Abstract

Background and Aims: The measuring tools used for assessment of neuropathy include various questionnaires, monofilament testing, Biothesiometry, and the gold standard test, nerve conduction studies (NCS). This study aims to evaluate the diagnostic accuracies of Michigan Neuropathy Screening Instrument (MNSI), Biothesiometry, Semmes Weinstein Monofilament (SWMF), Sural Radial Amplitude Ratio (SRAR) and minimal F wave latency as compared to conventional NCS and arrive at a simple diagnostic algorithm for early detection of Diabetic Peripheral Neuropathy (DPN).

Methods: In a cross-sectional observational study on 48 Type 2 diabetes mellitus patients, MNSI, Biothesiometry, SWMF and NCS including F waves and SRAR were done and diagnostic accuracies (sensitivity, specificity, positive and negative predictive values) calculated taking NCS as gold standard.

Results: MNSI, Biothesiometry, SWMF, SRAR and minimal F wave latency had a sensitivity of 64.3%, 78.6%, 14.3%, 100% and 78.6% and specificity of 67.7%, 52.9%, 94.1%, 23.53% and 76.47% respectively, with reference to NCS. Based on combined sensitivities and specificities, we arrived at a simple algorithm for early diagnosis of DPN, which showed that DPN could either be diagnosed or ruled out in 75% of the patients by a combination of the Biothesiometry, SRAR and left lower limb minimal F wave latency results.

Conclusions: In the setting of an outpatient, multidisciplinary diabetic clinic, simple tests such as questionnaires, monofilament testing and biothesiometer could be performed with greater ease while considering NCS as the gold standard. This algorithm, combining Biothesiometry, SRAR and left lower limb minimal F wave latency would be less time consuming and help in early diagnosis of DPN.

Keywords: Biothesiometry, diabetic peripheral neuropathy, F wave, nerve conduction studies, sural radial amplitude ratio

Introduction

Diabetic Peripheral Neuropathy (DPN) is a common complication of Diabetes Mellitus, often leading to foot ulcers, foot infections, Charcot arthropathy and amputation. Early diagnosis will help in prevention of complications through lifestyle changes and optimal interventions.[1]

Various questionnaires, monofilament testing, Vibration perception threshold testing by Biothesiometry and nerve conduction studies (NCS) are used for assessment of neuropathy. The aim of our study was hence to evaluate the diagnostic accuracies of Michigan Neuropathy Screening Instrument (MNSI), Semmes Weinstein

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Monofilament (SWMF), Biothesiometry, Sural Radial Amplitude Ratio (SRAR) and minimal F wave latency when compared to conventional NCS and arrive at a simple diagnostic algorithm for the early detection of DPN in patients with Type 2 Diabetes Mellitus (T2DM).

Materials and Methods

This was a cross-sectional observational study conducted in a tertiary care teaching hospital after obtaining approval from the Institutional Review Board and Ethical committee (IRB no: 9468/05.06.2015).

Forty-eight patients with T2DM, aged between 30-65 years were enrolled into the study after getting informed consent. Patients with ulcers, amputations, Charcot foot, obesity, cardiac pacemakers, rhythm abnormalities as well as other diseases which affect the peripheral nerve function such as malnutrition, alcoholism, chronic liver and kidney disease and patients with clinical evidence of any other peripheral nerve lesions, lumbosacral radiculopathy and lumbar canal stenosis were excluded from the study. Patients with ulcers, amputations and Charcot’s foot were excluded since the primary focus of this study was the early diagnosis of diabetic peripheral neuropathy.

Baseline demographic parameters such as age, sex, BMI and duration of diabetes were assessed. A clinical proforma, which included a detailed history and examination, was administered.

The Michigan Neuropathy Screening instrument (MNSI) consists of a history score (self-administered questionnaire) and an examination score. The examination score includes foot inspection, presence/absence of foot ulcer, assessment of vibration sense, grading of the ankle reflex and monofilament testing using a Semmes Weinstein 10 g monofilament testing (SWMF).

