

A Prospective Study on the use of the Indicator Test Neuropad® for the Early Diagnosis of Peripheral Neuropathy in type 2 Diabetes

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Key words

- diabetes mellitus
- diabetic neuropathy
- diagnosis
- neuropad

Abstract

Aim: The aim of this prospective study was to evaluate the contribution of the indicator test for sudomotor function Neuropad® to the early diagnosis of peripheral neuropathy in patients with type 2 diabetes mellitus. Included were 109 type 2 diabetic patients (55 men, mean age 56.15 ± 6.14 years), whose initial clinical examination (Neuropathy Disability Score, NDS) was negative for neuropathy. Patients were first examined between January and June 2004 and re-examined 5 years later by the NDS and Neuropad®. Initially, 70 patients (64.22%) had normal and 39 (35.78%) patients had abnormal Neuropad® (groups A and

B, respectively). NDS was significantly higher in group B on both examinations ($p < 0.001$). On the second examination, 2 patients (2.86%) in group A and 10 patients (25.64%) in group B had developed neuropathy ($p = 0.001$). Neuropad® had 83.33% sensitivity and 68.04% specificity for neuropathy. There was a modest but significant agreement ($\kappa = 0.259$, $p < 0.001$) between Neuropad® and NDS for neuropathy.

Conclusions: Among type 2 diabetic patients with normal NDS, development of neuropathy is significantly more frequent in those with abnormal Neuropad®. These results suggest a potential utility of Neuropad® for the earlier diagnosis of neuropathy in type 2 diabetes.

Introduction

Peripheral neuropathy is a major chronic complication of diabetes (Boulton et al., 2005; Várkonyi and Kempler, 2008). In everyday practice, clinical examination is the cornerstone of diagnosis (Boulton et al., 2005). However, neuropathy may often be unrecognised or diagnosed late (Herman and Kennedy, 2005). Nerve Conduction Study (NCS) enables earlier diagnosis of neuropathy (Valk et al., 1992; Krarup 2003; Rota et al., 2005). Nonetheless, this modality is not widely accessible and requires experienced personnel, so that it cannot be employed as a screening test (Boulton et al., 2005; Papanas and Ziegler, 2009). Alternatively, early diagnosis of neuropathy may be facilitated by nerve biopsy and measurement of intra-epidermal nerve fibre density through skin biopsy (Smith et al., 2001; Malik et al., 2005; Quattrini et al., 2008). Clearly, these techniques are suitable for research, rather than widespread clinical application.

New tests to improve the diagnosis of neuropathy have been developed (Ziegler et al., 2005; Papanas and Ziegler, 2009). The indicator test for sudomotor function Neuropad® has been repeatedly

shown to have a very high sensitivity for the diagnosis of neuropathy (Papanas et al., 2005; Liatis et al., 2007; Papanas et al., 2007; Papanas et al., 2008; Spallone et al., 2009; Kamenov et al., 2010). Moreover, the test has excellent reproducibility (Papanas et al., 2005b) and is suitable for patient self-examination (Tentolouris et al., 2008). So far, the test has been shown to yield modestly high specificity due to its false positive rates (Papanas et al., 2005; Liatis et al., 2007; Papanas et al., 2007; Papanas et al., 2008; Spallone et al., 2009). It has been proposed that these false positives may be attributable to early detection of neuropathy (Papanas et al., 2005; Papanas et al., 2007). Given that biopsy has confirmed that abnormal Neuropad® response indicates both functional and structural small fibre denervation in the foot (Quattrini et al., 2008), it is conceivable that the test could help towards timely detection of neuropathy on the basis of such early small fibre changes. However, this assumption has not been documented. Indeed, the potential utility of Neuropad® for the early diagnosis of neuropathy can only be addressed with a prospective study. Therefore, the aim of the present prospective study was to evaluate the

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contribution of Neuropad® to the early diagnosis of peripheral neuropathy in patients with type 2 diabetes mellitus (Quattrini et al., 2004; Yagihashi et al., 2007).

Patients and Methods

This study included 109 type 2 diabetic patients (55 men, 54 women, mean age 56.15 ± 6.14 years and mean diabetes duration 3.51 ± 1.09 years) whose initial clinical examination was negative for peripheral neuropathy. Patients were recruited from the Outpatient Clinic of Obesity, Diabetes and Metabolism in the Second Department of Internal Medicine at Democritus University of Thrace, Greece. The study was conducted in accordance with the Helsinki Declaration of Human Rights and all patients gave their informed consent. Recruitment was consecutive and performed in a tertiary care setting.

The following exclusion criteria were used: age <17 years or >75 years, peripheral arterial occlusive disease, other potential causes of neuropathy (end-stage renal failure, alcohol abuse, Vitamin B₁₂ depletion, malignancy, peripheral nerve lesions), thyroid disease, drugs (corticosteroids, antihistaminic and psychoactive drugs, which may affect sweating), as well as skin diseases (neurodermatitis, psoriasis, scleroderma, allergy to metals, Raynaud syndrome, hyperhidrosis, acrocyanosis) (Papanas et al., 2005).

