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Greater small nerve fibre damage in the skin and cornea of type 1 diabetic patients with painful compared to painless diabetic neuropathy

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Abstract

Background and aim: Damage to small nociceptive fibres may contribute to painful diabetic neuropathy. We aimed to compare large and small nerve fibre measurements together with skin biopsy and corneal confocal microscopy (CCM) in patients with type 1 diabetes and painful or painless diabetic neuropathy.

Methods: We have assessed the McGill pain questionnaire, neuropathy disability score (NDS), vibration perception threshold (VPT), warm and cold sensation thresholds (WST, CST), electrophysiology, corneal confocal microscopy (CCM) and skin biopsy in participants with type 1 diabetes and painful (n=41) or painless (n=50) diabetic neuropathy and control subjects (n=50).

Results: The duration of diabetes, BMI, HbA1c, blood pressure and lipid profile did not differ between subjects with painful and painless neuropathy. Neuropathy disability score, and vibration perception threshold were higher and sural nerve conduction velocity was lower but sural nerve amplitude, peroneal nerve amplitude and conduction velocity and cold and warm sensation thresholds did not differ between patients with painful compared to painless diabetic neuropathy. However, intra-epidermal nerve fibre density, corneal nerve fibre density, corneal nerve branch density and corneal nerve fibre length were significantly lower in subjects with painful compared to painless diabetic neuropathy.

Conclusions: There is evidence of more severe neuropathy, particularly small fibre damage in the skin and cornea of patients with painful compared to painless diabetic neuropathy.

Introduction

Approximately 20% of patients with diabetic peripheral neuropathy (DPN) develop painful neuropathy which is associated with significant morbidity and reduced quality of life [1]. The efficacy of current therapies is highly variable [2,3], which may in part reflect diverse etiological mechanisms operating at the peripheral nociceptor, spinal and supraspinal levels in painful DPN [4]. It has been suggested that phenotyping patients based on their symptom complex and identifying the primary site and mechanism of their symptoms may allow a more tailored therapeutic approach and improved response to treatment [5]. However, sensory testing, skin punch biopsy and brain imaging have been deemed to have insufficient evidence to support their use as biomarkers in pain clinical trials [6]. Furthermore, a retrospective analysis of 7 clinical trials showed a limited impact of sensory phenotyping on predicting the efficacy of drugs to relieve neuropathic pain [7].

Given that painful diabetic neuropathy is considered to arise from dysfunction and damage to nociceptive C-fibres and autonomic fibres [8,9], it is no surprise that standard neurophysiology does not differentiate diabetic patients with painful compared to painless neuropathy [10]. Indeed, whilst neurophysiological parameters such as the F-wave and H-reflex identify patients with sub-clinical diabetic neuropathy, they cannot differentiate patients with and without painful neuropathy [11,12]. However, a recent metanalysis has shown a greater abnormality in heat pain thresholds in patients with painful compared to painless diabetic neuropathy [13]. But, a skin biopsy study demonstrated no difference in IENFD in patients with and without painful neuropathy [14]. Similarly, another detailed immunohistochemical skin biopsy study found no difference in IENFD, but patients with painful diabetic neuropathy had an increased density of dermal peptidergic fibers containing SP and CGRP compared to patients with painless DPN and healthy controls [15]. We have previously shown a reduction in intraepidermal nerve fibre length in patients with painful compared to painless diabetic neuropathy [16]. A recent study has shown increased axonal regeneration and swelling [17], whilst another study has shown no relationship between the severity of axonal swelling [18] and painful diabetic neuropathy. Furthermore, exercise reduced painful neuropathic symptoms but was associated with an increase in IENF branch density [19].

Corneal confocal microscopy (CCM) is a rapid, non-invasive technique, which can be used to image and quantify small fibre pathology [20] and is comparable to IENFD for the diagnosis of diabetic peripheral neuropathy [21-23]. We have shown a significant reduction in corneal nerve fibre

length in patients with painful compared to painless diabetic neuropathy [16] and more recently we have shown a reduction in central and inferior whorl corneal nerve fibre length in patients with painful diabetic neuropathy [24]. Furthermore, in a recent study from China there was greater corneal nerve fibre damage in diabetic patients with painful compared to painless diabetic neuropathy [25].

