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Role of peripheral nerve ultrasound in pre-clinical prediction of diabetic neuropathy: Hospital-based cross-sectional study

Dr Suha Hani, Dr Rajesh Malik, Jitendra Sharma, Abhinav C Bhagat, Aman Kumar, Ankur Patel, Dr Radha Sarawagi

Department of Radiodiagnosis, All India Institute of Medical Sciences, Bhopal, MP. India

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ABSTRACT

Purpose:

Nerve involvement in diabetes is a result of chronic microvascular insult, and it can precede the onset of neurological signs/symptoms in months. Nerve conduction studies (invasive) are the only known lab test to detect neuropathy. Nerve ultrasound can diagnose changes in neuropathy, particularly in peripheral nerves. The study aimed to assess sonography features of the sural nerve in patients with diabetic neuropathy and to determine whether ultrasound of the sural nerve can be used as a screening tool to detect early neuropathy changes in otherwise asymptomatic known diabetics.

Material and methods: This was a hospital-based cross-sectional observational analytical study. Subjects were recruited by consecutive sampling in three groups based on their diabetic status and symptoms: Diabetic Neuropathic, Diabetic non-neuropathic and Healthy controls. All subjects underwent ultrasound of the sural nerve to assess three parameters: cross-sectional area (CSA), thickness-to-width ratio and maximum fascicle thickness. A nerve conduction study of the sural nerve was done for 2nd group. Data was analysed in SPSS V25, and group findings were compared using multiple analysis methods. P<0.05 was considered statistically significant.

Results:

Among three USG parameters, CSA of the sural nerve was found to be the most sensitive and specific for neuropathy. Within the 2nd group, CSA showed a significant difference (p-value 0.02) between individuals with normal and abnormal Nerve conduction study results (sensitivity 86.4%; specificity 54.8% for the cut-off value 2.31mm2).

Conclusion:

Sural nerve ultrasound is comparable to nerve conduction studies in detecting precedent neuropathy in otherwise asymptomatic known diabetics.

CORRESPONDING AUTHOR, GUARANTOR

Dr. Jitendra Sharma, MD: Assistant Professor; Department of Radiodiagnosis, All India Institute of Medical Sciences, Bhopal, INDIA
Email ID jeet.imsbhu@gmail.com

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KEY WORDS

Ultrasound, peripheral nerve, sural nerve, Diabetic neuropathy, nerve conduction studies

Introduction

Diabetes is one of the most significant global health emergencies of this century; about 90–95% of diabetes cases are Type 2 Diabetes mellitus. Diabetes causes pathognomonic alterations in the microvasculature, increasing the Thickness of the capillary basement membrane and damaging arterioles in the glomeruli, retina, heart, skin, and muscle. This causes diabetic microangiopathy, which in turn causes microvascular problems such as Diabetic retinopathy, Diabetic nephropathy, and Diabetic neuropathy [1].

Diabetic neuropathy is described by the American Diabetes Association (ADA) as "the presence of symptoms and signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes" [2]. Distal symmetric diabetic polyneuropathy (DSPN) is the most prevalent type of diabetic neuropathy (DN) and likely makes up 75% of all DNs. According to estimates, neuropathy may be present in up to 10% of people with type 2 diabetes and up to 50% of people with long-term diabetes [3] Nerve conduction study (NCS) is used chiefly to diagnose diabetic neuropathy, an invasive, laborious, and relatively costly test.

High-resolution ultrasound is emerging as a promising modality in the auxiliary diagnosis of diabetic polyneuropathy and also in its prognosis because it is non-invasive and repeatable [4]. Various studies have proved that the sonographic appearance of diabetic neuropathic nerves shows particular changes [5,6]. The sural nerve (SN) is a pure sensory nerve and is susceptible to diabetic neuropathy because distal symmetric involvement is the most prevalent form of diabetic neuropathy; due to its superficial location, sonographic assessment is fast and easy.

Since neuropathy in diabetes is a result of chronic microvascular insult of long-term hyperglycemia, the pathological changes of neuropathy should precede symptoms and clinical signs in months or years [3]. Early detection in this phase can only be possible with an NCS. However, this invasive and time-consuming test cannot be applied to a larger population as a routine screening /regular checkup method.

This study had the following objectives: -

• To study ultrasonography (USG) parameters of the sural nerve in diabetics and their variation in patients with and without neuropathy and the control group.

• To correlate USG parameters of the sural nerve with clinical severity and duration of diabetes.

