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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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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

Dr. Sujaya Chattopadhyay [a], Dr. Soumik Goswami [b], Dr. Nilanjan Sengupta [c], Dr. Arjun Baidya[d]

[a] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; 97.sujaya@gmail.com

[b] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; dr.soumikgoswami@gmail.com

[c] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; drnilanjansengupta@gmail.com

[d] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; arjun.baidya@gmail.com

CORRESPONDING AUTHOR

Dr. Sujaya Chattopadhyay

97.sujaya@gmail.com

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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 cut off of >25V with the biothesiometer 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. A p value <0.05 was considered as statistically significant.

Results: The timed tuning fork test showed a strong correlation with the VPT measurements (r=0.5, p<0.001). Using the VPT findings as a reference, a timed tuning fork cut-off of 4.8 seconds 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.

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Conclusions: The 128 Hz tuning fork can be employed for accurate diagnosis of diabetic neuropathy as well as quantification of its severity.

Strengths and limitations:

- This study aims to measure the approximation of results between the erstwhile standards and the tuning fork.
- We hope this study can pave the way for more research into simplifying and streamlining routine screening of diabetic neuropathy at early stages of diagnosis and prognosis without being limited by logistic constraints.
- Limitations of the study include absence of cost-effective analysis and establishment of correlation rather than causation.

Introduction

The prevalence of diabetic neuropathy has assumed significant proportions in India (1). Peripheral polyneuropathy is one of the major factors responsible for increased risk of amputation (2) and other microvascular complications(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

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neuropathy is not widely practised in India (5)which, coupled with poor foot care practices, have led to under diagnoses 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 clinical tests are the 5.07/10g Semmes-Weinstein monofilament, the pin prick test, the biothesiometer and the 128 Hz tuning fork (8).

The biothesiometer, monofilament and the tuning fork tests assess the vibration perception through the large-fiber dorsal column-medial lemniscal system(9), while the pin prick test is an indirect indicator of the transmission of pain sensation through the small fiber spino-thalamic 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 test(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

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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 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(23), (24), (8), (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 with regard to 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 with relation to biothesiometer findings.

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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 data from the study by Jasmine A et al(1) who found the prevalence of diabetic neuropathy to be 44.9% in Indian patients. A sample size of 95 was estimated and considering an anticipated attrition rate of 10%, the final sample size was found to be 110. However, we could include as many as 149 patients in the final analysis.

We included patients with type 2 diabetes mellitus of any duration or type 1 diabetes mellitus for at least 5 years. Exclusion criteria included patients with prediabetes, 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.)

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

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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. Voltage began at zero and was then manually increased 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 128Hz 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. Participants also instructed to state “now” when they stopped feeling the vibration after providing a “yes” response when they first felt a vibratory sensation. The time elapsed between application of the tuning

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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 upon 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.

The data was analysed using SPSS Version 26 (IBM, Chicago). Correlations were assessed with Spearman's correlation coefficient while the sensitivity and specificity of timed tuning fork test in relation to the biothesiometer finding was determined using receiver operating characteristic (ROC) curve, 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.

We used the STARD checklist when writing our report(30). Patients were involved in the conduct of the research from design till analysis.

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 are given in Table 1.

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

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	MEAN±S.D.
Age (years)	51.8 ± 9.41 (18-72)
Sex (M:F)	68:81
Duration of DM (years)	8.12±6.59
BMI (kg/m²)	24.05±3.55
FPG(mg/dl)	167.10±78.64
PPPG(mg/dl)	251.35±118.56

*Values in SI. M, Males; F, Females; DM, Diabetes Mellitus; BMI, body mass index; FPG, fasting plasma glucose; PPPG, post-prandial plasma glucose

A strong and significant correlation was found between VPT score and the tuning fork test (r= 0.5, p<0.005).

Taking 25V score on the VPT as the criterion for severe diabetic neuropathy, a timed tuning fork value of 0 second had 70% sensitivity and 90% specificity for diagnosing the same (Tables 2, 3; Supplemental Figure 1).

Area Under the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

			Asymptotic 95% Confidence Interval	
Area	Std. Error[a]	Asymptotic Sig.[b]	Lower Bound	Upper Bound
.751	.103	.008	.550	.953

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The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Table 2: Area under the curve for VPT>25V

Coordinates of the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

Positive if Less Than or Equal To[a]	Sensitivity	1 – Specificity
-1.0000	.000	.000
.2050	.700	.108
.5000	.700	.115
.8350	.700	.122
1.1500	.700	.129
1.2550	.700	.137
1.3150	.700	.144
1.5450	.700	.151
1.7600	.700	.158
1.8150	.700	.165
1.9100	.700	.173
1.9950	.700	.180
2.0750	.700	.187
2.1750	.700	.194
2.3050	.700	.201
2.4150	.700	.209
2.5050	.700	.216
2.6100	.700	.223
2.6850	.700	.230
2.8050	.700	.237
2.9500	.700	.245
3.0700	.700	.252
3.1500	.700	.259
3.2300	.700	.266
3.3250	.700	.273
3.3900	.700	.281
3.4950	.700	.288
3.5700	.700	.295
3.6950	.700	.317

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3.8200	.700	.324
3.8700	.700	.338
3.9150	.700	.345
3.9750	.700	.353
4.0400	.700	.360
4.0600	.700	.367
4.1200	.700	.374
4.1950	.700	.381
4.2450	.700	.388
4.2800	.700	.396
4.3400	.700	.403
4.4050	.800	.403
4.4600	.800	.410
4.5300	.800	.417
4.6100	.800	.432
4.6650	.800	.439
4.6800	.800	.446
4.7350	.800	.453
4.7900	.800	.460
4.8050	.800	.468
4.8200	.800	.475
4.8500	.800	.489
4.9350	.800	.496
5.0200	.800	.504
5.0450	.800	.525
5.0650	.800	.532
5.1350	.800	.540
5.1950	.800	.554
5.2600	.800	.561
5.3450	.800	.568
5.3850	.800	.576
5.4200	.800	.590
5.4550	.800	.597
5.4850	.800	.604
5.5100	.800	.612
5.5550	.800	.619
5.6300	.800	.626
5.6750	.800	.640
5.7550	.800	.647
5.8950	.800	.655

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5.9900	.800	.662
6.0350	.800	.669
6.0700	.800	.676
6.1050	.800	.683
6.1450	.800	.698
6.1800	.800	.705
6.2950	.800	.712
6.4350	.800	.719
6.4850	.800	.727
6.6050	.800	.734
6.7350	.800	.741
6.7950	.800	.748
6.8800	.800	.770
6.9550	.800	.777
6.9900	.800	.784
7.0200	.800	.806
7.1750	.800	.813
7.3200	.800	.820
7.3400	.800	.827
7.5050	.800	.835
7.6800	.900	.835
7.7050	.900	.842
7.8550	.900	.849
8.1300	.900	.856
8.3400	.900	.863
8.4750	.900	.871
8.6000	1.000	.871
8.7500	1.000	.878
8.9150	1.000	.885
9.0350	1.000	.892
9.2100	1.000	.899
9.4250	1.000	.906
9.5450	1.000	.914
9.7800	1.000	.921
9.9850	1.000	.928
10.1650	1.000	.935
10.4450	1.000	.942
10.8650	1.000	.950
11.5600	1.000	.957
12.3000	1.000	.964

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13.1050	1.000	.971
13.7200	1.000	.978
15.5050	1.000	.986
23.6650	1.000	.993
31.2000	1.000	1.000

The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cut-off value is the minimum observed test value minus 1, and the largest cut-off value is the maximum observed test value plus 1. All the other cut-off values are the averages of two consecutive ordered observed test values.

Table 3: Co-ordinates of the curve for VPT >25V

Using a ROC curve, a timed tuning fork value of 4.8 seconds showed 76% sensitivity and 77% specificity for detection of mild diabetic peripheral neuropathy using a VPT score above 15V score as an indicator of the same (Tables 4,5 ; Supplemental Figure 2).

Area Under the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

			Asymptotic 95% Confidence Interval	
Area	Std. Error[a]	Asymptotic Sig.[b]	Lower Bound	Upper Bound
.789	.039	.000	.713	.866

The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Table 4: Area under the curve for VPT>15V

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Coordinates of the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

Positive if Less Than or Equal To[a]	Sensitivity	1 – Specificity
-1.0000	.000	.000
.2050	.284	.013
.5000	.297	.013
.8350	.311	.013
1.1500	.324	.013
1.2550	.338	.013
1.3150	.351	.013
1.5450	.365	.013
1.7600	.378	.013
1.8150	.392	.013
1.9100	.392	.027
1.9950	.405	.027
2.0750	.405	.040
2.1750	.419	.040
2.3050	.419	.053
2.4150	.432	.053
2.5050	.446	.053
2.6100	.459	.053
2.6850	.473	.053
2.8050	.486	.053
2.9500	.500	.053
3.0700	.514	.053
3.1500	.527	.053
3.2300	.541	.053
3.3250	.554	.053
3.3900	.554	.067
3.4950	.568	.067
3.5700	.581	.067
3.6950	.595	.093
3.8200	.608	.093
3.8700	.622	.107
3.9150	.635	.107
3.9750	.635	.120
4.0400	.635	.133
4.0600	.649	.133
4.1200	.649	.147
4.1950	.662	.147

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4.2450	.676	.147
4.2800	.676	.160
4.3400	.689	.160
4.4050	.703	.160
4.4600	.703	.173
4.5300	.703	.187
4.6100	.716	.200
4.6650	.716	.213
4.6800	.730	.213
4.7350	.743	.213
4.7900	.757	.213
4.8050	**.757**	**.227**
4.8200	.757	.240
4.8500	.770	.253
4.9350	.770	.267
5.0200	.770	.280
5.0450	.770	.320
5.0650	.770	.333
5.1350	.770	.347
5.1950	.770	.373
5.2600	.770	.387
5.3450	.784	.387
5.3850	.784	.400
5.4200	.784	.427
5.4550	.784	.440
5.4850	.784	.453
5.5100	.784	.467
5.5550	.784	.480
5.6300	.784	.493
5.6750	.797	.507
5.7550	.797	.520
5.8950	.797	.533
5.9900	.811	.533
6.0350	.811	.547
6.0700	.811	.560
6.1050	.824	.560
6.1450	.824	.587
6.1800	.838	.587
6.2950	.838	.600
6.4350	.838	.613

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6.4850	.838	.627
6.6050	.838	.640
6.7350	.851	.640
6.7950	.851	.653
6.8800	.878	.667
6.9550	.878	.680
6.9900	.878	.693
7.0200	.878	.733
7.1750	.878	.747
7.3200	.878	.760
7.3400	.878	.773
7.5050	.892	.773
7.6800	.905	.773
7.7050	.905	.787
7.8550	.905	.800
8.1300	.905	.813
8.3400	.905	.827
8.4750	.905	.840
8.6000	.919	.840
8.7500	.919	.853
8.9150	.919	.867
9.0350	.932	.867
9.2100	.932	.880
9.4250	.946	.880
9.5450	.959	.880
9.7800	.959	.893
9.9850	.959	.907
10.1650	.959	.920
10.4450	.959	.933
10.8650	.959	.947
11.5600	.973	.947
12.3000	.973	.960
13.1050	.986	.960
13.7200	1.000	.960
15.5050	1.000	.973
23.6650	1.000	.987
31.2000	1.000	1.000

The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group.

