Received: 4 March 2020 | Revised: 12 May 2020 | Accepted: 18 May 2020

DOI: 10.1002/dmrr.3361

WILEY

RESEARCH ARTICLE

Vitamin D deficiency is associated with painful diabetic neuropathy

Uazman Alam[1,2] | Ioannis N. Petropoulos[3] | Georgios Ponirakis[3] | Maryam Ferdousi[4] | Omar Asghar[4] | Maria Jeziorska[4] | Andrew Marshall[5] | Andrew J. M. Boulton[4] | Nathan Efron[6] | Rayaz A. Malik[3,4]

[1]Department of Cardiovascular & Metabolic Medicine and the Pain Research Institute, Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool, UK

[2]Department of Diabetes, Endocrinology & Gastroenterology, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK

[3]Weill Cornell Medicine-Qatar, Research Division, Qatar Foundation, Doha, Qatar

[4]Institute of Cardiovascular Sciences, Cardiac Centre, Faculty of Medical and Human Sciences, University of Manchester and NIHR Clinical Research Facility, Manchester, UK

[5]Pain Research Institute, Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool, UK

[6]Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

Correspondence
Rayaz A. Malik, Weill Cornell Medicine-Qatar, Qatar Foundation, Education City, PO Box 24144-Doha, Qatar.
Email: ram2045@qatar-med.cornell.edu

Uazman Alam, University of Liverpool, 3rd Floor Clinical Sciences Centre Aintree University Hospital. Longmoor Lane, Liverpool. L9 7ALEmail: Uazman.alam@liverpool.ac.uk

Funding information
Center for Scientific Review, Grant/Award Number: R105991; Juvenile Diabetes Research Foundation International, Grant/Award Number: 8-2008-362; National Institutes of Health

Abstract

Background: The aetiology of painful diabetic neuropathy is unclear. We have evaluated vitamin D levels in diabetic patients with and without painful neuropathy.

Methods: Forty-three patients with type 1 diabetes and painless (DPN) (n = 20) or painful (PDN) (n = 23) neuropathy and 14 non-diabetic healthy control subjects (C) underwent assessment of neurologic deficits, quantitative sensory testing (QST), electrophysiology, skin biopsy, corneal confocal microscopy (CCM) and measurement of serum 25(OH)D.

Results: There were no significant differences for age, BMI, HbA_{1c}, lipids, neurological deficits, QST, electrophysiology, intra-epidermal nerve fibre density (IENFD) and corneal nerve morphology between patients with DPN and PDN. Both positive (hyperalgesia and allodynia) and negative symptoms (paraesthesia and numbness) of diabetic neuropathy were greater in PDN compared with DPN (P = .009 and P = .02, respectively). Serum 25(OH)D levels were significantly lower in PDN (24.0 ± 14.1 ng/mL) compared with DPN (34.6 ± 15.0 ng/mL, P = .01) and controls (34.1 ± 8.6 ng/mL, P = .03). The odds ratio in favour of painful diabetic neuropathy was 9.8 [P = .003 (95% CI, 2.2–76.4)] for vitamin D deficiency (<20 ng/mL) and 4.4 [P = .03 (95% CI, 1.1-19.8)] for vitamin D insufficiency (<30 ng/mL).

Conclusions: This study suggests that vitamin D deficiency and insufficiency are associated with painful diabetic neuropathy.

KEYWORDS

diabetic neuropathy, small fibre neuropathy, vitamin D, Painful neuropathy

1 | BACKGROUND

The prevalence of painful diabetic neuropathy is ~21% and painful symptoms are more prevalent in patients with type 2 diabetes, females and South Asians.[1-4] Painful diabetic neuropathy is characterized by symmetrical lower limb paraesthesiae, dysaesthesiae, lancinating pains and allodynia with nocturnal exacerbation[5] and is associated with significant sleep disturbance and reduced quality of life.[6]

Observational studies in people with diabetes demonstrate a significant association between vitamin D deficiency with paraesthesiae and numbness,[7] the severity of DPN using the neuropathy symptom score, neurological deficits and electrophysiology,[8] impaired nerve conduction velocity[9] and reduced parasympathetic function.[10] In a recent study there was an inverse U-shaped association between serum vitamin D levels and the E/I ratio, 30/15 ratio and three heart rate variability indices.[11] Furthermore, a recent systematic review and meta-analysis showed a significant association between vitamin D deficiency and the development of DPN, particularly in Asian patients.[12] In another study, vitamin D deficiency was related to diabetic painful neuropathy in Greek but not Bangladeshi patients.[13] Shillo et al[14] have recently demonstrated a significantly lower 25(OH)D levels in people with painful compared with painless diabetic neuropathy.

In relation to a mechanistic link between vitamin D and neuropathic pain, nociceptive calcitonin gene-related peptide (CGRP)-positive neurones have a distinct vitamin D phenotype with hormonally regulated ligand and receptor levels.[15] Vitamin D deficiency results in increased numbers of axons containing CGRP, and in culture, vitamin D receptor (VDR) expression is increased in growth cones, and sprouting appears to be regulated by VDR-mediated rapid response signalling pathways.[16] Nerve growth factor (NGF) is depleted in experimental diabetes[17] and a preservation of NGF expression was shown in sciatic nerves of diabetic animals treated with a vitamin D analogue (CB1093). Similarly, tacalcitol, active vitamin D3, induces NGF production in human epidermal keratinocytes.[18] Treatment with vitamin D3 has been shown to limit demyelination in a cuprizone experimental model of demyelination[19] and in a spinal cord compression model, it has been shown to induce axonal regeneration.[20]

In a prospective study of 51 patients with type 2 diabetes and painful neuropathy treated with 2000 IU of cholecalciferol daily for 3 months, there was a ~50% decrease in pain scores as measured by the Visual Analogue Score (VAS). More recently in a placebo-controlled study of 112 patients with type 2 diabetes randomized to 50 000 IU of cholecalciferol once weekly for 8 weeks, there was a significant increase in 25OHD and an improvement in the neuropathy symptom score, but no change in NDS or neurophysiology.[21] In a study of people with diabetes given 400 IU of vitamin D daily for 12 weeks, there was a significant improvement in pain, numbness and weakness.[22] We have also shown a significant improvement in neuropathic symptoms[23,24] and quality of life in patients with painful diabetic neuropathy treated with 600 000 IU of vitamin D.