• To correlate USG parameters with NCS results in the diabetic non-neuropathy group. To identify the most sensitive nerve ultrasound parameter for detecting subclinical neuropathy in otherwise asymptomatic diabetics.

Material and methods

The study was conducted following the Declaration of Helsinki and was approved by the institute's local ethics committee (IHEC). Informed written consent was obtained from all patients before enrolling in this study.

Study design: A hospital-based, cross-sectional, observational study with descriptive and analytical components comparing findings among three groups.

Study setting: This study was conducted in the department of radiodiagnosis of a tertiary-level hospital and research institute in central India. The study was 18 months, from February 2021 to August 2022. Measurements/data from each participant were obtained only once during the study period with no follow-up measurement.

Participants: This study required the recruitment of subjects in three groups: Diabetic neuropathy (Group 1), Diabetic non-neuropathy (Group 2), and Healthy controls (Group 3). Consecutive sampling was done to recruit participants in these three groups, in which all diabetic neuropathic and diabetic non-neuropathic people were referred from the diabetic clinic. Diabetic patients were diagnosed based on ADA criteria (5). Based on the Diabetic neuropathy symptom score (DNS score), patients with a score = or > one were put in group 1 of the diabetic neuropathy group. Diabetic patients with a score of 0 were put in group 2 of the diabetic non-neuropathy group. Healthy non-diabetic controls were selected from a generally healthy population after matching for Age and sex.

Exclusion Criteria (common to all groups) were: Patient

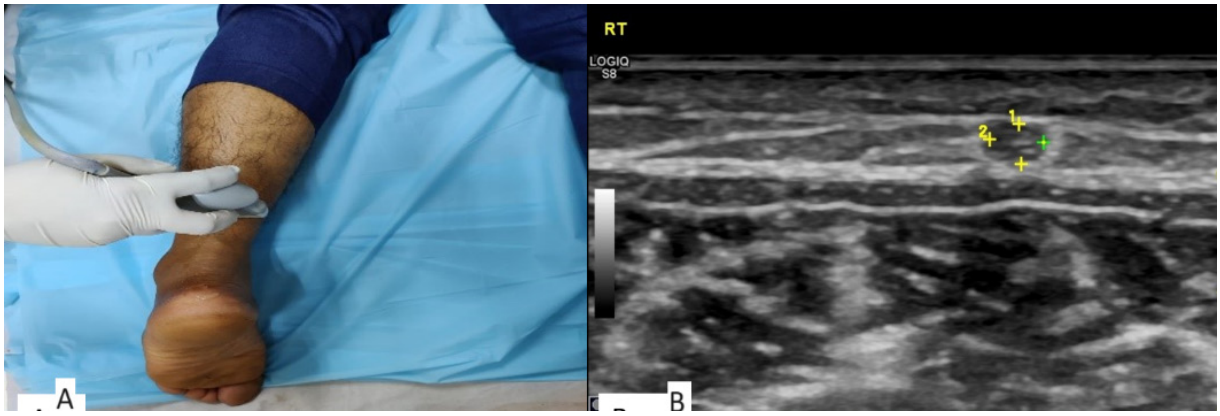

Figure 1: *A; Patient position and probe placement for sonographic examination of sural nerve, B; Asterisk shows normal sural nerve showing an ovoid hypoechoic reticular structure with a hyperechoic rim near the short saphenous vein.*

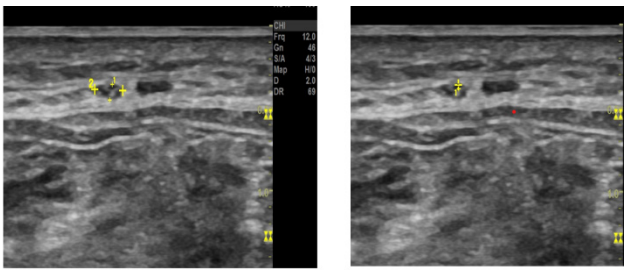

Figure 2*- Asterisk shows a sonography image of the sural nerve of a 60-year-old male who is diagnosed with type 2 diabetes mellitus for 6 years and has neuropathic symptoms. CSA of the sural nerve was 0.0329 cm², T/W was 0.535 and the maximum fascicle thickness (shown in the left half of the image by asterisks) was 0.3 mm. There is also blurring of the normal honeycomb pattern.*

