Original article

Comparison of stromal corneal nerves between normal and keratoconus patients using confocal microscopy

M. Ramírez Fernández*, E. Hernández Quintela, R. Naranjo Tackman

Servicio de Córnea y Cirugía Refractiva, Asociación Para Evitar la Ceguera en México (APEC), Hospital Luis Sánchez Bulnes, Universidad Nacional Autónoma de México, Mexico City, Mexico

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ABSTRACT

Objective: To evaluate the differences in stromal corneal nerves between normal patients and keratoconus patients.

Material and methods: A total of 140 eyes of 70 normal patients (group A) and 122 eyes of 87 keratoconus patients (group B) were examined with the confocal microscope, with a central scan of the total corneal thickness being taken. The morphology and thickness of the corneal stromal nerves were evaluated by using the Navis v. 3.5.0 software. Nerve thickness was obtained from the mean between the widest and the narrowest portions of each stromal nerve.

Results: Corneal stromal nerves were observed as irregular linear hyper-reflective structures with wide and narrow portions in all cases. Mean corneal stromal nerves thickness in group A was 5.7 ± 1.7 (range from 3.3 to 10.4 μm), mean corneal stromal nerves thickness in group B was 7.2 ± 1.9 (range from 3.5 to 12.0 μm). There was a statistical significant difference (p < .05) in stromal corneal nerves thickness between group A and group B.

Conclusion: Stromal corneal nerves morphology was similar in both groups, but stromal nerves were thicker in keratoconus patients.

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Análisis de nervios estromales en pacientes con queratocono

RESUMEN

Objetivo: Evaluar las diferencias de los nervios del estroma de la córnea entre sujetos normales y pacientes con queratocono.

Métodos: Un total de 140 ojos de 70 sujetos normales (grupo A) y 122 ojos de 87 pacientes con queratocono (grupo B), fueron evaluados con el microscopio confocal, realizando un rastreo central del espesor total de la córnea. La morfología y el espesor de los nervios fueron evaluados utilizando el programa Navis v. 3.5.0. El espesor de los nervios se obtuvo del promedio de la porción más delgada y la más gruesa de cada nervio.

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Introduction

Corneal confocal microscopy under normal conditions reveals epithelium, subepithelial nerve plexus (the name given to the nerve plexus beneath cornea epithelium), stroma, stroma nerves and endothelium.\textsuperscript{1} Regarding nerves, there have been many studies on the subepithelial nerve plexus of the cornea thanks to the ease of imaging on this tissue by confocal microscopy, including studies in keratoconus patients.\textsuperscript{2–6} However, corneal stroma nerves are much less abundant, and it is more difficult to take their images by confocal microscopy; therefore, the study was more limited.\textsuperscript{1,7,8}

Although corneal stroma nerves are not as abundant as those in the subepithelial nerve plexus, upon usual eye examination by slit lamp, due to their size, corneal stroma nerves are the only ones that can be seen; for years they have been described as more apparent and thicker in keratoconus patients than in normal patients or those without this condition\textsuperscript{7}; it has even been suggested that corneal stroma nerves are linked to keratoconus progression.\textsuperscript{9} This study aims to take images and thus be able to compare as many nerves in the corneal stroma, between those from normal subjects and those from keratoconus patients, using confocal microscopy.

Subjects, material and methods

All people involved signed the consent form; the study was divided into two groups: group A, control group where 140 eyes of 70 normal subjects without any ocular or systemic disease were studied; and group B where 122 eyes of 87 patients diagnosed with keratoconus using topography (Bausch & Lomb Surgical, Orbtek Inc., Salt Lake City, UT, USA) in stages II and III based on the Amsler-Krumeich, classification, which has been used in multiple keratoconus studies.\textsuperscript{11–14}