Biothesiometry was done in all patients with the biothesiometer applied perpendicular to the test site with constant and firm pressure. It was performed using a Vibrometer - Vibration Proprioception Threshold (VPT) machine, model number V114012706 (Diabetic Foot Care India Private Limited, India) The vibration proprioception was measured over the first Distal Interphalangeal (DIP) joint of both the legs. The voltage was slowly increased at the rate of 1 mV/sec and the vibration perception testing value was defined as the voltage at which the subject first felt the vibration sense. The mean of three records was taken. A vibration perception testing value of <15 mV was scored as normal, 15-25 mV as mild neuropathy, 25-40 mV as moderate neuropathy and >40 mV as severe neuropathy. The time taken to do this test bilaterally was three minutes approximately.

SWMF testing using 2, 4- and 10-gram monofilaments was performed in all patients. Following an initial pretest where four to six perpendicular applications were done to the dorsum of the examiner’s first finger, the filaments were applied to ten sites including nine plantar sites and one dorsal site with the foot supported. The plantar sites included the ventral aspect of digits one, three and five, metatarsal heads (1,3,5), medial and lateral mid-foot and heel. The dorsal site was the area between the base of digits one and two. The filament was applied perpendicularly and briefly for less than a second with an even pressure. Bending of the filament indicated that a force of 2/4/10 grams had been applied. If there were more than or equal to five incorrect responses out of ten in one foot with 2 gram monofilament, the test was considered abnormal. The same test was repeated using the 5 gram and 10 gram monofilaments and abnormal responses recorded.

NCS was performed in all patients using the Medelec synergy system (Multi sync LCD1770NX), using standard surface stimulating and recording techniques. Motor (median, ulnar, tibial and common peroneal nerves) and sensory (median, ulnar, radial, sural and superficial peroneal nerves) NCS were performed bilaterally. The attributes measured were distal latency, amplitude and conduction velocity of the Compound Muscle Action Potentials (CMAPs) and Sensory Nerve Action Potentials (SNAPs). F wave studies of median, ulnar, tibial and common peroneal nerves were performed and Minimal F wave latencies recorded. “Case definition criterion” for electro-diagnostic confirmation of distal symmetric polyneuropathy was an abnormality (99th or 1st percentile) of any attribute of nerve conduction in two separate nerves, one of them being the sural nerve. As per the protocol recommended for the above mentioned case definition criterion, unilateral (left sided) studies of sural sensory, median sensory and ulnar sensory nerves, and peroneal, tibial, median and ulnar motor nerves with F waves were taken into consideration for diagnosis of DPN based on conventional NCS. Each parameter was determined as normal or abnormal based on the upper and lower limits of normal for our laboratory.

Sural Radial Amplitude Ratio (SRAR) was calculated as the ratio of the SNAP amplitudes of sural and radial nerves. SRAR of >0.4 was considered as normal. In the case of minimal F wave latency, normal values were taken from an Indian study, published in 2013. The reference ranges are shown in the supplementary data. Studies have shown that tibial and common peroneal F waves are the most sensitive measures to detect diabetic peripheral neuropathy. Hence only left lower limb F waves were included in the algorithm.

Statistical analysis

Data entry was done using MS excel and analyzed using STATA/IC 13.1. Independent t test/Wilcoxon Rank-sum test was used for continuous variables and Chi square test for categorical variables. The diagnostic accuracies (sensitivity, specificity, positive predictive value and negative predictive value) were calculated for all clinical tools, based on NCS as the gold standard. Receiver Operating Characterististics (ROC) curves were used. A multivariate logistic adjustability for age and HbA1C was analyzed to study the influence of age and HbA1C on the occurrence of neuropathy.
Results

Baseline demographic characteristics
A total of 48 patients were recruited. The baseline characteristics are described in Table 1. The mean age was 51.31 years and the duration of diabetes ranged from two months to twenty years.

