Original article

# Quantitative sensory testing in type-1 diabetic patients with mild to severe diabetic neuropathy

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## ABSTRACT

**Background:** Small nerve fibre damage has been shown to precede large fibre damage and represents the early stage for diabetic neuropathy (DPN).

**Aims:** To assess small nerve fibre deficit in patients with mild to severe DPN using quantitative sensory testing (QST) and to correlate QST findings with other neuropathy assessment measures in patients with DPN.

**Materials and Methods:** Seventy-nine diabetic patients (49 with and 30 without diabetic neuropathy) and 35 healthy volunteers underwent neuropathy assessment. The evaluation comprised neuropathy symptoms profile, modified neuropathy disability score (NDS), quantitative sensory testing (QST) in form of cold and warm thresholds, vibration pressure threshold (VPT), nerve conduction studies (NCS), deep breathing heart rate variability (DB-HRV), and Neuropad staining scores. Patients were also stratified by their NDS scores into mild, moderate and severe DPN. Measures of QST were compared among various grades of DPN severity and correlated with other clinical and neurophysiological tests.

**Results:** Patients with DPN demonstrated higher vibration perception ($P<0.001$) and warm ($P<0.001$) thresholds and lower cold threshold ($P<0.001$) and these QST abnormalities related directly to the grade of DPN severity. Measures of QST also correlated with those of nerve conduction studies (r range 0.54-0.68) and autonomic function assessment(r range 0.33-0.48).

**Conclusion:** Quantitative sensory testing can identify small nerve fibres impairment which could represent the early stage of diabetic neuropathy and hence the optimal period for intervention.

**Keywords:** Diabetic neuropathy, quantitative sensory testing, cold threshold, warm threshold, vibration perception threshold

## INTRODUCTION

Chronic sensorimotor neuropathy is the most common type of diabetic neuropathy (DPN). The prevalence of DPN is about 8% in the recently diagnosed patients and can reach higher than 50% in long standing disease [1]. Early diagnosis and treatment of DPN is vital as more than half of patients can be asymptomatic with higher risk of foot injury and subsequent ulceration [2]. Additionally, diabetic patients may have non-diabetic neuropathies and also may have autonomic neuropathy that is associated with significant morbidity and mortality.

Validated scales and questionnaire have been established for assessing DPN including McGill pain questionnaire, the Neuropathic Pain Symptoms Inventory and Brief Pain Inventory [3]. Neural deficit of DPN can be assessed by composite scores like Neuropathy Disability Score (NDS) and neuropathic impairment score in the lower limbs (NIS-LL) [4]. Modified neuropathy disability score is easy to perform, assess all sensory modalities and have been linked to increased foot ulceration [5] and Intraepidermal nerve fibres (IENF) loss [6]. Cardiovascular autonomic neuropathy (CAN) is a serious complication of diabetes and can occur simultaneously with sensory neuropathy in diabetic patients. Assessment of CAN can be achieved by measuring heart rate variability in response to deep breathing and standing, alteration in blood pressure in response to standing and Valsalva Manoeuvre [7]. A recent work have also demonstrated a link between sweat gland nerve fibre density and clinical profile of DPN with amount of sweat produced by sweat glands [8]. Nerve conduction studies have been also used to evaluate large nerve fibre involvement clinical setting and as end points for follow up studies of DPN [9]. Yet, routine nerve

conduction studies cannot detect damage of thin unmyelinated C and thinly myelinated Aδ small nerve fibres but they help to exclude other causes of neuropathy [10].

The term small nerve fibre disease (SFD) has been applied to the selective degeneration of the distal terminations of small sensory and autonomic nerve fibres in diabetic patients [11]. Although the precise underlying mechanism for SFD is unknown, recent work have demonstrated a link between mutation of voltage-gated sodium channels and SFD [12]. Further, a significant proportion of asymptomatic SFD patients have been shown to have impaired glucose tolerance at the time of diagnosis or after one year of follow-up [10]. Establishing a reliable diagnostic tools and criteria for SFD is essential as damage to these small fibres could represent the early stage of DPN in which both clinical features and NCS findings are inconclusive. Moreover, the current therapeutic approach for DPN is relying on optimal glycaemic control and symptomatic treatment and there is no reliable neuroprotective therapy.

