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Small fiber neuropathy in women with fibromyalgia. An in vivo assessment using corneal confocal bio-microscopy

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ABSTRACT

Objective: A consistent line of investigation suggests that fibromyalgia is a neuropathic pain syndrome. This outlook has been recently reinforced by several controlled studies that describe decreased small nerve fiber density in skin biopsies of patients with fibromyalgia. The cornea receives the densest small fiber innervation of the body. Corneal confocal bio-microscopy is a new noninvasive method to evaluate small nerve fiber morphology. Our objective was to assess corneal small nerve fiber morphology in patients with fibromyalgia, and to associate corneal nerve microscopic features with neuropathic pain descriptors and other fibromyalgia symptoms.

Methods: We studied 17 female patients with fibromyalgia and 17 age-matched healthy control subjects. All the participants completed different questionnaires regarding the symptoms of fibromyalgia, including a neuropathic pain survey. A central corneal thickness scan was obtained with a confocal microscope. Nerve measurements were made by a single ophthalmologist without knowledge of the clinical diagnosis. Stromal nerve thickness was defined as the mean value between the widest and the narrowest portion of each analyzed stromal nerve. Corneal sub-basal plexus nerve density was also assessed.

Results: Patients with fibromyalgia had stromal nerve thickness of 5.0 $\pm$ 1.0 μm (mean $\pm$ standard deviation) significantly different from that of control's values (6.1 $\pm$ 1.3) $p = 0.01$. Patients also had decreased sub-basal plexus nerve density per square millimeter (85 $\pm$ 29) vs. 107 $\pm$ 26 of controls $p = 0.02$. When controls and patients were grouped together, there was an association between stromal nerve slenderness and neuropathic pain descriptors (Fisher's exact test $p = 0.007$).

Conclusion: Women suffering from fibromyalgia have thinner corneal stromal nerves and diminished sub-basal plexus nerve density when compared to healthy controls. Nerve scarcity is associated with neuropathic pain descriptors. Small fiber neuropathy may play a role in the pathogenesis of fibromyalgia pain. Corneal confocal microscopy could become a useful test in the study of patients with fibromyalgia.

Fibromyalgia is a stress-related syndrome that predominantly affects women. This illness is characterized by chronic widespread pain, insomnia, fatigue, and cognitive difficulties [1]. Fibromyalgia has neuropathic pain features: it is a stimulus-independent pain state, accompanied by allodynia and paresthesias [2]. The notion of fibromyalgia as a neuropathic pain syndrome has been recently reinforced by several controlled studies describing small fiber neuropathy in patients with fibromyalgia [3–8]. Small fiber neuropathy is a disorder of the peripheral nerves that mainly affects small sensory fibers and sympathetic fibers, resulting in pain, paresthesias, and autonomic dysfunction. The diagnosis of small fiber neuropathy is based on the functional tests (quantitative sensory or autonomic reflex testing) showing altered skin sensory or autonomic response plus a skin biopsy examination, demonstrating decreased small nerve fiber density [9].

The cornea receives the densest small fiber innervation of the body from the trigeminal nerve. Small fibers first innervate the corneal stroma, and then they divide and run parallel to the superficial corneal surface, configuring the sub-basal nerve plexus. Corneal confocal bio-microscopy is a new noninvasive method to assess small nerve fiber pathology [10]. It provides real time

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in vivo structural images of the corneal stromal nerves and the sub-basal nerve plexus [11,12].The confocal microscope has computer software that enables the analysis and the measurement of different structures. The software is able to estimate the total number of nerves per square millimeter lying in the sub-basal plexus [13,14].

Objective

The original main objective of this cross-sectional investigation was to assess the corneal stromal nerve fiber morphology in patients with fibromyalgia using confocal microscopy. The secondary goal was to correlate corneal nerve microscopic features with fibromyalgia severity parameters contained in several validated questionnaires, including a neuropathic pain survey and an autonomic symptom questionnaire. Subsequent to the stromal nerve analysis positive initial results, a post hoc measurement of sub-basal plexus small nerve fiber density was performed.

Patients and methods

We studied 17 female patients with fibromyalgia. The entry criteria required the following items: age ranging between 18 and 50 years; having fibromyalgia and fulfilling both versions of the criteria endorsed by the American College of Rheumatology in 1990 and in 2010 and; no concurrent diseases, particularly no evidence of coexisting autoimmune of endocrine illnesses. Patients were sourced from different rheumatology private clinics. A group of 17 age-matched ($\pm$ 3 years) healthy women acted as controls. The entry criteria for controls required that they consider themselves healthy and have a normal physical examination with 5 or less fibromyalgia tender points. The controls were medical and paramedical personnel. A written consent was obtained from all participants. The study was approved by the Institutional Ethics and Research Committees.