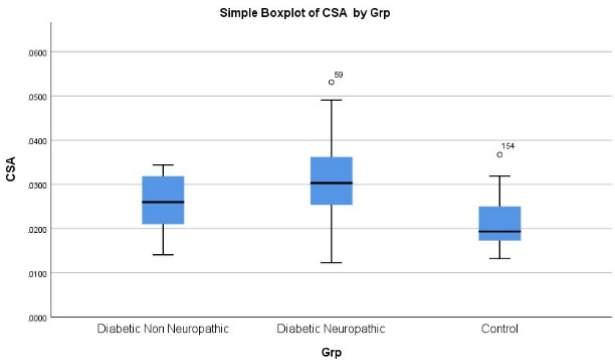

Figure 3*- Box plot depicting mean CSA of the bilateral sural nerve in diabetic neuropathic, non-neuropathic, and control group in cm². [CSA: Cross-sectional area]*

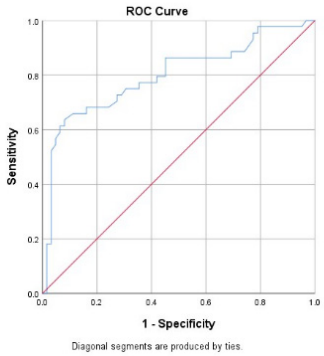

Figure 4. *Receiver operating curve using CSA to predict diabetic neuropathy in subclinical patients. [CSA: Cross-sectional area]*

not willing to participate in the study, Age <18 years or diagnosed with juvenile diabetes, Patient with type 1 diabetes mellitus, Patient who consumes alcohol and tobacco, Patient with peripheral vascular diseases, Patient diagnosed with other sensory neuron diseases, Patient diagnosed with thyroid disease, Patient undergoing chemotherapy/radiotherapy, Patient taking any neurotoxic medication.

Variables: This study included a total of 4 variables. Three variables were USG-based parameters recorded in all three groups (cross-sectional area of nerve (CSA), Thickness width ratio (T/W ratio), and maximum fascicle thickness (MFT)); the fourth variable was NCS, whose results were recorded as normal or abnormal.

Data sources/ measurements: While participants of all three groups were subjected to ultrasonography of bi-

Table 1-Correlation between the sonographic parameters among diabetic non neuropathic, diabetic neuropathic and control group. [CSA :Cross sectional area, T/W :Thickness/ Width.]

	Group						
	Diabetic Neuropathic		Diabetic Non-Neuropathic		Control		
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	P value
CSA (cm^2)	.0312	.0084	.0256	.0061	.0210	.0049	0.001
T/W RATIO	.5076	.0625	.5116	.0611	.4835	.0380	0.001
MAXIMUM FASCICLE THICKNESS in mm (MFT)	.24	.06	.19	.05	.20	.07	0.001

lateral sural nerves, NCS was performed only in group 2 participants, neither in group 1(these were all clinically confirmed cases of diabetic neuropathy, so NCS was not indicated), nor in group 3 (healthy non-diabetic controls Because of no suspicion of neuropathy and the invasive nature of test)

USG technique:

Ultrasonography was done using Siemens Acuson S-3000(Siemens Medical Solutions, mountain-view, CA) using a linear probe with a frequency of 7-17 MHZ. In all the patients, high-resolution ultrasonography was performed using a linear transducer with a 7-17 MHz frequency on the Siemens Acuson S-3000. All of the subjects were examined in the prone position. The transducer was placed in the transverse position on the lower section of the calf, ensuring the sural nerve was not compressed (Figure 1A). Care was taken to exert minimal pressure on the skin because any compression that could cause the lesser saphenous vein deformation was avoided. On the transverse sonograms, the sural nerve usually appears as an ovoid hypoechoic reticular structure with a hyperechoic rim near the lesser saphenous vein (Figure 1B). On the longitudinal sonograms, the sural nerve appears as multiple hypoechoic bands corresponding to neuronal fascicles, separated by hyperechoic lines corresponding to epineurium. The Thickness divided by width on the transverse image of the SN was defined as the thickness/width (T/W) ratio, an indicator of the SN's swelling and deformation level. The SN's cross-sectional areas (CSAs), which were traced along the outer hyperechoic rim on the transverse sonograms, were calculated using the formula for an ellipse. The maximum Thickness of the nerve fascicles (MFT) was measured along the short axis.

Nerve conduction study:

Only participants of group 2 were subjected to NCS of the sural nerve in bilateral lower limbs from the neurophysiology lab in the department of physiology. Nerve conduction velocity was measured in the sural nerve of bilateral limbs.

