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Research paper

Nerve ultrasonographic findings in diabetes mellitus are determined by anatomical location and type of diabetes

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ABSTRACT

Objective: A prospective ultrasound study to analyze nerve size and its modifying factors in type 1 and type 2 diabetes mellitus.
Methods: The cross-sectional areas (CSAs) of motor and sensory nerves in both upper and lower limbs were measured at 14 measurement points, using high resolution ultrasound in 26 patients with type 1 and 76 patients with type 2 diabetes, and in 50 control subjects. All diabetic patients underwent electrophysiological assessment to check for the presence of polyneuropathy.
Results: Significant mild/moderate diffuse nerve enlargement was demonstrated in type 2 diabetes, more pronounced at compression sites versus non-compression sites, and on the upper limbs versus lower limbs (p value for pooled DM2 v. control group: <0.001). In type 1 diabetes, nerve enlargement was found only at one compression site (median nerve wrist; p = 0.002). No significant difference was found between patients with or without polyneuropathy.
Conclusions: The primary predictors of nerve size in diabetes are anatomical location (i.e. compression sites versus non-compression sites, upper versus lower limbs) and type of diabetes. Changes occur before the electrophysiological signs of polyneuropathy are detected.
Significance: Nerve ultrasound may contribute to early recognition of the neuropathic complications of diabetes.

1. Introduction

Diabetes causes polyneuropathy and is also a risk factor for compression neuropathies such as carpal tunnel syndrome (Brown et al. 1984). In compression neuropathies, ultrasound can be a useful adjunctive diagnostic tool to electrophysiology, especially in coexisting diabetic sensorimotor polyneuropathy, but its clinical use in the diagnosis of polyneuropathy is less straightforward (Chen et al. 2020). Interpretation of study results is complicated by the fact that most reported data came from the examination of one or two nerves, only the upper or lower limb nerves, or just some segments of the nerves. The few studies which analyzed multiple segments of multiple upper and lower limb nerves (Arumugam 2016, Breiner et al., 2017, Singh 2019, Narayan 2021, Tandon 2021), had different protocols. Some of the studies (Arumugam 2016, Singh 2019, Narayan 2021) did not directly compare the non-polyneuropathic and polyneuropathic groups, or did not measure all compression and non-compression sites of the nerves (Narayan 2021, Tandon 2021). There were also differences in the definition of polyneuropathic groups depending on whether subclinical cases were included in the study or not.

The studies analysing the median nerve, reported mainly larger (Watanabe et al. 2010; Moon et al. 2014; Kim et al. 2014; Attah et al., 2019), rarely smaller (Chen et al. 2011) cross-sectional area at the wrist in diabetic patients, compared to controls. No significant correlation was found between nerve CSAs and polyneuropathy in lower limb nerves either (Riazi et al. 2012; Agirman et al. 2016; Pitarokoili et al. 2016). All studies reporting data of multiple nerves on the upper and lower limb in a large number of patients (Breiner et al., 2017, Singh 2019, Arumugam 2016, Narayan 2021, Tandon 2021), described larger CSAs in the diabetic group than in the control group. Studies which compared data of diabetic groups with and without polyneuropathy found larger CSAs in the polyneuropathic than in the non-polyneuropathic patients

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(Breiner et al., 2017, Tandon et al. 2021), with statistical significance in the upper limb nerves.

The aim of our prospective ultrasound study was to analyze patterns of nerve enlargement in diabetes, both in patients with and without diabetic polyneuropathy. We analyzed the factors influencing nerve size changes in diabetes, with particular regard to the effect of the type of diabetes.

2. Patients and methods

The study was approved by the Institutional Ethics Committee of Vaszary Kolos Hospital. Patients gave written informed consent. Altogether 102 patients with diabetes mellitus were recruited between July 2017 and December 2019. The total diabetic group (DM) consisted of 26 (13 men and 13 women) patients with type 1 diabetes (DM1) and 76 (36 men and 40 women) patients with type 2 diabetes (DM2).