In the present study, we have compared detailed measures of large and small fibre function together with skin biopsy and CCM in a large cohort of patients with type 1 diabetes and painful or painless diabetic neuropathy.

Research Design and Methods

Study Participants

91 participants with type 1 diabetes mellitus and 50 age-matched healthy control subjects were studied. The study was approved by the North Manchester Research Ethics committee and written informed consent was obtained from all participants. This research adhered to the tenets of the Declaration of Helsinki. Patients with a history of any systemic disease apart from diabetes associated with neuropathy were excluded from the study. In addition, patients with any history of systemic or ocular diseases associated with corneal involvement were excluded from the study.

Clinical and peripheral neuropathy assessments

All participants underwent a complete medical history and an assessment of body mass index (BMI), blood pressure, glycated haemoglobin (HbA1c), cholesterol, LDL, HDL and triglycerides. Symptoms of neuropathy were assessed using the neuropathy symptom profile (NSP) and short form McGill pain questionnaire (SF-MPQ) [26].

Detailed assessment of neuropathy included the neuropathy disability score (NDS), vibration perception threshold (VPT) on the hallux of both feet using a Neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilford, Nottingham, UK), cold and warm sensation threshold (CST & WST); and cold and heat induced pain threshold (CIP & HIP) using a TSA-II NeuroSensory Analyser (Medoc Ltd., Ramat-Yishai, Israel) on the dorsolateral aspect of the left foot. A Dantec "Keypoint" system (Dantec Dynamics Ltd, Bristol, UK) was used to measure sural nerve action potential amplitude (SNAP) and conduction velocity (SNCV) and peroneal nerve action potential amplitude (PMNAP) and conduction velocity (PMNCV).

Skin biopsy and IENFD assessment

Two 3-mm punch skin biopsies were taken from the dorsum of the dominant foot, 2 cm above the second metatarsal head under local anaesthesia (1% lidocaine). The biopsies were immediately fixed in 4% paraformaldehyde, cryoprotected in graded solutions of sucrose, frozen and cut on a cryomicrotome (HM450, Microm International, Germany). Six 50 micrometre sections per biopsy were immuno-stained using anti-human PGP 9.5 antibody (Abcam, Cambridge, U.K.), and nerve

fibres were demonstrated using SG chromogen (Vector Laboratories, Peterborough, U.K.) [27]. IENFD (no./mm) was quantified in accordance with established criteria and expressed as a number of nerve fibres per millimetre length of epidermis [28].

Corneal confocal microscopy

The central cornea of all study participants was scanned using a laser IVCCM (Heidelberg Retinal Tomograph III Rostock Cornea Module (HRT III RCM); Heidelberg Engineering GmbH, Heidelberg, Germany) [29]. The examination was performed according to our previously published protocol, by highly experienced optometrists and took approximately 5 minutes for both eyes of each participant [30]. Six images (3 from each eye) from the corneal sub-basal nerve plexus were exported and used for image analysis. Two experienced examiners analysed all the images manually using CCMetrics (MA Dabbah; Imaging Science and Biomedical Engineering, University of Manchester, Manchester, UK), while being masked from the outcome of medical and peripheral neuropathy assessments. Corneal nerve morphological parameters including corneal nerve fibre density (CNFD), the number of main nerve fibres/mm^2; corneal nerve branch density (CNBD), the number of branch points on the main nerves/mm^2; corneal nerve fibre length (CNFL), the total length of nerves mm/mm^2; and corneal nerve fibre tortuosity (CNFT), the tortuosity coefficient of main nerves (TC) were measured for each image and the average of the results from 6 images was used for data analysis.

Corneal sensation was measured in the centre of the cornea using non-contact corneal aesthesiometry (NCCA) (Glasgow Caledonian University, Glasgow, Scotland, UK) [31].