In the present study we have evaluated the levels of 25(OH)D in patients with painful compared with painless diabetic neuropathy matched for severity of DPN.

2 | RESEARCH DESIGN AND METHODS

2.1 | Selection of patients

Forty-three patients with type 1 diabetes were categorized into two groups: painless neuropathy (DPN) (n = 20) and painful neuropathy (PDN) (n = 23) using the McGill Visual Analogue Score (McGill VAS) and McGill pain score, and compared with 14 age, sex and ethnicity matched non-diabetic healthy control subjects (C). Participants with a history of neurologic conditions, ocular trauma or ocular surgery were excluded. Participants were randomly selected and not recruited based on any arbitrary bias such as PDN or vitamin D deficiency symptoms to ensure selection bias was minimized. The study was approved by the North West and Salford and Trafford Research Ethics committee, and written informed consent was obtained according to the Declaration of Helsinki.

2.2 | Assessment of neuropathy

All patients and control subjects underwent a detailed evaluation with the neuropathy symptom profile (NSP) and the McGill VAS was used to assess the severity of painful neuropathy. Neurologic deficits were assessed using the modified neuropathy disability score, which includes evaluation of vibration, pinprick and temperature perception as well as the presence or absence of ankle reflexes. Quantitative sensory testing included an assessment of vibration perception threshold (VPT), measured using a Neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilford, Nottingham, UK), cold sensation (CST) (Aδ fibres) and warm sensation (WST) (C fibres) thresholds using the method of limits with the MEDOC TSA II (Medoc, Ramat Yishai, Israel) on the dorsum of the left foot.[25] Computer-aided sensory evaluator (CASE IV) (WR Medical Electronic Ltd, Maplewood, Maine, USA) was used to measure the heart rate response to deep breathing (HRV-DB) over two eight-cycle breathing series interspersed by a 5-minutes period of normal breathing. Electro-diagnostic studies were undertaken using a Dantec 'Keypoint' system (Dantec Dynamics, Bristol, U. K.). Peroneal motor and sural sensory nerves were assessed in the right lower limb by a consultant neurophysiologist.

2.3 | Corneal confocal microscopy

Patients underwent examination with the Heidelberg retina tomography III in vivo corneal confocal microscope employing our established methodology for image acquisition.[26,27] Several scans of the entire depth of the central cornea were recorded using the section mode, which enables manual acquisition and storage of single images of all corneal layers. This provides en face two-dimensional images with a lateral resolution of ~2 mm/pixel and final image size of 400 × 400 pixels of the sub-basal nerve plexus of the cornea. Five images per patient from the centre of the cornea were selected and examined in a masked and randomized fashion.[28] Three corneal nerve parameters

were quantified: (a) CNFD, the total number of major nerves per square millimetre of corneal tissue (no mm^2); (b) corneal nerve branch density (CNBD), the number of branches emanating from all major nerve trunks per square millimetre of corneal tissue (no mm^2) and (c) corneal nerve fibre length (CNFL), the total length of all nerve fibres and branches (mm/mm^2) within the area of corneal tissue.

2.4 | Skin biopsy and immunohistochemistry

Intra-epidermal nerve fibre density (IENFD) was assessed in a subcohort of participants (Controls n = 10, DPN n = 11, and PDN n = 9) who agreed to undergo a 3-mm punch skin biopsy from the dorsum of the foot, 2 cm proximal to the second metatarsal head, after local anaesthesia (1% lidocaine). The biopsy specimen was immediately fixed in PBS-buffered 4% paraformaldehyde and after 18 to 24 hours rinsed in tris-buffered saline and soaked in 33% sucrose (2–4 hours) for cryoprotection. It was then embedded in optimal cutting temperature-embedding compound, rapidly frozen in liquid nitrogen, and cut into 50-μm sections using a cryostat (model OTF; Bright Instruments, Huntington, UK). Four floating sections per subject were subjected to melanin bleaching (0.25% $KMnO_4$ for 15 minutes followed by 5% oxalic acid for 3 minutes), a 4-hour protein block with a tris-buffered saline solution of 5% normal swine serum, 0.5% powdered milk and 1% Triton X-100, and overnight incubation with 1:1200 Biogenesis polyclonal rabbit anti-human PGP9.5 antibody (Serotec, Oxford, UK). Biotinylated swine anti-rabbit secondary antibody (1:300; DakoCytomation, Ely, UK) was then applied for 1 hour; sections were quenched with 1% H_2O_2 in 30% MeOH-PBS (30 minutes) before a 1-hour incubation with 1:500 horseradish peroxidase–streptavidin (Vector Laboratories, Peterborough, UK). Nerve fibres were demonstrated using 3, 3-diaminobenzidine chromogen (Sigma-Aldrich, Manchester, UK). Sections were mildly counterstained with eosin to better localize the basement membrane to identify nerve fibres passing through it. Negative control subjects consisted of replacing the anti-PGP9.5 antibody with rabbit immunoglobulin (DakoCytomation) at a concentration matching that of the primary antibody and showed no immunostaining. IENFD, that is, the number of fibres per millimetre of basement membrane, was quantified in accord with established criteria and techniques and expressed as number per millimetre.[29]