The inclusion criterion for the study was the diagnosis of diabetes mellitus. Exclusion criteria included endocrine, metabolic or connective tissue disorders (e.g. thyroid dysfunction, chronic kidney disease, rheumatoid arthritis) or known polyneuropathy from causes other than diabetes, and pregnancy. All patients underwent neurological, electrophysiological and nerve ultrasound examination. The diabetic groups were subdivided according to whether diabetes was associated with polyneuropathy or not (yes/no). The diagnosis of diabetic polyneuropathy was based on abnormal electrophysiological studies, irrespective of clinical symptoms and signs of polyneuropathy (asymptomatic and symptomatic). In the subgroups without polyneuropathy, electrophysiological or clinical signs of polyneuropathy were not present. Within the polyneuropathy group, subgroups were delineated as follows:

Signs of demyelination with or without signs of sensorimotor axon loss, shown by electrophysiological examination (yes/no), Electrophysiological severity of polyneuropathy: mild (sensory axon loss in the lower limbs)/moderate (sensory axon loss in the upper and lower limbs)/medium (severe sensory axon loss and/or mild motor axon loss in the lower limbs)/severe (severe sensorimotor axon loss in the upper and lower limbs).

For the control group, 50 age-matched healthy individuals (14 men and 33 women) were examined. Two equal groups of 25 individuals were created, whose mean age corresponded to the mean age of the DM1 and DM2 groups (Control 1 and Control 2 groups). Electrophysiological examination was not performed in control subjects, but none of the subjects had signs or symptoms of polyneuropathy or systemic disease, nor other neuromuscular diseases associated with polyneuropathy. Verbal informed consent was provided.

2.1. Nerve conduction studies (NCS)

NCS were performed at our institute using a Nicolet Viking Quest or EDX System (Carefusion Corporation) by one of the authors (M.T.), who are physicians trained in clinical neurophysiology. The following standard protocol for polyneuropathy was performed in all diabetic patients: median and ulnar nerve motor and sensory NCS with F-wave studies, fibular and tibial nerve motor NCS with F-wave studies, superficial radial and sural sensory NCS. Electromyography was performed at the discretion of the examiner, not on a routine basis. In most patients, the right side was examined. Axonal/demyelinating polyneuropathy was diagnosed using standard criteria (Sander 2003; Tankisi et al. 2005). The diagnosis of demyelination was based on the presence of abnormal temporal dispersion, decrease in conduction velocity in the demyelinating range, or increase of distal latency/F-wave latency in at least 2 motor nerves. Reference values for NCS were collected in our laboratory. The mean ± 2.5 SD (standard deviation) was used for limits of normality.

2.2. Ultrasonography

Ultrasonography was performed by one of the authors (M.T.), with six years of experience in nerve ultrasound, using a HITACHI HI VISION Avius UH ultrasound system and an L18–5 MHz linear array transducer. Settings were optimized for nerve imaging, including the use of compound imaging mode. The time interval between ultrasound and electrophysiological investigation was within 10 days. Nerve size measurements were performed in transverse section on the side of electrophysiological examination, usually on the right. CSAs were determined altogether at 14 sites: median nerve on the mid-upper arm, on the forearm 12 cm proximal to the wrist, at the wrist (carpal tunnel inlet), and at the palm (carpal tunnel outlet); the ulnar nerve on the mid-upper arm, at the elbow (at the level of the medial epicondyle), on the mid-forearm, and at the wrist (pisiform bone); the superficial radial nerve 7–8 cm proximal to the styloid process of the radius; the fibular nerve at the popliteal fossa, and at the fibular head; the tibial nerve in the popliteal fossa, and posterior to the medial malleolus; the sural nerve on the distal leg. In six patients (1 with type 1 and 5 with type 2 diabetes), the sural nerve could not be examined due to body habitus. The same measurements were done in all control subjects on the right side. According to the literature, there is no significant side difference of nerve size (Zaidman et al. 2009). Measurements were taken within the hyperechoic external rim of the nerve. Three measurements were taken at each site, and the average value was used to calculate group mean values and standard deviation. In the upper limbs, the arm-forearm ratio (AFR) was also determined.