Quantitative sensory testing (QST) is a psychophysical test that allow evaluation of both large and small nerve fibres and their neural pathways SFD in clinical practice and clinical trials [13]. Different sensory modalities can be assessed including vibration pressure threshold, thermal thresholds and thermal pain thresholds. Vibration perception threshold is used for evaluation of large sensory fibre damage in DPN while cold and warm thresholds can assess small nerve fibre function [14]. Measurements of QST in DPN patients have been recommended by many medical associations and recently normative data sets of QST have been published for a large population of age and gender-matched control groups [15]. Small nerve fibre damage can also be assessed on nerve biopsy [16], skin intraepidermal nerve fibre measures and recently corneal confocal microscopy [17]. Limitations of these tests, however, include invasiveness and the need for expensive equipment and special expertise.

The aim of the present study was to assess small nerve fibres deficit in patients with mild to severe DPN using quantitative sensory testing (QST) and to correlate QST findings with other neuropathy assessment tests in patients with DPN.

## MATERIALS AND METHODS

Seventy-nine type 1 diabetic patients (49 with and 30 without diabetic neuropathy) attending Manchester Diabetes Centre/ Wellcome Trust Manchester Clinical Research Facility and 30 age-matched healthy non-diabetic volunteers were enrolled in this case-control study. Diabetic neuropathy was defined according to the Toronto consensus [3] as the presence of abnormal personal motor nerve conduction velocity (<42m/sec) and the presence of abnormal symptoms and signs of DPN (Neuropathy Disability Score NDS score >2). Patients were excluded if they have non-diabetic causes of peripheral neuropathy, systemic disease like cancer, Addison's disease or heart failure. Study approval was obtained from Central Manchester Ethics Committee and all participants provided written informed consent in accordance with the declaration of Helsinki.

Patients and controls underwent detailed evaluation of their symptoms using neuropathy symptom profile (NSP)[18] and McGill visual analogue scale (McGill VAS) for pain[19]. Neurological deficits were assessed using validated methods. Modified neuropathy disability score (NDS)[4] was used and comprises pin prick, thermal and vibration sensation and ankle reflexes assessment. Patients with DPN were further stratified by their NDS scores into mild (NDS 3-5), moderate (NDS 6-8) and severe (NDS 9-10) neuropathy groups. Quantitative sensory testing (QST) involved the quantification of cold (CT) and warm (WT) thresholds using the Neuro Sensory Analyser TSA-II (Medoc Ltd., UK) [20] and Vibration Perception Threshold (VPT) using a Neuroaesthesiometer (Horwell, Scientific Laboratory Supplies, Wilford, UK)[20]. Cold and warm thresholds were measured on the dorsolateral aspect of the left foot using method of limits and VPT on the distal phalanx of the first toe with initial adaptation temperature of 30-32°C. Autonomic neuropathy was assessed by measuring deep breathing heart rate variability (DB-HRV) using a CASE IV machine (WR Medical Electronics, Inc, Stillwater, MN, USA) and sweat glands sudomotor function indicator Neuropad (Trigocare, Germany). Nerve conduction studies were undertaken by consultant neurophysiologist (Dr. Andrew Marshall) using Medtronic Keypoint™ EMG system using standardized protocol with skin temperature maintained at 32-35°C. Sural sensory and peroneal motor nerves amplitudes (SA, PA) and conduction velocities (SNCV, PMNCV) were assessed on the left foot at standardized sites. Blood pressure, body mass index, HbA1c, lipid fractions and estimated glomerular filtration rate (eGFR) were measured for all participants according to the local protocol.

### Statistical analysis

StatsDirect v. 2.7.8 (StatsDirect Ltd., Cheshire, UK) and SPSS 22.0 (SPSS, Chicago, IL) for Windows

software were used for statistical analysis. Data were assessed for normality distribution by Shapiro-Wilk test and histograms where appropriate. Data were presented as mean ± SD (parametric) or median and interquartile range (nonparametric). Comparison among groups were made by Analysis of variance (ANOVA) with Tukey test was used as a post hoc test to determine the significance of difference between pairs of groups for normally distributed data or Kruskal-Wallis test and Conover-Inman test for data that did not follow a normal distribution. Unpaired t-test was performed to compare the duration of diabetes between patients with and without neuropathy. Correlations were performed using Pearson's correlation coefficient. $P<0.05$ was considered statistically significant.

