


Corneal confocal microscopy detects severe small fiber neuropathy in diabetic patients with Charcot neuroarthropathy

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ABSTRACT

Aims/Introduction: The aim of the present study was to identify the extent of small fiber neuropathy in diabetic patients with Charcot neuroarthropathy (CN).

Materials and Methods: A total of 20 patients with CN were compared with 20 age- and diabetes duration-matched patients with type 2 diabetes and 20 age-matched control participants. All patients underwent corneal confocal microscopy with quantification of corneal nerve morphology and assessment for vibration perception threshold, and a subset of patients with CN underwent assessment of sudomotor function and neuropathic pain.

Results: In patients with CN compared with type 2 diabetes patients and control participants, there was a significant reduction in corneal nerve fiber density (14.94 ± 8.23 vs 23.86 ± 7.71 , $P = 0.004$ vs 34.84 ± 9.13 , $P < 0.001$), corneal nerve branch density (18.61 ± 16.7 vs 41.62 ± 22.67 , $P = 0.032$ vs 76.47 ± 38.44 , $P < 0.001$) and corneal nerve fiber length (8.40 ± 4.83 vs 14.87 ± 4.76 , $P = 0.001$ vs 21.24 ± 6.48 , $P < 0.001$), electrochemical skin conductance on the feet (20.57 ± 13.99 vs 61.50 ± 22.26 , $P < 0.001$ vs 76.23 ± 12.01 , $P < 0.001$) and hands (30.86 ± 18.10 vs 61.13 ± 19.14 , $P = 0.001$ vs 68.31 ± 11.96 , $P < 0.001$), and a significant increase in the vibration perception threshold in the feet (38.46 ± 15.10 vs 14.15 ± 10.25 , $P < 0.001$ vs 7.75 ± 4.01 , $P < 0.001$).

Conclusions: Patients with diabetes and CN have severe large and particularly small fiber neuropathy.

INTRODUCTION

Charcot neuroarthropathy (CN) can occur in 0.4–13% of people with diabetes and is an extremely debilitating complication¹. It typically presents with a hot swollen foot, and underlying bone disruption and destruction, resulting in significant disability, and is associated with a mortality rate of 28.3% within 5 years².

The clinical signs during acute CN include warmth and swelling, indicative of acute inflammation^{3–5}. Diagnosis is based on the history and clinical examination and timely imaging of the foot⁶. The pathogenesis of CN remains unclear. Much of the focus has been on inflammatory pathways affecting osteoblast^{7,8} and, more recently, osteoclast dysfunction^{9,10}. Pathways that have received the most attention in the pathogenesis of CN

include the receptor activator of nuclear factor- κ B, receptor activator of nuclear factor- κ B ligand and osteoprotegerin¹¹, and more recently, abnormalities in sclerostin, dickkopf-1, Wnt inhibitory factor-1 and Wnt ligand-1¹². The role of these pathways is complex, with a recent study showing initial suppression of receptor activator of nuclear factor- κ B ligand in peripheral blood mononuclear cells followed by increased levels in those with faster healing on magnetic resonance imaging¹³.

Charcot originally suggested the ‘neurovascular theory’ of sympathetic denervation, altered vasoregulation and bone resorption as a consequence of small fiber involvement, whereas Volkman and Virchow attributed a loss of proprioception to large fiber involvement in CN. Since then, the role of small fiber neuropathy has received little attention in relation to CN, despite the observation that there are significant numbers of substance P and calcitonin gene-related peptide (CGRP)-

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expressing nerves in the periosteum and bone marrow¹⁴. Indeed, there is a substantial literature on the role of non-synaptically released substance P and CGRP, regulating osteoblast and osteoclast proliferation¹⁵, and inhibiting pro-inflammatory cytokines¹⁶, key players in the pathophysiology of CN. Whereas in a study of 23 patients with CN, small fiber integrity, assessed by the hyperemic response to heating skin, was found to be preserved¹⁷, a subsequent study from the same group showed an impaired nerve–axon reflex in patients with CN¹⁸. More direct bone biopsy studies have shown reduced expression of CGRP in the bone of patients with CN compared to patients with diabetic neuropathy only¹⁹. Furthermore, in a study comparing patients with ankle osteoarthritis and CN, the density of sympathetic nerve fibers was significantly lower in the skin, synovium and bone of patients with CN²⁰. These studies show that the loss of small fibers, particularly autonomic sympathetic fibers, might be an important feature of CN.

