Journal of Diabetes Science and Technology
Volume 6, Issue 1, January 2012

Thermal Threshold: Research Study on Small Fiber Dysfunction in Distal Diabetic Polyneuropathy

Pedro Jimenez-Cohl, M.D.,[1] Carlos Grekin, M.D.,[1] Cristian Leyton, M.D.,[2] Claudio Vargas, M.D.,[3] and Roberto Villaseca, M.D.[4]

Abstract

Objective:

The most commonly used technique for diagnosis of diabetic neuropathy (DN) is nervous conduction (NC). Our hypothesis is that the use of the thermal threshold (TT) technique to evaluate small fiber damage, which precedes large fiber damage, could enable earlier diagnosis and diminish false negatives.

Research Design and Methods:

The study involved 70 asymptomatic patients with type 2 diabetes mellitus (T2DM) all being treated with oral hypoglycemic medication, and having negative metabolic control levels with glycosylated hemoglobin A1c greater than 7% and less than 8%. Diabetic neuropathy was their only evident complication. All other complications or other causes of neuropathy were discarded. Their time of evolution was 1 to 48 months since date of diagnosis of diabetes. Both thermal threshold and sensory and motor nervous conduction were determined in upper and lower limbs.

Results:

Nervous conduction was found normal in 81% and altered in 19% of patients (large fiber neuropathy). Thermal threshold was normal in 57% and altered in 43% of patients (small fiber neuropathy). In those with normal TTs, no case with an altered NC was found ($p < 0.001$). Patients with altered TTs could have normal (57%) or altered NC (43%). Thus, NC showed a high frequency of false negatives for DN (57% of 30 cases).

The frequency of small fiber neuropathy found with the TT test was higher than that of large fiber neuropathy found with the NC test ($p < 0.001$) and was found at an earlier age.

Conclusions:

The TT test demonstrated a higher frequency of neuropathy than the NC test in clinically asymptomatic T2DM patients. We suggest that small fiber should be studied before large fiber function to diagnosis distal and symmetrical DN.

J Diabetes Sci Technol 2012;6(1):177-183

Author Affiliations: [1]Hospital Militar de Santiago, Santiago, Chile; [2]Department of Neurology, Universidad de Santiago, Santiago, Chile; [3]Department of Statistics, Universidad de Santiago, Santiago, Chile; and [4]Department of Internal Medicine & Endocrinology, Hospital Militar de Santiago, Santiago, Chile

Abbreviations: (CI) confidence interval, (DM) diabetes mellitus, (DN) diabetic neuropathy, (DSPN) distal symmetrical polyneuropathy, (EMG) electromyography, (NC) nervous conduction, (SD) standard deviation, (SFN) subclinical small fiber neuropathy, (T2DM) type 2 diabetes mellitus, (TT) thermal threshold

Keywords: diabetic neuropathy, thermal threshold, nervous conduction

Corresponding Author: Pedro Jimenez-Cohl, M.D., Nueva Los Leones 07, Apt 606., Providencia, Santiago, Chile; e-mail address pejimco@yahoo.com

Introduction

Diabetic neuropathies (DNs) are heterogeneous in their clinical expression and localization.[1,2] The most common forms are autonomic neuropathy and distal symmetrical polyneuropathy (DSPN) the latter being the most diagnosed in patients with diabetes mellitus (DM).[1,3-5] Its prevalence fluctuates between 8 and 40% according to the clinical criteria or laboratory tests used for its diagnosis.[3-6] Most DM patients with DSPN are asymptomatic[3,7] and are symptomatic only in 15% of type 1 and 4–13% of type 2 (T2DM) patients.[6,8,9] Early diagnosis and treatment are of great importance because (1) DSPN is the chief risk factor for trophic lesions and lower limb amputations in DM patients,[6,8,10,11] and around 85–96% of patients with DSPN are asymptomatic; (2) on occasion, neuropathies due to other aetiologies occur, requiring differential diagnosis; and (3) treatment at an earlier stage should improve prognosis.[12]

