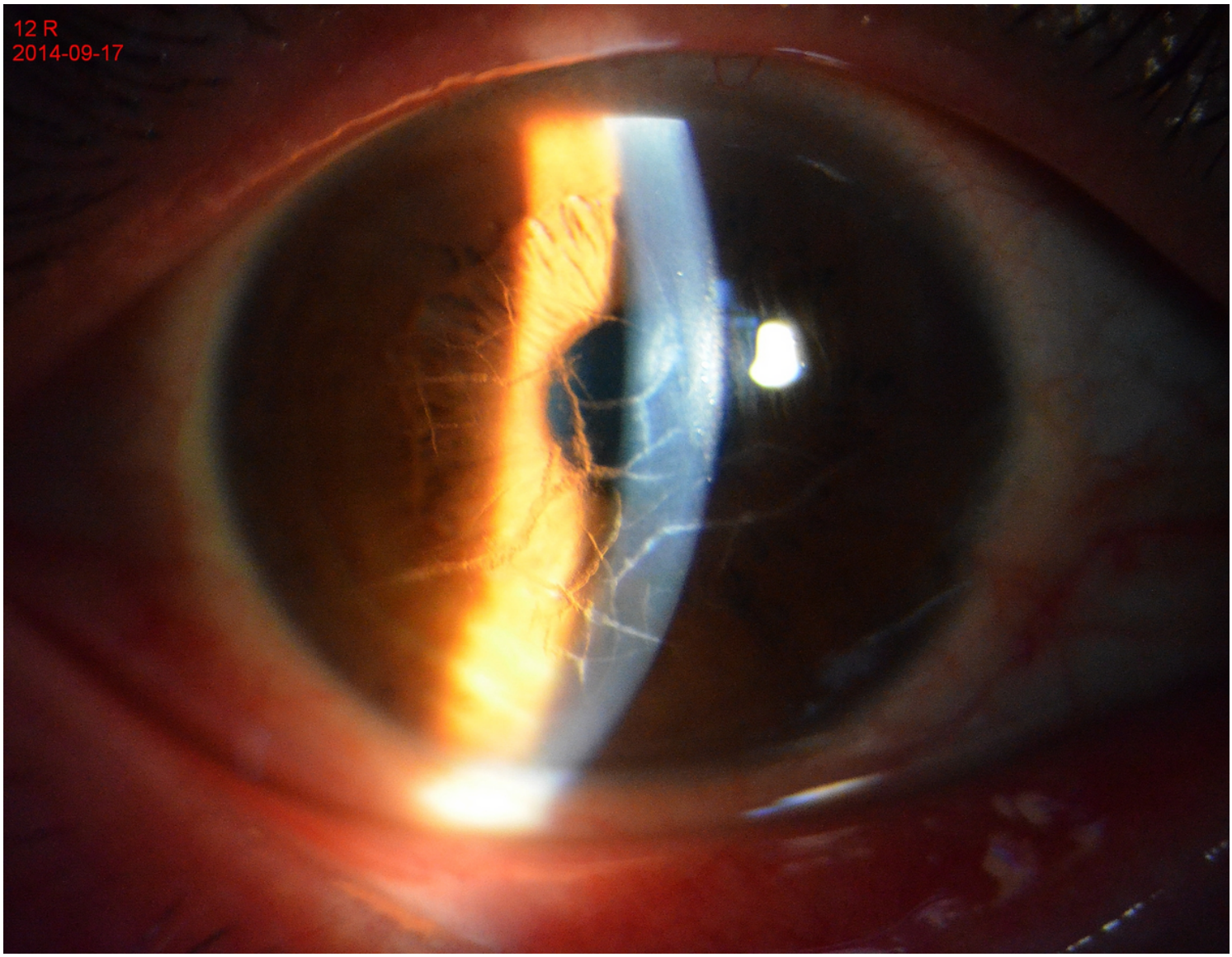

Figure 1

All patients underwent the conventional slit lamp examination by an ophthalmologist, the corneal stroma seen as lattice-like streaky turbidity, as indicated by the photograph of the anterior segment of the patient's eye with LCD (Figure1).