Results of the various screening tests
Based on MNSI, 20 (41.66%) patients had clinical neuropathy [Table 2]. The Biothesiometry examination found nine (18.75%) patients to have neuropathy, which was moderate in three (6.25%) patients and severe in six (12.5%) patients. Four patients (8.3%) had an abnormal Semmes Weinstein Monofilament testing (Two patients perceived less than five points in a 4 and 10 gram monofilament and two patients perceived less than five points on a 10 gram monofilament only). The remaining 44 patients (91.6%) could perceive more than five points with a 2 gram monofilament [Table 2].

With conventional NCS, 14 (29.16%) patients were found to have neuropathy. The nerves of the lower limb were affected more than those of the upper limb.

SRAR was abnormal in 40 (83.33%) patients and an F wave abnormality was observed in 19 (39.58%) patients. Left lower limb F wave abnormality was observed in 14 patients (29.1%).

Sensitivity and specificity of the screening tools
The sensitivities and specificities of all screening tools were calculated based on NCS, the gold standard. The test with the highest sensitivity was SRAR (100% sensitivity and 100% negative predictive value) [Table 3 and Figure 1]. However the specificity of this test was only 23.5%. Biothesiometry with 15V as cut off had a better specificity of 52.9% with sensitivity of 78.6% and negative predictive value of 85.7%. A cut off of 25V further increased the specificity of this test to 91.2% with sensitivity of 50% and a positive predictive value of 70%. [Table 3 and Figure 2]. The highest specificity, 95.1% however was for Semmes Weinstein monofilament, but the sensitivity was only 14.3%.

Diagnostic accuracies of different combinations of these tests were analyzed. A combination of SRAR and Biothesiometry results (15V as cut off), considerably increased the specificity to 64.7%, with only a slight decrease in sensitivity to 78.6%. The specificity was further increased to 97.1% with 50% sensitivity if 25V cut off was used for Biothesiometry in combination with SRAR.

The combinations with highest sensitivity were SRAR with Biothesiometer with 15V as cut off (78.6%) and SRAR with F wave (78.6%). SRAR and left lower limb F waves alone had slightly lower sensitivity of 71.4%and a better specificity of 94.1%. Biothesiometry value with SRAR and

| Variables                  | Mean  | SD    | Median | Min  | Max  |
|----------------------------|-------|-------|--------|------|------|
| Age (years)                | 51.31 | 7.89  | 50.5   | 31   | 65   |
| BMI (Kg/m²)                | 24.94 | 2.99  | 24.75  | 18.4 | 29.7 |
| HbA1C (%)                  | 8.21  | 1.97  | 7.7    | 5.4  | 13.2 |
| Duration of diabetes (years)| 5.95  | 4.81  | 4.5    | 0.2  | 20   |
| BMI ‑ Body Mass Index, SD ‑ Standard Deviation |

| Screening tool              | Abnormal (Number) | Abnormal (Percentage) |
|-----------------------------|-------------------|-----------------------|
| Michigan Neuropathy Screening Instrument (MNSI) | 20 | 41.6% |
| Semmes Weinstein Monofilament (SWMF) | 4 | 8.3% |
| Biothesiometry (BT) with 25V as cut off | 9 | 18.75% |
| Nerve Conduction Study (NCS) | 14 | 29.16% |
| Sural Radial Amplitude Ratio (SRAR) | 40 | 83.33% |
| F wave study                | 19 | 39.58% |

Table 1: Baseline demographic characteristics

Table 2: Results of the standard screening tests, nerve conduction studies, sural radial amplitude ratio and minimal F wave latency

Figure 1: ROC curve for sural radial amplitude ratio (with NCS as gold standard)

Figure 2: ROC Curve for biothesiometer (with NCS as Gold Standard)
F wave results combined together had a good specificity and sensitivity (sensitivity 71.4% and specificity 91.2%) [Table 4].

Based on the above results, an algorithm was derived which could easily be followed in the outpatient section for screening and early diagnosis of diabetic peripheral neuropathy.