2.5 | 25(OH) Vitamin D_3 Assay

The laboratory used for the biochemical assay measurements (Vitamin D Research Group Manchester Royal Infirmary, UK) was accredited to ISO 9001:2008 and ISO 13485:2003 by Lloyd's Register Quality Assurance certificate number LRQ 4001542 and participated successfully in the Vitamin D quality assurance scheme (DEQAS). Serum was separated from whole blood and stored at −20°C until assay. The assay used was an automated platform assay (ImmunoDiagnostic Systems Ltd, Bolden, Tyne and Wear, UK) and is based on chemiluminescence technology. Briefly, samples were subjected to a pre-treatment step to denature the vitamin D-binding protein. The treated samples were then neutralized in assay buffer and a specific anti-25(OH)D antibody labelled with acridinium was added. Following an incubation step, magnetic particles linked to 25(OH)D were added. Following a further incubation step, the magnetic particles were 'captured' using a magnet. After a washing step and addition of trigger reagents, the light emitted by the acridinium label was inversely proportional to the concentration of 25(OH)D in the original sample. The concentration of 25(OH)D was calculated automatically using a 4-point logistic curve. The cross reactivity for vitamin D^2 (of the assay) as per manufacturers assertion was 100% (relative to vitamin D^3) and the assay has excellent correlation to existing globally recognized assays, in combination with good sensitivity and precision.[30] The reportable range of the assay was 5–140 ng/mL. Inter- and intra-assay variation of the in-house control was 5.6% and 9.7%, respectively. Vitamin D deficiency (<20 ng/mL) and insufficiency (<30 ng/mL) were defined according to the Institute of Medicine (IOM) of the National Academies.[31]

2.6 | Statistical analysis

Statistical analyses were undertaken on StatsDirect (StatsDirect, Cheshire, UK). All values are presented as mean ± SD. ANOVA method or a non-parametric counterpart Kruskal-Wallis was used to assess differences between groups depending on normality of the data. Overall, the *P* value was maintained at .05 for multiple comparison tests (Bonferroni adjustment or Dwass-Steel-Chritchlow-Fligner pairwise comparison). Unpaired *t* test or Mann-Whitney *U* test were used for analysis for DPN vs PDN for the duration of diabetes and IENFD. Chi-squared analyses were used to assess frequencies of gender, ethnicity and aetiology of diabetes. Odds Ratios for painful symptoms were calculated by further delineating DPN and PDN groups based on the cut offs for vitamin D deficiency (<20 ng/mL) and insufficiency (<30 ng/mL).

3 | RESULTS

3.1 | Demographics, metabolic and anthropometric assessment

The participant demographics and metabolic and anthropometric measurements in people with diabetes and control subjects are summarized in Table 1. There were no significant differences in age, gender, BMI, duration or type of diabetes and all subjects were of white European origin. HbA_{1c} ($P < .0001$) was significantly higher in those with diabetes compared with control subjects with no difference between patients with PDN and DPN. The total cholesterol was significantly lower in participants with DPN ($P = .003$) and PDN ($P = .02$) compared with control subjects, due to greater statin use. HDL, triglycerides, systolic and diastolic blood pressure were comparable between diabetes groups (DPN and PDN) and control subjects. The estimated glomerular filtration rate was comparable between diabetes groups and control participants, but the albumin–creatinine ratio (ACR) was higher in the DPN ($P = .009$) and PDN ($P = .002$) groups compared

TABLE 1 Participant demographics and metabolic parameters in control subjects and patients with DPN and PDN, with statistically significant differences between groups

	C (n = 14)	DPN (n = 20)	PDN (n = 23)	DPN vs PDN
Age (years)	59.3 ± 7.8	57.1 ± 13.5	59.8 ± 11.7	0.41
Gender (male) (%)	57	60	53	0.61
Ethnicity (White European) (%)	100	100	100	>0.99
Aetiology of diabetes (type 1 DM) (%)	—	85	91	0.43
Duration of diabetes (years)	—	36.0 ± 17.5	35.5 ± 14.9	0.90
HbA_{1c} (%)	5.7 ± 0.2	8.1 ± 1.1*	8.0 ± 1.5**	0.41
HbA_{1c} (mmol/mol)	38.3 ± 2.5	65.2 ± 12.0*	63.7 ± 16.3**	
BMI (kg/m^2)	31.0 ± 4.2	28.1 ± 4.1	26.6 ± 4.9	0.24
T-CHL (mmol/L)	5.1 ± 1.2	4.2 ± 1.0†	4.4 ± 0.8††	0.40
HDL-C (mmol/L)	1.7 ± 0.5	1.7 ± 0.4	1.7 ± 0.6	0.73
Triglycerides	1.6 ± 0.7	1.2 ± 0.5	1.3 ± 0.8	0.97
Systolic BP (mmHg)	138 ± 15	141 ± 26	141 ± 23	0.99
Diastolic BP (mmHg)	77 ± 8	71 ± 7	71 ± 11	0.97
ACR (mg/mmol) ([Median]IQR)	**0.4 ± 0.3 (0.2[0.2-0.7])**	**7.1 ± 16.7¥ (0.2(0.2-0.7)**	**5.3 ± 6.7¥¥ (2.2(0.5-10.2)**	**0.009**
eGFR (mL/min/L.73)	85 ± 7	77 ± 16	78 ± 15	0.82
Serum vitamin B12 (ng/L)	247 ± 69	342 ± 121	317 ± 111	0.54

Note: HbA_{1c} *C vs DPN and **C vs PDN—$P < .05$. T-CHL †C vs DPN and ††C v vs s PDN—$P < .05$. ACR ¥C vs DPN and ¥¥C vs PDN—$P < .05$. Significantly different values are given in bold.