Neuropathy is usually suspected when subjective symptoms such as burning pain, paraesthesia, hyperesthesia and painful cramps preferentially involving the lower limbs are referred to by aware patients and/or when decreased vibratory (128 Hz tuning fork) and tactile sensation (10g monofilament) and/or derangements of thermal perception and an absent Achilles reflex are observed. In symptomatic patients (only 4–15% of DM patients with neuropathy), these methods allow clinical diagnosis of DSPN in 87% of the cases.[13]

In asymptomatic individuals with DSPN, the limitations of these methods may under diagnose 85–96% of these patients. To palliate this limitation and improve diagnostic sensitivity, electromyography (EMG) and nervous conduction (NC) are the most used techniques to quantitate peripheral large myelinated fiber function in symptomatic and asymptomatic DM patients,[14,15] but EMG/NC are bothersome and techniques using electric currents to measure NC and needles to study muscle innervation are uncomfortable. These techniques do not evaluate small fiber, which conduct autonomic function, sense cold, heat, and pain, and are altered earlier than thick fiber in DM patients and glucose intolerants.[15,16–20]

Hence our interest in comparing DSPN frequency in T2DM patients by using two different methods: (1) thermal threshold (TT) which studies small fiber function and (2) EMG and NC, which study large fiber function.

Patients and Methods

Because it is impossible to determine how many years of subclinical evolution the disease already has, and in order to improve the reliability of the TT technique, avoid distortion, and mark a difference regarding other reports and reviews,[21] an extremely select sample of 70 T2DM patients were chosen (39 men, 31 women) using the following criteria: no central nervous system damage, normal IQ, homogeneous educational level, no cognitive derangement (Wais Test), normal consciousness, and no psychiatric condition (Minnesota MultiPhasic Personality Inventory and Rorschach Test). In addition, other causes of peripheral neuropathy were ruled out using vitamin B12 and folic acid levels, thyroid hormones, electrolytes, calcium, phosphorus, magnesium, plasma protein and immunoglobulin electrophoresis, liver tests, chest x-rays, abdomen ultrasound, and gynecological evaluations.

All patients have T2DM and are clinically asymptomatic for small and large fiber neuropathy. They present no alteration in reflexes, sensitivity, or muscle strength. Diabetic neuropathy is their only evident complication. All other complications were discarded.

The patients had a clinic evolution time from 1 to 48 months since the date of diagnosis of diabetes. Their average age was 54 ± 10, ranging from 32 to 78 years old.

We considered the date of diagnosis as the day in which the diagnosis was confirmed with conclusive laboratory tests according to criteria of the American Diabetes Association or World Health Organization.[22,23] Of the 39 men, 18 were medically checked once a year, making it very probable that their T2DM was diagnosed at an early stage of the disease.

All patients were being treated with oral hypoglycemic medication and had negative metabolic control levels with hemoglobin A1c greater than 7% and less than 8%.

All were submitted to a neurological clinical examination, distal and bilateral TT and NC studies (NC velocity and measurements of latencies and amplitudes of sensory and motor potentials).

The NC test was performed with a Nicolet Viking IV® (Nicolet Biomedical, Memphis, TN) electromyography

system. The parameters studied were: motor conduction velocity, latency and amplitude of the distal motor potential in the median and peroneal nerves, sensory conduction velocity of the median nerve in the wrists and forearms, and conduction latency and amplitude of the distal sensory potential in the median, ulnar, and sural nerves on both sides.

For NC studies, criteria considered as diagnostic of DSPN were: (A) bilateral and symmetrically reduced sensory or sensorimotor conduction velocities in the lower limbs or in the upper and lower limbs simultaneously; (B) bilateral and symmetrically reduced sensory or motor amplitudes in the lower or in the upper and lower limbs simultaneously; or (C) concomitance of (A) and (B). **Table 1** shows normal values using similar criteria to those in literature.[24]

The TT study was performed with a computerized Nicolet Sensation® (Israel) equipment according to the technique described in Marstock[25] in which the temperature changes are issued through a contact probe (surface 3 × 4 cm) attached to the patient's skin. The velocity of the rise or falls of the probe's temperature as well as its basal temperature are pre-established and computerized. Standardization of the TT was achieved according to international standards[25,26] as was the evaluation of 80 healthy volunteers without evidence of intellectual derangement (Wais test) and/or neuropathy. Each center should establish its own normality criteria because parameters such as basal skin temperature, stimulation site, age, race and others can influence them.[27] In order to avoid the possibility of malingering, the test was performed twice on separate occasions in each patient.