2.3. Statistics

Statistical analysis was performed using Statistica for Windows v.12 program (StatSoft Tulsa OK, USA). A P value of $p < 0.05$ was set for statistical significance. Demographic data, clinical parameters of the patient population, CSA, and AFR values were described by descriptive statistics. Depending on normality, unpaired *t*-test or Mann–Whitney *U* test was used to compare CSA, AFR values between the patient and the control groups. The Shapiro–Wilk W test was used to check normality. Polyneuropathy and sex ratios were analyzed with Chi-Square test. Diabetic subgroups as well as severity categories of polyneuropathy were compared using Kruskal-Wallis test. To examine the association between CSA and age, height, body mass index (BMI), and HbA1c, the Spearman correlation coefficient was calculated.

3. Results

Table 1 shows the demographic and clinical data of the patient and control groups, including age, height, BMI, duration of diabetes, HbA1c level, and the presence of polyneuropathy. No significant differences were found between the patient and respective control groups except for BMI, which was larger in the diabetic (DM) than the control group. When the DM1 and DM2 subgroups were compared, mean age and BMI were significantly higher in the DM2, whereas the duration of diabetes and HbA1c levels were significantly higher in the DM1 group. There was no significant difference in sex ratio ($p = 0.8$). Sixty-seven % of diabetic patients (DM) showed electrophysiological signs of polyneuropathy, being slightly higher in the DM2 subgroup.

Table 1
Demographic and clinical data of the diabetic and the control groups.

Group	DM	DM1	DM2	Control	Control 1	Control 2	p			
	n = 102	n = 26	n = 76	n = 50	n = 25	n = 25	DM v. Control	DM1 v. Control 1	DM2 v. Control 2	DM1 v. DM2
Age, years mean (SD)	58.8 (14.9)	41.0 (14.9)	64.6 (9.4)	54.4 (16.7)	42.4 (11.8)	66.4 (11.5)	0.8	0.8	0.6	**<0.001***
Height, cm mean (SD)	168 (9.1)	171 (8.2)	167 (9.3)	170 (9.0)	172 (9.0)	169 (9.1)	0.3	0.3	0.5	0.3*
BMI mean (SD)	28.8 (5.31)	25.6 (4.23)	30.3 (4.98)	25.1 (5.18)	23.9 (4.93)	27.1 (5.18)	**0.001**	0.2	0.1	**<0.001***
Diabetes duration, years mean (SD)	3.8 (9.1)	21.6 (8.7)	10.9 (7.5)							**<0.001***
HbA1c, % mean (SD)	5.1 (4.6)	8.2 (1.2)	7.32 (1.45)							**0.001***
Presence of polyneuropathy n (%)	68 (67%)	15 (58%)	53 (70%)							0.3**
Symptomatic	50 (74%)	11 (73%)	39 (74%)							
Asymptomatic	18 (26%)	4 (27%)	14 (26%)							

DM: all diabetes; DM1: type 1 diabetes; DM2: type 2 diabetes; Control 1: control group of DM1; Control 2: control group of DM 2; pnp: polyneuropathy; BMI: body mass index; highlighted in bold: statistically significant difference; p: Mann-Whitney *U* test; p*: Krustal-Wallis test; p**: Chi-Square test.

Table 2
Mean CSA and SD values of patient and respective control groups, and statistical significance levels of group comparisons.