Abbreviations: ACR, Albumin–Creatinine Ratio; BMI, body mass index; BP, blood pressure; C, controls; DPN, diabetic peripheral neuropathy; eGFR, estimated Glomerular Filtration Rate; HbA_{1c}, Glycated Haemoglobin A1c; HDL, high density lipoprotein cholesterol; PDN, painful diabetic neuropathy; T-CHL, total cholesterol.

TABLE 2 Neuropathic symptoms and deficits in control subjects and diabetic patients with DPN and PDN, with statistically significant differences between groups

	C (n = 14)	DPN (n = 20)	PDN (n = 23)	DPN vs PDN
NDS (–/10) (Median(IQR)	1.4 ± 1.5 1(0-2)	3.9 ± 3.2* 3.5(1.5-5.5)*	4.5 ± 3.2** 4(2-6)**	0.49
NSP (–/38) (Median(IQR)	**1 ± 1.5 0(0-1)**	**2.2 ± 2.6 1(0.5-2.5)**	**6.3 ± 5.5 ^^5(2-11)^^**	**<0.0005**
+ve symptoms on NSP (–/6) (Median(IQR)	**0.5 ± 0.9 (0(0-1)**	**0.25 ± 0.6 (0(0-0)**	**1.6 ± 1.8€ (1(0-3)€**	**0.009**
–ve symptoms on NSP (–/4) (Median(IQR)	**0 ± 0 (0(0–0)**	**0.3 ± 0.4 (0.5(0–1)**	**1.1 ± 1.2¥ (1(0-2)¥**	**0.02**
McGill VAS (–/10 cm)	**0.5 ± 1.4**	**0 ± 0**	**5.7 ± 2.3†**	**<0.0001**
McGill pain score (Median (IQR)	**0.4 ± 0.9 (0(0–0)**	**0 ± 0 (0(0-0)**	**6.1 ± 6.5† (3(2–10)**	**<0.0001**

Note: NDS*C vs DPN and **C vs PDN—$P < .05$; NSP ^C vs PDN—$P < .05$; +ve symptomsC vs PDN—$P = .05$; –ve symptoms¥C vs PDN—$P < .05$; McGill VAS & †C vs PDN—$P < .05$; McGill pain score†C vs PDN—$P < 0.05$. Significantly different values are given in bold.

Abbreviations: C, controls; DPN, diabetic peripheral neuropathy; McGill VAS, McGill visual analogue score; NDS, neuropathy disability score; PDN, painful diabetic peripheral neuropathy; type 1 DM, type 1 diabetes mellitus.

with control subjects with a higher ACR in PDN compared with DPN. Serum vitamin B12 levels were comparable between groups.

3.2 | Symptoms and deficits

The NSP was significantly higher in PDN compared with DPN ($P < .0005$) and control subjects ($P < .0001$) (Table 2). Positive symptoms (out of 6) were significantly greater in PDN compared with DPN ($P = .009$) and control subjects ($P = .05$). Negative symptoms (out of 4) were significantly greater in PDN compared with DPN ($P = .02$) and control subjects ($P = .009$). The McGill pain score and McGill VAS were significantly greater in PDN compared with DPN ($P < .0001$) and control subjects ($P < .0001$). The NDS was significantly greater in patients with DPN ($P = .01$) and PDN ($P = .002$) compared with control subjects, but there were no difference between DPN and PDN.

3.3 | Quantitative sensory tests

VPT, CST and WST did not differ between diabetes groups and control subjects or between patients with DPN and PDN (Table 3).

TABLE 3 Small and large fibre tests of nerve structure and function in control subjects and diabetic patients with DPN and PDN, with statistically significant differences between groups

	C (n = 14)	DPN (n = 20)	PDN (n = 23)	DPN vs PDN
CNFD (no/mm^2)	34.6 ± 5.4	24.5 ± 8.4*	20.4 ± 10.0**	0.35
CNBD (no/mm^2)	75.9 ± 24.2	56.1 ± 31.8	45.5 ± 29.3†	0.36
CNFL (mm/mm^2)	24.3 ± 3.6	19.8 ± 5.7$^{¥}$	15.8 ± 7.2$^{¥¥}$	0.13
IENFD (no/mm)	7.6 ± 3.4 (n = 10)	5.2 ± 3.7 (n = 11)	3.9 ± 2.9^{a} (n = 9)	0.67
DB-HRV (beats/min)	23 ± 10	18 ± 9	18 ± 14	0.55
CST (°C)	27.2 ± 1.9	24.0 ± 6.6	23.5 ± 6.8	0.68
WST(°C)	39.8 ± 3.8	40.7 ± 4.7	42.0 ± 4.9	0.35
VPT (volts)	10.7 ± 6.6	17.0 ± 14.0	19.5 ± 13.1	0.35
Sural SNCV (m/s)	47.7 ± 5.1	41.9 ± 6.2°	42.2 ± 4.6$^{\circ\circ}$	0.61
Sural amplitude (μV)	9.6 ± 2.7	6.9 ± 4.8$^{€}$	5.3 ± 5.3$^{€€}$	0.46
Peroneal MNCV (m/s)	45.9 ± 3.3	38.3 ± 9.3^{a}	38.4 ± 8.7^{aa}	0.64
Peroneal amplitude (mV)	4.9 ± 1.0	2.6 ± 1.9§	2.6 ± 2.5§§	0.78

Note: CNFD*C vs DPN and **C vs PDN—P < .05; CNBD†C vs PDN—P < .05; CNFL¥C vs DPN and ¥¥C vs PDN—P < .05; IENFD ^{a}C vs PDN—P = .05; Sural SNCV$^{\circ}$C vs DPN and $^{\circ\circ}$C vs PDN—P < .05; Sural SNAmp$^{€}$C vs DPN and $^{€€}$C vs PDN—P < .05; Peroneal MNCV^{a}C vs DPN and ^{aa}C vs PDN—P < .05; Peroneal MNAmp§C vs DPN and §§C vs PDN—P < .05.