According to the algorithm shown in the flowchart [Figure 3], all patients diagnosed with diabetes mellitus should undergo an initial Biothesiometry evaluation, which is a simple tool, without need for technical expertise and can be done by the Diabetic clinic nurse. If Biothesiometry value is more than 25V, DPN can be diagnosed as the specificity of this test is very high (91.2%). If the Biothesiometry reading is <15V, neuropathy could be excluded due to a sensitivity of 78.6% and Negative predictive value of 85.7. If the patient has a biothesiometry value between 15 and 25V, then the patient should undergo an additional SRAR testing. An SRAR of >0.4, would exclude neuropathy due to the 100% sensitivity and negative predictive value. A reading of less than 0.4, would warrant an additional left lower limb F wave study, and an abnormal minimal F wave latency would suggest neuropathy due to its high specificity of 100%. Biothesiometry between 15 to 25V, SRAR less than 0.4 and normal left lower limb F wave studies would be an indication to do conventional NCS to confirm diagnosis of neuropathy. Thus, this flow chart shows that the combination of simple tests such as biothesiometry, SRAR and left lower limb F wave studies help in early diagnosis of DPN. Seventy five percent of DPN can either be ruled in or ruled out by Biothesiometry, SRAR and left lower limb F wave studies combined together.

**DISCUSSION**

The prevalence of DPN that has been reported in a number of studies is quite variable. While in certain studies, peripheral neuropathy was diagnosed in 29.16% patients with diabetes, others reported a prevalence of 52.6%[7] based on NCS, which is the gold standard test. Other studies showed similar prevalence of 29% with the gold standard being Neuropathy Symptom Score (NSS) & Neuropathy Disability Score (NDS) in one study and Biothesiometry in another[1,2].

Of the total sample of 48 patients, 41.66% had clinical neuropathy based on the MNSI examination score with 2 as cut off. Another study reported a slightly lower incidence of clinical neuropathy (32.07%) probably because of the higher MNSI cut off score (2.5) used by them.[13]

In our study, 18.75% of the patients had an abnormal Biothesiometry value of more than 25 volts and 56.25% had a Biothesiometry value of more than 15 volts. This is similar to that reported by Young et al.[9] on 469 patients in which 55.44% of the patients had a Biothesiometry value of more than 15 V.

As in other studies,[14-16] we found that upper limb motor and sensory nerves were affected to a lesser extent when compared to the lower limb nerves, explained by the length dependent peripheral neuropathy in diabetes mellitus. Common Peroneal Nerve was most commonly affected, followed by abnormalities of the Superficial Peroneal Nerve and Sural nerve. Other studies have also reported that both motor and sensory nerve parameters can be significantly affected, though it is commonly thought that motor nerves are rarely involved.[14,15] However the severity of involvement of sensory nerves (absent SNAPs) was more than motor nerves.

With NCS as gold standard, MNSI had a sensitivity of 64.3% and specificity of 67.6%, not unlike that reported by Mete et al.[13] where, among patients with clinical neuropathy, 58.8% had abnormal NCS.

The sensitivity and specificity of Semmes Weinstein monofilament testing was 14.3% and 94.1% respectively, based on NCS. A similar finding of a low sensitivity (6%) was seen in certain studies whereas other studies showed a high sensitivity and specificity of 90% and 85% respectively.[18] The reason for this wide variability in different studies could be the lack of standardization for the use of monofilament including the number and location of sites to be tested and the gold standard test used for comparison.

| Table 3: Diagnostic accuracies based on nerve conduction studies (NCS) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | MNSI            | SWMF            | BT (15V as cut-off) | BT (25V as cut-off) | SRAR            | F wave          |
| Sensitivity    | 64.3%           | 14.3%           | 78.6%              | 50%              | 100%           | 78.6%           |
| Specificity    | 67.6%           | 94.1%           | 52.9%              | 91.2%            | 23.5%          | 76.5%           |
| Positive Predictive Value | 45%            | 50%              | 40.7%              | 70%              | 35%            | 57.9%           |
| Negative Predictive Value | 82.1%          | 72.7%           | 85.7%              | 81.6%            | 100%           | 89.7%           |