Study definition of painful neuropathy

All 91 patients with type 1 diabetes were confirmed to have diabetic neuropathy based on an abnormality in at least two out of five neurological measures including IENFD (<3.3), CNFD (<24.4), CST (<23.8), VPT (>16.5) or PMNCV (<40) defined by results lying 2SD out with the mean of control subjects. Patients were divided into two groups based on the SF-MPQ & McGill pain index (PI) with a score >1/5 considered to have painful diabetic neuropathy and those with a score 0/5 were considered to have painless diabetic neuropathy.

Statistical analysis

IBM SPSS v19.0 (Chicago, IL, USA) for Windows was used to compute the results. Analysis included descriptive and frequency statistics and all data presented as Mean ± SD. Shapiro-Wilk test and Q-Q plots were used to evaluate whether the data were normally distributed or not. To evaluate the difference between the two groups, the Independent sample T test (Mann-Whitney U test for non-parametric) and to evaluate the difference among the groups, One-way ANOVA with Bonferroni adjustment were used.

Results

Demographic and clinical findings

Ninety-one patients with type 1 diabetes mellitus, 41 with painful neuropathy and 50 with painless neuropathy and 50 age-matched controls were studied. There was no difference in age, gender, ethnicity, smoking, alcohol consumption, BMI, lipid profile, blood pressure and HbA1c, but height was significantly lower (P=0.02) in patients with painful compared to painless diabetic neuropathy (Table 1).

Neuropathy assessments

SNCV, SNAP, PMNCV, PMNAP, CST and IENFD were significantly (P<0.01) lower and NSP, NDS, VPT and WST were significantly higher (P<0.01) in patients with type 1 diabetes compared to control subjects.

NSP (P=0.0001), NDS (P=0.003) and VPT (P=0.001) were higher and SNCV (P=0.009) was lower in patients with painful compared to painless neuropathy. IENFD was significantly lower (P=0.02) in patients with painful compared to painless neuropathy (Figure 1). There was no correlation between NDS, VPT, quantitative sensory testing and neurophysiology with the severity of painful neuropathic symptoms using the McGill visual analogue index (Table 2).

Corneal sensation and nerve fibre pathology

The corneal sensation threshold was significantly higher in patients with painful neuropathy compared to control subjects (P<0.001) and painless diabetic neuropathy (P=0.003). CNFD, CNBD and CNFL were significantly lower (P<0.001) in patients with type 1 diabetes compared to healthy controls and CNFD (P=0.005), CNFL (P=0.01) and CNBD (P=0.05) were significantly lower in patients with painful compared to painless diabetic neuropathy (Figures 2 and 3). CNFT

was significantly higher (P=0.03) in patients with painless neuropathy compared to controls but there was no difference between patients with painful and painless diabetic neuropathy. There was no correlation between corneal nerve parameters and the severity of neuropathic pain based on the McGill visual analogue index.

Discussion

The present study confirms our previous studies demonstrating that CCM can identify neuropathy in patients with diabetes [32-34]. Small fibre neuropathy is considered to underlie neuropathic pain [35], however, symptom assessment tools [36] as opposed to measures of small fibre dysfunction or damage are advocated in the diagnosis of neuropathic pain [37]. This is because there is controversy as to whether specific features of small fibre pathology in skin biopsies [38,39] may be related to painful neuropathy [17,18,40,41].

We demonstrate a more severe neuropathy in patients with painful neuropathy, which is consistent with our data from a large population based study [1] and from two recent detailed phenotyping studies [42]. Most neurophysiological measures and thermal thresholds did not differ between patients with painful and painless diabetic neuropathy, in agreement with previous data [9,16]. However, there was evidence of a greater reduction in IENFD, CNFD, CNBD and CNFL in patients with painful compared to painless diabetic neuropathy [16,43]. The lower corneal nerve branch density, suggests lower nerve regeneration, which contrasts with the results of a recent study showing increased axonal regeneration and axonal swelling in patients with painful diabetic neuropathy [17].