Abbreviations: C, controls; CNBD, corneal nerve branch density; CNFD, corneal nerve fibre density; CNFL, corneal nerve fibre length; CST, cold sensation threshold; DB-HRV, deep breathing—heart rate variability; DPN, diabetic peripheral neuropathy; IENFD, intra epidermal nerve fibre density; PDN, painful diabetic neuropathy; Peroneal MNAmp, peroneal motor nerve amplitude; Peroneal MNCV, peroneal motor nerve conduction velocity; Sural SNAmp, sural nerve sensory nerve amplitude; VPT, vibration perception threshold; WST, warm sensation threshold.

3.4 | Electrophysiology

Peroneal nerve conduction velocity and amplitude were significantly lower in DPN (P = .004, P = .003, respectively) and PDN (P = .0008, P = .001, respectively) compared with control subjects, but there was no difference between patients with DPN and PDN (Table 3). Sural nerve conduction velocity and amplitude were significantly lower in DPN (P = .02, P = .007, respectively) and PDN (P = .005, P = .04, respectively) compared with control subjects, but there was no significant difference between patients with DPN and PDN.

3.5 | Autonomic function, IENFD and CCM

HRV-DB did not differ between diabetes groups and control subjects or between patients with DPN and PDN (Table 3). IENFD did not differ between patients with DPN and control subjects but was significantly reduced in PDN compared with control subjects (P = .05) with no difference between DPN and PDN. CNFD was significantly reduced in patients with DPN (P = .0008) and PDN (P < .0001) compared with control subjects with no difference between DPN and PDN. CNBD was significantly reduced only in patients with PDN (P < .03) compared with control subjects with no difference between DPN and PDN. CNFL was significantly reduced in patients with DPN (P = .03) and PDN (P < .0009) compared with control subjects with no difference between DPN and PDN.

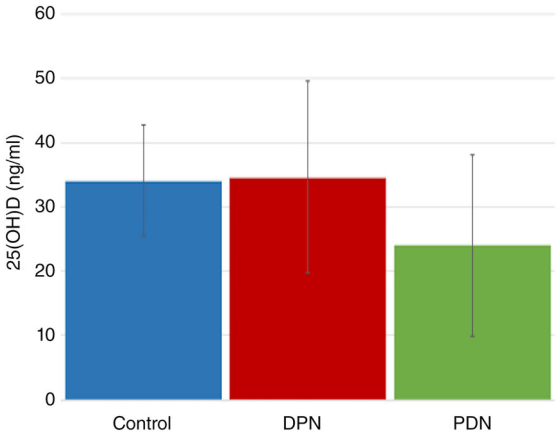

FIGURE 1 Graph showing 25(OH)D levels in ng/mL in controls and patients with DPN and PDN. C vs DPN—P = NS, *C vs PDN—P = .03, **DPN vs PDN—P = .01 (Overall P for Kruskal Wallis = .02)

3.6 | 25(OH)D status

The serum 25(OH)D level was significantly lower in PDN (24.0 ± 14.1 ng/mL) compared with DPN (34.6 ± 15.0 ng/mL, P = .01) and control subjects (34.1 ± 8.6 ng/mL, P = .03) (Figure 1). The odds ratio in favour of painful diabetic neuropathy was 9.8 (P = .003 [95% CI, 2.2-76.4]) for vitamin D deficiency (<20 ng/mL) and 4.4, (P = .03 (95% CI, 1.1-19.8]) for vitamin D insufficiency (<30 ng/mL).

4 | DISCUSSION

Painful diabetic neuropathy is an extremely disabling condition, which may be present in at least one-fifth of people with diabetes.[4] The treatment of this condition is unsatisfactory with a 'good' response to conventional medication rated at between 30% to 50% pain relief.[32] Available drugs are often moderately effective and their use is limited by side effects. Furthermore, recent studies of novel drugs in the treatment of painful diabetic neuropathy have failed to show efficacy.[33,34] There is an urgent need to explore new mechanisms and treatments for diabetic painful neuropathy.

The aetiology of painful diabetic neuropathy is not clear. Central changes comprising of increased thalamic vascularity,[35] Aβ fibre sprouting into lamina II of the dorsal horn and reduced inhibition via descending inhibitory pathways[5] together with axonal atrophy in peripheral nerves have been demonstrated in patients with painful diabetic neuropathy.[36] Painful diabetic neuropathy has also been associated with autonomic dysfunction.[37] Previously, we have shown that the LDI flare, a measure of small fibre function, is abnormal in patients with painful diabetic neuropathy, whereas conventional quantitative sensory testing and dermal nerve fibre density did not differ from those with painless diabetic neuropathy.[38]