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a. The smallest cut-off value is the minimum observed test value minus 1, and the largest cut-off value is the maximum observed test value plus 1. All the other cut-off values are the averages of two consecutive ordered observed test values.

Table 5: Co-ordinates of the curve for VPT>15V

Discussion

The tests considered for assessment of diabetic peripheral neuropathy in our study were the 128Hz 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. Regression analysis demonstrated excellent correlation between the results of the tuning fork test and the VPT measurements ($r=0.5$; $p<0.001$). This is in agreement 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: 16-25V and Grade II neuropathy: ≥25V (33), (34). A study found grade I severity in approximately 27% of patients with clinical neuropathy and in 50% asymptomatic patients (34), which indicates presence of subclinical

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neuropathic damage. In another study, nerve pain was experienced by the study population from a VPT score as low as 16V (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 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 seconds 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 seconds and ≤4 seconds have been shown to be a risk factor for lower limb injuries (39), and foot ulceration (40), respectively.

Taking 25V 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(41) , (42). The 5.07 (10g) monofilament test is the most recent recommendation for the detection of diabetic neuropathy by the American Diabetes Association (43).

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However, a study(44) 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 seconds 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 early 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 seconds) 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 (45), (38), (46), (33). However, it is an expensive machine, needs electricity to operate and is quite difficult to procure in primary health care 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 (47). The tuning fork has been shown to be considerably quicker

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than the VPT measurement (48). 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. Thus, 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(49). Cost-effective analyses of the tuning fork test were not done in the study, which can further strengthen the justification of its use. We appreciate the cross-sectional study design allows for demonstration of correlation, more than causality. However, more studies exploring this possibility might pave the way for strengthening the evidence for this hypotheses.

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.

List of abbreviations

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VPT- Vibration perception threshold; g- gram; V-volt; Hz-Hertz; Std-Standard; Sig-Significance; DPN- Diabetic peripheral neuropathy; ROC- Receiver operator characteristic; NCS-Nerve conduction studies

Declarations

Ethics approval and consent to participate

After deliberations and review the Institutional Ethics Committee, Nil Ratan Sircar Medical College and Hospital, took the decision "APPROVED" regarding the study proposal (Memo No. NRSMC/IEC/18/2022).

Consent for publication

Informed consent was obtained from all the participants by the principal author.

Sources of funding

None

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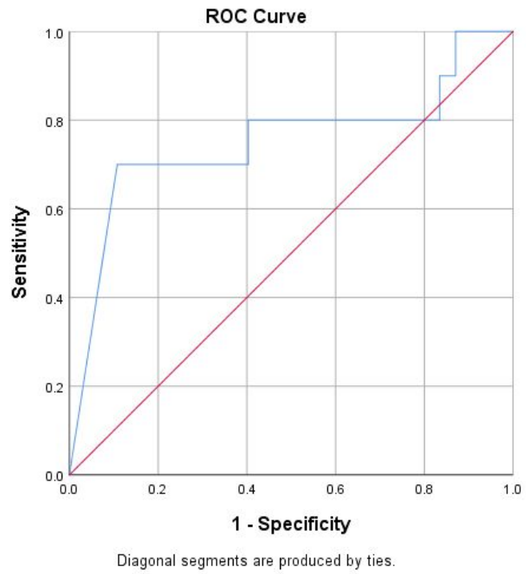
Case Processing Summary

Severe DPN	Valid N (listwise)
Positive[a]	10
Negative	139
Missing	1

Smaller values of the test result variable(s) indicate stronger evidence for a positive actual state.

a. The positive actual state is Abnormal.



Diagonal segments are produced by ties.

Area Under the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.751	.103	.008	.550	.953

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The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Coordinates of the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

Positive if Less Than or Equal To[a]	Sensitivity	1 - Specificity
-1.0000	.000	.000
.2050	.700	.108
.5000	.700	.115
.8350	.700	.122
1.1500	.700	.129
1.2550	.700	.137
1.3150	.700	.144
1.5450	.700	.151
1.7600	.700	.158
1.8150	.700	.165
1.9100	.700	.173
1.9950	.700	.180
2.0750	.700	.187
2.1750	.700	.194
2.3050	.700	.201
2.4150	.700	.209
2.5050	.700	.216
2.6100	.700	.223
2.6850	.700	.230
2.8050	.700	.237
2.9500	.700	.245
3.0700	.700	.252
3.1500	.700	.259
3.2300	.700	.266
3.3250	.700	.273
3.3900	.700	.281
3.4950	.700	.288
3.5700	.700	.295

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3.6950	.700	.317
3.8200	.700	.324
3.8700	.700	.338
3.9150	.700	.345
3.9750	.700	.353
4.0400	.700	.360
4.0600	.700	.367
4.1200	.700	.374
4.1950	.700	.381
4.2450	.700	.388
4.2800	.700	.396
4.3400	.700	.403
4.4050	.800	.403
4.4600	.800	.410
4.5300	.800	.417
4.6100	.800	.432
4.6650	.800	.439
4.6800	.800	.446
4.7350	.800	.453
4.7900	.800	.460
4.8050	.800	.468
4.8200	.800	.475
4.8500	.800	.489
4.9350	.800	.496
5.0200	.800	.504
5.0450	.800	.525
5.0650	.800	.532
5.1350	.800	.540
5.1950	.800	.554
5.2600	.800	.561
5.3450	.800	.568
5.3850	.800	.576
5.4200	.800	.590
5.4550	.800	.597
5.4850	.800	.604
5.5100	.800	.612
5.5550	.800	.619
5.6300	.800	.626

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5.6750	.800	.640
5.7550	.800	.647
5.8950	.800	.655
5.9900	.800	.662
6.0350	.800	.669
6.0700	.800	.676
6.1050	.800	.683
6.1450	.800	.698
6.1800	.800	.705
6.2950	.800	.712
6.4350	.800	.719
6.4850	.800	.727
6.6050	.800	.734
6.7350	.800	.741
6.7950	.800	.748
6.8800	.800	.770
6.9550	.800	.777
6.9900	.800	.784
7.0200	.800	.806
7.1750	.800	.813
7.3200	.800	.820
7.3400	.800	.827
7.5050	.800	.835
7.6800	.900	.835
7.7050	.900	.842
7.8550	.900	.849
8.1300	.900	.856
8.3400	.900	.863
8.4750	.900	.871
8.6000	1.000	.871
8.7500	1.000	.878
8.9150	1.000	.885
9.0350	1.000	.892
9.2100	1.000	.899
9.4250	1.000	.906
9.5450	1.000	.914
9.7800	1.000	.921
9.9850	1.000	.928

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10.1650	1.000	.935
10.4450	1.000	.942
10.8650	1.000	.950
11.5600	1.000	.957
12.3000	1.000	.964
13.1050	1.000	.971
13.7200	1.000	.978
15.5050	1.000	.986
23.6650	1.000	.993
31.2000	1.000	1.000

The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

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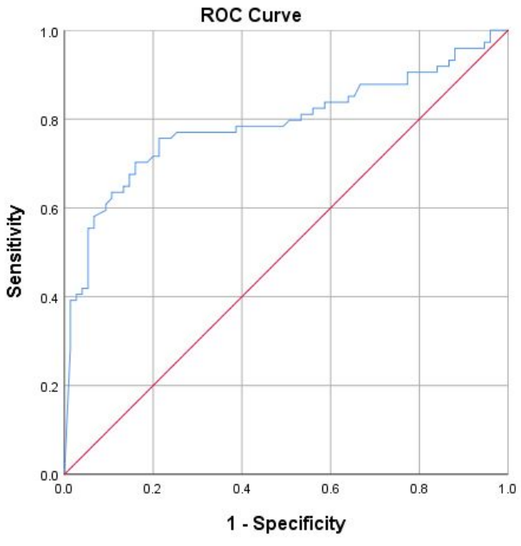
Case Processing Summary

Mild DPN	Valid N (listwise)
Positive[a]	74
Negative	75

Smaller values of the test result variable(s) indicate stronger evidence for a positive actual state.

a. The positive actual state is Abnormal.



Diagonal segments are produced by ties.