## RESULTS

**Table 1: Demographics and biochemical characteristics of controls and patients with and without diabetic neuropathy**

| | Controls | No DPN | DPN |
|---|---|---|---|
| **n** | 35 | 30 | 49 |
| **Age (years)** | 45.75± 11.86 | 46.06± 12.37 | 52.07± 12.85 |
| **Duration of diabetes (years)** | | 31.06± 12.04 | 35.57± 13.69 |
| **HbA1c (%)‡** | 5.61±0.37 | 8.14±1.04¶ | 8.085± 2.20¶ |
| **SBP (mm Hg)†** | 128.03± 17.42 | 128.83± 16.64 | 143.11± 22.87¶‖ |
| **DBP (mm Hg)** | 73.54± 9.43 | 69.66± 7.55 | 74.25± 10.84 |
| **BMI (Kg/m2)** | 26.65± 3.66 | 25.90 ± 3.37 | 25.35± 5.16 |
| **eGFR (mls/min/1.73m2)‡** | 82.96± 9.55 | 85.76± 11.58 | 66.27± 27.22¶‖ |
| **Cholesterol (mmol/l)‡** | 5.20± 0.79 | 4.47± 1.08¶ | 4.23± 0.92¶ |
| **HDL(mmol/l)** | 1.51± 0.40 | 1.75± 0.37 | 1.64± 0.49 |
| **TRIG(mmol/l)†** | 1.42± 0.72 | 0.92± 0.41¶ | 1.22± 0.65 |
| **LDL(mmol/l)‡** | 2.95± 0.86 | 2.30± 0.97¶ | 2.03± 0.72¶ |

Results are expressed as mean ± SD or median (interquartile range). Statistically significant differences using ANOVA or Kruskal Wallis test: † $P<0.01$, ‡ $P<0.001$. ¶ Post hoc (Tukey or Conover Inman test) results significantly different from control subjects, ‖Post hoc results significantly different from no neuropathy group. DPN, diabetic peripheral neuropathy; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; BMI, Body Mass Index; eGFR, estimated Glomerular Filtration Rate; HDL, High-Density Lipoproteins; TRIG, Triglycerides; LDL, Low-Density Lipoproteins.

**Table 2: Neuropathy assessment in controls and patients with and without neuropathy**

| | Controls | No DPN | DPN |
|---|---|---|---|
| **n** | 35 | 30 | 49 |
| **NSP(0-37)‡** | 0(0-0) | 1(0-2)¶ | 6(2-13)¶‖ |
| **McGill VAS 0-10)‡** | 0(0-0) | 0(0-0) | 3(0-7)¶‖ |
| **VPT R(V) ‡** | 4.94±3.61 | 8.14±5.45 | 27.36±14.00¶‖ |
| **CT(°C)‡** | 28.37±2.54 | 27.36±1.96 | 15.59± 10.65¶‖ |
| **WT(°C)‡** | 36.94± 2.38 | 37.80± 2.93 | 44.60± 4.96¶‖ |
| **SA (uV)‡** | 20.96± 9.49 | 11.88± 6.93¶ | 3.61± 4.21¶‖ |
| **SNCV (m/s)‡** | 51.04± 4.66 | 44.53± 5.21¶ | 35.26± 7.25¶‖ |
| **PA (m/s)‡** | 5.63± 2.02 | 3.87± 1.79¶ | 1.10 ± 1.31¶‖ |
| **PMNCV (m/s)‡** | 49.12± 3.85 | 42.77± 5.17¶ | 30.82± 9.29¶‖ |
| **DB-HRV (beats per min)‡** | 29.86(23.33-40.13) | 29.89(18.94-42.57) | 8.51(6.11-15.63)¶‖ |
| **Neuropad R (%)‡** | 100(85-100) | 100(50-100) | 50(10-90)¶‖ |

Results are expressed as mean ± SD or median (interquartile range). Statistically significant differences using ANOVA or Kruskal Wallis test: ‡ $P<0.001$. ¶ Post hoc (Tukey or Conover Inman test) results significantly different from control subjects, ‖Post hoc results significantly different from no neuropathy group. DPN, diabetic peripheral neuropathy; NSP, Neuropathy Symptom Profile; McGill VAS, McGill Visual Analogue Scale; NDS, Neuropathy Disability Score; VPT, Vibration Perception Threshold; WT, Warm Threshold; CT, Cold Threshold; CIP, Cold Induced Pain; HRV-DB, Heart Rate Variability to Deep Breathing; LA, Sural Nerve Amplitude; SNCV, Sural Nerve Conduction Velocity; PA; Peroneal Nerve Amplitude; PMNCV; Peroneal Motor Nerve Conduction Velocity.

Diabetes duration and HbA1c were comparable between patients with and without neuropathy (35.57± 13.69 vs. 31.06± 12.04 years, 8.085± 2.20 vs. 8.14±1.04 % respectively) although HbA1c was higher than the target level for patients with diabetes (Table 1). Systolic blood pressure was significantly higher in patients with DPN as compared to controls and patients without DPN. Renal function estimated by eGFR was significantly lower in patients with DPN in comparison with controls and those without neuropathy but it was comparable between controls and no neuropathy group. Both serum Cholesterol and LDL were significantly lower in patients with and without DPN as compared to healthy controls. HDL, however, did not differ significantly among patients and controls.