The TT parameters studied were: (1) sensory thresholds to cold and heat and (2) pain thresholds induced by cold and heat on both palms and on the dorsum and soles of both feet.

Thermal threshold criteria considered for the diagnosis of small fiber distal and symmetrical DN were: (1) when the standardized reference value for each of these thresholds from our laboratory were surpassed in more than two standard deviations (SDs) bilaterally and symmetrically in lower limbs or in lower and upper limbs simultaneously and/or (2) when hyperalgesia to heat or cold or paradoxical reactions to heat or cold appeared bilaterally and symmetrically. (See **Table 2**.)

We used the latest update of expert panels (Toronto, Canada 2009) classification. This new classification defines minimal criteria for typical diabetic polyneuropathy in possible, probable, confirmed, and subclinical distal DN. According to this classification,[28] we classified our results of NC in (A) normal without DSPN, and (B) abnormal with subclinical DSPN, corresponding to subclinical DSPN.[28]

The results applying the TT allowed the classification of patients into two groups: (A) normal and (B) altered: with small fiber dysfunction [subclinical small fiber neuropathy (SFN)].

All our patients were 100% asymptomatic. Expressions of SFN damage were evident only as a result of the application of TT. This technique is presumably vouched for by the experts panel in Toronto[28] that suggest the use of a quantitative methodology as abnormal quantitative sensory testing TTs of the foot and/or skin biopsy and intraepidermal nerve fiber density at the ankle.

Table 2.
Thermal Thresholds Elicited by Cold, Heat, Pain: Normal Values in 80 Healthy Volunteers

	Cold (°C)	Heat (°C)	Pain by cold (°C)	Pain by heat (°C)
Tenar (hand)	≥29	≤35.5	0–4	44–50
Hypotenar (hand)	≥27	≤39	0–5	44–50
Dorsum (feet)	≥23	≤40	0–3	45–50
Soles (feet)	≥25	≤43	0–2	46.5–50

Table 1.
Motor and Sensory Conduction: Normal Values in 80 Volunteers

Nerve	Motor conduction velocity (m/s)	Motor distal latency (msec)	Motor distal amplitudes (mV)	Sensory conduction velocity (m/s)	Sensory distal latency (msec)	Sensory distal amplitudes (microV)
Median	≥50	≤4.5	≥4.0	≥50	≤3.9	≥15
Peroneal	≥40	≤6.0	≥2.0	—	—	—
Ulnar	—	—	—	≥50	≤3.7	≥10
Sural	—	—	—	≥40	≤4.2	≥5.0

Analysis of data was performed using χ^2 statistics for categorical variables, Student's t statistics for independent samples to compare continuous variables, the trend test to analyze a variable against another and the McNemar Test for one sample in which two repeated measurements of each technique are compared separately. All confidence intervals (CIs) were estimated at 95% confidence level. The data are shown as average and SD.

All patients provided informed signed consent and the Ethics Committee of our hospital approved the protocol.

Results

In the NC test, 57 patients were normal (81% of the sample) and only 13 were diagnosed with distal and symmetric large fiber DN (19%). In 2 cases we found a sensorimotor neuropathy (alterations in the motor amplitude of the peroneal nerve and sensory amplitude of the sural nerve), in 11 cases we found only a sensory neuropathy (alterations in the sensory amplitude of the sural nerve). No case with alterations in the upper limbs.

In the TT study, 30 patients were diagnosed with distal and symmetric SFN (43%) whereas the remaining 40 patients (57%) were normal **(Table 3)**. In the 30 cases, alterations of the TT were found in the dorsum and soles of the feet. There were no patients with abnormalities in the upper limbs.

The frequency of SFN studied with the TT test is significantly greater ($p < 0.001$) than the frequency of large fiber neuropathy studied with the NC test **(Table 3)**.

Regarding age, the T2DM patients with a normal NC averaged 52 years compared to those with altered NC, who averaged 61 years ($p < 0.001$).