Area Under the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.789	.039	.000	.713	.866

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The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Coordinates of the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

Positive if Less Than or Equal To[a]	Sensitivity	1 - Specificity
-1.0000	.000	.000
.2050	.284	.013
.5000	.297	.013
.8350	.311	.013
1.1500	.324	.013
1.2550	.338	.013
1.3150	.351	.013
1.5450	.365	.013
1.7600	.378	.013
1.8150	.392	.013
1.9100	.392	.027
1.9950	.405	.027
2.0750	.405	.040
2.1750	.419	.040
2.3050	.419	.053
2.4150	.432	.053
2.5050	.446	.053
2.6100	.459	.053
2.6850	.473	.053
2.8050	.486	.053
2.9500	.500	.053
3.0700	.514	.053
3.1500	.527	.053
3.2300	.541	.053
3.3250	.554	.053
3.3900	.554	.067
3.4950	.568	.067
3.5700	.581	.067

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3.6950	.595	.093
3.8200	.608	.093
3.8700	.622	.107
3.9150	.635	.107
3.9750	.635	.120
4.0400	.635	.133
4.0600	.649	.133
4.1200	.649	.147
4.1950	.662	.147
4.2450	.676	.147
4.2800	.676	.160
4.3400	.689	.160
4.4050	.703	.160
4.4600	.703	.173
4.5300	.703	.187
4.6100	.716	.200
4.6650	.716	.213
4.6800	.730	.213
4.7350	.743	.213
4.7900	.757	.213
4.8050	**.757**	**.227**
4.8200	.757	.240
4.8500	.770	.253
4.9350	.770	.267
5.0200	.770	.280
5.0450	.770	.320
5.0650	.770	.333
5.1350	.770	.347
5.1950	.770	.373
5.2600	.770	.387
5.3450	.784	.387
5.3850	.784	.400
5.4200	.784	.427
5.4550	.784	.440
5.4850	.784	.453
5.5100	.784	.467
5.5550	.784	.480
5.6300	.784	.493

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5.6750	.797	.507
5.7550	.797	.520
5.8950	.797	.533
5.9900	.811	.533
6.0350	.811	.547
6.0700	.811	.560
6.1050	.824	.560
6.1450	.824	.587
6.1800	.838	.587
6.2950	.838	.600
6.4350	.838	.613
6.4850	.838	.627
6.6050	.838	.640
6.7350	.851	.640
6.7950	.851	.653
6.8800	.878	.667
6.9550	.878	.680
6.9900	.878	.693
7.0200	.878	.733
7.1750	.878	.747
7.3200	.878	.760
7.3400	.878	.773
7.5050	.892	.773
7.6800	.905	.773
7.7050	.905	.787
7.8550	.905	.800
8.1300	.905	.813
8.3400	.905	.827
8.4750	.905	.840
8.6000	.919	.840
8.7500	.919	.853
8.9150	.919	.867
9.0350	.932	.867
9.2100	.932	.880
9.4250	.946	.880
9.5450	.959	.880
9.7800	.959	.893
9.9850	.959	.907

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10.1650	.959	.920
10.4450	.959	.933
10.8650	.959	.947
11.5600	.973	.947
12.3000	.973	.960
13.1050	.986	.960
13.7200	1.000	.960
15.5050	1.000	.973
23.6650	1.000	.987
31.2000	1.000	1.000

The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

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Reporting checklist for diagnostic test accuracy study.

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		Reporting Item	Page Number
Title or abstract			
None	#1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	10
Abstract			
None	#2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts https://www.equator-network.org/reporting-guidelines/stard-abstracts/)	2
Introduction			
None	#3	Scientific and clinical background, including the intended use and clinical role of the index test	3

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None	#4	Study objectives and hypotheses	6
Methods			
Study design	#5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6
Participants	#6	Eligibility criteria	6
Participants	#7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	6
Participants	#8	Where and when potentially eligible participants were identified (setting, location and dates)	6
Participants	#9	Whether participants formed a consecutive, random or convenience series	6
Test methods	#10	Index and reference tests in sufficient detail to allow replication	7,8
Test methods	#11	Rationale for choosing the reference standard (if alternatives exist)	5
Test methods	#12	Definition of and rationale for test positivity cut-offs or result categories of the index and reference tests, distinguishing pre-specified from exploratory	10-18
Test methods	#13	Whether clinical information and reference standard results were available to the performers / readers of the index test; Whether clinical information and index test results were available to the assessors of the reference standard	8
Analysis	#14	Methods for estimating or comparing measures of diagnostic accuracy	10-18
Analysis	#15	How indeterminate index test or reference standard results were handled	n/a; no indeterminate variable
Analysis	#16	How missing data on the index test and reference standard were handled	n/a, no

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			missing variable
Analysis	#17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	10-18
Analysis	#18	Intended sample size and how it was determined	6
Results			
Participants	#19	Flow of participants, using a diagram	n/a; cross-sectional study design and independent participation
Participants	#20	Baseline demographic and clinical characteristics of participants	9
Participants	#21	Distribution of severity of disease in those with the target condition, and distribution of alternative diagnoses in those without the target condition	11,15
Participants	#22	Time interval and any clinical interventions between index test and reference standard	n/a; no clinical intervention
Test results	#23	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	9,14
Test results	#24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	9
Test results	#25	Any adverse events from performing the index test or the reference standard	n/a; no adverse events
Discussion			
None	#26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	21
None	#27	Implications for practice, including the intended use and clinical role of the index test	21

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Other information			
None	#28	Registration number and name of registry	n/a; cross-sectional observational study
None	#29	Where the full study protocol can be accessed	n/a- completely detailed in the study
None	#30	Sources of funding and other support; role of funders	22

Notes:

- 15: n/a; no indeterminate variable
- 16: n/a, no missing variable
- 19: n/a; cross-sectional study design and independent participation
- 22: n/a; no clinical intervention
- 25: n/a; no adverse events
- 28: n/a; cross-sectional observational study
- 29: n/a- completely detailed in the study The STARD checklist is distributed under the terms of the Creative Commons Attribution License CC-BY. This checklist was completed on 10. November 2023 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

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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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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

Dr. Sujaya Chattopadhyay [a], Dr. Soumik Goswami [b], Dr. Nilanjan Sengupta [c], Dr. Arjun Baidya[d]

[a] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; 97.sujaya@gmail.com

[b] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; dr.soumikgoswami@gmail.com

[c] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; drnilanjansengupta@gmail.com

[d] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; arjun.baidya@gmail.com

CORRESPONDING AUTHOR

Dr. Sujaya Chattopadhyay

97.sujaya@gmail.com

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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 >25V with 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. A p value <0.05 was considered as statistically significant.

Results: The timed tuning fork 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 seconds was 76% sensitive and 77% specific in diagnosing mild neuropathy while absent tuning fork sensation

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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 seconds can be a useful indicator of the early stages of onset of the condition.

Strengths and limitations:

- This paper highlights the utility of a simple bedside test for diagnosing and grading the severity of diabetic peripheral neuropathy.
- This study evaluates the diagnostic test both qualitatively and quantitatively.
- Limitation of the study includes establishment of correlation rather than causation and lack of HbA1c to assess the glycemic 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

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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, have led to under diagnoses 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 pin prick test and temperature testing is an indirect indicator of the transmission through the small fibre spino-thalamic 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

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studies exploring the reliability of the pin prick test demonstrated its weaker performance than the VPT and the tuning fork test(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 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(23), (24), (8), (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 regarding the biothesiometer.

Research Design and Methods:

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The objective of our study was to determine a cut-off (in seconds) for the tuning fork test to detect diabetic peripheral neuropathy 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 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 or type 1 diabetes mellitus for at least 5 years. Exclusion criteria included patients with prediabetes, 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.)

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

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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. Voltage began at zero and was then manually increased 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 128Hz 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. Participants also instructed to state "now" when they

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stopped feeling the vibration after providing a “yes” response when they first felt a vibratory sensation. 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 upon 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 Version 26 (IBM, Chicago). Correlations were assessed with 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 was determined using receiver operating characteristic (ROC) curve, 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 STARD checklist when writing our report(30).

Patient and Public involvement:

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Consenting patients were involved in the conduct of the research from design till analysis, recruited according to the inclusion and exclusion criteria. 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 SD in Table 1.

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

	MEAN±S.D.
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

*Values in SI. M, Males; F, Females; DM, Diabetes Mellitus; BMI, body mass index; FPG, fasting plasma glucose; PPPG, post-

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prandial plasma glucose (FPG was collected after 8 hours of overnight fasting and PPPG was collected 2 hours after the start of a meal. The samples were collected via venipuncture in a fluoride oxalate tube)

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

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

		Timed Tuning Fork (seconds)	VPT (volts)
Timed Tuning Fork (seconds)	Pearson Correlation	1	-.500**
	Sig. (2-tailed)		.000
	N	149	149
VPT (volts)	Pearson Correlation	-.500**	1
	Sig. (2-tailed)	.000	
	N	149	149

**. Correlation is significant at the 0.01 level (2-tailed).

Taking 25V score on the VPT as the criterion for severe diabetic neuropathy, a timed tuning fork value of 0 second had 70% sensitivity and 90% specificity for diagnosing the same (Supplementary table 1, Supplementary table 2 in Appendix). It had a positive predictive value of 33.6% and a negative predictive value of 97.7% for severe DPN.

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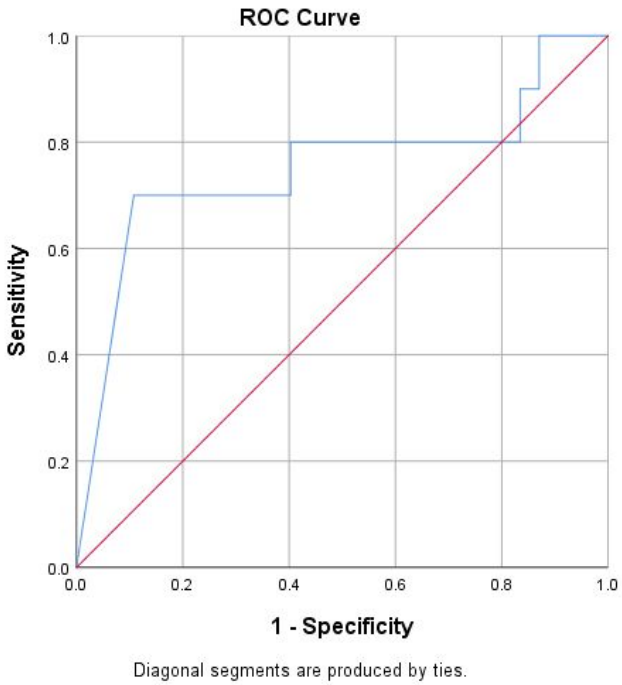

Using a ROC curve, a timed tuning fork value of 4.8 seconds showed 76% sensitivity and 77% specificity for detection of mild diabetic peripheral neuropathy using a VPT score above 15V score as an indicator of the same (Supplementary table 3, Supplementary table 4 in appendix). It had a positive predictive value of 76% and a negative predictive value of 76.9% for mild DPN detection.