Bias: No obvious bias could be identified in this study.

Study size: A complete enumeration process was used for sample size calculation. This study assumed a medium effect size of 0.5, setting the type 1 error as 0.05 and the power of study as 80% with a critical t value of 1.660; the sample size comes to be 53 for each group.

Statistical methods: Data was collected using a preformed patient Case Data Sheet, which included demographic data, clinical details, ultrasonographic findings, and NCS findings (copy enclosed). Data collected were primarily entered into Excel sheets (Microsoft Excel version 16.51) and analysed in SPSS V25. Descriptive statistics were represented with percentages for qualitative data and mean with SD for quantitative data. An Independent t-test was applied for comparison between means and medians. ANOVA test was applied to find significance. Karl Pearson

Table 2-Correlation of cross sectional area (CSA) , thickness to width ratio (T/W ratio) and Maximum fascicle thickness (MFT) in Diabetic neuropathic, Diabetic cnon neuropathic and healthy control group.[CSA: Cross sectional area]

Dependent Variable	Group	Group	Significance
CSA	Diabetic Non-Neuropathic	Diabetic Neuropathic	.001
		Control	.001
	Diabetic Neuropathic)	Diabetic Non-Neuropathic	.000
		Control	.000
T/W	Diabetic Non neuropathic	Diabetic Neuropathic	0.334
		Control	0.00
	Diabetic Neuropathic	Diabetic Non neuropathic	0.334
		Control	0.008
Maximum Fascicle Thickness	Diabetic Non-Neuropathic	Diabetic Neuropathic	0.000
		Control	0.620
	Diabetic Neuropathic	Diabetic Non-Neuropathic	0.000
		Control	0.003

correlation was applied. A simple linear regression model was fitted. P<0.05 was considered statistically significant.

Results

- The total number of participants was 159 (100 male and 59 female), with 53 participants in each group.
- The mean Age of participants was 56 years for group 1, 50 years for group 2, and 45 years for group 3.
- The main findings of sural nerve ultrasound in group 1 were increased nerve diameter, blurring of honeycomb structure, and increased MFT (Figure 2).
- Outcomes of all three USG variables, CSA, T/W ratio, and MFT, are described in Table 1 for each group. All showed statistically significant differences with a value of 0.001.
- The mean value of CSA of the sural nerve in group 1 was 0.0312 cm2 +/- 0.0084, in group 2 was 0.0256 cm2 +/- 0.0061, and in group 3 was 0.0210 cm2 +/- 0.0049. (Figure 3). The difference in mean CSA values among all three groups was statistically significant. (Table 2)
- Mean value of T/W of sural nerve in group 1 was 0.5076 +/- 0.0625, in group 2 was 0.5116 +/- 0.0611 and in group 3 was 0.4835 +/- 0.0380. T/W of the sural nerve in group 3 showed a significant statistical difference with group 1 and group 2 (p-<0.05). (Table 2)
- The mean value of MFT of the sural nerve in group 1 was 0.24mm +/- 0.06, in group 2 was 0.19mm +/- 0.05, and in group 3 was 0.20 mm +/- 0.07. There was a statistically significant difference observed between group 1 - group 3 and between group 1-group 2 (p-<0.05). (Table 2)
- Correlation with severity of diabetic neuropathy: - All three sonographic parameters, CSA, T/W, and MFT,

Table 3- Correlation of DNS score with sonographic parameters of sural nerve. [CSA :Cross sectional area, T/W :Thickness/ Width, DNS score: Diabetic neuropathy symptom score]

			DNS SCORE
Spearman's rho	CSA	Correlation Coefficient	.502**
		Sig. (2-tailed)	.000
		N	159
	T/W RATIO	Correlation Coefficient	.206**
		Sig. (2-tailed)	.009
		N	159
	MAXIMUM FASCICLE THICKNESS	Correlation Coefficient	.291**
		Sig. (2-tailed)	.000
		N	159

Table 4 – correlation of USG parameters of sural nerve with nerve conduction result in group 2 of diabetic non-neuropathy.