Corneal confocal microscopy (CCM) is a rapid non-invasive ophthalmic imaging technique, which allows high-resolution imaging of corneal nerves. We have pioneered its use to identify axonal loss in diabetic^{21,22} and other peripheral neuropathies^{23,24}, and have shown that it has a very high sensitivity and specificity for identifying diabetic autonomic neuropathy¹³. Furthermore, we have recently shown a rapid decline in corneal nerve fibers, with no change in neurophysiology or quantitative sensory testing, before the development of CN in a patient with diabetes; suggesting that small fiber neuropathy might be critical in the development of CN²⁵.

The aim of the present study was to establish the extent of small fiber pathology in a large cohort of patients with diabetes and CN.

METHODS

Patients with CN ($n = 20$) and age- and diabetes duration-matched patients with type 2 diabetes ($n = 20$) were recruited from the podiatric and diabetes departments at Hamad General Hospital in Doha, Qatar.

Charcot neuroarthropathy participants were assessed from December 2014 to April 2017, and type 2 diabetes participants were assessed from July 2016 to April 2017. Age-matched healthy control participants ($n = 20$) were recruited and assessed from October 2016 to April 2017, from Rumailah Hospital in Doha, Qatar.

Patients were diagnosed with CN on the basis of clinical and radiological findings, and were excluded if they had autoimmune disease, ongoing infection, osteomyelitis, acute or critical limb ischemia, or any other cause of peripheral neuropathy. Participants were also excluded if they had a known history of ocular trauma or surgery, or any corneal or anterior segment pathology. No sample power analysis was carried out, as there are no previous studies evaluating small fiber damage using CCM in CN.

The present study adhered to the tenets of the declaration of Helsinki, and was approved by the institutional review boards

of Weill Cornell Medicine-Qatar (no. 942389-1), Hamad General Hospital (no. 942389-1) and Rumailah Hospital (no. 15-00019). Informed, written consent was obtained from all participants before participation in the study.

Body mass index, blood pressure, glycated hemoglobin, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, albumin, alanine transaminase, aspartate transaminase, creatinine, vitamin D and corrected calcium were assessed on the day of their visit.

Vibration perception threshold was assessed using a neurothesiometer (Horwell; Scientific Laboratory Supplies, Wilfrod, Nottingham, UK). Three consecutive readings were taken on the great toe of each foot and averaged.

The Douleur Neuropathique 4 interview questionnaire was used to identify neuropathic pain in eight of the participants with CN. Sudoscan™ (Impeto Medical, Paris, France) was used to measure sudomotor function based on sweat chloride concentrations through reverse iontophoresis and chronoamperometry²⁶. Patients simultaneously placed both hands and feet on two sets of large-area nickel electrode plates for 2 min without movement. The electrochemical skin conductance, expressed in microSiemens (μ S), is the ratio between the current generated and the constant DC stimulus (≤ 4 V) applied to the electrodes. Sudomotor dysfunction was defined according to the electrochemical skin conductance measured on the feet: >60 μ S, no dysfunction; 40–60 μ S, moderate dysfunction; and <40 μ S, severe dysfunction. Results were only available in seven patients with CN, because the instrument was not available when we commenced the study.

Central corneal sub-basal plexus nerve images were captured using a Heidelberg Retinal Tomograph III Rostock Cornea Module (Heidelberg Engineering GmbH, Heidelberg, Germany) after instilling two drops of local anesthetic (0.4% benoxinate hydrochloride; Chauvin Pharmaceuticals, Chefaro, UK) to anesthetize each eye and Viscotears (Carbomer 980, 0.2%; Novartis, Grimsby, UK) as the coupling agent between the cornea and the applanation cap. Based on depth, contrast and focus position, four to six images per participant were selected and analyzed using validated, purpose-written software (CCMetrics®, MA Dabbah, ISBE; University of Manchester, Manchester, UK)²¹. The specific parameters measured per frame were those we have previously established: corneal nerve fiber density, corneal nerve branch density and corneal nerve fiber length.

Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics software version 24 for Windows (Armonk, New York, USA). Normality of the distribution of data was examined using the Kolmogorov–Smirnov test and by visual inspection of the histogram and normal Q–Q plot. Data is expressed as the mean \pm standard deviation (Table 1).