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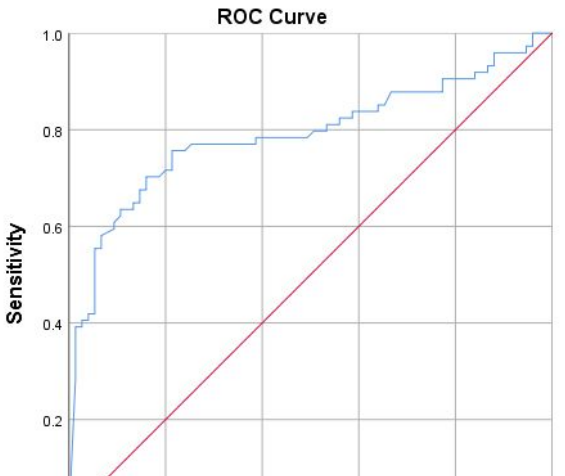

The maximum sum of sensitivity and specificity was chosen as the cut off at which point the Youden's index (Se + Sp – 1) was also maximum.

Discussion

The tests considered for assessment of diabetic peripheral neuropathy in our study were the 128Hz 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.

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The grading of VPT scores is done as follows: Normal: ≤15 V, Grade I neuropathy: 16-25V and Grade II neuropathy: ≥25V (33), (34). A study found grade I severity in approximately 27% of patients with clinical neuropathy and in 50% asymptomatic patients (34), which indicates presence of subclinical neuropathic damage. In another study, nerve pain was experienced by the study population from a VPT score as low as 16V (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 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 seconds 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 seconds and ≤4 seconds have been shown to be a risk factor for lower limb injuries (39), and foot ulceration (40), respectively.

Taking 25V 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

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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(41) , (42). The 5.07 (10g) monofilament test is the most recent recommendation for the detection of diabetic neuropathy by the American Diabetes Association (43). However, a study(44) 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 seconds 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 early 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 seconds) 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 (45), (38), (46), (33). However, it is an expensive machine, needs electricity to operate and is quite difficult to procure in primary health care and rural settings. The entire procedure demands a significant amount of time which can be quite

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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 (47). The tuning fork has been shown to be considerably quicker than the VPT measurement (48). 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. Thus, 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(49). We also appreciate the cross-sectional study design allows for demonstration of correlation, more than causality. Another limitation was the lack of HbA1c to assess the glycemic 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.

List of abbreviations

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VPT- Vibration perception threshold; g- gram; V-volt; Hz-Hertz; Std-Standard; Sig-Significance; DPN- Diabetic peripheral neuropathy; ROC- Receiver operator characteristic; NCS-Nerve conduction studies

Declarations

Ethics approval and consent to participate

After deliberations and review the Institutional Ethics Committee, Nil Ratan Sircar Medical College and Hospital, took the decision "APPROVED" regarding the study proposal (Memo No. NRSMC/IEC/18/2022).

Consent for publication

Informed consent was obtained from all the participants by the principal author.

Sources of funding

None

Data availability statement

Available upon request in the form of Microsoft Excel spreadsheets.

Contributorship statement:

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SC: Data Collection, manuscript initial draft preparation, correspondence, ethical approval; SG: Analysis, Final draft preparation; NS: Supervision, proof-reading; AB: Supervision, proof reading

Competing interests:

None

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APPENDIX:

Area Under the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.751	.103	.008	.550	.953

The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Supplementary Table 1: Area under the curve for VPT>25V

Coordinates of the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

Positive if Less Than or Equal To[a]	Sensitivity	1 – Specificity
-1.0000	.000	.000
.2050	.700	.108
.5000	.700	.115
.8350	.700	.122
1.1500	.700	.129
1.2550	.700	.137
1.3150	.700	.144
1.5450	.700	.151
1.7600	.700	.158
1.8150	.700	.165
1.9100	.700	.173
1.9950	.700	.180
2.0750	.700	.187
2.1750	.700	.194
2.3050	.700	.201
2.4150	.700	.209
2.5050	.700	.216
2.6100	.700	.223
2.6850	.700	.230
2.8050	.700	.237

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2.9500	.700	.245
3.0700	.700	.252
3.1500	.700	.259
3.2300	.700	.266
3.3250	.700	.273
3.3900	.700	.281
3.4950	.700	.288
3.5700	.700	.295
3.6950	.700	.317
3.8200	.700	.324
3.8700	.700	.338
3.9150	.700	.345
3.9750	.700	.353
4.0400	.700	.360
4.0600	.700	.367
4.1200	.700	.374
4.1950	.700	.381
4.2450	.700	.388
4.2800	.700	.396
4.3400	.700	.403
4.4050	.800	.403
4.4600	.800	.410
4.5300	.800	.417
4.6100	.800	.432
4.6650	.800	.439
4.6800	.800	.446
4.7350	.800	.453
4.7900	.800	.460
4.8050	.800	.468
4.8200	.800	.475
4.8500	.800	.489
4.9350	.800	.496
5.0200	.800	.504
5.0450	.800	.525
5.0650	.800	.532
5.1350	.800	.540
5.1950	.800	.554
5.2600	.800	.561
5.3450	.800	.568
5.3850	.800	.576

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5.4200	.800	.590
5.4550	.800	.597
5.4850	.800	.604
5.5100	.800	.612
5.5550	.800	.619
5.6300	.800	.626
5.6750	.800	.640
5.7550	.800	.647
5.8950	.800	.655
5.9900	.800	.662
6.0350	.800	.669
6.0700	.800	.676
6.1050	.800	.683
6.1450	.800	.698
6.1800	.800	.705
6.2950	.800	.712
6.4350	.800	.719
6.4850	.800	.727
6.6050	.800	.734
6.7350	.800	.741
6.7950	.800	.748
6.8800	.800	.770
6.9550	.800	.777
6.9900	.800	.784
7.0200	.800	.806
7.1750	.800	.813
7.3200	.800	.820
7.3400	.800	.827
7.5050	.800	.835
7.6800	.900	.835
7.7050	.900	.842
7.8550	.900	.849
8.1300	.900	.856
8.3400	.900	.863
8.4750	.900	.871
8.6000	1.000	.871
8.7500	1.000	.878
8.9150	1.000	.885
9.0350	1.000	.892
9.2100	1.000	.899

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9.4250	1.000	.906
9.5450	1.000	.914
9.7800	1.000	.921
9.9850	1.000	.928
10.1650	1.000	.935
10.4450	1.000	.942
10.8650	1.000	.950
11.5600	1.000	.957
12.3000	1.000	.964
13.1050	1.000	.971
13.7200	1.000	.978
15.5050	1.000	.986
23.6650	1.000	.993
31.2000	1.000	1.000

The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cut-off value is the minimum observed test value minus 1, and the largest cut-off value is the maximum observed test value plus 1. All the other cut-off values are the averages of two consecutive ordered observed test values.

Supplementary Table 2: Co-ordinates of the curve for VPT >25V

Area Under the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

			Asymptotic 95% Confidence Interval	
Area	Std. Error[a]	Asymptotic Sig.[b]	Lower Bound	Upper Bound
.789	.039	.000	.713	.866

The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Supplementary Table 3: Area under the curve for VPT>15V

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Coordinates of the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

Positive if Less Than or Equal To[a]	Sensitivity	1 – Specificity
-1.0000	.000	.000
.2050	.284	.013
.5000	.297	.013
.8350	.311	.013
1.1500	.324	.013
1.2550	.338	.013
1.3150	.351	.013
1.5450	.365	.013
1.7600	.378	.013
1.8150	.392	.013
1.9100	.392	.027
1.9950	.405	.027
2.0750	.405	.040
2.1750	.419	.040
2.3050	.419	.053
2.4150	.432	.053
2.5050	.446	.053
2.6100	.459	.053
2.6850	.473	.053
2.8050	.486	.053
2.9500	.500	.053
3.0700	.514	.053
3.1500	.527	.053
3.2300	.541	.053
3.3250	.554	.053
3.3900	.554	.067
3.4950	.568	.067
3.5700	.581	.067
3.6950	.595	.093
3.8200	.608	.093
3.8700	.622	.107
3.9150	.635	.107
3.9750	.635	.120
4.0400	.635	.133

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4.0600	.649	.133
4.1200	.649	.147
4.1950	.662	.147
4.2450	.676	.147
4.2800	.676	.160
4.3400	.689	.160
4.4050	.703	.160
4.4600	.703	.173
4.5300	.703	.187
4.6100	.716	.200
4.6650	.716	.213
4.6800	.730	.213
4.7350	.743	.213
4.7900	.757	.213
4.8050	**.757**	**.227**
4.8200	.757	.240
4.8500	.770	.253
4.9350	.770	.267
5.0200	.770	.280
5.0450	.770	.320
5.0650	.770	.333
5.1350	.770	.347
5.1950	.770	.373
5.2600	.770	.387
5.3450	.784	.387
5.3850	.784	.400
5.4200	.784	.427
5.4550	.784	.440
5.4850	.784	.453
5.5100	.784	.467
5.5550	.784	.480
5.6300	.784	.493
5.6750	.797	.507
5.7550	.797	.520
5.8950	.797	.533
5.9900	.811	.533
6.0350	.811	.547
6.0700	.811	.560
6.1050	.824	.560
6.1450	.824	.587

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6.1800	.838	.587
6.2950	.838	.600
6.4350	.838	.613
6.4850	.838	.627
6.6050	.838	.640
6.7350	.851	.640
6.7950	.851	.653
6.8800	.878	.667
6.9550	.878	.680
6.9900	.878	.693
7.0200	.878	.733
7.1750	.878	.747
7.3200	.878	.760
7.3400	.878	.773
7.5050	.892	.773
7.6800	.905	.773
7.7050	.905	.787
7.8550	.905	.800
8.1300	.905	.813
8.3400	.905	.827
8.4750	.905	.840
8.6000	.919	.840
8.7500	.919	.853
8.9150	.919	.867
9.0350	.932	.867
9.2100	.932	.880
9.4250	.946	.880
9.5450	.959	.880
9.7800	.959	.893
9.9850	.959	.907
10.1650	.959	.920
10.4450	.959	.933
10.8650	.959	.947
11.5600	.973	.947
12.3000	.973	.960
13.1050	.986	.960
13.7200	1.000	.960
15.5050	1.000	.973
23.6650	1.000	.987
31.2000	1.000	1.000

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The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cut-off value is the minimum observed test value minus 1, and the largest cut-off value is the maximum observed test value plus 1. All the other cut-off values are the averages of two consecutive ordered observed test values.

