Useful Application of the Neuropad Test for Assessment of Diabetic Polyneuropathy

Keiji Yoshioka¹ and Hiroshi Okada²

Abstract

Objective  Examination of sudomotor function is now recommended to assess peripheral autonomic dysfunction. The aim of this study was to evaluate the clinical usefulness of Neuropad, a simple visual indicator test, for assessment of diabetic polyneuropathy (DPN).

Methods  This study examined 87 diabetic patients with a mean age of 61.1±8.8 years, a mean diabetes duration of 13.0±7.5 years and a mean HbA1c of 8.8±1.7%. Diagnosis of DPN was based on clinical examinations using modified Toronto Clinical Neuropathy Score (mTCNS). The patients also underwent 4-g monofilament test and heart rate variability by coefficient of variation of R-R intervals (CVR-R) was determined with the patients at rest. The Neuropad test was applied on the plantar aspect of the great toe and removed after 10 minutes to evaluate the color change as normal (blue to completely pink), patchy (patches of blue and pink) and abnormal (remained blue).

Results  Twenty-eight patients showed a normal, 45 patchy and 14 abnormal response to the Neuropad test. Patients with an abnormal response had significantly longer diabetes duration than those with a normal or a patchy response, but HbA1c levels were similar among the three groups. The CR-R at rest was significantly lower in patients with an abnormal response than those of normal and patchy response, respectively. Abnormal responders showed significantly higher mTCNS and lower monofilament results as well as higher prevalence of orthostatic hypotension, retinopathy or nephropathy than normal responders.

Conclusion  The Neuropad test is a useful screening test for detecting DPN.

Key words: Neuropad test, sudomotor function, modified Toronto Clinical Neuropathy Score, diabetic polyneuropathy

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Introduction

Diabetic polyneuropathy (DPN) is the most common complication of diabetes, reaching a prevalence of 45-50% (1). The autonomic nervous system as well as the sensory motor can be affected in DPN. Chronic damage of peripheral sympathetic nerves manifests clinically as dry skin of the feet and may result in foot problems such as callus formation and foot ulceration (2). The examination of sudomotor function is now recommended to evaluate peripheral autonomic dysfunction and hence prevent diabetic foot (3). Diabetic autonomic dysfunction has been principally evaluated centrally by assessing heart rate variability (4), and peripherally by assessing sweating using complex and expensive equipment (5). A new Neuropad test (6-8) for sudomotor function has been developed as a simple visual indicator test to evaluate DPN. On the other hand, several instruments (9, 10) have developed for use in clinical practice because clinical evaluation of DPN is complex. The modified Toronto Clinical Neuropathy Score (mTCNS) (11) has recently proven to have sufficient reliability and validity to capture mild to moderate symptoms and signs of DPN. The aim of this study was to evaluate clinical usefulness of Neuropad test for assessment of DPN diagnosed through mTCNS.
Table 1. General Clinical Characteristics of the Patients according to the Categories of Neuropad Responses

<table>
<thead>
<tr>
<th></th>
<th>Neuropad normal</th>
<th>Neuropad patchy</th>
<th>Neuropad abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>n [%]</td>
<td>28 (32.2)</td>
<td>45 (51.7)</td>
<td>14 (16.1)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>59.4 ± 9.3</td>
<td>63.0 ± 8.4</td>
<td>58.2 ± 8.6</td>
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<tr>
<td>Duration of diabetes (yrs)</td>
<td>10.9 ± 6.9</td>
<td>11.7 ± 7.0</td>
<td>19.9 ± 7.9**</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4 ± 3.9</td>
<td>22.9 ± 3.4</td>
<td>22.7 ± 4.3</td>
</tr>
<tr>
<td>Medication of ARI [n (%)]</td>
<td>4(14.3)</td>
<td>12(26.7)</td>
<td>9(64.3)%</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.5 ± 1.6</td>
<td>9.1 ± 2.1</td>
<td>8.4 ± 1.2</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>133 ± 52</td>
<td>158 ± 77</td>
<td>119 ± 36</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>58 ± 15</td>
<td>51 ± 12</td>
<td>68 ± 19</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>110 ± 21</td>
<td>116 ± 29</td>
<td>116 ± 25</td>
</tr>
<tr>
<td>Retinopathy [n(%)]</td>
<td>3 (10.7)</td>
<td>16 (35.5)</td>
<td>13 (29.2)%</td>
</tr>
<tr>
<td>Nephropathy [n(%)]</td>
<td>5 (17.9)</td>
<td>19 (42.2)</td>
<td>10 (71.4)%</td>
</tr>
</tbody>
</table>

Data are means ± SD. LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, ARI: aldose reductase inhibitor
*p<0.05, **p<0.005 by ANOVA
*¹p<0.05, ¹¹p=0.01 by χ² test

**Subjects**

A total of 87 subjects were recruited from type 2 diabetic inpatients (55 men, 32 women). The mean age was 61.1±8.8 years and the mean diabetes duration was 13.0±7.5 years. Exclusion criteria were peripheral arterial occlusive disease, as well as chronic alcohol abuse, thyroid disease, vitamin B₁₂ deficiency, lumbar spine disorders or any other cause of peripheral neuropathy. The control group included 19 healthy volunteers (12 men, 7 women; mean age, 45.2±9.0 years). Written informed consent was obtained from all participants.