Diagnosis and exclusion of peripheral neuropathy was based on the Neuropathy Disability Score (NDS) (Young et al., 1993). NDS represents a standardised examination of ankle reflexes, 128 Hz tuning fork sensation, pin-prick and temperature sensation at the hallux, as described earlier (Young et al., 1993). Patients with an NDS score ≥ 6 were diagnosed with neuropathy (Young et al., 1993; Papanas et al., 2007).

Examination with Neuropad® was performed as previously described (Papanas et al., 2005b; Papanas et al., 2008). Patients were allowed a ten-minute acclimatisation period in constant room temperature (25°C) after they had removed their socks and shoes. Indicator tests were applied to both soles at the level of the 1st-2nd metatarsal heads. Time until complete colour change of the test from blue to pink was recorded in seconds with an exactitude of 10 s (Papanas et al., 2005b; Papanas et al., 2008). Abnormal Neuropad® diagnostic of sudomotor dysfunction was defined as time until complete colour change exceeding 600 s in at least one foot (Zick et al., 2003; Papanas et al., 2005; Papanas et al., 2008). Examination with Neuropad® was carried out by an operator who was blinded to the patients' neuropathy status.

Patients were examined twice. The first examination was performed between January and June 2004. Patients were re-examined 5 years later, between January and June 2009. On both occasions, patient evaluation comprised clinical examination for neuropathy by means of NDS, application of Neuropad® and general physical examination.

Statistical analysis was performed using the SPSS (Statistical Package for Social Sciences, Chicago, Illinois) 13.0. Normally distributed quantitative variables were analysed by unpaired t-test. Qualitative variables were compared by Fisher's exact test (after Yates' correction where appropriate). The correlation between time until complete colour change of the test and NDS was evaluated by Spearman's rank co-efficient (r_s). Agreement between NDS and Neuropad® in the diagnosis of neuropathy was evaluated by the Kappa coefficient. Data were expressed as mean \pm 1

Standard Deviation ($\bar{X} \pm 1SD$). Significance was defined at a level of 5% ($p < 0.05$).

Results

Initially, 70 patients (64.22%) had normal and 39 (35.78%) patients had abnormal Neuropad® results (defined as groups A and B, respectively). On both examinations, there was 100% agreement between abnormal Neuropad® response on the right and left foot. There was no change of Neuropad® from abnormal to normal between the first and second examination. 2 patients from group A developed sudomotor dysfunction (abnormal Neuropad®) on re-examination, without developing neuropathy. NDS was significantly higher in group B vs. group A, on both first (4.23 ± 0.99 vs. 2.97 ± 0.72 , $p < 0.001$) and second examination (4.63 ± 1.33 vs. 3.39 ± 0.91 , $p < 0.001$). On both occasions, there was a significant positive correlation between time to colour change of Neuropad® and NDS (first examination: $r_s = 0.552$, $p < 0.001$ on the right and $r_s = 0.595$, $p < 0.001$ on the left foot; second examination: $r_s = 0.436$, $p < 0.001$ on the right and $r_s = 0.496$, $p < 0.001$ on the left foot).

On the second examination, 2/70 patients (2.86%) in group A and 10/39 patients (25.64%) in group B had developed neuropathy (Table 1). Neuropathy was significantly ($p = 0.001$) more frequent in group B.

On the second examination, Neuropad® had 83.33% sensitivity and 68.04% specificity for neuropathy, as evaluated by NDS. There were 31 false positives and 2 false negatives. There was a modest but significant agreement ($\kappa = 0.259$, $p < 0.001$) between Neuropad® and NDS for the diagnosis of neuropathy.

Discussion

The present prospective study attempted to examine the contribution of the indicator test for sudomotor function Neuropad® to the early diagnosis of peripheral neuropathy in patients with type 2 diabetes mellitus. It included subjects whose initial examination was negative for neuropathy and who were re-examined after 5 years. The major finding is that development of neuropathy on the second examination was significantly more frequent in those with abnormal Neuropad®. It has hitherto been suggested that Neuropad® may facilitate earlier diagnosis of neuropathy than standardised clinical examination (Papanas et al., 2005; Papanas et al., 2007). The novel finding that initial abnormal Neuropad® was associated with significantly more frequent neuropathy 5 years later provides some evidence that this test may, indeed, contribute to earlier detection of neuropathy. It is plausible that the earlier detection of neuropathy may be attributed to the principle that Neuropad® assesses sudomotor function, which is mediated by small fibres (Low et al., 2006; Provitera et al., 2010). The latter may, according to pathological

Table 1 Neuropathy status in the 2 groups on the second examination ($p = 0.001$, Yates corrected).