Confocal microscopy: At the corneal imaging unit of the Cornea and Refractive Surgery Department of the hospital of the Association to Prevent Blindness in Mexico, after topical anesthesia of cornea with tetracaine hydrochloride 5.0 mg per ml (Ponti Ofteno, Laboratorios Sophia, S.A. Guadalajara, Mexico), all study subjects in both groups underwent a central scan of the total cornea thickness using Confoscan 4 confocal microscope (Fortune Technologies, Vigonza, Italy). Each confocal microscopy test rendered scanned images in JPEG format, consisting of 2 consecutive scans of total central cornea thickness depth; this scan is equivalent to the endothelium and epithelium and back to endothelium imaging scan, i.e., from posterior to anterior and back to posterior, to allow movement in the Z axis of central cornea thickness. A Z-Ring Scan (Confoscan, Fortune Technologies, Italy) was used; this device maintains contact with the cornea surface to obtain reliable thickness measurements without anteroposterior eyeball movement.

An average of 350 images per scan were obtained; they were 340\textmu m \times 255\textmu m at axes X, Y; they are automatically saved to a computer hard disk for further analysis using Navis v. 3.5.0 microscopic image analysis software (NIDEK, Multi-Instrument Diagnostic System, Japan).

Each image from every scan obtained by confocal cornea microscopy was checked by searching for those with pictures of nerves in stroma. Only nerves in focus and with sharp edges were assessed and measured. Stroma nerve morphology and thickness were analyzed; they were measured using Navis v. 3.5.0 microscopic image analysis software (NIDEK, Multi-Instrument Diagnostic System, Japan). Nerve thickness was obtained from the average of the thickest and narrowest portion of each nerve tested (Figs. 1 and 2). Nerves with bifurcations (Fig. 3) were not measured. Only corneal stroma nerves were analyzed. Subepithelial nerve plexus nerves were not measured.

Fig. 1 – Corneal stroma confocal microscopy image 340\textmu m \times 255\textmu m. Corneal stroma nerve of group A is shown, seen as a linear, highly reflective structure with thick and narrow portions (indicated by arrows) surrounded by keratinocytes.
Therefore, surrounded by keratocytes, the latter with moderate reflection (Figs. 1 and 2). Corneal stroma nerves were arranged in an oblique direction in 100% of all images assessed, also in all cases with coarse and narrow morphology in some portions (Figs. 1 and 2).

Mean stroma nerve thickness in group A, i.e. patients without disease (Fig. 1) was $5.7 \pm 1.7$ ranging from 3.3 to 10.4 $\mu$m, mean stroma nerves thickness in group B, keratoconus patients (Fig. 2) was $7.2 \pm 1.9$ ranging from 3.5 to 12.0 $\mu$m. The difference in the thickness of corneal stroma nerves between group A and group B was statistically significant ($p<0.05$, unpaired t test) (Fig. 4).

### Discussion

Under normal conditions, various structures such as corneal surface epithelium, basal epithelial cells, nerve plexus subepithelial, cores stromal keratocytes and endothelial cells may be assessed by confocal microscopy. Stroma nerves are no exception. In this study, they show as highly reflective linear structures with thick and thin portions, as already reported in the literature, surrounded by keratinocytes nuclei with moderate reflection.

Most studies of corneal nerves using confocal microscopy have focused mainly on subepithelial nerve plexus because it is across the cornea, beneath its epithelium, which makes it very easy to take images of the plexus. Furthermore, there have been studies of the subepithelial nerve plexus under normal conditions (or changes the nerve plexus undergoes resulting from surgical procedures such as corneal transplantation or refractive surgery) and diseases such as dry eye. Subepithelial nerve plexus studies have been conducted on keratoconus patients, including linking them to corneal sensitivity. Furthermore, it is harder to find corneal stroma nerves, since it is not a plexus, and given that images by confocal microscopy are $340 \mu m \times 255 \mu m$ in the axes X, Y (Confoscan 4 Fortune Technologies, Vigonza, Italy), it has to match the path of a corneal stroma nerve with the tiny image taken in the scan. This study focused on corneal stroma nerves.

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**Fig. 2** – Corneal stroma confocal microscopy image of $340 \mu m \times 255 \mu m$. Corneal stroma nerve of group B is shown, seen as a linear, highly reflective structure with thick and narrow portions (indicated by arrows) surrounded by keratinocytes.

