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Can the 128-Hz tuning fork be an alternative to the biothesiometer for diabetic peripheral neuropathy screening? A cross-sectional study in a tertiary hospital in East India

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ABSTRACT

Introduction Diabetic neuropathy is frequently underdiagnosed and undertreated. Logistic problems accompany the routine use of the biothesiometer. Hence, we attempted to find a more easily available alternative.

Research design and methods 149 patients with diabetes visiting the outpatient endocrinology clinic were assessed for vibration sense using a 128-Hz tuning fork (absolute timing method) and a biothesiometer. A reading of >25 V on the biothesiometer (known as vibration perception threshold or VPT) was taken as the diagnostic criterion for severe neuropathy while >15 V was used as an indicator of the mild form. The sensitivity and specificity were calculated by constructing the receiver operating characteristic curve (ROC). A p value of <0.05 was considered as statistically significant.

Results The timed tuning fork (TTF) test showed a statistically significant correlation with the VPT measurements (r=−0.5, p=0.000). Using the VPT findings as a reference, a timed tuning fork cut-off of 4.8 s was 76% sensitive and 77% specific in diagnosing mild neuropathy while absent tuning fork sensation demonstrated 70% sensitivity and 90% specificity in detecting severe neuropathy.

Conclusions The tuning fork test demonstrated significant sensitivity and specificity in diagnosing diabetic peripheral neuropathy when compared against the biothesiometer. A cut-off of 4.8 s can be a useful indicator of the early stages of onset of the condition.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The inclusion criteria comprised both type 1 and type 2 diabetes.
⇒ The sample size exceeded the estimated figure, thus making provisions for wider extrapolation and applicability of the results.
⇒ This study evaluates the diagnostic test both qualitatively and quantitatively.
⇒ The study establishes correlation rather than causation.
⇒ Another limitation of the study is the lack of glycated haemoglobin (HbA1c) to assess the glycaemic control.

INTRODUCTION

The prevalence of diabetic neuropathy has assumed significant proportions in India.[1] Peripheral polyneuropathy is one of the major factors responsible for increased risks of amputation[2] and positively correlates with the development of other microvascular complications like retinopathy[3] in patients with diabetes. Primary care physicians play a crucial role in preventing diabetic foot complications by initiating prompt screening and patient education from the first point of contact in the rural health clinics.[4] However, screening for peripheral neuropathy is not widely practised in India[5] which, coupled with poor foot care practices, has led to underdiagnoses of the condition in a significant proportion of the population.[6] Several studies have concluded that it is crucial to assess for sensory neuropathic changes for better evaluation and management of these patients.[7]

> The commonly used modalities are the 5.07/10g Semmes-Weinstein monofilament, the pin prick test, temperature sensation, lower extremity reflexes, and the biothesiometer and the 128 Hz tuning fork for vibration testing.[8]

The biothesiometer, and the tuning fork tests assess the vibration perception through the large-fibre dorsal column-medial lemniscal system,[9] while the pinprick test and temperature testing is an indirect indicator of the transmission through the small fibre spinothalamic tract.[10] Previous research has shown that the monofilament may not be ideal for screening patients at risk of foot ulcers and that the 128-Hz tuning fork tested at fewer number of sites has the same

accuracy as the monofilament,[11] alone or in combination with the appearance of the feet and presence of ulcers.[12] Two studies exploring the reliability of the pin prick test demonstrated its weaker performance than the VPT and the tuning fork tests.[13 14] This has led to several researchers advising the use of the tuning fork either alone,[15] or by the absolute timing method.[16] Biothesiometer, used to measure vibration perception threshold, has been reliably used in some settings to screen for diabetic neuropathy, even in children with diabetes mellitus.[17] Previous studies have exhibited its usefulness in the context when the erstwhile gold standard Nerve conduction studies (NCS)[18 19] might be cumbersome due to the techniques and the costs involved,[20 21] and complicate large sample screening.[22] This has prompted considerable research comparing the bedside tests, including absent tuning fork sensation, with biothesiometer as the standard.[8 23–25] The use of the biothesiometer requires electricity and hands-on training by a specialist or an expert operator, besides incurring significant additional costs, all of which can preclude its use in less-equipped primary healthcare settings.[26] In a previous study, the sensitivity of the biothesiometer was equal to that of the non-graduated tuning fork.[27] However, there are lacunae in existing literature looking at the relevance of the absolute timing method using a conventional 128-Hz tuning fork against the biothesiometer.