MNSI - Michigan Neuropathy Screening Instrument; SWMF - Semmes Weinstein Monofilament; BT - Biothesiometer; SRAR - Sural Radial Amplitude Ratio

| Table 4: Sensitivity and Specificity of combined parameters based on nerve conduction studies |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                | SRAR + BT (15V as cut-off)      | SRAR + BT (25V as cut-off)      | SRAR + F wave                  | SRAR + F wave + BT (15V as cut-off) | SRAR + Lt LL F wave + BT (15V as cut-off) |
| Sensitivity                    | 78.6%                          | 50%                            | 78.6%                          | 71.4%                          | 71.4%                          | 64.3%                          |
| Specificity                    | 64.7%                          | 97.1%                          | 88.2%                          | 91.2%                          | 94.1%                          | 94.1%                          |

SRAR - Sural Radial Amplitude Ratio; BT - Biothesiometer; Lt LL - Left Lower limb
Biothesiometry with a cut off of 15V was found to have a sensitivity of 78.6% and specificity of 52.9%. An increase in the cut off to 25V decreased the sensitivity to 50% and increased the specificity to 91.2%. Pourhamidi et al.\textsuperscript{[17]} observed a slightly higher sensitivity and specificity of 82% and 70% respectively, probably due to the stricter criteria used to define cases with peripheral neuropathy (NDS and NCS).

SRAR, a derived ratio from NCS had a very high sensitivity of 100% (cut off of <0.4) and specificity of 92.86% (cut off of <0.2) [Figure 1]. Rutkove et al.\textsuperscript{[3]} also has previously described the usefulness of Sural/radial amplitude ratio in the diagnosis of mild axonal polyneuropathy as compared to Sural SNAP amplitude alone.

Another parameter in NCS useful in the subclinical diagnosis of peripheral neuropathy is minimal F wave latency, the sensitivity and specificity of which were 78.6% and 76.5% respectively. Weisman et al.\textsuperscript{[19]} reported comparable sensitivity and specificity of 78.6% & 63% for tibial F wave latency and 74% & 70% for peroneal F wave latency.

Figure 3: Flow chart showing simple outpatient based algorithm for early diagnosis of diabetic peripheral neuropathy

Diagnostic approaches and strategies for early detection of DPN, including symptoms, signs and simple screening tools followed by conventional NCS for confirmation of diagnosis have been described.\textsuperscript{[20,21]} A recent study has evaluated the use
of Point of Care devices, namely DPN check (to evaluate sural nerve amplitude and conduction velocity) and Sudoscan (for assessment of sudomotor function) and arrived at a diagnostic algorithm for clinical application of “point of care” devices and early diagnosis of DPN based the sensitivity and specificity of each test.\[22] However these devices are expensive.

Akin to other studies,\[17,23\] we found that combining various screening tools resulted in a better specificity with only a slight decrease in sensitivity. Our algorithm for early diagnosis of DPN based on the combined sensitivity and specificity of various screening tools could easily be implemented in the setting of a multidisciplinary diabetic outpatient clinic for screening and diagnosis of diabetic peripheral neuropathy. This would be cost-effective and easy to perform as 75% percent of DPN can either be ruled in or ruled out by Biothesiometry, SRAR and left lower limb minimal F wave latency combined together.

Limitations
The cross sectional nature of the study, with its small sample size and the fact that small fiber neuropathy was not taken into consideration could be limitations of the study. Normal NCS is affected significantly by age and height, which was not taken into consideration. A larger study with follow up to observe development of ulcers would give a more accurate sensitivity and specificity of the various tools.

Future perspective
The diagnostic algorithm can be tested prospectively in a larger sample of patients with diabetes, in order to diagnose diabetic peripheral neuropathy early.

Conclusions
In the setting of a multidisciplinary outpatient diabetic clinic, simple tests like a questionnaire; monofilament testing and biothesiometer could be performed more easily while considering NCV as a gold standard. SRAR adds credibility in the diagnosis of length dependent neuropathy and minimal F wave latency is useful for early diagnosis of DPN. Our algorithm combining Biothesiometry, SRAR and F wave studies increases the diagnostic accuracy and can be used for early diagnosis of DPN while considering NCS as gold standard.

Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest
There are no conflicts of interest.

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