**Laboratory examinations**

Blood samples were obtained in the morning after the subjects fasted for at least 12 hours. HbA1c was measured by high performance liquid chromatography (HPLC) using an ADAMS-A1c HA-8160 (Arkray Inc., Kyoto, Japan). The value for HbA1c (%) estimated by the National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula HbA1c (%) = HbA1c (Japan Diabetes Society: JDS) (%) + 0.4%, based on the relational expression of HbA1c (JDS) (%) measured by the previous Japanese standard substance and measurement methods and HbA1c (NGSP) (12). The lipid profile was determined using a Hitachi 7600 biochemistry autoanalyzer. LDL-C was calculated according to the Friedewald formula.

**Assessment of diabetic neuropathy**

Evaluation of DPN was based on clinical examinations using the modified mTCNS, as described previously (11). It consists of graded symptoms (foot pain, numbness, tingling, weakness, ataxia and upper limb symptoms) and a sensory test (pinprick, temperature, light touch, vibration and position sense) score associated with DPN in the judgment of the examiner. The scale varies from 0 (no signs or symptoms) to 33 (maximal symptoms and signs). The test was not performed during winter and summer. The patients were allowed a 10-minute acclimatization period in constant room temperature (24±1°C) after they had removed their socks and shoes. The Neuropad test (mioVerbandstoffe, Wiehl-Drabenderhöhe, Germany) was applied on the plantar aspect of both great toes and removed after 10 minutes to evaluate the color change as normal (blue to completely pink), patchy (patches of blue and pink) and abnormal (remained blue). In addition, the study examined the 4-g Semmes-Weinstein monofilament perception three times on three plantar sites (under the great toe and first and fifth metatarsal heads). Inability to perceive the 4-g monofilament at any site was considered abnormal. Orthostatic hypotension was defined if the systolic blood pressure dropped at least 20 mmHg within 2 minutes of standing. Heart rate variability by the coefficient of variation of R-R intervals (CVR-R) at rest was also examined as a measure of autonomic dysfunction.

**Statistical analysis**

All statistical analyses were performed using the StatView software package version 5.0 (Abacus Concept, Berkeley, CA). Data are presented as the mean ± SD. Post hoc test was conducted with Fisher’s protected least significant difference (PLSD) method after analysis of variance (ANOVA) for quantitative variables and by χ² test for qualitative variables. A p value less than 0.05 was considered to be statistically significant.

**Results**

Twenty-eight of the 87 diabetic patients studied (32.2%) showed normal, 45 (51.7%) patchy and 14 (16.1%) abnormal Neuropad responses. On the other hands, 18 of the 19 healthy control subjects were normal (94.7%), 1 (5.3%) patchy and 0 (0%) abnormal in the Neuropad test. A comparison between the diabetic patients and healthy controls showed that the diabetic patients showed significantly higher prevalence of patchy or abnormal responses in the Neuropad
Table 2. mTCNS, ATR, Orthostatic Hypotension, Monofilament Insensation, CV_{R-R}, CAVI and ABI according to the Categories of Neuropad Responses

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<td>28 (32.2)</td>
<td>45 (51.7)</td>
<td>14 (16.1)</td>
</tr>
<tr>
<td>mTCNS</td>
<td>2.1 ± 2.0</td>
<td>4.5 ± 3.4*</td>
<td>9.4 ± 5.0**</td>
</tr>
<tr>
<td>ATR decrease [%]</td>
<td>9 (32.1)</td>
<td>21 (46.7)</td>
<td>14 (100)*</td>
</tr>
<tr>
<td>Orthostatic hypotension [%]</td>
<td>1(3.6)</td>
<td>3(6.7)</td>
<td>6(42.8)**</td>
</tr>
<tr>
<td>Monofilament [%]</td>
<td>2 (7.1)</td>
<td>4 (8.9)</td>
<td>7 (50)</td>
</tr>
</tbody>
</table>

Data are means ± SD. mTCNS: modified Toronto Clinical Neuropathy Score
ATR: Achilles tendon reflex, CV_{R-R}: coefficient of variation of R-R intervals,
CAVI: cardio ankle vascular index, ABI: ankle brachial index
*p<0.05, **p<0.005 by ANOVA
†p<0.05, ‡p<0.01 by χ² test

test than healthy volunteers (χ² test, p<0.001). Table 1 presents the general clinical characteristics of the diabetic patients in relation to the three categories of Neuropad responses. Age, body mass index (BMI), HbA1c and lipids profiles did not differ between patient groups with different Neuropad responses, whereas the duration of diabetes, Epalrestat, an aldose reductase inhibitor (ARI), was administered to abnormal Neuropad responders much more than to the normal responders. None of the enrolled patients took any β-blockers. Table 2 shows that the mTCNS was significantly higher in patients with a patchy (4.5±3.4) and an abnormal (9.1±5.0) Neuropad response in comparison to those with a normal response (2.1±2.0). The lack of an Achilles tendon reflex, orthostatic hypotension and insensation to 4-g monofilament were more frequently observed in patients with an abnormal Neuropad response than those with a normal response. Heart rate variability assessed by CV_{R-R} at rest was also significantly decreased in abnormal Neuropad responders in comparison to normal responders. Abnormal Neuropad responders had a significantly higher percentage of retinopathy and nephropathy, and showed a higher cardio ankle vascular index (CAVI) than did normal responders.