**Table 3: Demographics and biochemical characteristics of controls and patients with mild to severe diabetic neuropathy**

| | Controls | No DPN | Mild DPN | Moderate DPN | severe DPN |
|---|---|---|---|---|---|
| **n** | 35 | 30 | 17 | 17 | 15 |
| **Age (years)** | 45.75± 11.86 | 46.06± 12.37 | 52.83± 11.82 | 52.58± 14.41 | 51.69± 13.21 |
| **Duration of Diabetes (years)** | | 31.06± 12.04 | 35.21± 12.77 | 40.01± 12.96 | 30.96± 14.76 |
| **HbA1c (%)‡** | 5.61±0.37 | 8.14±1.04¶ | 8.07±1.86¶ | 7.62±1.46¶ | 8.68±3.18¶ |
| **SBP (mm Hg)‡** | 128.03± 17.42 | 128.83± 16.64 | 132.25± 22.62 | 141.75± 24.72 | 156.45± 14.29¶‖§ |
| **DBP (mm Hg)*** | 73.54± 9.43 | 69.66± 7.55 | 70.75± 9.75 | 71.50± 11.54 | 81.09± 8.58‖ |
| **BMI (Kg/m2)** | 26.65± 3.66 | 25.90 ± 3.37 | 25.99± 3.77 | 26.74 ± 3.80 | 22.95± 7.08 |
| **eGFR (mls/min/1.73m2)†** | 82.96± 9.55 | 85.76± 11.58 | 80.16± 19.36 | 67.33±13.09¶‖ | 72.33± 9.30 |
| **Cholesterol (mmol/l)‡** | 5.20± 0.79 | 4.47± 1.08¶ | 4.38± 0.66¶ | 4.30± 1.02¶ | 3.95± 1.12¶ |
| **HDL(mmol/l)** | 1.51± 0.40 | 1.75± 0.37 | 1.67± 0.30 | 1.52± 0.40 | 1.74 ± 0.74 |
| **TRIG(mmol/l)*** | 1.42± 0.72 | 0.92± 0.41¶ | 1.16± 0.49 | 1.36 ± 0.89 | 1.15± 0.55 |
| **LDL(mmol/l)‡** | 2.95± 0.86 | 2.30± 0.97¶ | 2.18± 0.57¶ | 2.16± 0.84¶ | 1.69± 0.70¶ |

Results are expressed as mean ± SD or median (interquartile range). Statistically significant differences using ANOVA or Kruskal Wallis test: * P<0.05, † P<0.01, ‡ P<0.001. ¶ Post hoc (Tukey or Conover Inman test) results significantly different from control subjects, ‖Post hoc results significantly different from no neuropathy group.§ Post hoc results significantly different from mild neuropathy group. DPN, diabetic peripheral neuropathy; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; BMI, Body Mass Index; eGFR, estimated Glomerular Filtration Rate; HDL, High-Density Lipoproteins; TRIG, Triglycerides; LDL, Low-Density Lipoproteins.