There were no significant differences between these two groups regarding time of clinical evolution since the date of diagnosis of diabetes **(Table 3).**

The group with the altered TT had an average age of 56 years and those with a normal TT had an average of 52 years (statistically not significant). The time elapsed since the date of diagnosis did not show significant differences between these two groups **(Table 3)**.

The subgroup of patients with an altered TT but with normal NC was in average 9 years younger than the group of patients with both altered TT and NC (52 versus 61 years, statistically significant, $p < 0.005$).

There was no significant difference between the groups regarding time of evolution since the date of diagnosis of diabetes **(Table 3).**

Forty patients had a normal TT (57%) and none of them showed derangements in NC. Thirty patients were found to have an altered TT (43%) and 17 of these had normal NC (**Table 4**).

If a bilaterally and symmetrically altered TT was always an expression of DN, then the 17 cases found in this group with an altered TT and normal NC should be considered as "false negative of the NC for DN," CI 95% (45-69%) **(Table 4).**

If the capability of the TT test to detect true bearers of DN was 100%, it would be only 43% for NC (13 altered NC/30 altered TT), CI 95% (26-61%).

The probability that a patient with a normal NC has no neuropathy (40 true negatives/57 cases with a normal NC)

Table 3.
Frequency of Diabetic Neuropathy according to Used Method, Age, and Time of Evolution in 70 Cases

Nervous conduction	No. (%)	Average age	Time of evolution
Altered	13 (19%)[a]	61 years[b]	18 months
Normal	57 (81%)	52 years[b]	13 months
Thermal threshold	No. (%)	Average age in years	Time of evolution
Altered	30 (43%)[a]	56 years	15 months
Normal	40 (57%)	52 years	12 months

[a] $p < 0.001$ (CI 95%: 0.31–0.54 vs 0.09–0.28).
[b] $p < 0.001$

Table 4.
Relationship between Thermal Threshold and Nervous Conduction in 70 Cases

	Normal NC			Altered NC		
	n	%	CI[a]	*n*	%	CI[a]
Normal TT (n = 40)	40	100%	0.928–1.00	0	0%	0–0.072
Altered TT (n =30)	17	57%	0.374–0.745	13	43%	0.254–0.626

[a] Exact 95% binomial CIs.

in the sample is 70%, CI 95% (58–82%), thus implying that, if the NC is normal, then there is a 30% probability of having neuropathy.

Regarding the small fiber, the most common alterations found in measured parameters are shown in **Table 5**.

The number of altered parameters of TT is directly correlated to age ($p < 0.05$) and not to the time of evolution of diabetes since the date of diagnosis ($p > 0.10$) **(Table 6).**

Normal values found in 80 volunteers in repeated TT (first and second tests) are shown in **Table 7**.

Discussion

In other reports,[21] we found that the groups of patients and the methods used were not comparable to each other. We feel that a single computerized methodology of TT should be used in order to minimize the theoretical limitations of the different techniques (computerized or not) evaluated in the American Neurological Association[21] report, where different kinds of patients as well as different times of clinical and subclinical evolution of the disease were mixed. The same is supported by other authors who value the use of the TT test in diagnosis of DN prior to large fiber damage.[30–33]

The results of our research show a 19% frequency of distal large fiber sensory or sensorimotor neuropathy when the NC technique was applied and 43% of SFN when the TT technique was used ($p < 0.001$). Both types of damage would be the expression of distal and symmetric DN in different stages of the disease.[34]

If **Table 1** is analyzed, it can be seen that small fiber alterations appear at earlier ages and that large fiber damage appears later. Even though it can be affirmed that age itself plays a permissive role in the expression of the type of damaged fiber, probably, the compromise is more related to a longer time of evolution of T2DM in the subclinical stage.[3,5,35]

Although ample information supports the influence of the time of evolution and metabolic control of the disease on fiber damage,[3,5,36,37] our study design (patients of up to 4 years of evolution since the date of diagnosis) does not allow us to demonstrate the relationship between neuropathy and the actual clinical and subclinical evolution.