USG parameter		Result of Nerve conduction study of bilateral sural nerves				p value
		Normal		Abnormal		
		Mean	SD	Mean	SD	
CSA in cm^2	Right	0.0245	0.0073	0.0315	0.0082	0.002
	Left	0.02056	0.00538	0.2860	0.00508	0.01
T/W ratio	Right	0.481	0.133	0.528	0.057	0.09
	Left	0.521	0.037	0.525	0.047	0.178
MFT in mm	Right	0.20	0.02	0.2	0.01	0.135
	Left	0.20	0.07	0.19	0.07	0.392

showed a positive correlation with the DNS score (correlation coefficient of 0.502, 0.206, and 0.291, respectively). Amongst them, CSA has shown the strongest positive correlation with the DNS score. (Table 3)

- Correlation with duration of diabetes and HbA1c levels: Out of the three USG parameters, only CSA showed a weak positive correlation with the duration of diabetes (correlation coefficient of 0.191). However, no correlation

was observed between CSA values and serum HbA1c levels.

Correlation with nerve conduction studies:

- Out of three USG parameters, only CSA showed a significant correlation with abnormal results of NCS (Table 4).
- On deriving the odds ratio for CSA in the diabetic non-neuropathic group (group 2) for predictability of subclinical diagnosis of diabetic neuropathy, the odds of having neuropathy were higher in patients with higher CSA than those with lesser CSA.

Using the data obtained from bilateral sural nerves of group 2 participants, a cut-off value of CSA was derived by applying the Receiver Operating Characteristic (ROC) curve. For a cut-off value of 2.31mm2, CSA showed a sensitivity of 86.4% and specificity of 54.8% for diagnosing subclinical neuropathy in diabetics (Figure 4).

Discussion

Diabetes mellitus contributes to a significant number in burden of chronic diseases worldwide. Diabetic neuropathy, being one of the common microvascular complications of diabetes mellitus, leads to conditions such as foot ulcers, which can affect a patient's daily activity and even lead to limb amputation.

Nerve biopsy is the gold standard investigation for diagnosing diabetic neuropathy; however, due to its invasive nature, NCS is widely used in diagnosing diabetic neuropathy. However, it is costly and not commonly available, raising the need for an alternative investigation that can easily detect changes in neuropathy.

High-resolution sonography, being a commonly available, accessible, and affordable investigation, has been used in our study for assessing changes in neuropathy in nerves. The sural nerve, being a purely sensory nerve and superficial in location, was used in our study.

In our study, among the patients examined, the prevalence of diabetes was higher in men than women. This is in concordance with other studies done on the Indian population by Sujata and Ramna Thakur in 2021[7] and in studies assessing the global prevalence of diabetes mellitus by Moien Abdul Basith Khan [8], in which prevalence was higher in the male population. The mean age of the diabetic neuropathic and diabetic nonneuropathic groups in our study was in the sixth decade, which shows results similar to those of global studies conducted on the prevalence of diabetes mellitus [8].

Out of the three sonographic parameters studied, the CSA of the sural nerve showed a statistically significant difference between all three groups (with a mean value of CSA of the sural nerve in Group 1, 0.0312 cm2 with SD of 0.0084, in Group 2 0.0256 cm2 with SD of 0.0061 and in Group 3, 0.0210 cm2 with a standard deviation of 0.0049). A similar statistically significant difference in CSA of the sural nerve was reported by Faang Liu et al. [9] and Singh et al. [6]. Similar results between CSA of diabetic neuropathic patients and healthy individuals were also proved by Arumugam et al. [10] and Kanav Goyal et al. [11].

The mean CSA of the sural nerve in healthy controls and diabetic neuropathic patients in our study were comparable with previous studies conducted by Habid Ebadi et al. in which the mean CSA of the neuropathic group was 3.2mm2 with SD 0.8 and the mean CSA of controls was 2.7mm2 with SD of 0.6 [12]. The possible explanation for this discrepancy could be ethnicity difference and also that they used an extended range of probe frequency up to 22 MHz.

The T/W ratio, which was used to detect the level of sural nerve deformation, was also used to evaluate the morphological changes in our study. There was a significant difference between the T/W of the sural nerve in the diabetic group (mean T/W 0.5096 and SD of 0.0615 and healthy controls (mean 0.4835 with SD of 0.0380). Both diabetic neuropathic and diabetic nonneuropathic groups showed statistically significant differences in T/W in comparison to controls. Similar mean values of T/W and correlation were obtained in previous studies by Singh et al. among diabetic patients and normal controls [13].

Our study found no statistically significant difference between the T/W ratio of diabetic neuropathic and diabetic nonneuropathic patients. This is contradictory to a study conducted by Liu et al. [9] in which there was a significant difference between the T/W ratio of diabetic neuropathic, diabetic nonneuropathic, and control group (T/W was 0.5960 with SD 0.09 for patients in the neuropathic group compared to 0.5660 with SD 0.10 for patients in the nonneuropathic group and 0.5160 with SD 0.07 in control group). These differences may be due to the number of subjects examined in the study; in the previously mentioned study, 300 subjects were examined; however, in our study, we could examine 159 patients only, with 53

patients in every group.