**Table 4: Neuropathy assessment in controls and patients with mild to severe neuropathy**

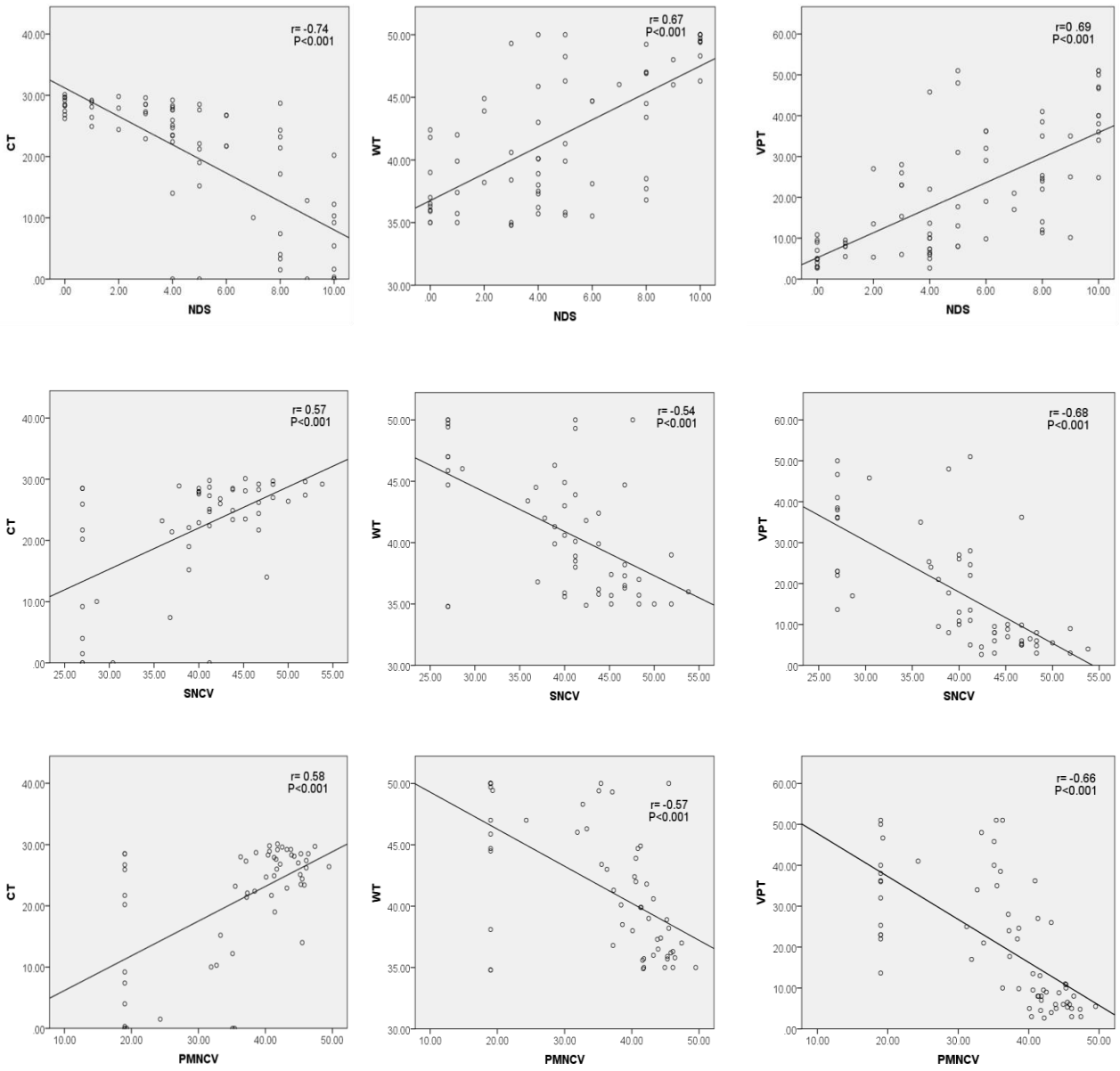
| | Controls | No NP | mild | moderate | severe NP |
|---|---|---|---|---|---|
| **n** | 35 | 30 | 17 | 17 | 15 |
| **NSP(0-37)‡** | 0(0-0) | 1(0-2)¶ | 3(1-6)¶‖ | 6.5(3-13)¶‖§ | 13.5(4-18)¶‖§ |
| **McGill VAS 0-10)‡** | 0(0-0) | 0(0-0) | 1.2(0-6)¶‖ | 2(0-4)¶‖ | 7(0-9)¶‖ |
| **VPT R(V) ‡** | 4.94±3.61 | 8.14±5.45 | 21.41±15.22¶‖ | 24.88± 9.96¶‖ | 37.76±11.74¶‖§ |
| **CT(°C)‡** | 28.37±2.54 | 27.36±1.96 | 22.53±7.71¶‖ | 16.98± 9.68¶‖§ | 5.53±6.73¶‖§ |
| **WT(°C)‡** | 36.94± 2.38 | 37.80± 2.93 | 42.57 ±5.08¶‖ | 42.86± 4.55¶‖ | 48.97±1.41¶‖§ |
| **SA (uV)‡** | 20.96± 9.49 | 11.88± 6.93¶ | 5.48± 5.35¶ | 2.67± 2.81¶‖ | 1.38± 2.19¶‖ |
| **SNCV (m/s)‡** | 51.04± 4.66 | 44.53± 5.21¶ | 29.84± 6.35¶‖ | 34.89± 7.53¶‖ | 37.90 ± 6.44¶‖ |
| **PA (m/s)‡** | 5.63± 2.02 | 3.87± 1.79¶ | 0.35± 0.32¶‖ | 1.04± 1.01¶‖ | 1.73± 1.73¶‖ |
| **PMNCV (m/s)‡** | 49.12± 3.85 | 42.77± 5.17¶ | 34.69± 9.75¶‖ | 30.20± 8.7426.60± 8.07¶‖ | 26.60± 8.07¶‖§ |
| **DB-HRV (beats per min)‡** | 29.86(23.33-40.13) | 29.89(18.94-42.57) | 16.62(7.85-22.08)¶‖ | 6.77(5.86-10.16)¶‖ | 8.92(6.77-9.5)¶‖§ |
| **Neuropad R (%)‡** | 100(85-100) | 100(50-100) | 85(55-97.5) | 20(10-85)¶§ | 5(0-80)¶‖§ |