Studies using skin biopsies describing SFN in DM and glucose intolerant patients[17–19,38,39] support the fact

Table 5.
Thermal Threshold Findings in 30 Cases[a]

Paradoxical response to cold + hypoesthesia to heat	6/30 cases (20 %)
Hypoesthesia to cold	6/30 cases (20%)
Paradoxical response to cold + hypoesthesia to cold and heat	6/30 cases 20%
Isolated paradoxical response to cold	5/30 cases (17%)
Loss of intense heat pain (always associated to others TT alterations)	5/30 cases (17%)
Isolated hypoesthesia to heat.	4/30 cases (13%)
Hypoesthesia + paradoxical response to cold	2/30 cases (6%)
Hypoesthesia to cold and heat	1/30 cases (3%)

[a] A patient may have more than one parameter altered in thermal sensory threshold.

Table 6.
Number of Altered Parameters in the Thermal Threshold Related to Age, Time of Evolution since Date of Diabetes Diagnosis and Number of Cases

Number of altered parameters in the thermal threshold	Average age[a]	Time of evolution[b]	Number of patients
1	52 years	17 months	13
2	56 years	14 months	9
3	60 years	16 months	6
4	70 years	12 months	2

[a] Trend test: $p < 0.05$
[b] Trend test: $p > 0.10$

Table 7.
Normal Values Found in 80 Volunteers in Repeated Thermal Threshold Tests. Thermal Sensory Thresholds Found by Stimulating Cold, Heat, Cold Pain, or Heat Pain[a]

	Cold (°C)	Heat (°C)	Cold pain (°C)	Heat pain (°C)
Thenar region (first test)	30.5 ± 1.0	34.0 ± 1.0	2.0 ± 2.0	47.0 ± 3.0
Thenar region (second test)	30.3 ± 1.2	34.1 ± 0.9	2.2 ± 2.1	46.8 ± 2.8
Dorsum of the foot (first test)	28.0 ± 3.0	35.5 ± 2.0	1.5 ± 0.8	47.5± 0.5
Dorsum of the foot (second test)	27.5 ± 3.2	35.7 ± 2.2	1.7 ± 1.0	47.2 ± 2.3

[a] Values are expressed as mean ± SD.

that small fiber damage, also distal and symmetrical, precedes large fiber damage found with the NC test.

An explanation for early damage of small fiber is based upon a greater vulnerability of these to the metabolic damage of oxidative and vascular stress in the axon and endoneurium induced by glyco and lipotoxicity, which will finally lead to axonal degeneration and atrophy.[36,40,41]

Puncture skin biopsy and intraepidermal nerve fiber density count should be the reference standard[28] in indicating structural small fiber damage, but it is an invasive, complex, and expensive technique[25–28] that is difficult to apply on a great scale. Thus, less invasive and cheaper techniques to study small fiber function should be considered, among which the TT technique could be a reasonable choice.[30] Further research must be carried out to compare both techniques.

Our results show the following: (1) of all our patients with confirmed DN, 57% have only distal and symmetric small fiber damage demonstrated with the TT method; (2) an altered NC always has an altered TT; (3) a normal TT always has a normal NC; and (4) a higher number of altered parameters exist in TT with higher age.

Based on bibliographic evidence that show that (1) alterations in small fiber (A delta and C) found with the TT test have a progressive and evolving character confirmed by skin biopsy and intraepidermal fiber count[30–34] and (2) small and not large fiber damage exists in glucose intolerants,[17–19] we propose that in the study of subclinical DN (having discarded other causes of neuropathy) TT can be a more useful technique than NC because it would allow the diagnosis of small fiber damage that may precede large fiber damage, allowing earlier diagnosis especially in clinically asymptomatic patients.

We support the efforts[28] to review the clinical and laboratory criteria used[13,42] for diagnosis of DN. The place given to TT as a technique for diagnosis of small fiber damage in asymptomatic and symptomatic patients remains to be determined. Hence the existence of literature that supports this methodology, and supports the belief that small fiber damage precedes large fiber damage.[30–34]

We recommend the TT test for diagnosis of diabetic SFN in asymptomatic, cooperative and lucid patients, with slight or no cognitive derangement and in every newly diagnosed T2DM patient, including those in which EMG/NC are normal.