Maximum fascicle thickness of the sural nerve in Diabetic neuropathic patients showed significant statistical differences with Diabetic nonneuropathic patients and healthy controls. (p-<0.05). Similar results were obtained in a study conducted by Liu et al. [9]. However, the maximum fascicle thickness of the sural nerve in Diabetic nonneuropathic patients did not show a significant statistical difference with healthy controls in our study. (p->0.05). There was no significant difference between the maximum fascicle thickness of the sural nerve between the diabetic and non-diabetic groups, contrary to the study conducted by Singh et al. [13]. This might be because, in a previous study, they measured MFT up to three decimal points.

In our study, we found a significant correlation between CSA and NCS results. Using this correlation, the ROC curve was plotted to obtain the cut-off value of CSA to predict subclinical diagnosis of neuropathy. Since the aim of our study was to get a cut-off value that can be used as a sensitive screening tool in the diabetic population, CSA values showing higher sensitivity were considered for the calculation of the cut-off value. For the sensitivity at 86.4%, a CSA cut-off value of 2.31mm2 could be established for predicting neuropathy in diabetic patients. Similar values were obtained in a study conducted by Arumugam, the cut-off value for the sural nerve at two mm2 (area under the curve 0.88) between the presence and absence of DSP (sensitivity 0.90; specificity 0.74). [10]

Different CSA cut-off values were obtained in several other studies. In a study conducted by Kanav Goyal et al., the cut-off value was 4.41mm2 [11]. However, in this study, the area was measured at the superior border of the lateral malleolus, whereas in our study, the nerve was measured 5cm proximal to the lateral malleolus. In a study conducted by Fang Liu et al., the cut-off value obtained for CSA was 1.658mm2 [9]. This difference could be due to ethnicity differences, higher sample size, and usage of an extended range of probe frequency up to 22MHz.

In our study, CSA and MFT showed statistically significant differences between group 1 and group 2. However, on comparing with NCS results, only CSA showed a significant positive correlation. Hence, it can be stated that CSA is the most sensitive parameter that can be used for screening/detection of sub-clinical neuropathy in diabetic nonneuropathy groups.

Among the three sonographic parameters evaluated, all of them showed a positive correlation with the DNS score, among which CSA showed the strongest positive correlation, indicating its relation with the severity of diabetic neuropathy.

Among the three sonographic parameters evaluated, CSA shows a mild positive correlation with the duration of diabetes. Similar results showing a positive correlation between CSA and duration of diabetes arrived in the study conducted by Arumugam in the median and ulnar nerve [10]. However, this contradicts a previous study conducted by Sharmendra in which no correlation was found between the duration of diabetes and CSA [14]. No significant correlation was found between the T/W ratio and maximum fascicle thickness and duration of diabetes mellitus.

In our study, no significant correlation was found between sonographic parameters and HbA1c. Similar observations were observed by Riazi et al. and Dharmendra. [14,15].

Limitations

In our study, the diabetic group of patients was inhomogeneous as they had different disease profiles in terms of duration of diabetes and control of blood sugar levels.

ü Due to technical differences among the various ultrasound machines used worldwide, variations in assessed measurements could be observed in comparison to other studies.

ü Since sonography is an operator-dependent imaging modality, intra-observer variability is known to exist with such investigation methods.

Conclusion

From our study, it can be concluded that USG features of the sural nerve showed a good correlation with the diagnosis and clinical severity of diabetic neuropathy and the results of the nerve conduction study. Among the three ultrasound parameters examined, CSA turned out to be the most sensitive sonographic measurement for diagnosis of diabetic neuropathy, and a cut-off value of 2.31mm2 can be used as a good sensitive parameter (sensitivity 86.4%) to detect subclinical neuropathy in otherwise asymptomatic diabetics population.

High-resolution ultrasound of the sural nerve can be used as a non-invasive, readily available, rapid, and affordable method for screening/regular checkups in the Type 2 diabetic population on an OPD basis. Invasive and more labori-

ous tests such as nerve conduction studies or detailed clinical evaluation can be saved for patients showing suspicious results on nerve ultrasound for early initiation of neuropathy preventive measures. This would be particularly helpful in countries like India, where resources are limited and diabetic populations are ever-growing. **R**

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