Identifying small fibre pathology may help in the diagnosis and identification of patients who may respond optimally to a particular therapy. Indeed in two randomized placebo controlled studies the best response to treatment with oxcarbazepine [44] and lignocaine 5% patch [45] was achieved in those patients with an irritable nociceptor phenotype. We have recently shown that patients with altered rate dependent depression, indicating reduced spinal inhibition and those with a reduction in corneal nerve fibre density may respond optimally to therapies such as duloxetine [43].

This is a cross sectional study which prevents us from concluding a causal relationship between small fibre damage in the skin and cornea in patients with painful diabetic neuropathy. The study was also only undertaken in patients with type 1 diabetes and the findings may not be applicable to

the majority of patients with type 2 diabetes. Although, greater corneal nerve fibre damage has been shown in patients with type 2 diabetes and painful compared to painless neuropathy [25]. In addition, previous studies have assessed patients with type 1 and type 2 diabetes and shown greater small nerve fibre damage in painful compared to painless neuropathy [9,16,24].

Corneal confocal microscopy represents a rapid non-invasive ophthalmic imaging technique to objectively quantify small fibre pathology and differentiate patients with painful from painless diabetic neuropathy.

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Dr Mitra Tavakoli undertook corneal confocal microscopy and Dr Hassan Fadavi undertook clinical assessment and quantitative sensory testing in a proportion of the patients and control subjects.

Data availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest: There is no conflict of interest related to this work for any of the authors.

Author contributions

M.F. researched data, performed analysis and wrote the manuscript, S.A. researched data and reviewed the manuscript, A.K. researched data and reviewed the manuscript, I.N.P. researched data, G.P. researched data, O.A researched data, U.A. researched data, A.M. researched data, A.J.M.B. reviewed and revised the manuscript, N.E. reviewed and revised the manuscript, H.S. reviewed and revised the manuscript, M.J. researched data and reviewed the manuscript, R.A.M. designed the study and reviewed and revised the manuscript.

R.A.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Figure 1. Representative examples of 50 μm sections from skin biopsies immunostained for PGP9.5 In healthy control (A), a diabetic patient with painless neuropathy (B) and a diabetic patient with painful neuropathy (C). Healthy control (A) shows numerous long branching intraepidermal nerve fibres reaching upper levels of the epidermis (red arrow heads) and well-developed sub-epidermal nerve plexus (yellow arrow heads). Biopsy from diabetic patient with painless neuropathy shows scant but well developed intraepidermal nerve fibres compared to a biopsy from diabetic patient with painful neuropathy (C) showing scant short intraepidermal nerve fibres. A-C at the same magnification, scale bar = 100 μm.

Figure 2. CCM images of corneal sub basal nerve plexus in a healthy control, patient with type 1 diabetes in painless and painful neuropathy (Red arrows: main nerve, yellow arrows: branches).

Figure 3. Corneal nerve fibre density (CNFD) (A), corneal nerve branch density (CNBD) (B) and corneal nerve fibre length (CNFL) (C) and Intra epidermal nerve fibre density (D) was reduced significantly in diabetic patients compared to control subjects and was further reduced in diabetic patients with painful compared to painless diabetic neuropathy.

Table 1. Demographic and clinical findings in healthy controls and patients with type 1 diabetes with and without painful neuropathy. Data are presented as Mean ± SD. All symbols indicate statistically significant difference, * $P<0.01$ compared to controls and # $P<0.05$ compared to patients with painless neuropathy.

Table 2. Neuropathy symptoms, deficits, quantitative sensory testing, neurophysiology, skin biopsy, corneal sensitivity and confocal microscopy in healthy controls and patients with type 1 diabetes with painful and painless diabetic neuropathy. Data are presented as Mean ± SD. All symbols indicate statistically significant difference, * $P<0.01$ compared to controls and # $P<0.05$ compared to the patients with painless neuropathy.