Patient Group	with Neuropathy	without Neuropathy
GROUP A (n = 70)	2	68
GROUP B (n = 39)	10	29

GROUP A: Patients with normal Neuropad® on the first examination

GROUP B: Patients with abnormal Neuropad® on the first examination

studies, be early injured in diabetic patients with normal clinical or electrophysiological findings, or even earlier in patients with impaired glucose tolerance (Malik et al., 2005; Smith et al., 2001; Sumner et al., 2003; Provitera et al., 2010). Of note, the association between abnormal Neuropad® response and structural small fibre injury has been confirmed by skin biopsy (Quattrini et al., 2008), providing the histopathological basis for the early detection of neuropathy by means of the indicator test.

NDS was significantly higher in patients with abnormal than in those with normal Neuropad® results. This finding is in agreement with previous studies reporting that patients with sudomotor dysfunction have higher scores of clinical neuropathy (Papanas et al., 2005; Papanas et al., 2007; Papanas et al., 2007b; Papanas et al., 2008; Spallone et al., 2009). So far, however, the association of sudomotor dysfunction with worse clinical neuropathy status has only been demonstrated for diabetic patients in general. This work extends previous findings and shows that the aforementioned association also holds true for patients without clinical neuropathy.

On both patient examinations, time to colour change of Neuropad® had a significant positive correlation with NDS. Again, this has been shown in type 2 diabetic patients (Papanas et al., 2008), but the present observation adds that the positive correlation may also be found in non-neuropathic patients. Taken together, the new findings and the previous observations suggest that there is a continuum of deteriorating sudomotor function (as evaluated by Neuropad® time to colour change) in parallel with aggravating clinical neuropathy scores (NDS).

Interestingly, Neuropad® on the second examination had a high sensitivity and a modestly high specificity for the diagnosis of neuropathy. Sensitivity and specificity were in the same range as those already reported for diabetic patients in general (Papanas et al., 2005; Liatis et al., 2007; Papanas et al., 2007; Papanas et al., 2007b; Papanas et al., 2008; Spallone et al., 2009).

Moreover, there was a modest but significant agreement between Neuropad® and NDS for the diagnosis of neuropathy on patients' re-examination. The agreement was only modest, because Neuropad® was abnormal in a substantial number of patients with normal clinical examination. These patients may be described as false positives. In the light of the new findings, however, it may be argued that at least some of these so-called false positive patients will develop neuropathy at a later stage. In this context, it would be very useful to re-examine such patients after 5 additional years, in order to provide potential further confirmation of the argument on early diagnosis.

The limitations of this work may be outlined as follows. First, patients' follow-up was not long. Indeed, neuropathy usually develops insidiously over a long time (Boulton et al., 2005; Várkonyi and Kempler, 2008; Ziegler et al., 1991), and so it would be very interesting to obtain results on neuropathy status after 10 years. Even so, our results are useful because they represent, to the best of our knowledge, the first prospective study on the potential utility of Neuropad® for the early diagnosis of neuropathy. Secondly, we recruited patients in a tertiary care setting, and so our results may be not directly applicable to the general diabetic population.

The present study may have the following practical implications. In type 2 diabetic patients with clinical examination negative for neuropathy, abnormal results with the indicator test for sudomotor function Neuropad® are associated with significantly more frequent development of neuropathy in the future. Thus,

abnormal Neuropad® results may alert the clinician to the possibility of developing neuropathy. Consequently, the clinical importance of abnormal Neuropad® is twofold. First, it may contribute to the early recognition of patients gradually developing neuropathy, thereby helping towards improved diagnosis of this complication, which is of vital importance for the reduction of morbidity (Herman and Kennedy, 2005; Papanas and Ziegler, 2009). The early diagnosis of neuropathy is based on the detection of small fibre injury, as documented by the correlation of the indicator test with biopsy (Quattrini et al., 2008). Secondly, Neuropad® diagnoses sudomotor dysfunction, which has recently been confirmed as an independent risk factor for foot ulceration (Sun et al., 2008; Tentolouris et al., 2009). By identifying patients with sudomotor dysfunction, an abnormal Neuropad® response correlates very strongly with the presence of foot ulceration, as demonstrated on multivariate logistic regression analysis (Tentolouris et al., 2010). All in all, the early detection of neuropathy, along with the evaluation of sudomotor dysfunction, render Neuropad® a very useful screening test, as already suggested (Papanas et al., 2008; Quattrini et al., 2008).

In conclusion, we have provided evidence that development of neuropathy at 5 years among type 2 diabetic patients with normal NDS is significantly more frequent in those with abnormal Neuropad®. There is a modest but significant agreement between Neuropad® and NDS for the diagnosis of neuropathy. These results suggest a potential utility of Neuropad® for the earlier diagnosis of neuropathy in type 2 diabetes. Additional prospective studies should now be encouraged to fully elucidate the utility of Neuropad® in the timely diagnosis of neuropathy in everyday practice.

Conflict of Interest: None.

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