Discussion

The present study clearly demonstrated that sudomotor dysfunction assessed by the Neuropad test progressed with the severity of DPN rated by the mTCNS. Numerous sudomotor tests have been devised to evaluate sudomotor dysfunction (5, 13), but they are not generally available because they require of expensive equipment and training. The new Neuropad test has been developed as a simple visual indicator test for diagnosis of sudomotor function (6-8). Papanas et al. (6, 7) reported that the sensitivity of the Neuropad test for diagnosing DPN was 94.4% and specificity was 69.7%. The lower specificity in comparison to the sensitivity was thought to be due to the fact that sudomotor dysfunction develops early in the course of diabetes and can be detected even in patients with normal clinical examinations and nerve conduction studies. Quattrini et al. (14) used skin biopsies to demonstrate the correlation between the Neuropad response and the intra-epidermal nerve fiber density (IENFD) in the dorsum of the foot, a gold standard measure of skin denervation and neuropathy. Although IENFD may not specifically reflect small-fiber injury, which mediates sudomotor dysfunction, small-fiber injury may occur early in diabetic patients that show normal clinical examination (15).

Several screening instruments such as the Michigan Neuropathy Score (MDNS) (9) or the Neuropathy Disability Score (NDS) (10) have been extensively used in research and clinical practice for evaluation of DPN. Nonetheless, investigations continue to determine whether these previous clinical scoring systems are sufficiently sensitive to detect the early pathophysiological changes of DPN. The mTCNS has been developed as clinical tool to capture symptoms and signs of DPN because of its ease of use, acceptability by patients, and its ability to classify the severity of DPN with reliability and validity (11). The present study applied this instrument to evaluate DPN and found that the mTCNS was significantly higher in patients with an abnormal and a patchy Neuropad response in comparison to those with a normal response. The time to complete the color change has been reported to be significantly associated with the clinical staging of DPN (8, 16) diagnosed through NDS, showing that patients with time to change between 600 and 1,000 s, which may correspond to the patchy Neuropad responders in the current study, could have mild or moderate DPN diagnosed through the NDS. Likewise, patients with a time to color change between 1,000 and 1,200 s have moderate DNP and those scoring >1,200 s appear to have severe DNP. The current findings are consistent with previous reports suggesting that the patchy Neuropad responders may have mild DNP and abnormal responders have moderate to severe DPN.
Reduced sweating and dry skin caused by sudomotor dysfunction results in callus and fissure formation and hence develops into foot ulceration (17). Identification of the risk of foot ulceration by the combined use of simple tests including pinprick, temperature, vibration, and monofilament perception as well as ankle reflexes are recommended in diabetics (18). The components of this recommendation are included in the mTCNS and this study method. The present study found that lack of sensation of a 4-g monofilament, which is reported to detect relatively early DPN (19), was more frequently observed in abnormal Neuropad responders than normal responders, although a 4-g monofilament, not a 10-g monofilament, may overestimate the prevalence of sensory deficits. Tentolouris et al. (20) demonstrated that an abnormal Neuropad response correlates with foot ulceration in diabetic subjects and suggest that it may be a screening test for the prediction of foot ulceration. The visual reinforcement of the Neuropad test may make diabetic patients more aware of foot care, in addition to conventional evaluations, because the Neuropad test can be self-performed and is easy to carry out by the patients (21).

Abnormal Neuropad responders had decreased CVR-R at rest in comparison to normal responders in the current study, although it was not examined during breathing. This suggests that peripheral autonomic neuropathy may develop in association with cardiovascular autonomic neuropathy. Indeed, orthostatic hypotension was more frequently observed in abnormal Neuropad responders than in normal responders. Moreover, the prevalence of microvascular complications of retinopathy and nephropathy was significantly higher in abnormal Neuropad responders than normal responders. These findings are consistent with a recent report showing that the Neuropad response is different in the presence of macrovascular diseases, nephropathy and retinopathy (22).

The present study has some limitations. First, the study was cross-sectional and the sample size was relatively small. Second, some diabetic patients had been taking ARI, which is used for treatment of diabetic neuropathy. The severity of DPN increased in abnormal Neuropad responders in comparison to normal responders, in spite of the large number of patients taking ARI. The longer duration of diabetes may account for these results.

In summary, the Neuropad test is a useful in clinical practice for detecting DPN and gives patients awareness of foot care because of its simplicity and high sensitivity. Furthermore, large scale prospective studies are needed to predict early diagnosis of DPN by Neuropad test.

The authors state that they have no Conflict of Interest (COI).

References

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