Nerve	Site	DM			DM1			DM2		
		CSA	CSA	p	CSA	CSA	p	CSA	CSA	p
		All (n = 102)	Co (n = 50)	All/Co	All (n = 26)	Co1 (n = 25)	All/Co1	All (n = 76)	Co2 (n = 25)	All/Co2
Median	***Palm***	12.51 (5.39)	8.62 (1.49)	**<0.001**	10.86 (5.02)	8.42 (1.95)	0.19	13.1 (5.42)	8.80 (0.96)	**<0.001**
	Wrist	12.41 (3.92)	8.82 (1.60)	**<0.001**	12.42 (5.15)	8.87 (1.66)	**0.002**	12.4 (3.42)	8.77 (1.57)	**0.001**
	Forearm	5.97 (1.29)	5.29 (0.88)	**0.005**	5.56 (1.14)	5.27 (0.99)	0.42	6.11 (1.32)	5.30 (0.78)	**0.004**
	Arm	8.69 (2.01)	7.53 (1.33)	**0.01**	8.71 (2.21)	7.76 (1.41)	0.18	8.65 (1.96)	7.08 (1.04)	**0.005**
	AFR	1.50 (0.35)	1.43 (0.26)	0.77	1.59 (0.38)	1.49 (0.28)	0.51	1.47 (0.33)	1.33 (0.19)	0.23
Ulnar	**Wrist**	4.08 (1.18)	3.67 (1.04)	0.05	3.75 (0.99)	3.78 (1.22)	0.60	4.18 (1.23)	3.54 (0.78)	0.06
	Forearm	5.46 (1.34)	4.70 (0.93)	**<0.001**	5.12 (1.11)	4.84 (0.99)	0.36	5.56 (1.37)	4.42 (0.79)	**0.005**
	Elbow	10.29 (3.27)	7.83 (1.76)	**<0.001**	8.73 (2.28)	7.70 (1.79)	0.18	10.76 (3.44)	8.03 (1.76)	**<0.001**
	Arm	7.29 (1.75)	6.18 (1.59)	**0.002**	6.75 (1.46)	6.40 (1.66)	0.89	7.46 (1.98)	5.77 (1.42)	**0.002**
	AFR	1.38 (0.29)	1.30 (0.26)	**0.04**	1.34 (0.27)	1.32 (0.25)	0.73	1.40 (0.30)	1.27 (0.27)	0.15
Spf radial	**Forearm**	2.13 (0.84)	1.74 (0.63)	**0.0.23**	1.68 (0.56)	1.96 (0.61)	0.16	2.28 (0.87)	1.31 (0.43)	**<0.001**
Tibial	**Popl**	21.38 (7.82)	19.3 (6.64)	0.16	18.84 (6.30)	18.96 (5.71)	0.87	22.70 (8.13)	19.87 (8.14)	0.10
	Ankle	7.43 (2.56)	6.66 (2.07)	0.07	6.32 (2.10)	6.96 (1.81)	0.18	7.93 (2.53)	6.08 (2.46)	**0.007**
Fibular	**Popl**	8.04 (2.25)	7.27 (1.74)	0.09	7.84 (2.37)	7.60 (1.50)	0.83	8.08 (2.18)	6.75 (1.99)	**0.02**
	Fib	7.71 (2.57)	7.09 (1.87)	0.17	7.24 (1.88)	7.08 (1.63)	0.86	7.86 (2.98)	7.09 (2.25)	0.09
Sural	**Leg**	2.15 (0.76)	1.76 (0.67)	**0.005**	1.92 (0.49)	2.00 (0.64)	0.93	2.40 (1.10)	1.31 (0.48)	**0.001**
	All sites			P **< 0.001**			P = 0.56			P **< 0.001**

CSA: cross-sectional areas are shown in mm^2 with respective SD values in parentheses; Co: Control; spf: superficial; Popl: popliteal fossa; Fib: fibular head; AFR: arm-forearm ratio; highlighted in bold: statistically significant difference; p: Mann-Whitney *U* test. P: pooled significance value; Measurement sites with italics are considered compression sites.

Table 2 shows the mean CSA values at all measurement sites in the patient and control groups, and the p values of statistical comparisons between the patient and control groups. Data were analyzed stratified according to the following categories: a.