RESEARCH DESIGN AND METHODS

The objective of our study was to determine a cut-off (in seconds) for the tuning fork test to detect diabetic peripheral neuropathy (DPN) with relation to biothesiometer findings.

This observational, cross-sectional study was conducted at the Diabetes Clinic of Endocrinology department of Nil Ratan Sircar Medical College and Hospital, Kolkata, West Bengal, India. Convenience sampling was done and the sample size was calculated by the appropriate formula.[28] Using the calculator at www.riskcalc.org/samplesize/ for determining area under ROC curve with an alpha error of 0.05, power of 90%, null hypothesis Area under Curve (AUC) value of 0.5 and considering the prevalence of DPN to be 0.45 in diabetic Indian participants,[1] the sample size required was 42. However, we could include as many as 149 patients in the final analysis.

We included patients with type 2 diabetes mellitus of any duration and/or type 1 diabetes mellitus for at least 5 years. Exclusion criteria included patients with pre-diabetes, gestational diabetes, amputated feet, undergoing treatment with drugs-modifying neuropathy (anti-arrhythmics, chemotherapeutic drugs, etc) or suffering from other diseases known to cause peripheral neuropathy (hypothyroidism, chronic renal disease, malignancy, etc). Fasting plasma glucose was collected after 8 hours of overnight fasting and post-prandial plasma glucose was collected 2 hours after the start of a meal. The samples were collected via venipuncture in a fluoride oxalate tube.

Clinical examination of the study participants was done to reveal any paralysis of the body, amputation of the feet or any visible deformity, ulcer or callus.

Vibration perception threshold (VPT) was measured with a biothesiometer in a standardised fashion by a single trained observer[29] with the subject in supine position and eyes closed. All VPT exams were first performed on a bony prominence on the dorsal aspect of the participant's hand prior to examining the feet. After placing the probe on the hand, the vibratory stimulus was alternately turned off and on and the participant asked to discriminate between vibratory and pressure sensation. The actual VPT assessment of the feet was done once the participant gained familiarity with vibratory sensation on the hand. The head of the probe was placed over the bony prominence at the distal pulp of the hallux. The voltage was then manually increased from zero until the patient said 'yes', confirming that they can sense the vibration. This process was repeated thrice and the average amplitude (V) was recorded.

Assessment of vibration sense was also done by the 128-Hz tuning fork. While being held at its proximal end by one hand of the examiner, the distal end of a 128-Hz tuning fork was forcefully struck against the palm of the examiner's other hand with consistent force for each examination. Once the fork was struck, it was placed onto the dorsal aspect of the distal phalanx of the great toe (hallux) just proximal to the nail bed (after demonstrating the sensation on the dorsal aspect of the participant's hand). Prior to applying the tuning fork, the participant was instructed to give a verbal response of 'yes' if/when they initially felt the vibration, followed by 'now' when they stopped feeling the same. The time elapsed between application of the tuning fork and a subsequent 'now' response was measured with a digital stopwatch (in seconds up to two decimal places). If participants were unable to feel vibratory sensation on initial contact of the tuning fork, the duration of examination was recorded as zero. This process was repeated thrice and the mean time to conduct the test (seconds) was recorded.

Statistical analysis

The data was analysed using SPSS V.26 (IBM, Chicago). Correlations were assessed using the Pearson's correlation coefficient while the positive predictive value, negative predictive value, sensitivity and specificity of timed tuning fork test in relation to the biothesiometer finding were determined by ROC curves, using VPT scores >25 V and >15 V as the cut-offs for severe and mild neuropathy, respectively. P <0.05 was considered as statistically significant. The continuous variables were checked for normality using the Shapiro-Wilk test.