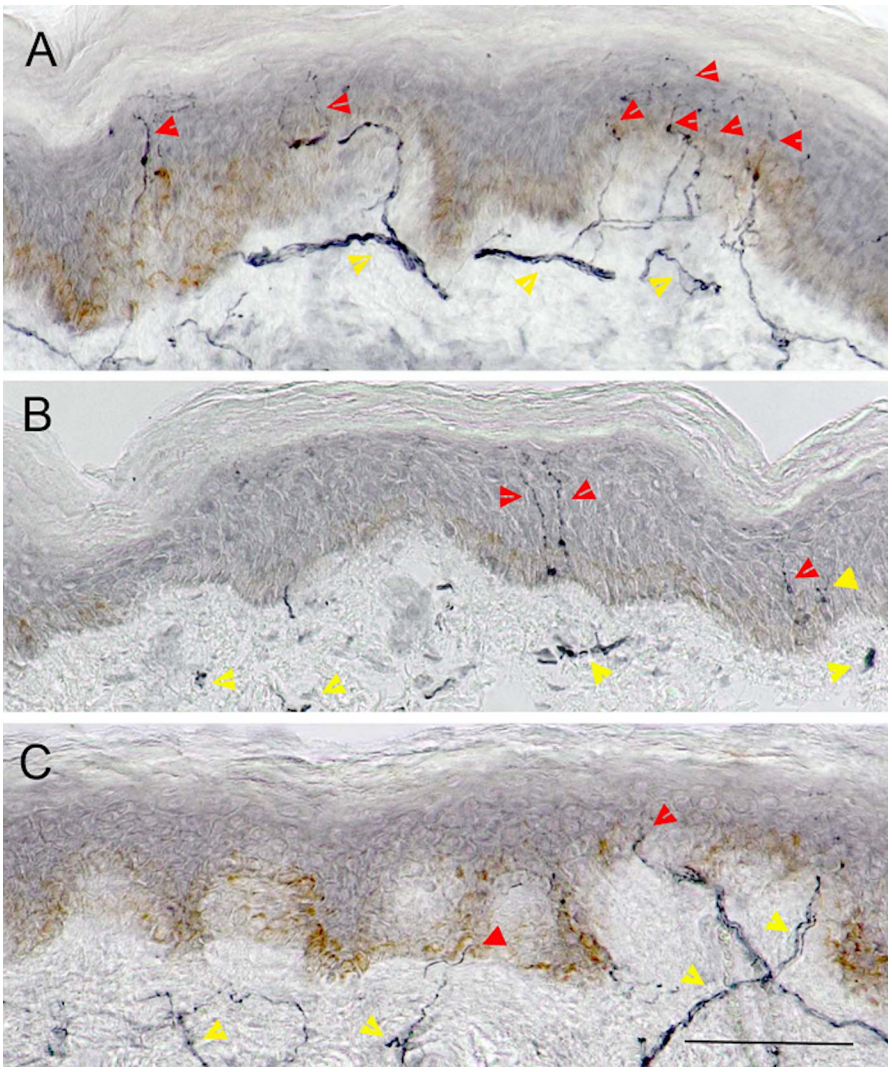
Parameters	Control	Type 1 diabetes Painless Neuropathy	Type 1 diabetes Painful Neuropathy
Number	50	50	41
Age (years)	51.5±12.7	47.6±14.4	52.7±14.4
Gender (F/M)	26/24	20/30	24/17
Ethnicity (Asian/European)	18/32	4/46	3/38
Duration of diabetes (years)	_	30.8±17.0	33.6±16.1
Smoking (no per day)	0.7±2.4	1.8±4.9	1.7±5.5
Alcohol consumption (units per week)	5.1±7.6	5.6±8.1	3.2±5.9
HbA1c (%)	5.6±0.3	8.2±1.2*	8.5±1.7*
IFCC (mmol/mol)	37.7±3.6	64.7±18.1*	69.6±18.5*
BMI (kg/m^2)	26.7±4.5	26.7±4.1	27.03±4.8
Height (cm)	166.0±10.7	171.6±9.2*	166.1±9.4$^{\#}$
Waist circumference (cm)	90.3±14.9	92.3±13.5	89.8±18.9
Cholesterol (mmol/l)	5.0±0.7	4.4±0.9	4.2±0.7
HDL (mmol/l)	1.6±0.5	1.6±0.4	1.7±0.5
Triglycerides (mmol/l)	1.4±0.7	1.1±0.6	1.2±0.7
LDL (mmol/l)	2.7±0.7	2.3±0.9*	2.1±0.4*
BP Sys /Dia (mm Hg)	128.7±17.9/ 73.0±9.9	133.6±20.5/ 70.4±7.8	137.7±24.6/ 72.1±11.2