Supplementary Table 4: Co-ordinates of the curve for VPT>15V

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		Reporting Item	Page Number
Title or abstract			
None	#1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	10
Abstract			
None	#2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts https://www.equator-network.org/reporting-guidelines/stard-abstracts/)	2
Introduction			
None	#3	Scientific and clinical background, including the intended use and clinical role of the index test	3

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None	#4	Study objectives and hypotheses	6
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Study design	#5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6
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Participants	#9	Whether participants formed a consecutive, random or convenience series	6
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Test methods	#13	Whether clinical information and reference standard results were available to the performers / readers of the index test; Whether clinical information and index test results were available to the assessors of the reference standard	8
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Analysis	#15	How indeterminate index test or reference standard results were handled	n/a; no indeterminate variable
Analysis	#16	How missing data on the index test and reference standard were handled	n/a, no

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			missing variable
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Other information			
None	#28	Registration number and name of registry	n/a; cross-sectional observational study
None	#29	Where the full study protocol can be accessed	n/a- completely detailed in the study
None	#30	Sources of funding and other support; role of funders	22

Notes:

- 15: n/a; no indeterminate variable
- 16: n/a, no missing variable
- 19: n/a; cross-sectional study design and independent participation
- 22: n/a; no clinical intervention
- 25: n/a; no adverse events
- 28: n/a; cross-sectional observational study
- 29: n/a- completely detailed in the study The STARD checklist is distributed under the terms of the Creative Commons Attribution License CC-BY. This checklist was completed on 10. November 2023 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

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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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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

Dr. Sujaya Chattopadhyay [a], Dr. Soumik Goswami [b], Dr. Nilanjan Sengupta [c], Dr. Arjun Baidya[d]

[a] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; 97.sujaya@gmail.com

[b] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; dr.soumikgoswami@gmail.com

[c] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; drnilanjansengupta@gmail.com

[d] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; arjun.baidya@gmail.com

CORRESPONDING AUTHOR

Dr. Sujaya Chattopadhyay

97.sujaya@gmail.com

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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 >25V with 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. A p value <0.05 was considered as statistically significant.

Results: The timed tuning fork 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 seconds was 76% sensitive and 77% specific in diagnosing mild neuropathy while absent tuning fork sensation

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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 seconds can be a useful indicator of the early stages of onset of the condition.

Strengths and limitations:

- The study population 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 HbA1c to assess the glycemic 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

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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, have led to under diagnoses 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 pin prick test and temperature testing is an indirect indicator of the transmission through the small fibre spino-thalamic 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

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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 test(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 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(23), (24), (8), (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 regarding the biothesiometer.

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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 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 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 or type 1 diabetes mellitus for at least 5 years. Exclusion criteria included patients with prediabetes, 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 (FPG) was collected after 8 hours of overnight fasting and post-prandial plasma glucose (PPPG) was collected 2 hours after the start of a meal. The samples were collected via venipuncture in a fluoride oxalate tube.

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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. Voltage began at zero and was then manually increased 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 128Hz 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

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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. Participants also instructed to state "now" when they stopped feeling the vibration after providing a "yes" response when they first felt a vibratory sensation. 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 upon 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 Version 26 (IBM, Chicago). Correlations were assessed with 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 was determined using receiver operating characteristic (ROC) curve, 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.

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We used the STARD checklist when writing our report(30).

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 SD in Table 1.

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

	MEAN±S.D.
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

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FPG(mg/dl)	167.10±78.64
PPPG(mg/dl)	251.35±118.56

*Values in SI. M, Males; F, Females; DM, Diabetes Mellitus; BMI, body mass index; FPG, fasting plasma glucose; PPPG, post-prandial plasma glucose

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

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

Table 2: Correlation between timed tuning fork and VPT

		Timed Tuning Fork (seconds)	VPT (volts)
Timed Tuning Fork (seconds)	Pearson Correlation	1	-.500**
	Sig. (2-tailed)		.000
	N	149	149
VPT (volts)	Pearson Correlation	-.500**	1
	Sig. (2-tailed)	.000	
	N	149	149

**. Correlation is significant at the 0.01 level (2-tailed).

Taking 25V score on the VPT as the criterion for severe diabetic neuropathy, a timed tuning fork value of 0 second had 70% sensitivity and 90% specificity for diagnosing the same (Supplementary table 1, Supplementary table 2 in Appendix). It had a positive predictive value of 33.6% and a negative

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predictive value of 97.7% for severe DPN [0.751(0.550,0.953)]. The ROC curve is depicted in Figure 1.

Using a ROC curve (Figure 2), a timed tuning fork value of 4.8 seconds showed 76% sensitivity and 77% specificity for detection of mild diabetic peripheral neuropathy using a VPT score above 15V score as an indicator of the same (Supplementary table 3, Supplementary table 4 in appendix). 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 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 128Hz 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.

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The grading of VPT scores is done as follows: Normal: ≤15 V, Grade I neuropathy: 16-25V and Grade II neuropathy: ≥25V (33), (34). A study found grade I severity in approximately 27% of patients with clinical neuropathy and in 50% asymptomatic patients (34), which indicates presence of subclinical neuropathic damage. In another study, nerve pain was experienced by the study population from a VPT score as low as 16V (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 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 seconds 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 seconds and ≤4 seconds have been shown to be a risk factor for lower limb injuries (39), and foot ulceration (40), respectively.

Taking 25V 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

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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(41) , (42). The 5.07 (10g) monofilament test is the most recent recommendation for the detection of diabetic neuropathy by the American Diabetes Association (43). However, a study(44) 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 seconds 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 early 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 seconds) 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 (45), (38), (46), (33). However, it is an expensive machine, needs electricity to operate and is quite difficult to procure in primary health care and rural settings. The entire procedure demands a significant amount of time which can be quite

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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 (47). The tuning fork has been shown to be considerably quicker than the VPT measurement (48). 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. Thus, 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(49). We also appreciate the cross-sectional study design allows for demonstration of correlation, more than causality. Another limitation was the lack of HbA1c to assess the glycemic 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.

List of abbreviations

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VPT- Vibration perception threshold; g- gram; V-volt; Hz-Hertz; Std-Standard; Sig-Significance; DPN- Diabetic peripheral neuropathy; ROC- Receiver operator characteristic; NCS-Nerve conduction studies

Declarations

Ethics approval and consent to participate

After deliberations and review the Institutional Ethics Committee, Nil Ratan Sircar Medical College and Hospital, took the decision "APPROVED" regarding the study proposal (Memo No. NRSMC/IEC/18/2022).

Consent for publication

Informed consent was obtained from all the participants by the principal author.

Sources of funding

None

Data availability statement

Deidentified participant data is 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

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and translated questionnaires, informed consent, and participant information sheets.

Contributorship statement:

SC: Data Collection, manuscript initial draft preparation, correspondence, ethical approval; SG: Analysis, Final draft preparation; NS: Supervision, proof-reading; AB: Supervision, proof reading

Competing interests:

None

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FIGURE LEGEND:

Figure 1: ROC curve for VPT>25V

Figure 2: ROC curve for VPT>15V

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Figure 1: ROC curve for VPT>25V

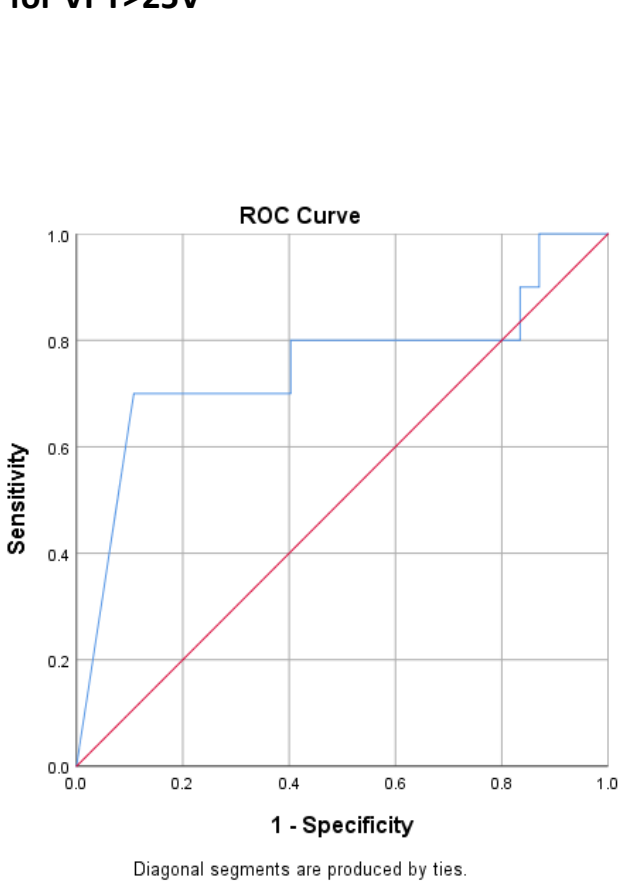

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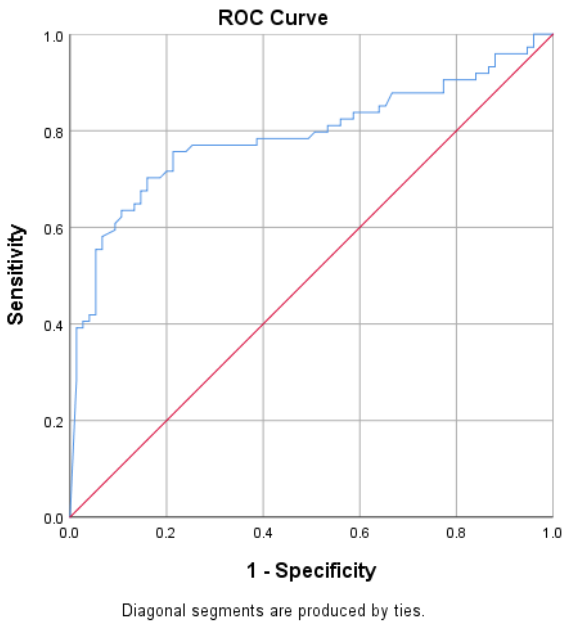
Figure 2: ROC curve for VPT>15V