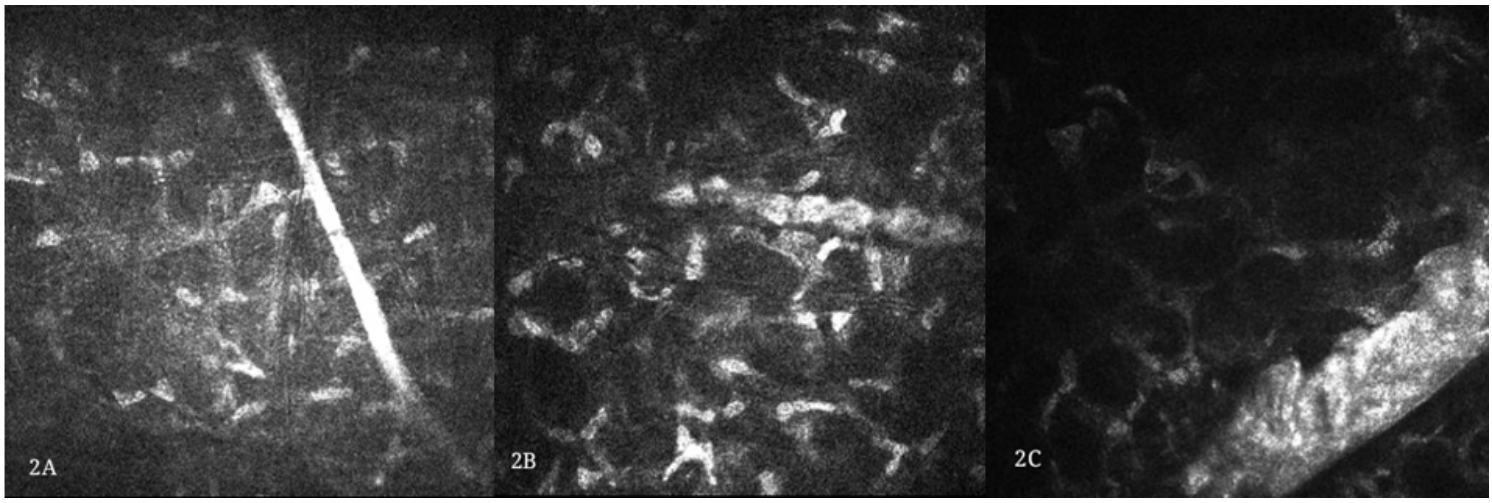

Figure 2

For the convenience of description, the corneal stromal nerve fibers that were not affected by LCD lesions were divided into three categories according to the pathway and thickness of the nerve in the corneal stroma by IVCM: The first type was composed of the straight nerve fibers (Fig 2A);

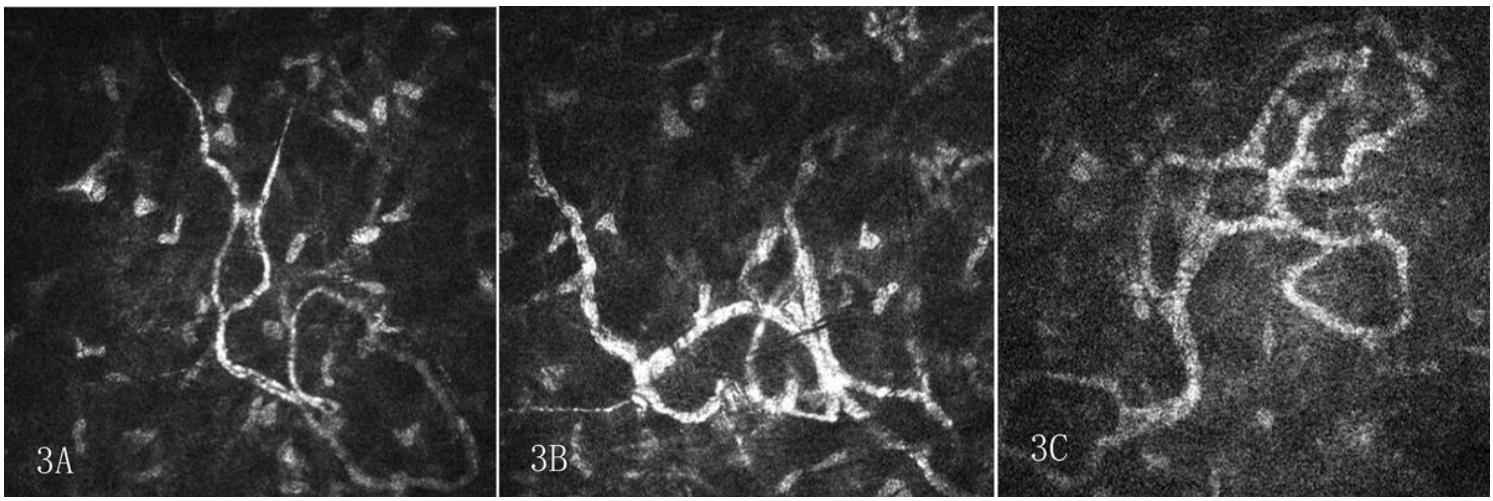

Figure 3

showing a clear structure, smooth border and highly reflective strip-like structure running straight. As shown in Figure 3A