We used the Standard for Reporting Diagnostic Accuracy (STARD) checklist when writing our report.[30]

Table 1 Baseline characteristics of the study population (n=149)

	Mean±SD
Age (years)	51.8±9.41 (18–72)
Sex (M:F)	68:81
Duration of DM (years)	8.12±6.59
BMI (kg/m^2)	24.05±3.55
FPG (mg/dL)	167.10±78.64
PPPG (mg/dL)	251.35±118.56

BMI, body mass index; DM, diabetes mellitus; F, females; FPG, fasting plasma glucose; M, males; PPPG, postprandial plasma glucose.

Patient and public involvement

Consenting patients were involved in the conduct of the research, recruited according to the inclusion and exclusion criteria. The study questionnaire was prepared in both English and the local language and corrected according to the feedback provided by the patients on the ease of understanding. It was agreed that dissemination of the results would be through gradual review and publication followed by incorporation in clinical practice.

RESULTS

A total of 149 patients (100% with type 2 diabetes) were included with a mean age of 51.8±9.41 years (18–72 years). Baseline characteristics of the study population namely, the continuous variables, are presented as mean and standard deviation (SD) in table 1.

42.3% (63) of the sample population demonstrated a VPT score between 15 and 25 V and 6.7% (10) demonstrated a score of ≥25 V.

Pearson's correlation coefficient testing shows a significant negative correlation between TTF and VPT values (r=−0.5, p=0.000) (table 2)

Taking 25 V score on the VPT as the criterion for severe diabetic neuropathy, a timed tuning fork value of 0 s had 70% sensitivity and 90% specificity for diagnosing the same (online supplemental table 1). It had a positive predictive value of 33.6% and a negative predictive value of 97.7% for severe DPN (0.751 (0.550, 0.953)). The ROC curve is depicted in figure 1.

Table 2 Correlation between timed tuning fork and VPT

		Timed tuning fork (s)	VPT (V)
Timed tuning fork (s)	Pearson correlation	1	−0.500*
	Sig. (two-tailed)		0.000
	N	149	149
VPT (V)	Pearson correlation	−0.500*	1
	Sig. (two-tailed)	0.000	
	N	149	149

*Correlation is significant at the 0.01 level (two-tailed).
VPT, vibration perception threshold.

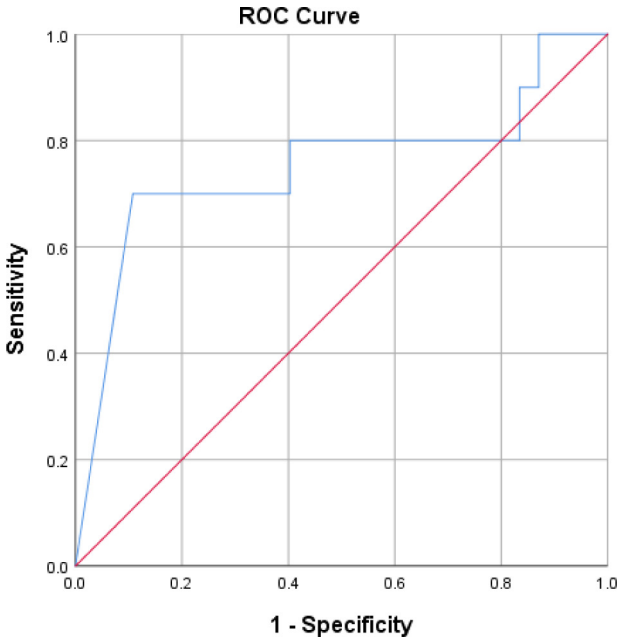

Figure 1 Receiver operating characteristic (ROC) curve for vibration perception threshold >25 V (0.751 (0.550, 0.953)). Diagonal segments are produced by ties.