Table 1. Demographic and clinical findings in healthy controls and patients with type 1 diabetes with and without painful neuropathy. Data are presented as Mean ± SD. All symbols indicate statistically significant difference, * $P<0.01$ compared to controls and # $P<0.05$ compared to patients with painless neuropathy. HbA1c – glycated haemoglobin, BMI – body mass index, HDL – high density lipoprotein, LDL – low density lipoprotein, BP – blood pressure.

Parameters	Control	Type 1 diabetes Painless	Type 1 diabetes Painful
McGill VAS (0-10)	0	0.04±0.2	5.51±2.7*#(P<0.0001)
NSP (0-38)	0.3±0.8	1.6±2.9	8.2±6.4# (P=0.0001)
NDS (0-10)	0.6±1.2	2.6±1.4	4.5±2.2# (P=0.003)
VPT (volts)	6.5±5.0	12.4±10.7	21.1±14.1# (P=0.001)
CST (°C)	28.2±2.2	25.3±5.3*	22.3±8.2*
WST (°C)	36.9±3.1	39.8±3.5*	41.6±4.9*
CIP (°C)	11.8±9.4	7.3±7.2*	7.4±8.9
HIP (°C)	44.9±3.2	45.9±3.3	47.3±3.1*
SNCV (m/s)	50.2±4.5	42.7±6.0*	38.5±7.9*# (P=0.009)
SNAP (μV)	19.4±9.6	9.8±7.7*	6.6±6.8*
PMNCV (m/s)	48.6±4.1	40.3±7.3*	37.7±8.7*
PMNAP (mV)	5±2.2	3.7±2.5	2.7±4.1*
IENFD (no./mm)	9.1±2.9	6.6±4*	4.4±4.2*#(P=0.02)
NCCA (mbars)	0.5±0.3	0.7±0.5	1.5±1.8*# (P=0.003)
CNFD (no./mm^2)	37.0±6.3	26.2±8.0*	20.2±10.7*# (P=0.005)
CNBD (no./mm^2)	87.1±34.4	58.1±30.0*	46.4±32.5*# (P=0.05)
CNFL (mm/mm^2)	26.1±5.2	19.8±5.6*	15.7±7.8*# (P=0.01)
CNFT (TC)	15.6±3.6	19.2±6.9*	17.5±8.6

Table 2. Neuropathy symptoms, deficits, quantitative sensory testing, neurophysiology, skin biopsy, corneal sensitivity and confocal microscopy in healthy controls and T1DM patients with painful and painless diabetic neuropathy. Data are presented as Mean ± SD. All symbols indicate statistically significant difference, * P<0.01 compared to controls and # P<0.05

compared to the patients with painless neuropathy. McGill VAS – visual analogue scale, NSP – neuropathy symptom profile, NDS – neuropathy disability score, VPT – vibration perception threshold, CST – cold sensation threshold, WST – warm perception threshold, CIP – cold induced pain threshold, HIP – heat induced pain threshold, SNCV – sural nerve conduction velocity, SNAP – sural nerve action potential amplitude, PMNCV – peroneal motor nerve conduction velocity, PMNAP – peroneal nerve action potential amplitude, IENFD – intra epidermal nerve fibre density, NCCA – non-contact corneal aesthesiometry, CNFD – corneal nerve fibre density, CNBD – corneal nerve branch density, CNFL – corneal nerve fibre length, CNFT – corneal nerve fibre tortuosity, TC – tortuosity coefficient.