Comparisons of the characteristics between different groups were assessed using one-way ANOVA with the Bonferroni *post-hoc* test (for normally distributed variables) and with the non-

Table 1 | Clinical, demographic, metabolic and peripheral neuropathy assessments in the participants

Characteristics	Control participants	Type 2 diabetes patients	CN
No. participants	20	20	20
Age (years)	62.04 ± 9.76	60.45 ± 9.38	59.15 ± 9.26
Sex (male/female)	14/6	09/11	13/07
Diabetes duration (years)	–	18.26 ± 7.33	19.42 ± 7.76
BMI (kg/m ²)	28.12 ± 2.89	32.27 ± 5.28	32.19 ± 7.33
Systolic BP (mmHg)	151.45 ± 44.02	137.10 ± 14.80	137.29 ± 36.33
Diastolic BP (mmHg)*	86.45 ± 21.04	73.40 ± 6.80	77.53 ± 7.95
HbA _{1c} (%) (mmol/mol)***	5.6 ± 0.5 (37 ± 5)	8.8 ± 1.8 (73 ± 18) [†]	8.8 ± 2.0 (73 ± 21) [†]
Albumin (mg/mmol)***	44.20 ± 3.03	38.05 ± 4.08 [†]	34.53 ± 5.10 ^{†‡}
Creatinine (mg/mmol)	78.88 ± 22.87	98.16 ± 92.93	118.95 ± 131.18
ALT (mg/L)	21.75 ± 9.20	26.79 ± 14.52	21.00 ± 12.08
AST (μmol/L)	21.00 ± 6.37	24.05 ± 15.28	19.79 ± 6.58
Total Cholesterol (mmol/L)**	5.08 ± 1.09	4.34 ± 1.19	3.80 ± 1.37 [†]
HDL (mmol/L)	1.37 ± 0.42	1.11 ± 0.26	1.22 ± 0.48
LDL (mmol/L)*	3.11 ± 1.03	2.44 ± 0.96	2.04 ± 1.06 [†]
Triglycerides (mmol/L)	1.33 ± 0.64	1.76 ± 1.08	1.60 ± 0.73
Vitamin D (ng/mL)	24.85 ± 8.63	25.74 ± 10.44	22.41 ± 9.73
Calcium corrected (mmol/L)	0.59 ± 0.04	0.60 ± 0.02	0.59 ± 0.03
Neuropathy assessments			
DN4 (score/10)***	0.00 ± 0.00	2.81 ± 2.26 [†]	6.13 ± 2.80 ^{†‡}
VPT (V)***	7.75 ± 4.01	14.15 ± 10.25	38.46 ± 15.10 ^{†‡}
ESC feet (μS)***	76.23 ± 12.01	61.50 ± 22.26	20.57 ± 13.99 ^{†‡}
ESC hands (μS)***	68.31 ± 11.96	61.13 ± 19.14	30.86 ± 18.10 ^{†‡}
CNFD (no/mm ²)***	34.84 ± 9.13	23.86 ± 7.71 [†]	14.94 ± 8.23 ^{†‡}
CNBD (no/mm ²)***	76.47 ± 38.44	41.62 ± 22.67 [†]	18.61 ± 16.79 ^{†‡}
CNFL (mm/mm ²)***	21.24 ± 6.48	14.87 ± 4.76 [†]	8.40 ± 4.83 ^{†‡}

Results are expressed as mean ± standard deviation. Statistically significant differences between groups using ANOVA: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. [†]Post-hoc results differ significantly from the control group ($P < 0.05$). [‡]Post-hoc results differ significantly from the type 2 diabetes group ($P < 0.05$). Type 2 diabetes with Charcot neuroarthropathy (CN). ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; BP, blood pressure; CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; DN4, Douleur Neuropathique 4; ESC, electrochemical skin conductance; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; VPT, vibration perception threshold.

parametric Kruskal–Wallis test (for non-normally distributed variables). A P -value of <0.05 was considered to be significant. We used Prism 6 (version 6.0 g; GraphPad Software Inc., La Jolla, California, USA) for plotting the graphs.

RESULTS

The clinical, metabolic and demographic profiles of the participants in the study are presented in Table 1. A total of 20 patients with type 2 diabetes and CN were compared with 20 patients with type 2 diabetes and 20 healthy control participants. There was no significant difference in age, body mass index, systolic blood pressure, creatinine, high-density lipoprotein cholesterol, triglycerides, vitamin D and corrected calcium between the control participants, patients with type 2 diabetes and patients with CN. Total cholesterol ($P = 0.005$) and low-density lipoprotein cholesterol ($P = 0.02$) were significantly lower in patients with CN compared with control participants. Albumin was lower in patients with CN compared with control participants ($P < 0.001$) and type 2 diabetes ($P = 0.037$),

whereas diastolic blood pressure was lower in type 2 diabetes compared with control participants ($P = 0.013$). There was no significant difference in glycated hemoglobin ($P = 1.000$) or diabetes duration between patients with CN and patients with type 2 diabetes ($P = 0.639$).