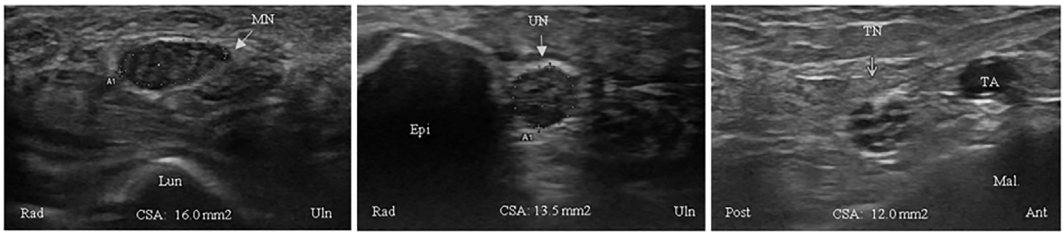

Fig. 1. Nerve cross-sectional scans of a patient with type 2 diabetes at compression sites, with no clinical symptoms of compression neuropathy. The image shows the median nerve at the wrist (left), the ulnar nerve at the elbow (middle) and the tibial nerve at the ankle (right). Note the enlargement of the nerves with loss of fascicular structure and hypoechogenicity in the axial plane in median (left) and ulnar (middle) nerves. The fascicular structure of the tibial nerve is preserved, but the nerve appears slightly hypoechogenic (right). MN: median nerve; UN: ulnar nerve; TN: tibial nerve; Rad: radial; Uln: ulnar; Ant: anterior; Post: posterior; Lun: lunate bone; Epi: medial epicondyle; Mal.: medial malleolus; TA: tibial artery. CSA: cross-sectional area.

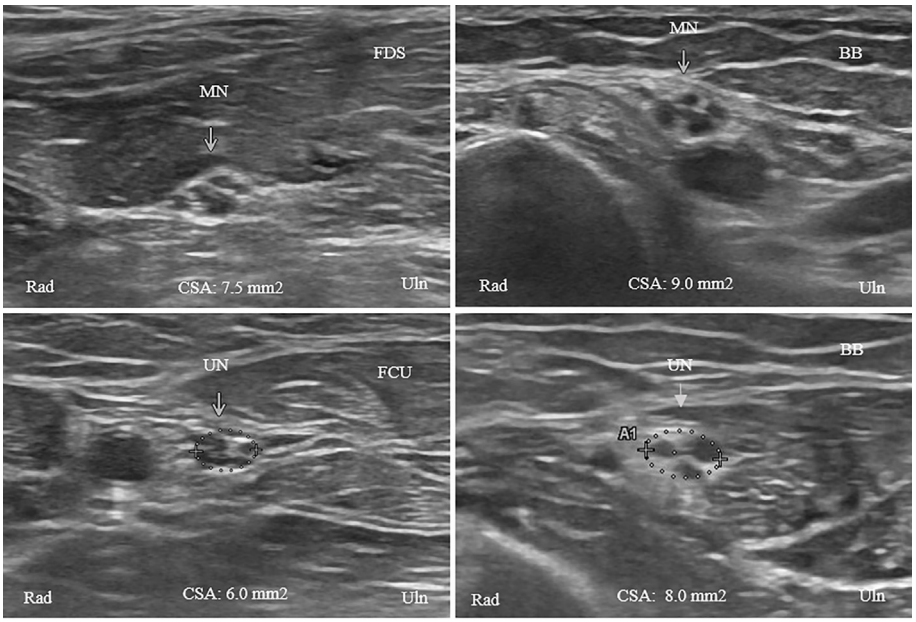

Fig. 2. Nerve cross-sectional scans of a patient with type 2 diabetes at non-compression sites. The upper images show the median nerve, the lower images the ulnar nerve at the forearm (left) and at the upper arm (right). Note that nerve size is mildly increased with the enlargement of individual fascicles. MN: median nerve; UN: ulnar nerve; Rad: radial; Uln: ulnar; FDS: flexor digitorum superficial muscle; FCU: flexor carpi ulnar muscle; BB: biceps brachii muscle; CSA: cross-sectional area.

anatomical distribution; b. type of diabetes; c. presence of polyneuropathy; d. severity of polyneuropathy; e. presence of demyelination; f. clinical characteristics.