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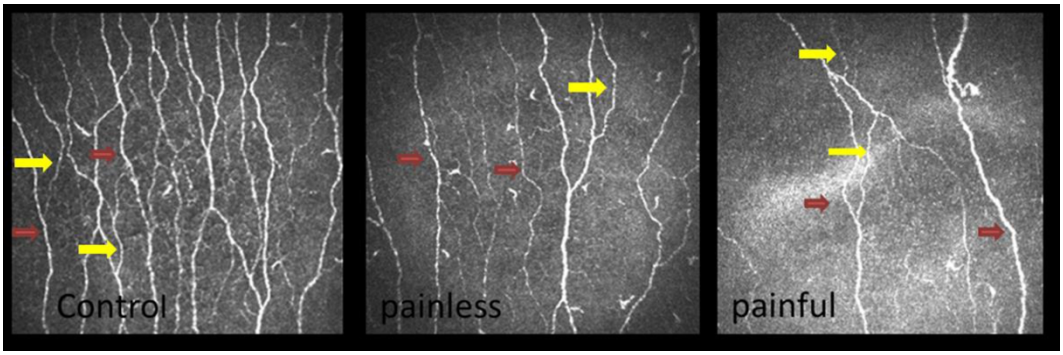

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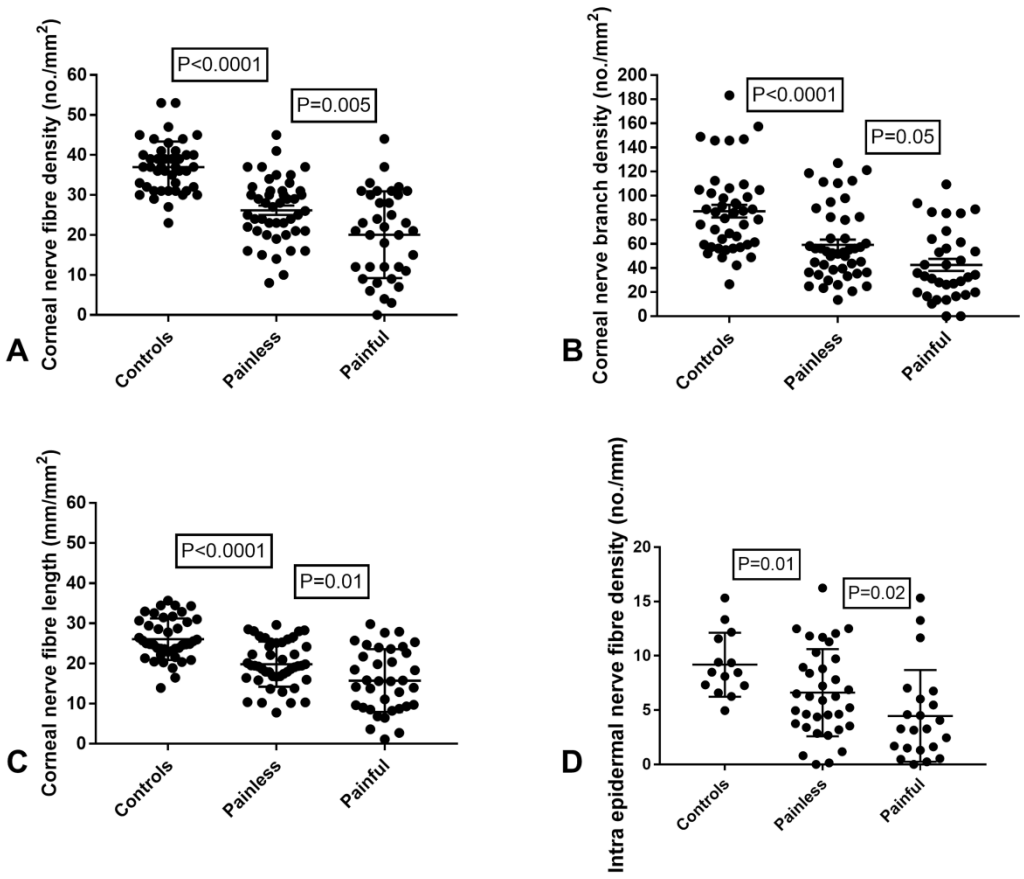

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