References:

1. Kelkar P. Diabetic neuropathy. Semin Neurol. 2005;25(2):168–73.
2. Duby JJ, Campbell RK, Setter SM, White JR, Rasmussen KA. Diabetic neuropathy: an intensive review. Am J Health Syst Pharm. 2004;61(2):160–73.
3. Pirart J. Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973. Diabete Metab. 1977;3(4):245–56.
4. Partanen J, Niskanen L, Lehtinen J, Mervaala E, Siitonen O, Uusitupa M. Natural history of peripheral neuropathy in patients with non-insulin-dependent diabetes mellitus. N Engl J Med. 1995;333(2):89–94.
5. Orchard TJ, Dorman JS, Maser RE, Becker DJ, Ellis D, LaPorte RE, Kuller LH, Wolfson SK, Drash AL. Factors associated with avoidance of severe complications after 25 years of IDDM: Pittsburgh epidemiology of diabetes complications Study I. Diabetes Care. 1990;13(7):741–7.
6. Dyck PJ, Kratz KM, Karnes JL, Litchy WJ, Klein R, Pach JM, Wilson DM, O'Brien PC, Melton LJ 3rd, Service FJ. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester Diabetic Neuropathy Study. Neurology. 1993;43(4):817–24.
7. Dyck PJ, Karnes JL, Daube J, O'Brien P, Service FJ. Clinical and neuropathological criteria for the diagnosis and staging of diabetic polyneuropathy. Brain. 1985:108(4):861–80.
8. O'Hare JA, Abuaisha F, Geoghegan M. Prevalence and forms of neuropathic morbidity in 800 diabetics. Ir J Med Sci. 1994;163(3):132–5.
9. Lehtinen JM, Uusitupa M, Siitonen O, Pyörälä K. Prevalence of neuropathy in newly diagnosed NIDDM and nondiabetic control subjects. Diabetes. 1989;38(10):1307–13.
10. Boulton AJ, Malik RA, Arezzo JC, Sosenko JM. Diabetic somatic neuropathies. Diabetes Care. 2004;27(6):1458–86.
11. Edmonds ME, Blundell MP, Morris ME, Thomas EM, Cotton LT, Watkins PJ. Improved survival of the diabetic foot: the role of a specialized foot clinic. Q J Med. 1986;60:763–71.
12. Smith AG, Russell J, Feldman EL, Goldstein J, Peltier A, Smith S, Hamwi J, Pollari D, Bixby B, Howard J, Singleton JR. Lifestyle intervention for pre-diabetic neuropathy. Diabetes Care. 2006;29(6):1294–9.
13. Boulton AJ, Vinik AI, Arezzo JC, Bril V, Feldman EL, Freeman R, Malik RA, Maser RE, Sosenko JM, Ziegler D, American Diabetes Association: Diabetic neuropathies: a statement by The American Diabetes Association. Diabetes Care. 2005;28(4):956–62.
14. Dorfman LJ, Cummins KL, Reaven GM, Ceranski J, Greenfield MS, Doberne L. Studies of diabetic polyneuropathy using conduction velocity distribution (DCV) analysis. Neurology. 1983; 33(6):773–9.
15. Levy D, Abraham R, Reid G. A comparison of two methods for measuring thermal thresholds in diabetic neuropathy. J Neurol Neurosurg Psychiatry. 1989;52:1072–77.
16. Hendriksen PH, Oey PL, Wieneke GH, Bravenboer B, Van Huffelen AC. Subclinical diabetic polyneuropathy: early detection of involvement of different nerve fibre types. J Neurol Neurosurg Psychiatry. 1993;56(5):509–14.
17. Sumner CJ, Sheth S, Griffin JW, Cornblath DR, Polydefkis M. The spectrum of neuropathy in diabetes and impaired glucose tolerance. Neurology. 2003;60(1):108–11.
18. Russell JW, Feldman EL: Impaired glucose tolerance–Does it cause neuropathy? Muscle Nerve. 2001;(24):1109–12.
19. Singleton JR, Smith AG, Bromberg MB. Painful sensory polyneuropathy associated with impaired glucose tolerance. Muscle Nerve. 2001;24(9):1225–8.

20. Levy DM, Abraham RR, Abraham RM. Small- and large-fiber involvement in early diabetic neuropathy: a study with medial plantar response and sensory thresholds. Diabetes Care. 1987;10(4):441–7.