Results are expressed as mean ± SD or median (interquartile range). Statistically significant differences using ANOVA or Kruskal Wallis test: ‡ P<0.001. ¶ Post hoc (Tukey or Conover Inman test) results significantly different from control subjects, ‖Post hoc results significantly different from no neuropathy group.§ Post hoc results significantly different from mild neuropathy group. DPN, diabetic peripheral neuropathy; NSP, Neuropathy Symptom Profile; McGill VAS, McGill Visual Analogue Scale; NDS, Neuropathy Disability Score; VPT, Vibration Perception Threshold; WT, Warm Threshold; CT, Cold Threshold; CIP, Cold Induced Pain; HRV-DB, Heart Rate Variability to Deep Breathing; LA, Sural Nerve Amplitude; SNCV, Sural Nerve Conduction Velocity; PA; Peroneal Nerve Amplitude; PMNCV; Peroneal Motor Nerve Conduction Velocity.

Neuropathy assessment in controls and patients with and without DPN is shown in table 2. For all measures of neuropathy, patients with DPN demonstrated significantly different values than controls and patients without DPN. Patients with DPN demonstrated higher NSP and McGill VAS than controls and patients without DPN. As for quantitative sensory testing, VPT (P<0.001, P<0.001) and CT (P<0.001) were lower and WT was higher (P<0.001) in patients with neuropathy in comparison with controls and no neuropathy groups. Mean VPT, CT and WT in patients with and without DPN were 27.36±14.00 vs. 8.14±5.45 V, 15.59± 10.65 vs. 27.36±1.96°Cand 44.60±4.96 vs. 37.80± 2.93°C respectively. Patients with DPN exhibited subnormal values of nerve conduction study and were significantly lower (P<0.001) as compared to controls and patients without DPN. Sural and peroneal nerves amplitudes and velocities decreased significantly in DPN patients as

compared to other groups. Patients without DPN also demonstrated modestly lower NCS values than

**Figure 1: Correlations between quantitative sensory tests and neuropathy disability score and sural and peroneal conduction velocities.r, Pearson's correlation coefficient. NDS, Neuropathy Disability Score; CT, Cold Threshold; WT, Warm Threshold; SNCV, Sural Nerve Conduction Velocity; PMNCV, Peroneal Motor Nerve Conduction Velocity**




controls. DB-HRV and Neuropad staining scores followed those of QST and were significantly lower (P<0.001) in patients with neuropathy as compared to controls and patients without neuropathy.

We have then stratified patients with neuropathy by their NDS scores into mild (n=17, 35%), moderate (n=17, 35%) and severe (n=15, 30%) neuropathy to compare findings of different grades of DPN severity. Duration of diabetes and HbA1c were not significantly different among patients with different grades of severity and in comparison with controls and no neuropathy groups (Table 3). Systolic and diastolic blood pressure gradually increased and BMI, eGFR, Cholesterol, HDL, Triglycerides and LDL gradually decreased with increasing severity of DPN. Patients with severe neuropathy exhibited significantly higher systolic blood pressure as compared to controls, no neuropathy and mild-moderate NP groups.

Table 4 illustrates neuropathy assessment in patients with mild to severe neuropathy as compared to other groups. There was gradual increase of NSP and QST tests (VPT, CT and WT), nerve conduction study tests (LPA,LPV,LPA,PMNCV) and autonomic nerve function tests (DB-HRV, Neuropad staining) with increasing severity of DPN and the values were significantly different from controls and patients with no neuropathy. Patients with mild neuropathy exhibited significantly lower NSP (P<0.01), VPT

(P<0.01), WT (P<0.05) and significantly higher CT (P<0.01), PMNCV (P<0.01), DB-HRV (P<0.01) and Neuropad staining score (P<0.001) as compared to patients with severe DPN. Mean VPT and CT in patients with mild and severe DPN were 21.41±15.22 vs. 37.76±11.74 V and 22.53±7.71 vs. 5.53±6.73 °Crespectively while mean sural and peroneal nerves velocities were 29.84± 6.35 vs. 37.90 ± 6.44 and 34.69± 9.75 vs. 26.60± 8.07 respectively.