**Fig. 3** – Corneal stroma confocal microscopy image of $340 \mu m \times 255 \mu m$. Corneal stroma nerve bifurcation of group A is shown (indicated by arrows) surrounded by keratinocytes.

**Fig. 4** – Mean ± standard deviation of corneal stroma nerve thickness of group A, normal subjects, and group B, keratoconus patients. Difference in thickness between the two groups was statistically significant ($p<0.05$, unpaired t test).
Keratoconus is a disease in which the cornea undergoes a usually progressive ectasia, which may appear in childhood or later in life and often leads to the need for a corneal transplant to correct the problem. Early diagnosis of keratoconus today is of utmost importance due to the rise of refractive surgery, since it is a major contraindication for it. Development and evolution of corneal topography equipment have allowed the diagnosis of keratoconus at increasingly early stages. However, for years it has been shown that there are subtle details such as observing corneal stroma nerves, most evident in keratoconus patients undergoing ophthalmic examination by slit lamp, which could prove incipient keratoconus and thus support diagnosis in cases only suspected via corneal topography. There is even speculation on the role corneal nerves may have on keratoconus progression. Even Al-Alqaba et al. reported a case series of 14 corneal buttons disease in keratoconus patients, comparing them to 6 corneal buttons in patients without this condition, where the corneal stroma nerves were thicker. This study was performed to compare the thickness of corneal stroma nerves by confocal microscopy in vivo and in a larger group.

Studies have been conducted using confocal microscopy in corneal stroma keratoconus patients, mainly focused on changes in density and reflection of stromal keratocytes, due to their easy capture by confocal microscopy since keratocytes are found across stroma thickness. This study aimed to compare morphological characteristics and thickness of corneal stroma nerves using confocal microscopy, between normal subjects and patients diagnosed with keratoconus. We found no morphological differences between the two groups; both showed corneal stroma nerves and well-defined highly reflective linear structures, surrounded by keratocytes as shown in confocal microscopy images of corneal stroma; they were arranged in an oblique direction in 100% of all images assessed, with various degrees of tortuosity of the thick and narrow portions in both groups. We did find a statistically significant difference regarding thickness of corneal stroma nerves; it was thicker in patients diagnosed with keratoconus, which underpins the classical idea that corneal stroma nerves are more apparent in ophthalmic examination by slit lamp in keratoconus patients.

Corneal stroma nerves thickness has been studied in other diseases where they are known to be abnormally thick, such as the Mocan et al. study, in which mean corneal stroma thickness in diabetic patients was 8.99 ± 2.32 μm. In that study, 35 eyes were assessed in the group of patients with diabetes and 24 in the healthy subject control group; this study assessed 122 in the keratoconic group, and 140 in the control group without disease, and, even so, the latter shows corneal stroma nerve thickness of 5.7 ± 1.7 μm, similar to the control group reported by Mocan et al. of 5.69 ± 2.32 μm.

In this study, stromal nerve with bifurcations were not measured, to avoid overestimating their thickness or altering the actual mean thickness of measured nerves. Only patients with a diagnosis of keratoconus in stages I and III based on the Amsler-Krumeich classification were included because stage IV patients already have scarring that limits the taking and acquisition of quality corneal stroma images using confocal microscopy. Likewise, for stage I patients, keratoconus diagnosis may be doubtful, and bias the study by measuring nerves in non-keratoconus patients, as we needed to be certain to measure corneal stroma nerves in patients with unquestionable keratoconus diagnosis.

Thanks to confocal microscopy it is possible to evaluate corneal stroma nerves; although they are more difficult to capture in confocal microscopy images than subepithelial nerve plexus, a higher number of patients will yield enough images for analysis and measurement. This study can confirm that keratoconus patients have corneal stroma nerves thicker than those of normal subjects, and the aforementioned notion that, under ophthalmologic examination with slit lamp, corneal stroma nerves are more evident in keratoconus patients than in those without the disease. The latter may provide additional data on clinical suspicion of keratoconus.

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**Conflicts of interest**

The authors declare that they have no conflicts of interest.

**REFERENCES**