Vibration perception threshold was significantly higher in patients with CN compared with healthy control participants ($P < 0.001$) and patients with type 2 diabetes ($P < 0.001$). Douleur Neuropathique 4 was significantly higher in patients with CN compared with healthy control participants ($P < 0.001$) and patients with type 2 diabetes ($P < 0.001$). Electrochemical skin conductance on the hands ($P = 0.001$, $P < 0.001$) and feet ($P < 0.001$, $P < 0.001$) was significantly lower in diabetic patients with CN compared to patients with type 2 diabetes and control participants, respectively (Table 1). CCM showed severe small fiber damage in diabetic patients with Charcot compared to patients with type 2 diabetes and control participants (Table 1; Figure 1). There was a significant reduction in corneal nerve fiber density, corneal nerve branch density and

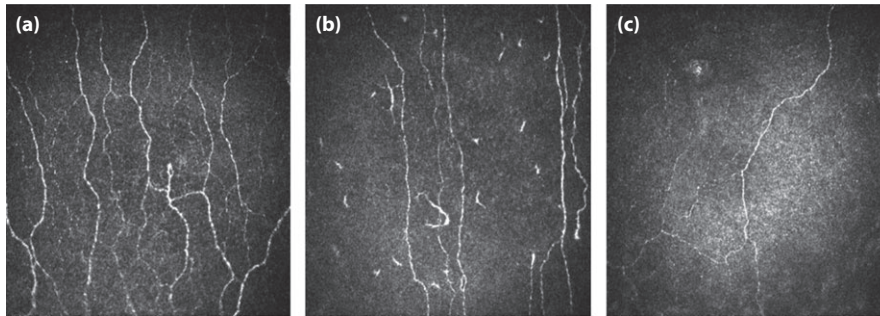


Figure 1 | Representative images of the sub-basal nerve plexus of (a) a healthy control participant, (b) with loss of corneal nerve fibers in a patient with type 2 diabetes and (c) marked loss in a diabetic patient with Charcot neuroarthropathy.

corneal nerve fiber length in diabetic patients with CN compared to patients with type 2 diabetes ($P < 0.001$, for all) and control participants ($P < 0.001$, for all; Figure 2).

DISCUSSION

The present study shows very severe and widespread small fiber neuropathy as evidenced by marked corneal nerve loss and sudomotor dysfunction in patients with type 2 diabetes and Charcot neuroarthropathy, compared with age-, diabetes

duration- and glycated hemoglobin-matched patients with type 2 diabetes.

It is widely accepted that neuropathy and local inflammation are important in the development of CN. However, most studies have focused on the role of large fiber neuropathy and showed elevated vibration perception in patients with CN^{17,18}. We confirm a significantly elevated vibration perception threshold in patients with CN compared to patients with type 2 diabetes and control participants. However, patients with CN have