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APPENDIX:

Area Under the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.751	.103	.008	.550	.953

The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Supplementary Table 1: Area under the curve for VPT>25V

Coordinates of the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

Positive if Less Than or Equal To[a]	Sensitivity	1 – Specificity
-1.0000	.000	.000
.2050	.700	.108
.5000	.700	.115
.8350	.700	.122
1.1500	.700	.129
1.2550	.700	.137
1.3150	.700	.144
1.5450	.700	.151
1.7600	.700	.158
1.8150	.700	.165
1.9100	.700	.173
1.9950	.700	.180
2.0750	.700	.187
2.1750	.700	.194
2.3050	.700	.201
2.4150	.700	.209
2.5050	.700	.216
2.6100	.700	.223
2.6850	.700	.230
2.8050	.700	.237

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2.9500	.700	.245
3.0700	.700	.252
3.1500	.700	.259
3.2300	.700	.266
3.3250	.700	.273
3.3900	.700	.281
3.4950	.700	.288
3.5700	.700	.295
3.6950	.700	.317
3.8200	.700	.324
3.8700	.700	.338
3.9150	.700	.345
3.9750	.700	.353
4.0400	.700	.360
4.0600	.700	.367
4.1200	.700	.374
4.1950	.700	.381
4.2450	.700	.388
4.2800	.700	.396
4.3400	.700	.403
4.4050	.800	.403
4.4600	.800	.410
4.5300	.800	.417
4.6100	.800	.432
4.6650	.800	.439
4.6800	.800	.446
4.7350	.800	.453
4.7900	.800	.460
4.8050	.800	.468
4.8200	.800	.475
4.8500	.800	.489
4.9350	.800	.496
5.0200	.800	.504
5.0450	.800	.525
5.0650	.800	.532
5.1350	.800	.540
5.1950	.800	.554
5.2600	.800	.561
5.3450	.800	.568
5.3850	.800	.576

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5.4200	.800	.590
5.4550	.800	.597
5.4850	.800	.604
5.5100	.800	.612
5.5550	.800	.619
5.6300	.800	.626
5.6750	.800	.640
5.7550	.800	.647
5.8950	.800	.655
5.9900	.800	.662
6.0350	.800	.669
6.0700	.800	.676
6.1050	.800	.683
6.1450	.800	.698
6.1800	.800	.705
6.2950	.800	.712
6.4350	.800	.719
6.4850	.800	.727
6.6050	.800	.734
6.7350	.800	.741
6.7950	.800	.748
6.8800	.800	.770
6.9550	.800	.777
6.9900	.800	.784
7.0200	.800	.806
7.1750	.800	.813
7.3200	.800	.820
7.3400	.800	.827
7.5050	.800	.835
7.6800	.900	.835
7.7050	.900	.842
7.8550	.900	.849
8.1300	.900	.856
8.3400	.900	.863
8.4750	.900	.871
8.6000	1.000	.871
8.7500	1.000	.878
8.9150	1.000	.885
9.0350	1.000	.892
9.2100	1.000	.899

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9.4250	1.000	.906
9.5450	1.000	.914
9.7800	1.000	.921
9.9850	1.000	.928
10.1650	1.000	.935
10.4450	1.000	.942
10.8650	1.000	.950
11.5600	1.000	.957
12.3000	1.000	.964
13.1050	1.000	.971
13.7200	1.000	.978
15.5050	1.000	.986
23.6650	1.000	.993
31.2000	1.000	1.000

The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cut-off value is the minimum observed test value minus 1, and the largest cut-off value is the maximum observed test value plus 1. All the other cut-off values are the averages of two consecutive ordered observed test values.

Supplementary Table 2: Co-ordinates of the curve for VPT >25V

Area Under the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

			Asymptotic 95% Confidence Interval	
Area	Std. Error[a]	Asymptotic Sig.[b]	Lower Bound	Upper Bound
.789	.039	.000	.713	.866

The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Supplementary Table 3: Area under the curve for VPT>15V

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Coordinates of the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

Positive if Less Than or Equal To[a]	Sensitivity	1 – Specificity
-1.0000	.000	.000
.2050	.284	.013
.5000	.297	.013
.8350	.311	.013
1.1500	.324	.013
1.2550	.338	.013
1.3150	.351	.013
1.5450	.365	.013
1.7600	.378	.013
1.8150	.392	.013
1.9100	.392	.027
1.9950	.405	.027
2.0750	.405	.040
2.1750	.419	.040
2.3050	.419	.053
2.4150	.432	.053
2.5050	.446	.053
2.6100	.459	.053
2.6850	.473	.053
2.8050	.486	.053
2.9500	.500	.053
3.0700	.514	.053
3.1500	.527	.053
3.2300	.541	.053
3.3250	.554	.053
3.3900	.554	.067
3.4950	.568	.067
3.5700	.581	.067
3.6950	.595	.093
3.8200	.608	.093
3.8700	.622	.107
3.9150	.635	.107
3.9750	.635	.120
4.0400	.635	.133

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4.0600	.649	.133
4.1200	.649	.147
4.1950	.662	.147
4.2450	.676	.147
4.2800	.676	.160
4.3400	.689	.160
4.4050	.703	.160
4.4600	.703	.173
4.5300	.703	.187
4.6100	.716	.200
4.6650	.716	.213
4.6800	.730	.213
4.7350	.743	.213
4.7900	.757	.213
4.8050	**.757**	**.227**
4.8200	.757	.240
4.8500	.770	.253
4.9350	.770	.267
5.0200	.770	.280
5.0450	.770	.320
5.0650	.770	.333
5.1350	.770	.347
5.1950	.770	.373
5.2600	.770	.387
5.3450	.784	.387
5.3850	.784	.400
5.4200	.784	.427
5.4550	.784	.440
5.4850	.784	.453
5.5100	.784	.467
5.5550	.784	.480
5.6300	.784	.493
5.6750	.797	.507
5.7550	.797	.520
5.8950	.797	.533
5.9900	.811	.533
6.0350	.811	.547
6.0700	.811	.560
6.1050	.824	.560
6.1450	.824	.587

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6.1800	.838	.587
6.2950	.838	.600
6.4350	.838	.613
6.4850	.838	.627
6.6050	.838	.640
6.7350	.851	.640
6.7950	.851	.653
6.8800	.878	.667
6.9550	.878	.680
6.9900	.878	.693
7.0200	.878	.733
7.1750	.878	.747
7.3200	.878	.760
7.3400	.878	.773
7.5050	.892	.773
7.6800	.905	.773
7.7050	.905	.787
7.8550	.905	.800
8.1300	.905	.813
8.3400	.905	.827
8.4750	.905	.840
8.6000	.919	.840
8.7500	.919	.853
8.9150	.919	.867
9.0350	.932	.867
9.2100	.932	.880
9.4250	.946	.880
9.5450	.959	.880
9.7800	.959	.893
9.9850	.959	.907
10.1650	.959	.920
10.4450	.959	.933
10.8650	.959	.947
11.5600	.973	.947
12.3000	.973	.960
13.1050	.986	.960
13.7200	1.000	.960
15.5050	1.000	.973
23.6650	1.000	.987
31.2000	1.000	1.000

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The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cut-off value is the minimum observed test value minus 1, and the largest cut-off value is the maximum observed test value plus 1. All the other cut-off values are the averages of two consecutive ordered observed test values.

Supplementary Table 4: Co-ordinates of the curve for VPT>15V

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Reporting checklist for diagnostic test accuracy study.

Based on the STARD guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STARDreporting guidelines, and cite them as:

Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, LijmerJG Moher D, Rennie D, de Vet HCW, Kressel HY, Rifai N, Golub RM, Altman DG, Hooft L, Korevaar DA, Cohen JF, For the STARD Group. STARD 2015: An Updated List of Essential Items for Reporting Diagnostic Accuracy Studies.