Using an ROC curve (figure 2), a timed tuning fork value of 4.8 s showed 76% sensitivity and 77% specificity for detection of mild diabetic peripheral neuropathy with a VPT score above 15 V score as an indicator of the same (online supplemental table 2). It had a positive predictive value of 76% and a negative predictive value of 76.9% for mild DPN detection (0.789 (0.713, 0.866)). The maximum sum of sensitivity and specificity was chosen as

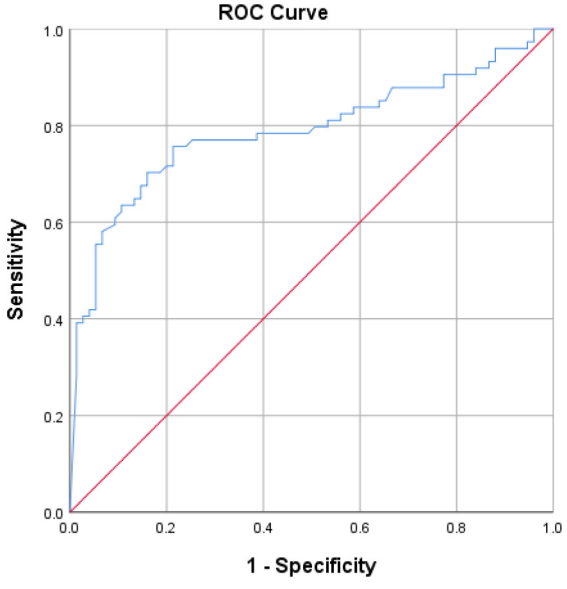

Figure 2 Receiver operating characteristic (ROC) curve for vibration perception threshold >15 V (0.789 (0.713,0.866)). Diagonal segments are produced by ties.

the cut-off at which point the Youden's index (Se+Sp – 1) was also maximum.

DISCUSSION

The tests considered for assessment of diabetic peripheral neuropathy in our study were the 128-Hz tuning fork test and the biothesiometer, which are some of the simplest bedside screening tools available for diabetic neuropathy.[31]

The 128-Hz tuning fork test is a convenient method of bedside screening of diabetic neuropathy. Statistical analysis demonstrated moderate correlation between the results of the tuning fork test and the VPT measurements. This agrees with the study conducted by Jayaprakash *et al* (r=0.59, p<0.001).[8] In a study conducted by J O'Neill *et al*,[32] the test proved to be unreliable, but the sample size (n=21) was too small to reach a definitive conclusion.

The grading of VPT scores is done as follows: normal: ≤15 V, grade I neuropathy: 16–25 V and grade II neuropathy: ≥25 V.[33 34] A study found grade I severity in approximately 27% of patients with clinical neuropathy and in 50% asymptomatic patients,[34] which indicates the presence of subclinical neuropathic damage. In another study, nerve pain was experienced by the study population from a VPT score as low as 16 V.[35] On comparing patients with and without diabetes, the mean VPT was found to be 16.14 for the former, showing a significant difference.[36] A VPT cut-off of 10.54 demonstrated a favourable diagnostic outcome when compared with the Nerve Conduction Velocity (NCV) examination.[37] In this study, we also attempted to look at timed tuning fork score as a marker for the detection of presence and severity of diabetic neuropathy. In our study, a cut-off of 4.8 s with the timed tuning fork test showed good sensitivity and specificity for the detection of grade I neuropathy.[38] In previous studies, tuning fork scores <2 s and ≤4 s have been shown to be a risk factor for lower limb injuries,[29] and foot ulceration,[39] respectively.

Taking 25 V on VPT as the threshold for severe diabetic neuropathy, a cut-off of zero seconds with the timed tuning fork showed a sensitivity of 70% and specificity for 90%. Absent tuning fork sensation has previously been found to correlate significantly with VPT scores by Tanveer *et al*,[24] who estimated a sensitivity of 75% but a specificity of 25% for the test. The values were 53% and 99%, respectively, for the tuning fork test in two other studies.[4 40] The 5.07 (10 g) monofilament test is the most recent recommendation for the detection of diabetic neuropathy by the American Diabetes Association.[41] However, a study[42] comparing the timed tuning fork test and the monofilament testing found the latter to be normal in 50% of patients with a vibration perception of 4 s or less. It concluded that the tuning fork test was a more reproducible, accurate and sensitive test to detect diabetic neuropathy and future risk of ulceration in the initial stages of the disease when the monofilament may show normal results. These findings, along with those from our study, highlight the probable need for modifying the current guidelines.