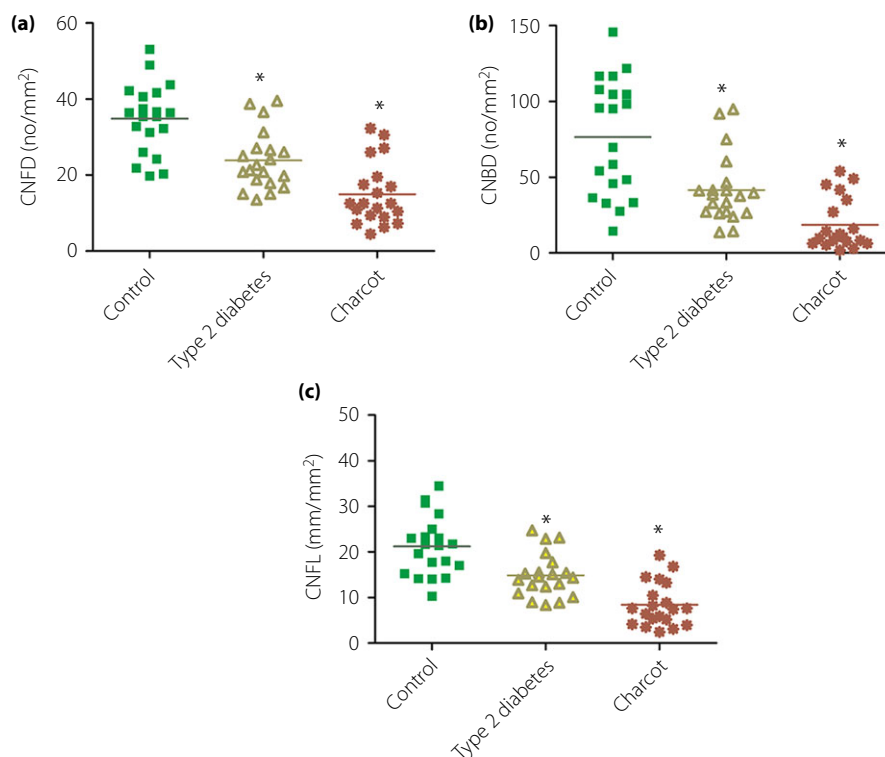


Figure 2 | Graphs showing a significant reduction in (a) corneal nerve fiber density (CNFD), (b) corneal nerve branch density (CNBD) and (c) corneal nerve fiber length (CNFL) in diabetic patients with Charcot neuroarthropathy compared to patients with type 2 diabetes and control participants, expressed as the mean (horizontal line) and individual data. * $P < 0.001$.

a more complex neuropathy phenotype with varying degrees of both large and small fiber involvement, as Stevens *et al.*²⁷ have shown preserved warm and light touch perception with abnormal cold and vibration perception, as well as cardiovascular autonomic function in patients with CN. Furthermore, abnormal cutaneous and skeletal hyperalgesia with altered cutaneous, but normal deep pressure pain, thresholds have been shown in patients with CN^{28,29}.

The importance of small fiber neuropathy in CN merits consideration, as recent studies have suggested that small fiber neuropathy could contribute to altered walking strategy and increased unsteadiness³⁰, with an increased risk of falls, joint capsule trauma and joint dislocation³¹ including the typical Lisfranc fracture dislocation³². This hypothesis is further strengthened by the demonstration of a reduction in neuropeptides released from unmyelinated nerve terminals in patients with CN¹⁹. Indeed, non-synaptically released substance P and CGRP regulate osteoblast and osteoclast proliferation¹⁵ and inhibit pro-inflammatory cytokines¹⁶, key players in the pathophysiology of CN. In a bone biopsy study, there was reduced expression of CGRP in the bone of patients with CN¹⁹, and sympathetic nerve fiber density was significantly lower in the skin, synovium and bone of patients with CN²⁰.

Previous studies have assessed small fiber dysfunction indirectly by assessing abnormalities in skin blood flow, and although some have shown an abnormality¹⁸, others have not^{17,33}. In the present study, we have shown widespread small fiber damage with a marked loss of corneal nerve fibers and sudomotor dysfunction in both the hands and feet of patients with CN compared to patients with type 2 diabetes. In addition, the Douleur Neuropathique 4 was also significantly elevated in patients with CN, suggesting the presence of neuropathic pain. The underlying reason for the more severe large and particularly small fiber neuropathy in patients with CN is not clear, as traditional risk factors for diabetic neuropathy, such as diabetes duration, glycemic control, lipids, blood pressure and renal function, were comparable between patients with CN and patients with type 2 diabetes.

Vitamin D deficiency is associated with diabetic neuropathy³⁴, and treatment with vitamin D can improve painful diabetic neuropathy³⁵. Vitamin D deficiency has also been related to reduced lower limb muscle strength and volume in patients with type 2 diabetes, which might predispose to unsteadiness and falls, contributing to foot trauma³⁶. Although Yoho *et al.*³⁷ found a lower level of vitamin D in diabetic patients compared to healthy control participants, there was no difference between diabetic patients with and without CN. In the present study, vitamin D levels were equally reduced in patients with CN, patients with type 2 diabetes and control participants.

Corneal confocal microscopy has emerged as a powerful non-invasive ophthalmic imaging technique to detect small fiber damage in patients with minimal and more advanced neuropathy. In the current study, we utilized CCM to identify

a marked loss of corneal nerve fibers, indicating a severe small fiber neuropathy in patients with CN. Longitudinal studies are required to determine the prognostic ability of CCM in predicting the development and progression of Charcot neuroarthropathy.

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DISCLOSURE

The authors declare no conflict of interest.

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