In vivo confocal microscopy

A single ophthalmologist expert in corneal pathology evaluated all cases. A ConfoScan 4 confocal microscope (Nidek Technologies, Artigianato, Italy) was used to obtain a central corneal scan of each eye. The front lens of the microscope was disinfected with 70% isopropyl alcohol wipes before and after each examination. A drop of gel (Viscotears, Cibavision Ltd., SA) was placed on the tip of the front lens to provide an immersion liquid within which the front lens could move forward and backward. A Z-ring adapter (ConfoScan, Fortune Technologies, Italy) was placed to maintain corneal contact in order to obtain a central corneal scan without anterior–posterior eye movement. Each corneal study consisted of 4 full-thickness corneal sequence of 350 images, which were digitized using NAVIS software V.3.1.2. (Nidek, Multi-Instrument Diagnostic, Japan). Each image represented a coronal section of approximately 340 $\times$ 255 µm. Scans were stored in the computer's memory [11,12]. All in-focus stromal nerves were studied. Corneal nerve thickness was defined as the mean between the widest and the narrowest portion of each analyzed stromal nerve. The average corneal nerve thickness per person was defined as the average value of all analyzed nerves in each individual. The nerve smoothness was defined as the difference between the widest and the narrowest portions of each analyzed nerve. The average nerve smoothness per person was defined as the average value of all analyzed nerves in each individual. Nerve thickness and nerve smoothness are closely interrelated measurements. NAVIS software (Nidek, Multi-Instrument Diagnostic, Japan) was use to quantify nerve thickness and smoothness [15].

Corneal sub-basal plexus nerve density was also assessed. Nerve density was defined as the total number of sub-basal plexus nerves and their branches per square millimeter. Nerve density measurements did not take into account nerve thickness or nerve length. The ophthalmologist judged, in each case, which was the single best in-focus sub-basal plexus image. Then, the number of nerves and their branches were manually calculated, carefully avoiding duplicate tallies. Based on this 340 $\times$ 255-µm image assessment, NAVIS software automatically calculated sub-basal plexus nerve density per square millimeter. All corneal nerve measurements were done by the same ophthalmologist blinded to the clinical diagnosis.

All participants completed validated Spanish versions of 7 questionnaires related to fibromyalgia symptoms, including autonomic dysfunction, sleep quality, anxiety, depression, fatigue, neuropathic pain, and general well-being. These 7 questionnaires were: Fibromyalgia Impact Questionnaire (FIQ) [16], Medical Outcome Sleep Scale (MOS) [17], Composite Autonomic Symptoms and Signs (COMPASS) [18], Hospital Anxiety and Depression Scale (HADS) [19], Multidimensional Assessment of Fatigue Scale (MAF) [20], Health Survey Short Form-36 (SF-36) [21], and Leeds assessment of neuropathic symptoms and signs (LANSS) [22]. FIQ is an instrument designed to estimate the overall impact of fibromyalgia over many dimensions (e.g., function, pain level, fatigue, sleep disturbance, and psychological distress). It is scored from 0 to 100, with the latter number being the worst case. The average score for the patients seen in tertiary care settings is about 50. MOS measures the 6 dimensions of sleep: initiation, maintenance, quantity, adequacy, somnolence, and respiratory impairment. COMPASS explores symptoms related to the 9 different autonomic function domains: orthostatic, secretomotor, male sexual dysfunction (not used in this study), urinary, gastrointestinal, pupillomotor, vasomotor, syncope, and sleep function. HADS has 14 intermingled anxiety and depression items. MAF is a 16-item scale that measures fatigue according to the 4 dimensions: degree and severity, the distress it causes, timing of fatigue, and their impact on various activities of daily living. SF-36 is a multi-purpose, short-form health survey with 36 questions. It yields an 8-scale profile of functional health and well-being scores as well as psychometrically based physical and mental health scores. LANSS questionnaire contains abnormal pain-associated descriptors related to dysesthesias, thermal, paroxysmal, evoked , or autonomic symptoms. A score of 12 is used as a cutoff point. Scores higher than 12, suggest a neuropathic component in the pain perception. However, it should be mentioned that this cutoff point of 12 has been validated in patients with a single painful location and may therefore misclassify symptoms when there is widespread pain [23].

Statistical analysis

Clinical and confocal microscopy quantitative data are expressed as mean $\pm$ standard deviation. Qualitative data are expressed as percentage. Normal distribution was confirmed by Kolmogorov–Smirnov test. Student's *t* test or Mann–Whitney *U* test analyzed inter-group significant differences. Pearson's or Spearman's methods were used to search for the correlations between confocal microscopy parameters and fibromyalgia symptoms severity. Chi-square or Fisher's exact test analyzed inter-group qualitative variables. The best cutoff point for nerve morphology parameters was determined from receiver-operating characteristic (ROC) curves. LANSS questionnaire has a previously validated cutoff point of 12.