In the present study, we carefully phenotyped diabetic patients into those with painful and painless diabetic neuropathy and undertook detailed assessment of large and small fibre neuropathy. There was no difference for electrophysiology, quantitative sensory testing and autonomic function between painful and painless neuropathy. Previously, we have shown a greater reduction in both intraepidermal nerve and corneal nerve fibre length in painful diabetic neuropathy[39] and a detailed immunophenotyping study has shown increased axonal growth (higher GAP43/PGP) and axonal swellings, positive for tropomyosin-receptor-kinase A and substance P in patients with painful compared with painless neuropathy.[40] In the present study, there was no significant difference in autonomic function, IENFD or corneal nerve morphology between painful and painless neuropathy. Indeed, we have recently shown that corneal nerve length at the inferior whorl as opposed to more central corneal nerve parameters differ between patients with and without painful diabetic neuropathy.[41]

Given that painful neuropathic symptoms vary in their severity over time, particularly with nocturnal exacerbation, it is difficult to reconcile these 'hard wired' changes with the fluctuating symptoms. Therefore, changes in sodium channel distribution and expression, altered peripheral blood flow and glycaemic flux have also been implicated in painful diabetic neuropathy.[5] Given the potential link between vitamin D and pain, together with the high prevalence of vitamin D deficiency in diabetic populations,[42] we have explored the link with painful diabetic neuropathy. Previous studies have shown a relationship between vitamin D deficiency and diabetic neuropathy,[7,8] but did not specifically assess the relationship to painful diabetic neuropathy. In the present study, we show a markedly increased risk of painful diabetic neuropathy particularly in patients with vitamin D deficiency but also in those with insufficiency. These data are in keeping with those of a recently published study.[14] A large epidemiological study has shown a higher prevalence of painful diabetic neuropathy in South Asians compared with Europeans.[4] In our previous study, we showed that 55% of South Asians were severely vitamin D deficient with a 25(OH)D < 10 ng/mL.[42] The cohort of subjects evaluated in this study was exclusively white European thus minimising ethnicity as a confounding factor. However, a large population-based study (n = 1461) from China indicated a low vitamin D concentration to be a risk factor for diabetic neuropathy in older adults (≥65 years) ($P < .05$).[43] There was no such relationship in the youth or middle-aged groups suggesting an age-related differential effect.[43]

Although vitamin D has been used to treat pain in rheumatological conditions,[44] a Cochrane review concluded that there was poor evidence for the efficacy of vitamin D in the treatment of chronic pain.[45] Valensi et al[46] showed an improvement in painful neuropathic symptoms with a topical compound (QR-333) containing quercetin, ascorbyl palmitate and vitamin D3. Lee et al[47] showed that oral cholecalciferol significantly improved symptoms of painful diabetic neuropathy, however, this study lacked randomization and a placebo group. Treatment with vitamin D produced a dramatic improvement in the symptoms of painful diabetic neuropathy in a type 1 patient, refractory to a range of standard therapies.[48] We have also shown that treatment with high-dose vitamin D results in a significant reduction in neuropathic symptoms[23] and improves the quality of life[24] in patients with painful diabetic neuropathy. More recently, a study using low-level laser therapy in people with painful diabetic neuropathy raised 25(OH)D levels and there were improvements in neuropathic pain and quality of life.[49]

Limitations of the current study are the small sample size, lack of quantification of sunlight exposure or daily activity and comparison with a non-neuropathic diabetic cohort. However, a major strength is the detailed phenotyping undertaken to ensure that diabetic patients with and without painful neuropathy were absolutely matched for all clinical and metabolic variables as well as the severity of neuropathy.

We demonstrate a relationship between vitamin D deficiency and painful diabetic neuropathy. A well-constructed clinical trial of vitamin D in painful diabetic neuropathy is required to assess the effectiveness of a potentially simple treatment with no obvious side effects.

ACKNOWLEDGEMENTS

This research was kindly supported by the JDRF (8-2008-362) and the NIH (R105991). Support from the NIHR/Wellcome Trust Clinical Research Facility (Manchester) and for Dr Mitra Tavakoli and Dr Hassan Fadavi for their part in clinical assessments is acknowledged.

CONFLICT OF INTEREST

No potential conflicts of interest relevant to this article were reported.

AUTHOR CONTRIBUTION

Uazman Alam undertook clinical and neurological assessment, skin biopsy and QST, researched and analysed the data and wrote the manuscript. Ioannis N. Petropoulos and Maryam Ferdousi researched data,

analysed CCM images and wrote the manuscript. Georgios Ponirakis was the study coordinator, researched and analysed the data and wrote the manuscript. Omar Asghar undertook clinical and neurological assessment, skin biopsy and QST, researched and analysed the data and wrote the manuscript. Maria Jeziorska undertook IENFD assessments. Andrew Marshall undertook neurophysiology researched and analysed the data. Andrew J. M. Boulton reviewed and revised the manuscript. Nathan Efron reviewed and revised the manuscript. Rayaz A. Malik supervised the project, undertook IENFD assessment, and reviewed and revised the manuscript. Rayaz A. Malik 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.

ORCID

Uazman Alam https://orcid.org/0000-0002-3190-1122
Georgios Ponirakis https://orcid.org/0000-0002-6936-1248

