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Original article

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



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ARTICLE INFO

Article history:

Received 24 July 2013

Accepted 24 February 2014

Available online 26 September 2014

Keywords:

Cornea

Stromal

Keratoconus

Nerves

Confocal

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 $10.4 \mu\text{m}$), mean corneal stromal nerves thickness in group B was 7.2 ± 1.9 (range from 3.5 to $12.0 \mu\text{m}$). 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.

Palabras clave:

Córnea

Estroma

Queratocono

Nervios

Confocal

[☆] Please cite this article as: Ramírez Fernández M, Hernández Quintela E, Naranjo Tackman R. Análisis de nervios estromales en pacientes con queratocono. Arch Soc Esp Ophthalmol. 2014;89:308–312.

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Resultados: Los nervios del estroma se observaron como estructuras lineales de alta reflexión e irregulares, con porciones gruesas y angostas en todos los casos. El promedio del espesor de los nervios en el grupo A fue de $5,7 \pm 1,7$ (rango de 3,3 a $10,4 \mu$), en el grupo B fue de $7,2 \pm 1,9$ (rango de 3,5 a $12,0 \mu$). La diferencia en el espesor de los nervios entre el grupo A y el grupo B fue estadísticamente significativa ($p < 0,05$).

Conclusiones: La morfología de los nervios del estroma de la córnea fue similar en ambos grupos; el espesor de estos fue mayor en los pacientes con queratocono.

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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.¹ Regarding nerves, there have been many studies on the subepithelial nerve plexus of the cornea thanks to the ease of imaging on this plexus by confocal microscopy, including studies in keratoconus patients.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.^{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 condition⁹; it has even been suggested that corneal stroma nerves are linked to keratoconus progression.¹⁰ 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, Ortek 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.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 $340 \mu\text{m} \times 255 \mu\text{m}$ 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.

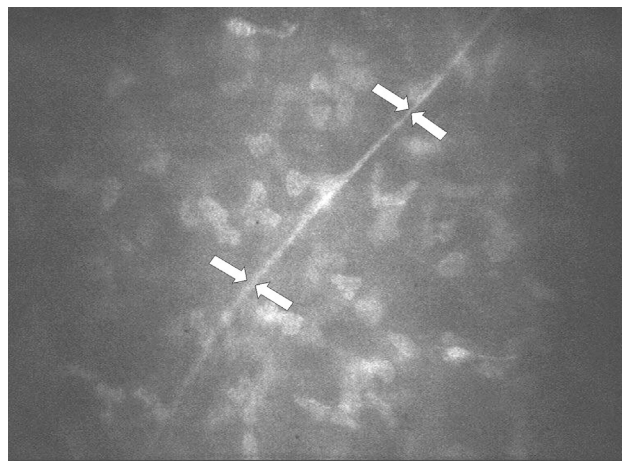


Fig. 1 – Corneal stroma confocal microscopy image $340 \mu\text{m} \times 255 \mu\text{m}$. Corneal stroma nerve of group A is shown, seen as a linear, highly reflective structure with thick and narrow portions (indicated by arrows) surrounded by keratinocytes.

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