21. Shy ME, Frohman EM, So YT, Arezzo JC, Cornblath DR, Giuliani MJ, Kincaid JC, Ochoa JL, Parry GJ, Weimer LH; Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Quantitative sensory testing. Reports of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology. 2003;60(6):898–904.

22. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 1997;20(7):1183–97.

23. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998;15(7):539–53.

24. Kimura J. Electrodiagnosis in diseases of nerve and muscle: principle and practice. F.A Davis/Philadelphia Eds 1985;pg 105–41.

25. Fruhstorfer H, Lindblom U, Schmidt WC. Method for quantitative estimation of thermal thresholds in patients. J Neurol Neurosurg Psychiatry. 1976;39(11):1071–75.

26. Gibbons C, Freeman R. The evaluation of small fiber function-autonomic and quantitative sensory testing. Neurol Clin. 2004;22(3):683–702, vii.

27. Defrin R, Sachal- Schiffer M, Hadgadg M, Peretz C. Quantitative somatosensory testing of warm and heat-pain thresholds: the effect of body region and testing method. Clin J Pain. 2006;22(2):130–6.

28. Tesfaye S, Boulton AJM, Dyck PJ, Freeman R, Horowitz M, Kempler P, Lauria G, Malik RA, Spallone V, Vinik A; Bernardi L; Valensi P, on behalf of the Toronto Diabetic Neuropathy Expert Group. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care. 2010;33(10):2285–93.

29. Selvin S. Practical Biostatistics Methods, 1995 Wadsworth Publishing Company/Duxbury Press An International Thomson Publishing Company.

30. Dyck LE, O'Brien PC. Quantitative sensory testing: Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Correspondence to the Editor. Neurology. 2003;61:1628–30.

31. Løseth S; Mellgren S; Jorde R; Lindal S; Stålberg E. Polyneuropathy in type 1 and type 2 diabetes: comparison of nerve conduction studies, thermal perception thresholds and intraepidermal nerve fibre densities. Diabetes Metab Res Rev. 2010;26(2):100–6.

32. Devigili G, Tugnoli V, Penza P, Camozzi F, Lombardi R, Melli G, Broglio L, Granieri E ,Lauria G. The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology. Brain. 2008;131(7):1912–25.

33. Gibbons CH, Freeman R, Veves A. Diabetic neuropathy: a cross-sectional study of the relationships among tests of neurophysiology. Diabetes Care. 2010;33(12):2629–34.

34. Vinik AI. Diabetic neuropathy: a small-fiber disease. Medscape Conference Coverage: 61st scientific sessions of the American Diabetes Association; June 22-26, 2001, Philadelphia Pennsylvania. http:// www.medscape.com/view article/418568. Accessed July 8, 2002.

35. Garfunkel CG: Pie diabético: Consideraciones médicas. In: Tratado de Patología arterial y venosa. Sociedad de Cirujanos de Chile & Raúl Poblete Silva Eds, Santiago, 1994;504-21.

36. Palumbo PJ, Elveback LR, Whisnant JP. Neurologic complications of diabetes mellitus: transient ischemic attack, stroke and peripheral neuropathy. Adv Neurol. 1978;19:593–601.

37. Dahl-Jorgensen K, Brinchmann-Hansen O, Hansen KF, Ganes T: Effect of near normoglycaemia for two years on progression of early diabetic retinopathy, nephropathy, and neuropathy: the Oslo Study. Br Med J (Clin Res Ed). 1986;293(6556):1195–9.

38. Polydefkis M, Griffin JW, McArthur J. New insights into diabetic polyneuropathy. JAMA. 2003;290(10):1371–76.

39. Singleton JR, Smith AG, Bromberg MB: Increased prevalence of impaired glucose tolerance in patients with painful sensory neuropathy. Diabetes Care. 2001;24(8):1448–53.

40. Vinik AI, Freeman R, Erbas T. Diabetic autonomic neuropathy. Semin Neurol. 2003;23(4):365–72.

41. Siemionow M, Demir Y. Diabetic neuropathy: Pathogenesis and treatment. A review. Journal of Reconstructive Microsurgery. 2004;20(3):241–52.

42. Consensus statement: Report and recommendations of the San Antonio conference on diabetic neuropathy. American Diabetes Association American Academy of Neurology. Diabetes Care 1988;11:592–7.