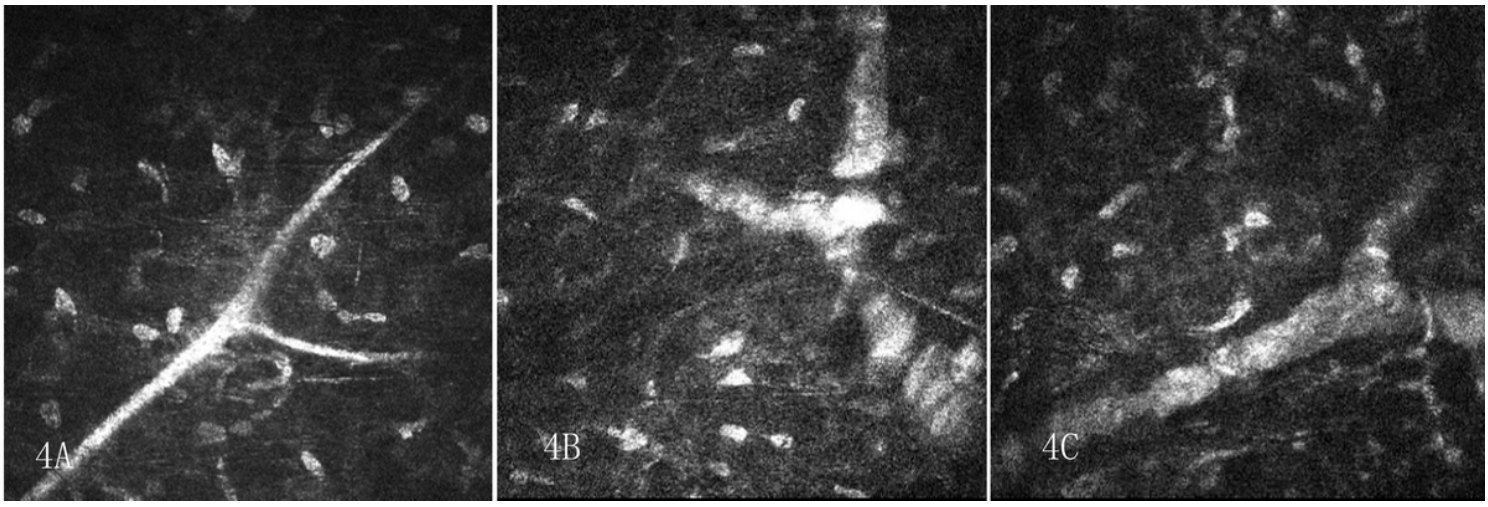

Figure 4

Some of the amyloid-coated nerve fibers were bifurcated in the corneal stroma, the nerve fibers being segmental (Figure 4).

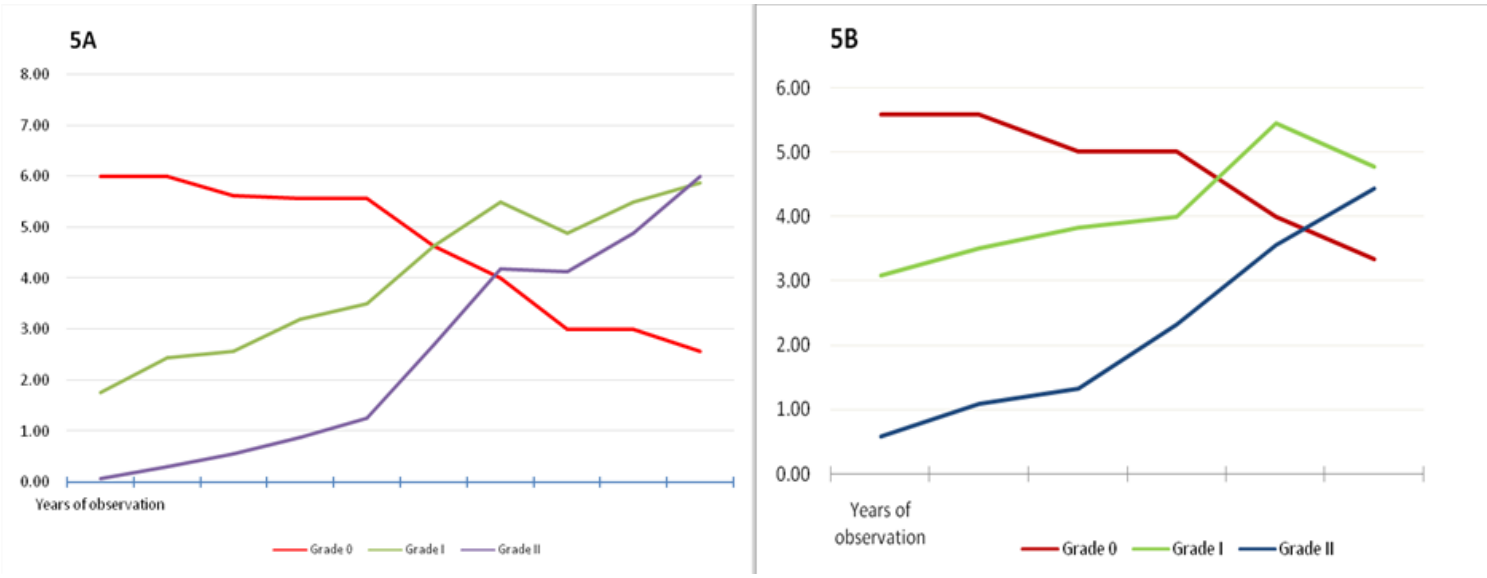

Figure 5

Out of the corneal neuropathic data averaged came a trend chart, as shown in Figure 5

Supplementary Files

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