3.1. Anatomical distribution

At compression sites (wrist and palm for the median nerve, elbow for the ulnar nerve, and ankle for the tibial nerve), the median nerve-wrist CSA of all diabetic groups (DM, DM1, DM2) was significantly larger than in controls. In the DM2 subgroup, all other compression site measurements were also significantly larger (Table 2, Fig. 1). *At non-compression sites*, no significant enlargement was seen in the DM1 subgroup, but most measurements were significantly larger in the DM2 subgroup (Table 2, Fig. 2). The arm-forearm ratio (AFR) calculated for the upper limb nerves showed no significant difference in any comparisons ($p > 0.23$).

3.2. Type of diabetes

It follows from the above that in the DM1 group only the median nerve-wrist CSA was significantly enlarged. On the other hand, most measurements were significantly larger in the DM2 group than in controls (Table 2, Fig. 3*a*).

3.3. Polyneuropathy

Table 3 shows the p values of statistical comparisons of patient subgroups with or without polyneuropathy within the DM, DM1 and DM2 categories to the control groups, and the comparisons of patient subgroups with polyneuropathy to those without polyneuropathy. It is seen that no major difference was found between subgroups with or without polyneuropathy, even when compared to each other (Table 3, Fig. 3*b*).

3.4. Severity of polyneuropathy

Fig. 4 shows the analysis of median and ulnar nerve forearm CSA values in DM patients with polyneuropathy according to the severity of polyneuropathy. Nerve sizes showed a gradual increase from mild to moderate/medium severity with no further significant changes in severe polyneuropathy. No such tendency was demonstrated on the upper arm or other measurement sites.

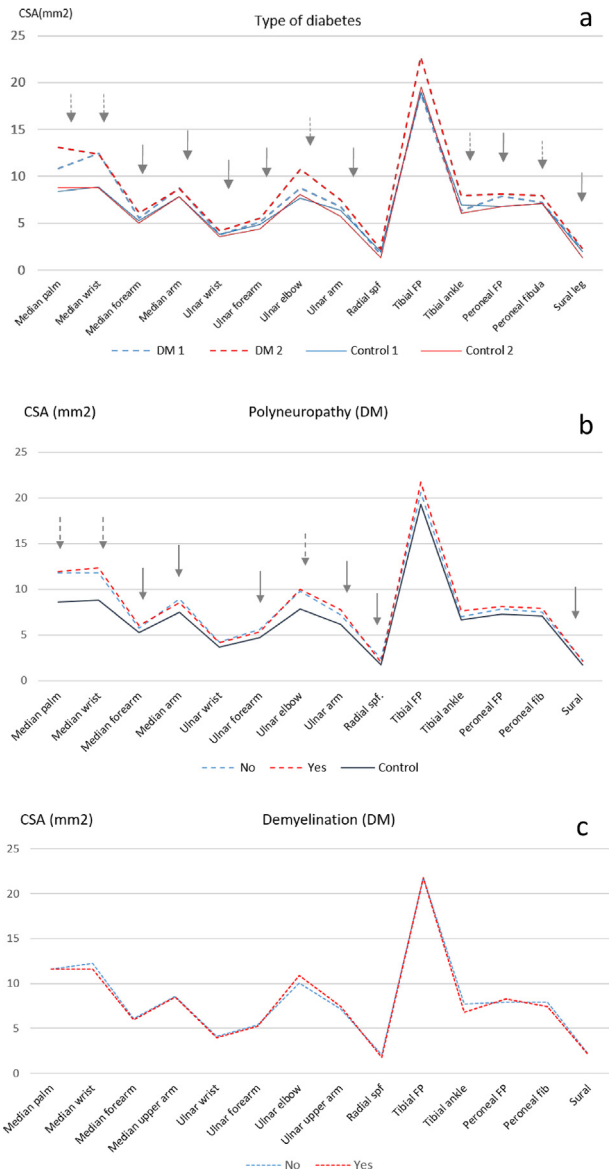

Fig. 3. Mean CSA values of the patient and control groups at 14 measurement sites in the subgroups delineated by the type of diabetes (a), the presence of polyneuropathy (b) and the presence of demyelination (c). Arrows point to the sites with statistically significant difference. Dashed arrows: compression sites; continuous arrows: non-compression sites. Yes: polyneuropathy/demyelination is present; No: no polyneuropathy/demyelination is present; Co: control; CSA: cross-sectional area; spf: superficial; FP: popliteal fossa; fib: fibula.