		Reporting Item	Page Number
Title or abstract			
None	#1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	10
Abstract			
None	#2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts https://www.equator-network.org/reporting-guidelines/stard-abstracts/)	2
Introduction			
None	#3	Scientific and clinical background, including the intended use and clinical role of the index test	3

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None	#4	Study objectives and hypotheses	6
Methods			
Study design	#5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6
Participants	#6	Eligibility criteria	6
Participants	#7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	6
Participants	#8	Where and when potentially eligible participants were identified (setting, location and dates)	6
Participants	#9	Whether participants formed a consecutive, random or convenience series	6
Test methods	#10	Index and reference tests in sufficient detail to allow replication	7,8
Test methods	#11	Rationale for choosing the reference standard (if alternatives exist)	5
Test methods	#12	Definition of and rationale for test positivity cut-offs or result categories of the index and reference tests, distinguishing pre-specified from exploratory	10-18
Test methods	#13	Whether clinical information and reference standard results were available to the performers / readers of the index test; Whether clinical information and index test results were available to the assessors of the reference standard	8
Analysis	#14	Methods for estimating or comparing measures of diagnostic accuracy	10-18
Analysis	#15	How indeterminate index test or reference standard results were handled	n/a; no indeterminate variable
Analysis	#16	How missing data on the index test and reference standard were handled	n/a, no

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			missing variable
Analysis	#17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	10-18
Analysis	#18	Intended sample size and how it was determined	6
Results			
Participants	#19	Flow of participants, using a diagram	n/a; cross-sectional study design and independent participation
Participants	#20	Baseline demographic and clinical characteristics of participants	9
Participants	#21	Distribution of severity of disease in those with the target condition, and distribution of alternative diagnoses in those without the target condition	11,15
Participants	#22	Time interval and any clinical interventions between index test and reference standard	n/a; no clinical intervention
Test results	#23	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	9,14
Test results	#24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	9
Test results	#25	Any adverse events from performing the index test or the reference standard	n/a; no adverse events
Discussion			
None	#26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	21
None	#27	Implications for practice, including the intended use and clinical role of the index test	21

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Other information			
None	#28	Registration number and name of registry	n/a; cross-sectional observational study
None	#29	Where the full study protocol can be accessed	n/a- completely detailed in the study
None	#30	Sources of funding and other support; role of funders	22

Notes:

- 15: n/a; no indeterminate variable
- 16: n/a, no missing variable
- 19: n/a; cross-sectional study design and independent participation
- 22: n/a; no clinical intervention
- 25: n/a; no adverse events
- 28: n/a; cross-sectional observational study
- 29: n/a- completely detailed in the study The STARD checklist is distributed under the terms of the Creative Commons Attribution License CC-BY. This checklist was completed on 10. November 2023 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

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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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Complete List of Authors:	Chattopadhyay, Sujaya; Portsmouth Hospitals University NHS Trust Goswami, Soumik; Nilratan Sircar Medical College, Department of Endocrinology Sengupta, Nilanjan; Nilratan Sircar Medical College, Department of Endocrinology Baidya, Arjun; Nilratan Sircar Medical College, Department of Endocrinology
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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

Dr. Sujaya Chattopadhyay [a], Dr. Soumik Goswami [b], Dr. Nilanjan Sengupta [c], Dr. Arjun Baidya[d]

[a] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; 97.sujaya@gmail.com

[b] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; dr.soumikgoswami@gmail.com

[c] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; drnilanjansengupta@gmail.com

[d] Department of Endocrinology, Nil Ratan Sircar Medical College and Hospital, 138, Acharya Jagadish Chandra Bose Road, Kolkata 700014, West Bengal, India; arjun.baidya@gmail.com

CORRESPONDING AUTHOR

Dr. Sujaya Chattopadhyay

97.sujaya@gmail.com

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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 >25V with 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. A p value <0.05 was considered as statistically significant.

Results: The timed tuning fork 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 seconds was 76% sensitive and 77% specific in diagnosing mild neuropathy while absent tuning fork sensation

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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 seconds can be a useful indicator of the early stages of onset of the condition.

Strengths and limitations:

- The study population 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 HbA1c to assess the glycemic 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

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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, have led to under diagnoses 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 pin prick test and temperature testing is an indirect indicator of the transmission through the small fibre spino-thalamic 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

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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 test(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 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(23), (24), (8), (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 regarding the biothesiometer.

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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 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 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 or type 1 diabetes mellitus for at least 5 years. Exclusion criteria included patients with prediabetes, 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 (FPG) was collected after 8 hours of overnight fasting and post-prandial plasma glucose (PPPG) was collected 2 hours after the start of a meal. The samples were collected via venipuncture in a fluoride oxalate tube.

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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. Voltage began at zero and was then manually increased 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 128Hz 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

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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. Participants also instructed to state "now" when they stopped feeling the vibration after providing a "yes" response when they first felt a vibratory sensation. 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 upon 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 Version 26 (IBM, Chicago). Correlations were assessed with 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 was determined using receiver operating characteristic (ROC) curve, 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.

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We used the STARD checklist when writing our report(30).

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 SD in Table 1.

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

	MEAN±S.D.
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

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FPG(mg/dl)	167.10±78.64
PPPG(mg/dl)	251.35±118.56

*Values in SI. M, Males; F, Females; DM, Diabetes Mellitus; BMI, body mass index; FPG, fasting plasma glucose; PPPG, post-prandial plasma glucose

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

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

Table 2: Correlation between timed tuning fork and VPT

		Timed Tuning Fork (seconds)	VPT (volts)
Timed Tuning Fork (seconds)	Pearson Correlation	1	-.500**
	Sig. (2-tailed)		.000
	N	149	149
VPT (volts)	Pearson Correlation	-.500**	1
	Sig. (2-tailed)	.000	
	N	149	149

**. Correlation is significant at the 0.01 level (2-tailed).

Taking 25V score on the VPT as the criterion for severe diabetic neuropathy, a timed tuning fork value of 0 second had 70% sensitivity and 90% specificity for diagnosing the same (Supplementary table 1 in Appendix). It had a positive

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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.

Using a ROC curve (Figure 2), a timed tuning fork value of 4.8 seconds showed 76% sensitivity and 77% specificity for detection of mild diabetic peripheral neuropathy using a VPT score above 15V score as an indicator of the same (Supplementary table 2 in appendix). 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 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 128Hz 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: 16-25V and Grade II neuropathy: ≥25V (33), (34). A study found

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grade I severity in approximately 27% of patients with clinical neuropathy and in 50% asymptomatic patients (34), which indicates presence of subclinical neuropathic damage. In another study, nerve pain was experienced by the study population from a VPT score as low as 16V (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 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 seconds 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 seconds and ≤4 seconds have been shown to be a risk factor for lower limb injuries (39), and foot ulceration (40), respectively.

Taking 25V 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(41) , (42).

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The 5.07 (10g) monofilament test is the most recent recommendation for the detection of diabetic neuropathy by the American Diabetes Association (43). However, a study(44) 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 seconds 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 early 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 seconds) 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 (45), (38), (46), (33). However, it is an expensive machine, needs electricity to operate and is quite difficult to procure in primary health care 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

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biothesiometer, the handy tuning fork test provides a simpler, easily available alternative (47). The tuning fork has been shown to be considerably quicker than the VPT measurement (48). 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. Thus, 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(49). We also appreciate the cross-sectional study design allows for demonstration of correlation, more than causality. Another limitation was the lack of HbA1c to assess the glycemic 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.

List of abbreviations

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VPT- Vibration perception threshold; g- gram; V-volt; Hz-Hertz; Std-Standard; Sig-Significance; DPN- Diabetic peripheral neuropathy; ROC- Receiver operator characteristic; NCS-Nerve conduction studies

Declarations

Ethics approval and consent to participate

After deliberations and review the Institutional Ethics Committee, Nil Ratan Sircar Medical College and Hospital, took the decision "APPROVED" regarding the study proposal (Memo No. NRSMC/IEC/18/2022).

Consent for publication

Informed consent was obtained from all the participants by the principal author.

Sources of funding

None

Data availability statement

Deidentified participant data is 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

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and translated questionnaires, informed consent, and participant information sheets.

Contributorship statement:

SC: Data Collection, manuscript initial draft preparation, correspondence, ethical approval; SG: Analysis, Final draft preparation; NS: Supervision, proof-reading; AB: Supervision, proof reading

Competing interests:

None

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FIGURE LEGEND:

Figure 1: ROC curve for VPT>25V [0.751(0.550,0.953)]

Figure 2: ROC curve for VPT>15V [0.789 (0.713,0.866)]

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Figure 1: ROC curve for VPT>25V

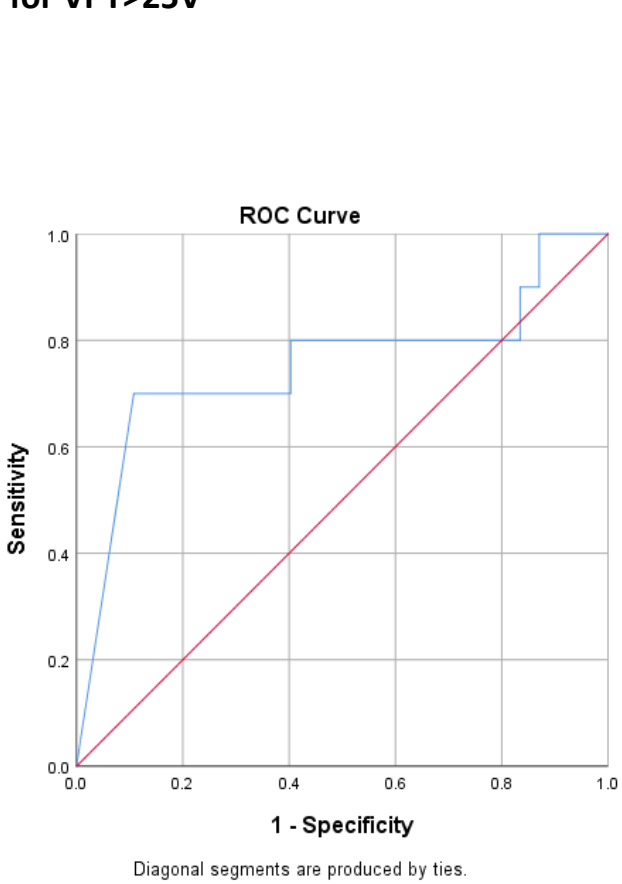

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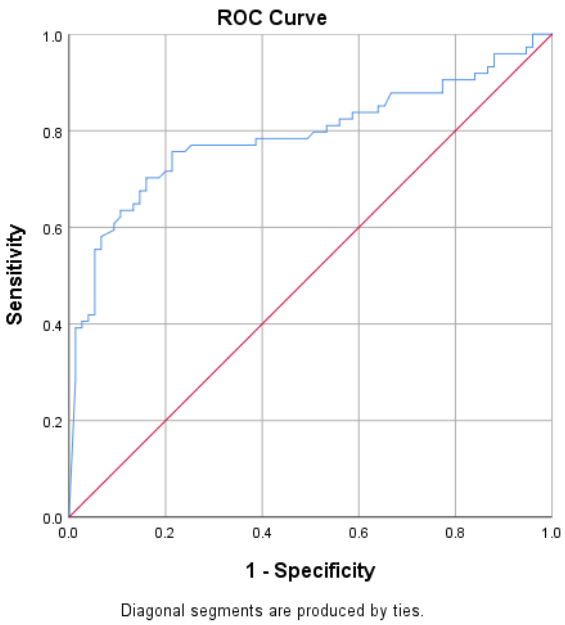
Figure 2: ROC curve for VPT>15V