The present study is unique in estimating a definite tuning fork score (4.8 s) to detect mild diabetic neuropathy besides reinforcing the utility of the test as a suitable surrogate for the biothesiometer.

Measurement of vibration perception threshold by the biothesiometer has been proven to be superior to all the other tests in several studies.[33 38 43 44] However, it is an expensive machine, needs electricity to operate and is quite difficult to procure in primary healthcare and rural settings. The entire procedure demands a significant amount of time which can be quite inconvenient at peak hours due to the immense workload of the healthcare professionals in developing countries. Hence, instead of investing in a biothesiometer, the handy tuning fork test provides a simpler, easily available alternative.[45] The tuning fork has been shown to be considerably quicker than the VPT measurement.[46] Therefore, a simple and accurate alternative like the tuning fork can be vital to improve the screening practices and gauge the severity and progression of diabetic neuropathy quite easily, both from the qualitative and the quantitative aspects. Hence, the present study recommends its use as a surrogate measure in less-equipped clinical settings.

Our study has some limitations. The study population comprised only adults with type 2 diabetes who were fit to attend the outpatient clinic.[47] We also appreciate that the cross-sectional study design allows for demonstration of correlation, more than causality. Another limitation was the lack of HbA1c to assess the glycaemic control.

CONCLUSION

Our study suggests that the tuning fork test can be an accurate, simple and easily available alternative to the biothesiometer for screening of diabetic neuropathy as well as in identifying the stage and progression of the disease.

Contributors SC: data collection, manuscript initial draft preparation, correspondence and ethical approval, guarantor. SG: analysis and final draft preparation. NS and AB: supervision and proofreading.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the Nil Ratan Sircar Medical College and Hospital Institutional Ethics Committee (Memo No. NRSMC/IEC/18/2022). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Deidentified participant data are available upon request in the form of Microsoft Excel spreadsheets from the corresponding author (ORCID ID 0000-0001-5737-

591X). Reuse is permitted only after prior intimation and deliberation with each of the contributors. Additional data that can be available include original and translated questionnaires, informed consent and participant information sheets.