REFERENCES

1. Abbott CA, Malik RA, van Ross ER, Kulkarni J, Boulton AJ. Prevalence and characteristics of painful diabetic neuropathy in a large community-based diabetic population in the U.K. *Diabetes Care*. 2011;34(10):2220-2224.
2. Almuhannadi H, Ponirakis G, Khan A, Malik RA. Diabetic neuropathy and painful diabetic neuropathy: Cinderella complications in South East Asia. *J Pak Med Assoc*. 2018;68(1):85-89.
3. Ponirakis G, Elhadd T, Chinnaiyan S, et al. Prevalence and risk factors for painful diabetic neuropathy in secondary healthcare in Qatar. *J Diabetes Investig*. 2019;10(6):1558-1564.
4. Abbott CA, Malik RA, van Ross ERE, Kulkarni J, Boulton AJM. Prevalence and characteristics of painful diabetic neuropathy in a large community-based diabetic population in the UK. *Diabetes Care*. 2011;34(10):2220-2224.
5. Tesfaye S, Vileikyte L, Rayman G, et al. Painful diabetic peripheral neuropathy: consensus recommendations on diagnosis, assessment and management. *Diabetes Metab Res Rev*. 2011;27:629-638.
6. Sadosky A, Schaefer C, Mann R, et al. Burden of illness associated with painful diabetic peripheral neuropathy among adults seeking treatment in the US: results from a retrospective chart review and cross-sectional survey. *Diabetes Metab Syndr Obes*. 2013;6:79-92.
7. Soderstrom LH, Johnson SP, Diaz VA, Mainous AG 3rd. Association between vitamin D and diabetic neuropathy in a nationally representative sample: results from 2001-2004 NHANES. *Diabet Med*. 2012;29(1):50-55.
8. Shehab D, Al-Jarallah K, Mojiminiyi OA, Al Mohamedy H, Abdella NA. Does vitamin D deficiency play a role in peripheral neuropathy in type 2 diabetes? *Diabet Med*. 2012;29(1):43-49.
9. Alamdari A, Mozafari R, Tafakhori A, et al. An inverse association between serum vitamin D levels with the presence and severity of impaired nerve conduction velocity and large fiber peripheral neuropathy in diabetic subjects. *Neurol Sci*. 2015;36(7):1121-1126.
10. Maser R, Lenhard M, Pohlig R. Vitamin D insufficiency is associated with reduced parasympathetic nerve fiber function in type 2 diabetes. *Endocr Pract*. 2015;21(2):174-181.
11. Hansen CS, Fleischer J, Vistisen D, Ridderstrale M, Jensen JS, Jorgensen ME. High and low vitamin D level is associated with cardiovascular autonomic neuropathy in people with type 1 and type 2 diabetes. *Diabet Med*. 2017;34(3):364-371.
12. Qu G-B, Wang L-L, Tang X, Wu W, Sun Y-H. The association between vitamin D level and diabetic peripheral neuropathy in patients with type 2 diabetes mellitus: an update systematic review and meta-analysis. *J Clin Trans Endocrinol*. 2017;9:25-31.
13. Zambelis T, Papadakis G, Kokotis P, Villiotou V, Dogkas N, Karandreas N. Lack of definite Association of Vitamin D Deficiency with diabetic neuropathy. Investigation in Greek and in Bangladeshi patients. *In Vivo (Athens, Greece)*. 2017;31(2):259-261.
14. Shillo P, Selvarajah D, Greig M, et al. Reduced vitamin D levels in painful diabetic peripheral neuropathy. *Diabet Med*. 2019;36(1):44-51.
15. Tague SE, Smith PG. Vitamin D receptor and enzyme expression in dorsal root ganglia of adult female rats: modulation by ovarian hormones. *J Chem Neuroanat*. 2011;41(1):1-12.
16. Tague SE, Clarke GL, Winter MK, McCarson KE, Wright DE, Smith PG. Vitamin D deficiency promotes skeletal muscle hypersensitivity and sensory hyperinnervation. *J Neurosci*. 2011;31(39):13728-13738.
17. Hellweg R, Wohrle M, Hartung HD, Stracke H, Hock C, Federlin K. Diabetes mellitus-associated decrease in nerve growth factor levels is reversed by allogeneic pancreatic islet transplantation. *Neurosci Lett*. 1991;125(1):1-4.
18. Fukuoka M, Sakurai K, Ohta T, Kiyoki M, Katayama I. Tacalcitol, an active vitamin D3, induces nerve growth factor production in human epidermal keratinocytes. *Skin Pharmacol Appl Skin Physiol*. 2001;14(4):226-233.
19. Wergeland S, Torkildsen O, Myhr KM, Aksnes L, Mork SJ, Bo L. Dietary vitamin D3 supplements reduce demyelination in the cuprizone model. *PLoS One*. 2011;6(10):e26262.
20. Bianco J, Gueye Y, Marqueste T, et al. Vitamin D(3) improves respiratory adjustment to fatigue and H-reflex responses in paraplegic adult rats. *Neuroscience*. 2011;188:182-192.
21. Shehab D, Al-Jarallah K, Abdella N, Mojiminiyi OA, Al MH. Prospective evaluation of the effect of short-term oral vitamin d supplementation on peripheral neuropathy in type 2 diabetes mellitus. *Med Princ Pract*. 2015;24(3):250-256.
22. Nadi M, Marandi SM, Esfarjani F, Saleki M, Mohammadi M. The comparison between effects of 12 weeks combined training and vitamin D supplement on improvement of sensory-motor neuropathy in type 2 diabetic women. *Adv Biomed Res*. 2017;6:55.
23. Basit A, Basit KA, Fawwad A, et al. Vitamin D for the treatment of painful diabetic neuropathy. *BMJ Open Diabetes Res Care*. 2016;4(1):e000148.
24. Alam U, Fawwad A, Shaheen F, Tahir B, Basit A, Malik RA. Improvement in neuropathy specific quality of life in patients with diabetes after vitamin D supplementation. *J Diabetes Res*. 2017;2017:7.
25. Bravenboer B, van Dam PS, Hop J, Vd Steenhoven J, Erkelens DW. Thermal threshold testing for the assessment of small fibre dysfunction: normal values and reproducibility. *Diabet Med*. 1992;9(6):546-549.
26. Petropoulos IN, Manzoor T, Morgan P, et al. Repeatability of in vivo corneal confocal microscopy to quantify corneal nerve morphology. *Cornea*. 2013;32(5):e83-e89.
27. Kalteniece A, Ferdousi M, Adam S, et al. Corneal confocal microscopy is a rapid reproducible ophthalmic technique for quantifying corneal nerve abnormalities. *PLOS One*. 2017;12(8):e0183040.
28. Tavakoli M, Malik RA. Corneal confocal microscopy: a novel non-invasive technique to quantify small fibre pathology in peripheral neuropathies. *J Vis Exp*. 2011;47:2194.
29. Lauria G, Bakkers M, Schmitz C, et al. Intraepidermal nerve fiber density at the distal leg: a worldwide normative reference study. *J Peripher Nerv Syst*. 2010;15(3):202-207.
30. ASBMR. 31st annual meeting MO0001–MO0445. *J Bone Miner Res*. 2009;24(S1):S370-S496.
31. Rosen CJ, Gallagher JC. The 2011 IOM report on vitamin D and calcium requirements for north america: clinical implications for providers treating patients with low bone mineral density. *J Clin Densitom*. 2011;14(2):79-84.