We have explored data further by correlating QST tests (VPT, CT and WT) with other parameters of the study. There was no significant correlations between QST measures and age or duration of diabetes apart from modest correlation between age and VPT (Pearson's correlation coefficient r=0.40, P<0.05). The correlations between QST tests and NDS, sural and peroneal velocities are shown in figure 1. There was direct and strong correlation between VPT (r=0.69, P<0.001) and WT (r=0.67, P<0.001) with NDS and also inverse and strong correlation between CT and NDS (r=-0.74, p<0.001). VPT and WT also exhibited inverse correlations and CT exhibited direct correlation with both sural and peroneal conduction velocities. Notably, these correlations were also significant but the strongest correlation was between VPT and sural (r=-0.68, P<0.001) and peroneal (r=-0.73, P<0.001) velocities. As for other neuropathy assessment tests, QST tests demonstrated weak correlations with McGill VAS and Neuropad staining scores. VPT (r=0.31, p<0.01) and WT (r=0.30,P<0.01) correlated directly while CT (r=-0.32, p<0.01) correlated inversely with McGill VAS. There was also inverse and significant correlation between VPT (r=-0.48, p<0.001) and WT (r=-0.37, P<0.01) and direct and significant correlation between CT (r=0.33, P<0.01) and Neuropad staining scores.

**DISCUSSION**

In the current study, duration of diabetes and glycaemic control were comparable between patients with and without neuropathy albeit HbA1c levels were higher than the recommended levels for diabetic patients (6.5-7.0%) [21]. Although optimal glycaemic control has been shown to reduce DPN in diabetic patients [22], recent study have demonstrated no relation between glycaemic control and diabetic chronic complications including DPN [23].

Patients with DPN demonstrated higher NSP and McGill VAS scores as compared to controls and patients without DPN. Previous studies have reported higher McGill VAS scores in patients with DPN [7,24]. Vibration perception threshold (VPT) was higher in patients with DPN. The perception of vibration stimulus can be assessed reliably with 128-Hz tuning fork but unlike QST, the measurement is not quantitative. Higher VPT have been demonstrated in patients with DPN [7] and in a large study that compared VPT to other bedside modalities in diabetic patients, VPT showed significant correlations with clinical measures of diabetic neuropathy, vibration assessed with tuning fork, monofilament test and ankle reflex [25]. Vibration pressure threshold assesses large nerve fibre function and have been shown to be sensitive and specific measure for diabetic foot ulcer and reproducible [26].

Cold and warm thresholds findings were consistent with VPT findings and patients with DPN exhibited lower cold threshold and higher warm thresholds as compared to patients without DPN. Quantitative sensory testing of thermal sensory modalities have been linked to small nerve fibre damage in patients with DPN [9]. Our data are congruent with previous reports that demonstrated an increase in VPT and reduction in cold threshold in DPN patients [6,27] and CT significantly correlated with reduced IENF assessed on foot skin biopsy [6]. Our results confirm increment of WT in patients with DPN demonstrated by other researchers [27,28]. In spite of lack of agreement on the cut-off values for QST tests, a large study have recently described the normative values for QST tests for subjects matched for age and sex [29]. Provided that QST procedure is standardized, QST is a powerful research tool in the diagnosis and assessment of DPN and SFD especially when NCS are normal. Indeed abnormalities of cold and warm thresholds have been reported in diabetic patients with asymptomatic SFD confirmed by skin biopsy IENF [30] suggesting that CT and WT might detect DPN at early stage. Early detection of DPN at early stage is crucial for optimal therapeutic intervention and as currently there is no effective treatment that can prevent or reverse neural injury in DPN.

In the current work, patients with DPN demonstrated lower sural and peroneal amplitudes and conduction velocities as compared to patients without DPN and controls which is consistent with the findings reported by other researchers [(6,27,31]. A recent follow up study of asymptomatic DPN type 1 patients revealed moderate reduction of peroneal and median nerve conduction velocities and sural nerve action potential [32]. Measurements of NCS are influenced by patients age, temperature of the nerve, and normal variability in nerve conduction and hence all these factors need to be taken into account when interpreting the results

[33]. Most importantly and unlike QST, NCS can identify large nerve fibre damage in DPN but they cannot detect small nerve fibre damage and therefore cannot aid in the diagnosis of SFD which could represent the early stage of DPN [14].

Patients with DPN also exhibited lower Deep breathing heart rate variability (DB-HRV) and Neuropad staining scores. Deep breathing heart rate variability is a measure of cardiac autonomic neuropathy which is associated with significant morbidity and increased mortality in diabetic patients [2] and has been shown to be reduced in patients with DPN [6,9,34]. Neuropad is an emerging visual indicator of sudomotor function that depends on colour change of cobalt II and has been identifed as a sensitive but less specific indicator for diagnosing of autonomic dysfunction in patients with DPN [35]. Recent studies have observed that Neuropad is sensitive test for detection of autonomic neuropathy in diabetic patients and Neuropad staining score correlated with IENF and CCM metrics as a measure of small nerve fibre damage [34,35]. Moreover,in a study on 127 diabetic patinets with and without DPN, Neuropad staining score has been shown to be sensitive measure (sensitivity range from 70%-83%) for small and large fibres diabetic neuropathy but with moderate specificity [36]. Obtaining Neuropad results is easy, quick and does not reuquire expensive equipements and the assesmsent can be performed automatically [34].