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REFERENCES

1 Jasmine A, G.v. A, Durai V, *et al*. Prevalence of peripheral neuropathy among type 2 diabetes mellitus patients in a rural health centre in South India. *Int J Diabetes Dev Ctries* 2021;41:293–300.
2 Mueller MJ, Zou D, Bohnert KL, *et al*. Plantar stresses on the neuropathic foot during barefoot walking. *Phys Ther* 2008;88:1375–84.
3 Wei WW, Yang XF, Gu H, *et al*. Association of diabetic retinopathy with diabetic peripheral neuropathy in type 2 diabetic patients: the Beijing Desheng diabetic eye disease study. *Zhonghua Yan Ke Za Zhi* 2017;53:509–13.
4 Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. *JAMA* 2005;293:217–28.
5 Shah VN, Kamdar PK, Shah N. Assessing the knowledge, attitudes and practice of type 2 diabetes among patients of Saurashtra region. *Int J Diabetes Dev Ctries* 2009;29:118–22.
6 Saurabh S, Sarkar S, Selvaraj K, *et al*. Effectiveness of foot care education among people with type 2 diabetes in rural Puducherry, India. *Indian J Endocrinol Metab* 2014;18:106–10.
7 Ledoux WR, Schoen J, Lovell M, *et al*. Clawed toes in the diabetic foot: neuropathy, intrinsic muscle volume, and plantar aponeurosis thickness. *J Foot Ankle Res* 2008;1:2.
8 Jayaprakash P, Bhansali A, Bhansali S, *et al*. Validation of bedside methods in evaluation of diabetic peripheral neuropathy. *Indian J Med Res* 2011;133:645–9.
9 Gilman S. Joint position sense and vibration sense: anatomical organisation and assessment. *J Neurol Neurosurg Psychiatry* 2002;73:473–7.
10 Haefeli J, Kramer JLK, Blum J, *et al*. Assessment of Spinothalamic tract function beyond pinprick in spinal cord lesions: a contact heat evoked potential study. *Neurorehabil Neural Repair* 2014;28:494–503.
11 Miranda-Palma B, Sosenko JM, Bowker JH, *et al*. A comparison of the monofilament with other testing modalities for foot ulcer susceptibility. *Diabetes Res Clin Pract* 2005;70:8–12.
12 Kanji JN, Anglin RES, Hunt DL, *et al*. Does this patient with diabetes have large-fiber peripheral neuropathy? *JAMA* 2010;303:1526–32.
13 McIllhatton A, Lanting S, Lambkin D, *et al*. Reliability of recommended non-invasive chairside screening tests for diabetes-related peripheral neuropathy: a systematic review with meta-analyses. *BMJ Open Diabetes Res Care* 2021;9:e002528.
14 Lanting S, Craike P, Spink M, *et al*. The reliability of non-invasive neurological examinations in people with diabetes. *J Foot Ankle Res* 2013;6:6.
15 Meijer J-WG, Smit AJ, Lefrandt JD, *et al*. Back to basics in diagnosing diabetic polyneuropathy with the tuning fork! diabetes care. *Diabetes Care* 2005;28:2201–5.
16 de Simone R, Rabitti PG, De Feo EM, *et al*. Proposal of a new method for the use of the rydel-seiffer tuning fork in the screening of diabetic polyneuropathy. A pilot study. *Diabetes Res Clin Pract* 2015;108:e21–4.
17 Liu F, Bao Y, Hu R, *et al*. Screening and prevalence of peripheral neuropathy in type 2 diabetic outpatients: a randomized multicentre survey in 12 city hospitals of China. *Diabetes Metab Res Rev* 2010;26:481–9.
18 Feng Y, Schlösser FJ, Sumpio BE. The semmes weinstein monofilament examination as a screening tool for diabetic peripheral neuropathy. *J Vasc Surg* 2009;50:675–82.
19 Pourhamidi K, Dahlin LB, Englund E, *et al*. Evaluation of clinical tools and their diagnostic use in distal symmetric polyneuropathy. *Prim Care Diabetes* 2014;8:77–84.
20 Bharathi C, Roopakala MS, Shivaprasad C, *et al*. Diagnostic value of vibration perception threshold in diabetic peripheral neuropathy. *Int J Physiol* 2018;6:84.
21 Bril V, Perkins BA. Comparison of vibration perception thresholds obtained with the neurothesiometer and the CASE IV and relationship to nerve conduction studies. *Diabet Med* 2002;19:661–6.
22 Callaghan BC, Price RS, Chen KS, *et al*. The importance of rare subtypes in diagnosis and treatment of peripheral neuropathy: a review. *JAMA Neurol* 2015;72:1510–8.
23 César Ernesto L-C, Néstor MZ, Raúl I-S, *et al*. Comparison of clinical tests for peripheral diabetic neuropathy in a type 1 diabetes cohort. *Endocr Pract* 2021;27:567–70.
24 Tanveer BA, Adil WH, Nahida WY, *et al*. Diagnostic accuracy of different bedside clinical methods in evaluation of peripheral neuropathy in patients of diabetes mellitus: experience at a tertiary care center in North India. *Nternational Journal of Scientific Study* 2019;7:7.
25 Arshad AR, Alvi KY. Diagnostic accuracy of clinical methods for detection of diabetic sensory neuropathy. *J Coll Physicians Surg Pak* 2016;26:374–9.
26 Hnit MW, Han TM, Nicodemus L. Accuracy and cost-effectiveness of the diabetic foot screen proforma in detection of diabetic peripheral neuropathy in Myanmar. *J ASEAN Fed Endocr Soc* 2022;37:31–7.
27 Wittenberg B, Svendsen TK, Gaist LM, *et al*. Test-retest and time dependent variation and diagnostic values of vibratory sensation determined by biothesiometer and the Rydel-Seiffer tuning fork. *Brain Behav* 2021;11:e2230.
28 Shrestha S, Gorhaly MP, Bajracharya MR. Diagnostic accuracy of Monofilament test to detect diabetic neuropathy. *J Adv Intern Med* 2021;10:20–5.
29 Thivolet C, el Farkh J, Petiot A, *et al*. Measuring vibration sensations with graduated tuning fork. simple and reliable means to detect diabetic patients at risk of neuropathic foot ulceration. *Diabetes Care* 1990;13:1077–80.
30 Bossuyt PM, Reitsma JB, Bruns DE, *et al*. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. *BMJ* 2015;351:h5527.
31 Yang Z, Zhang Y, Chen R, *et al*. Simple tests to screen for diabetic peripheral neuropathy. *Cochrane Database Syst Rev* 2018;2018:CD010975.
32 O'Neill J, McCann SM, Lagan KM. Tuning fork (128 Hz) versus neurothesiometer: a comparison of methods of assessing vibration sensation in patients with diabetes mellitus. *Int J Clin Pract* 2006;60:174–8.
33 Medakkel AA, Sheela P. Vibration perception threshold values and clinical symptoms of diabetic peripheral neuropathy. JCDR; 2018. Available: http://jcdr.net/article_fulltext.asp?issn=0973-709x&year=2018&volume=12&issue=5&page=LC20&issn=0973-709x&id=11549 [Accessed 06 Jul 2022].
34 Dash S, Thakur A. Perception of vibration threshold is a marker of diabetic neuropathy. *Natl J Physiol Pharm Pharmacol* 2017;1.
35 Narayana SRP. Study to assess neuropathy severity in patients with diabetic foot problems. *Int Surg J* 2020;7:3762.
36 Aruna B, Haragopal R. Role of Biothesiometry in the diagnosis of diabetic neuropathy. *Indian J Clin Anat Physiol* 2017;4:329–31.
37 Hou Y, Liu S, Zhu T, *et al*. Vibration perception threshold in diagnosing diabetic peripheral neuropathy by receiver operating characteristic curve. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2012;37:951–6.
38 Liu M, Gao Y, Chen D-W, *et al*. Quantitative vibration perception threshold in assessing diabetic polyneuropathy: should the cut-off value be adjusted for Chinese individuals with type 2 diabetes *J Diabetes Investig* 2021;12:1663–70.
39 Liniger C, Albeanu A, Bloise D, *et al*. The tuning fork revisited. *Diabet Med* 1990;7:859–64.
40 Perkins BA, Olaleye D, Zinman B, *et al*. Simple screening tests for peripheral neuropathy in the diabetes clinic. *Diabetes Care* 2001;24:250–6.
41 Boulton AJM, Vinik AI, Arezzo JC, *et al*. Diabetic neuropathies: a statement by the American diabetes association. *Diabetes Care* 2005;28:956–62.