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APPENDIX:

Coordinates of the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

Positive if Less Than or Equal To[a]	Sensitivity	1 – Specificity
-1.0000	.000	.000
.2050	.700	.108
.5000	.700	.115
.8350	.700	.122
1.1500	.700	.129
1.2550	.700	.137
1.3150	.700	.144
1.5450	.700	.151
1.7600	.700	.158
1.8150	.700	.165
1.9100	.700	.173
1.9950	.700	.180
2.0750	.700	.187
2.1750	.700	.194
2.3050	.700	.201
2.4150	.700	.209
2.5050	.700	.216
2.6100	.700	.223
2.6850	.700	.230
2.8050	.700	.237
2.9500	.700	.245
3.0700	.700	.252
3.1500	.700	.259
3.2300	.700	.266
3.3250	.700	.273
3.3900	.700	.281
3.4950	.700	.288
3.5700	.700	.295
3.6950	.700	.317
3.8200	.700	.324

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3.8700	.700	.338
3.9150	.700	.345
3.9750	.700	.353
4.0400	.700	.360
4.0600	.700	.367
4.1200	.700	.374
4.1950	.700	.381
4.2450	.700	.388
4.2800	.700	.396
4.3400	.700	.403
4.4050	.800	.403
4.4600	.800	.410
4.5300	.800	.417
4.6100	.800	.432
4.6650	.800	.439
4.6800	.800	.446
4.7350	.800	.453
4.7900	.800	.460
4.8050	.800	.468
4.8200	.800	.475
4.8500	.800	.489
4.9350	.800	.496
5.0200	.800	.504
5.0450	.800	.525
5.0650	.800	.532
5.1350	.800	.540
5.1950	.800	.554
5.2600	.800	.561
5.3450	.800	.568
5.3850	.800	.576
5.4200	.800	.590
5.4550	.800	.597
5.4850	.800	.604
5.5100	.800	.612
5.5550	.800	.619
5.6300	.800	.626
5.6750	.800	.640
5.7550	.800	.647
5.8950	.800	.655
5.9900	.800	.662

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6.0350	.800	.669
6.0700	.800	.676
6.1050	.800	.683
6.1450	.800	.698
6.1800	.800	.705
6.2950	.800	.712
6.4350	.800	.719
6.4850	.800	.727
6.6050	.800	.734
6.7350	.800	.741
6.7950	.800	.748
6.8800	.800	.770
6.9550	.800	.777
6.9900	.800	.784
7.0200	.800	.806
7.1750	.800	.813
7.3200	.800	.820
7.3400	.800	.827
7.5050	.800	.835
7.6800	.900	.835
7.7050	.900	.842
7.8550	.900	.849
8.1300	.900	.856
8.3400	.900	.863
8.4750	.900	.871
8.6000	1.000	.871
8.7500	1.000	.878
8.9150	1.000	.885
9.0350	1.000	.892
9.2100	1.000	.899
9.4250	1.000	.906
9.5450	1.000	.914
9.7800	1.000	.921
9.9850	1.000	.928
10.1650	1.000	.935
10.4450	1.000	.942
10.8650	1.000	.950
11.5600	1.000	.957
12.3000	1.000	.964
13.1050	1.000	.971

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13.7200	1.000	.978
15.5050	1.000	.986
23.6650	1.000	.993
31.2000	1.000	1.000

The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cut-off value is the minimum observed test value minus 1, and the largest cut-off value is the maximum observed test value plus 1. All the other cut-off values are the averages of two consecutive ordered observed test values.

Supplementary Table 1: Co-ordinates of the curve for VPT >25V

Coordinates of the Curve

Test Result Variable(s): Timed Tuning Fork (seconds)

Positive if Less Than or Equal To[a]	Sensitivity	1 – Specificity
-1.0000	.000	.000
.2050	.284	.013
.5000	.297	.013
.8350	.311	.013
1.1500	.324	.013
1.2550	.338	.013
1.3150	.351	.013
1.5450	.365	.013
1.7600	.378	.013
1.8150	.392	.013
1.9100	.392	.027
1.9950	.405	.027
2.0750	.405	.040
2.1750	.419	.040
2.3050	.419	.053
2.4150	.432	.053
2.5050	.446	.053

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2.6100	.459	.053
2.6850	.473	.053
2.8050	.486	.053
2.9500	.500	.053
3.0700	.514	.053
3.1500	.527	.053
3.2300	.541	.053
3.3250	.554	.053
3.3900	.554	.067
3.4950	.568	.067
3.5700	.581	.067
3.6950	.595	.093
3.8200	.608	.093
3.8700	.622	.107
3.9150	.635	.107
3.9750	.635	.120
4.0400	.635	.133
4.0600	.649	.133
4.1200	.649	.147
4.1950	.662	.147
4.2450	.676	.147
4.2800	.676	.160
4.3400	.689	.160
4.4050	.703	.160
4.4600	.703	.173
4.5300	.703	.187
4.6100	.716	.200
4.6650	.716	.213
4.6800	.730	.213
4.7350	.743	.213
4.7900	.757	.213
4.8050	**.757**	**.227**
4.8200	.757	.240
4.8500	.770	.253
4.9350	.770	.267
5.0200	.770	.280
5.0450	.770	.320
5.0650	.770	.333
5.1350	.770	.347
5.1950	.770	.373

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5.2600	.770	.387
5.3450	.784	.387
5.3850	.784	.400
5.4200	.784	.427
5.4550	.784	.440
5.4850	.784	.453
5.5100	.784	.467
5.5550	.784	.480
5.6300	.784	.493
5.6750	.797	.507
5.7550	.797	.520
5.8950	.797	.533
5.9900	.811	.533
6.0350	.811	.547
6.0700	.811	.560
6.1050	.824	.560
6.1450	.824	.587
6.1800	.838	.587
6.2950	.838	.600
6.4350	.838	.613
6.4850	.838	.627
6.6050	.838	.640
6.7350	.851	.640
6.7950	.851	.653
6.8800	.878	.667
6.9550	.878	.680
6.9900	.878	.693
7.0200	.878	.733
7.1750	.878	.747
7.3200	.878	.760
7.3400	.878	.773
7.5050	.892	.773
7.6800	.905	.773
7.7050	.905	.787
7.8550	.905	.800
8.1300	.905	.813
8.3400	.905	.827
8.4750	.905	.840
8.6000	.919	.840
8.7500	.919	.853

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8.9150	.919	.867
9.0350	.932	.867
9.2100	.932	.880
9.4250	.946	.880
9.5450	.959	.880
9.7800	.959	.893
9.9850	.959	.907
10.1650	.959	.920
10.4450	.959	.933
10.8650	.959	.947
11.5600	.973	.947
12.3000	.973	.960
13.1050	.986	.960
13.7200	1.000	.960
15.5050	1.000	.973
23.6650	1.000	.987
31.2000	1.000	1.000

The test result variable(s): Timed Tuning Fork (seconds) has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cut-off value is the minimum observed test value minus 1, and the largest cut-off value is the maximum observed test value plus 1. All the other cut-off values are the averages of two consecutive ordered observed test values.

Supplementary Table 2: Co-ordinates of the curve for VPT>15V

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Reporting checklist for diagnostic test accuracy study.

Based on the STARD guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

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		Reporting Item	Page Number
Title or abstract			
None	#1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	10
Abstract			
None	#2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts https://www.equator-network.org/reporting-guidelines/stard-abstracts/)	2
Introduction			
None	#3	Scientific and clinical background, including the intended use and clinical role of the index test	3

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None	#4	Study objectives and hypotheses	6
Methods			
Study design	#5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6
Participants	#6	Eligibility criteria	6
Participants	#7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	6
Participants	#8	Where and when potentially eligible participants were identified (setting, location and dates)	6
Participants	#9	Whether participants formed a consecutive, random or convenience series	6
Test methods	#10	Index and reference tests in sufficient detail to allow replication	7,8
Test methods	#11	Rationale for choosing the reference standard (if alternatives exist)	5
Test methods	#12	Definition of and rationale for test positivity cut-offs or result categories of the index and reference tests, distinguishing pre-specified from exploratory	10-18
Test methods	#13	Whether clinical information and reference standard results were available to the performers / readers of the index test; Whether clinical information and index test results were available to the assessors of the reference standard	8
Analysis	#14	Methods for estimating or comparing measures of diagnostic accuracy	10-18
Analysis	#15	How indeterminate index test or reference standard results were handled	n/a; no indeterminate variable
Analysis	#16	How missing data on the index test and reference standard were handled	n/a, no

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			missing variable
Analysis	#17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	10-18
Analysis	#18	Intended sample size and how it was determined	6
Results			
Participants	#19	Flow of participants, using a diagram	n/a; cross-sectional study design and independent participation
Participants	#20	Baseline demographic and clinical characteristics of participants	9
Participants	#21	Distribution of severity of disease in those with the target condition, and distribution of alternative diagnoses in those without the target condition	11,15
Participants	#22	Time interval and any clinical interventions between index test and reference standard	n/a; no clinical intervention
Test results	#23	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	9,14
Test results	#24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	9
Test results	#25	Any adverse events from performing the index test or the reference standard	n/a; no adverse events
Discussion			
None	#26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	21
None	#27	Implications for practice, including the intended use and clinical role of the index test	21

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Other information			
None	#28	Registration number and name of registry	n/a; cross-sectional observational study
None	#29	Where the full study protocol can be accessed	n/a- completely detailed in the study
None	#30	Sources of funding and other support; role of funders	22

Notes:

- 15: n/a; no indeterminate variable
- 16: n/a, no missing variable
- 19: n/a; cross-sectional study design and independent participation
- 22: n/a; no clinical intervention
- 25: n/a; no adverse events
- 28: n/a; cross-sectional observational study
- 29: n/a- completely detailed in the study The STARD checklist is distributed under the terms of the Creative Commons Attribution License CC-BY. This checklist was completed on 10. November 2023 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

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