32. Bril V, England J, Franklin GM, et al. Evidence-based guideline: treatment of painful diabetic neuropathy: report of the American Academy of Neurology, the American Association of Neuromuscular and Electrodiagnostic Medicine, and the American Academy of physical medicine and rehabilitation. *Neurology*. 2011;3(4):345-52.e21.
33. Selvarajah D, Gandhi R, Emery CJ, Tesfaye S. Randomized placebo-controlled double-blind clinical trial of cannabis-based medicinal product (Sativex) in painful diabetic neuropathy: depression is a major confounding factor. *Diabetes Care*. 2010;33(1):128-130.
34. Ziegler D, Hidvégi T, Gurieva I, et al. Efficacy and safety of Lacosamide in painful diabetic neuropathy. *Diabetes Care*. 2010;33(4):839-841.
35. Selvarajah D, Wilkinson ID, Gandhi R, Griffiths PD, Tesfaye S. Microvascular perfusion abnormalities of the thalamus in painful but not painless diabetic polyneuropathy: a clue to the pathogenesis of pain in type 1 diabetes. *Diabetes Care*. 2011;34(3):718-720.
36. Britland ST, Young RJ, Sharma AK, Clarke BF. Association of painful and painless diabetic polyneuropathy with different patterns of nerve fiber degeneration and regeneration. *Diabetes*. 1990;39(8):898-908.
37. Gandhi RA, Marques JL, Selvarajah D, Emery CJ, Tesfaye S. Painful diabetic neuropathy is associated with greater autonomic dysfunction than painless diabetic neuropathy. *Diabetes Care*. 2010;33(7):1585-1590.
38. Krishnan ST, Quattrini C, Jeziorska M, Malik RA, Rayman G. Abnormal LDIflare but normal quantitative sensory testing and dermal nerve fiber density in patients with painful diabetic neuropathy. *Diabetes Care*. 2009;32(3):451-455.
39. Quattrini C, Tavakoli M, Jeziorska M, et al. Surrogate markers of small fiber damage in human diabetic neuropathy. *Diabetes*. 2007;56(8):2148-2154.
40. Cheng HT, Dauch JR, Porzio MT, et al. Increased axonal regeneration and swellings in Intraepidermal nerve fibers characterize painful phenotypes of diabetic neuropathy. *J Pain*. 2013;14:941-947.
41. Kalteniece A, Ferdousi M, Petropoulos I, et al. Greater corneal nerve loss at the inferior whorl is related to the presence of diabetic neuropathy and painful diabetic neuropathy. *Sci Rep*. 2018;8(1):3283.
42. Alam U, Najam O, Al-Himdani S, et al. Marked vitamin D deficiency in patients with diabetes in the UK: ethnic and seasonal differences and an association with dyslipidaemia. *Diabet Med*. 2012;29(10):1343-1345.
43. Niu Y, Li J, Peng R, Zhao X, Wu J, Tang Q. Low vitamin D is associated with diabetes peripheral neuropathy in older but not in young and middle-aged patients. *Diabetes Metab Res Rev*. 2019;35(6):e3162.
44. Catalano A, Morabito N, Atteritano M, Basile G, Cucinotta D, Lasco A. Vitamin D reduces musculoskeletal pain after infusion of Zoledronic acid for postmenopausal osteoporosis. *Calcif Tissue Int*. 2012;90(4):279-285.
45. Straube S, Andrew Moore R, Derry S, McQuay HJ. Vitamin D and chronic pain. *Pain*. 2009;141(1–2):10-13.
46. Valensi P, Le Devehat C, Richard JL, et al. A multicenter, double-blind, safety study of QR-333 for the treatment of symptomatic diabetic peripheral neuropathy. A preliminary report. *J Diabetes Complications*. 2005;19(5):247-253.
47. Lee P, Chen R. Vitamin D as an analgesic for patients with type 2 diabetes and neuropathic pain. *Arch Intern Med*. 2008;168(7):771-772.
48. Burstein R, Henry NJ, Collison ML, et al. Mapping 123 million neonatal, infant and child deaths between 2000 and 2017. *Nature*. 2019;574(7778):353-358.
49. Anju M, Chacko L, Chettupalli Y, Maiya AG, Saleena UV. Effect of low level laser therapy on serum vitamin D and magnesium levels in patients with diabetic peripheral neuropathy–a pilot study. *Diabetes Metab Syndr Clin Res Rev*. 2019;13(2):1087-1091.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Alam U, Petropoulos IN, Ponirakis G, et al. Vitamin D deficiency is associated with painful diabetic neuropathy. *Diabetes Metab Res Rev*. 2020;e3361. https://doi.org/10.1002/dmrr.3361