42 Oyer DS, Saxon D, Shah A. Quantitative assessment of diabetic peripheral neuropathy with use of the clanging tuning fork test. *Endocr Pract* 2007;13:5–10.
43 Martin CL, Waberski BH, Pop-Busui R, *et al*. Vibration perception threshold as a measure of distal symmetrical peripheral neuropathy in type 1 diabetes. *Diabetes Care* 2010;33:2635–41.
44 Joseph MJ. Early detection of peripheral diabetic neuropathy - a correlative study of symptoms with digital biothesiometry. In: *Endocrine abstracts*. Bioscientifica, 2021. Available: https://www.endocrine-abstracts.org/ea/0077/ea0077p204
45 Paprzycki P. A tuning fork or a biothesiometer: which one to choose in the outpatient diabetes care? Diabetologia doswiadczalna i kliniczna, Available: https://www.academia.edu/22999932/A_tuning_fork_or_a_biothesiometer_Which_one_to_choose_in_the_outpatient_diabetes_care [Accessed 11 Sep 2022].
46 Raymond B, Steriovski J, Gillyard K, *et al*. Choosing a vibratory test to pair with semmes weinstein monofilament testing for evaluating lower extremity sensation in patients with diabetes: a comparison of three vibratory methodologies. *J Diabetes Sci Technol* 2020;14:8–15.
47 Sempere-Bigorra M, Julián-Rochina I, Cauli O. Differences and similarities in neuropathy in type 1 and 2 diabetes: a systematic review. *J Pers Med* 2021;11:230.