To explore the neurophysiological characteristics of patients with various grades of DPN severity, patients with DPN were further divided by their NDS scores into mild, moderate and severe DPN. Patients with mild DPN constituted 34.6%, moderate 34.6% and severe DPN constituted 30.8%. There is lack of agreement on the best approach to classify the severity of DPN but the severity of DPN represents a combination of symptoms, signs and electrophysiological tests and therefore sum scores could be used to grade the severity of DPN patients [3,37].

Quantitative sensory testing findings followed those of clinical assessment and vibration pressure threshold and warm thresholds gradually increased while cold threshold gradually decreased with higher grade of DPN severity. Our findings are consistent with previous studies which reported gradual increase in VPT, WT and gradual decrease in CT with increasing severity of DPN based on NDS scores [6,27] and correlated with morphological finding of reduction in foot skin biopsies IENFD [6] and CCM metrics [27]. A recent study have demonstrated a link between cold and warm thresholds and duration of diabetes in diabetic patient [38 ] but the duration of diabetes was comparable between our patients groups and therefore cannot be a contributing factor for the differences obtained. Our data suggests that small nerve fibre injury is directly related to the severity of DPN and mild injury to small fibres could represent the very early stage of DPN and the ideal period for potential therapies for DPN.

There was also a steady decrement of sural and peroneal nerves amplitudes and peroneal nerve conduction velocity was the only statistically significant one. Our findings are congruent with previous published studies which demonstrated reduction in sural and peroneal nerves amplitudes and velocities in patients with mild to severe DPN [6,27]. As for autonomic neuropathy assessment, both DB-HRV and Neuropad staining scores were lower in patients with severe DPN as compared to mild and moderate DPN groups. Measurement of DB-HRV has been shown to decrease with increasing severity of DPN [6] and associated with small and large nerve fibres measures [39]. Neuropad optimal cut-off was 90% for detection of DB-HRV and 80% for identifying diabetic patients with SFD [35] and the sensitivity improved with automatic quantification of staining [34]. Measurement of DB-HRV variability could be a marker of DPN and indicator of early stage of the disease [39].

In the current work, quantitative sensory measures correlated well with clinical and other neurophysiological tests. Cold threshold correlated inversely and warm threshold and vibration perception thresholds correlated directly with NDS scores. Previous work demonstrated that NDS the composite score is a reliable measure of both small and large nerve fibre sensory and motor impairment in patients with DPN and could be used for DPN staging [2]. In another study, however, NDS correlated with VPT but not with CT or WT in diabetic patients assessed for DPN [40]. Assessment of QST is reliable and reproducible predictor of the progression of diabetic foot ulceration [40]. In the current study, there was direct correlation between cold threshold and sural and peroneal nerve conduction velocities. Warm threshold and vibration perception threshold also correlated inversely with nerve conduction velocities and all these correlations were significant. Our results are congruent with previous data which showed moderate to strong correlations and clustering between QST measures and nerve conduction velocities [9,41]. The results of QST also correlated with Neuropad staining scores suggesting that QST measures could also help to

identify autonomic involvement in patients with DPN. The correlations observed in this study could be attributed to the simultaneous small and large nerve fibre damage due to common underlying biochemical mechanism. Unlike NCS, QST can detect small nerve fibre involvement in DPN and therefore could identify subclinical DPN which is invisible to NCS [30]. Further, abnormalities of CT and WT can be detected in the presence of normal VPT but the reverse might not be true [40]. A possible explanation is that small nerve fibres damage represents the early stage of DPN at which large fibres are still preserved and therefore detection of small nerve fibre damage is vital for early detection and treatment of DPN.

## CONCLUSION

Patients with DPN have higher vibration perception threshold and warm threshold and lower cold threshold and the magnitude of QST abnormalities is proportional to the grade of severity of DPN and correlate well with clinical and electrophysiological measures. Further, QST tests can identify small nerve fibre impairment which could represent the early stage of DPN at which therapeutic intervention are likely to be successful. Further studies are warranted to consolidate the evidence presorted in this study.

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### Conflict of interest

The author declares that he has no conflict of interest.

### Human and Animal Rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

### Informed Consent

Informed consent was obtained from all patients for being included in the study.

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