3.5. Demyelination

The DM subgroup with polyneuropathy (n = 68) was further divided into subgroups with (n = 23) or without (n = 45) signs of demyelination, as shown by electrophysiology. In the demyelinating group, 19 patients had mixed (demyelination and axon loss) electrophysiological abnormalities. No significant difference was found between the CSA values of the subgroups with demyelination or without demyelination *(Fig. 3c).*

3.6. Demographic/clinical characteristics

The duration of diabetes showed a mild negative correlation with the median nerve forearm CSA in DM2 group ($r = -0.23$, $p = 0.02$). No such tendency was demonstrated for other measurement sites. There was no significant correlation of nerve size with age or HbA1c levels in the patient group.

4. Discussion

The mechanism of diabetic neuropathy is unclear, but it is postulated that the accumulation of circulating inflammatory cytokines, the end products of glycolysis, may play a role in demyelination and axonal loss of peripheral nerves *(*Esposito et al. 2002; Yagihasi 2011*).* Abnormal endoneural microcirculation and microangiopathy may also contribute to nerve damage via increased vascular permeability and angiogenesis (Mojaddidi et al, 2014*).* Moreover, neuropathy renders the nerve vulnerable to compression injury *(*Thomsen et al. 2009*).* These functional and morphological abnormalities may cause sonographic visible changes of the peripheral nerves. The aim of our prospective study was to analyze the ultrasonography characteristics of diabetes-related changes in nerve morphology at 14 upper and lower limb measurement sites.

In our diabetic study population, significant nerve enlargement was seen only at one compression site in type 1 diabetes, whereas in type 2 diabetes nerve enlargement was diffuse, but more pronounced at compression sites. In general, nerve enlargement was of lesser degree for lower limb nerves than for upper limb nerves. Nerve enlargement was observed independent of the presence or absence of an associated diabetic polyneuropathy. Subgroup analysis of patients with associated polyneuropathy showed a significant increase of nerve size in the upper limb from mild to moderate/medium polyneuropathy severity, with no further difference in severe forms of polyneuropathy. Thus, nerve enlargement correlated with the severity of polyneuropathy only at the initial stages. Moreover, the presence of electrophysiological demyelination had no effect on nerve size, and no correlation was found between the duration of diabetes/mean HbA1c level and nerve size.

In our study, measurements were taken at multiple sites of multiple upper and lower limb nerves, amounting to 14 upper and lower limb measurement sites including both compression and non-compression sites. We found that nerve enlargement was more pronounced at compression sites versus non-compression sites, and in the upper limbs versus lower limbs. Most studies reported data of only one nerve, such as the median nerve (Chen et al. 2011; Moon et al. 2014; Kim et al. 2014; Attah et al. 2019*),* the tibial nerve (Lee et al. 2005; Riazi et al. 2012), the vagal nerve (Tawfik et al. 2017*),* two nerves (Watanabe et al. 2010; Hobson-Webb et al. 2013; Ishibashi et al. 2015; Agirman et al. 2016; Borire et al. 2018). The studies which analyzed data of multiple nerves did not perform measurements at all compression and non-compression sites of the nerves *(*Tandon et al. 2021; Narayan et al. 2021*).* One of the most detailed study *(*Breiner et al., 2017*)* reported data of 6 upper and lower limb nerves similar to our study, amounting to 11 measurement sites, including both compression and non-compression sites. Similar to our results, they found diffuse nerve enlargement in diabetic patients compared to controls, which was most pronounced at compression sites, such as the ulnar nerve at the elbow and the tibial nerve at the ankle. Moreover, comparing polyneuropathy and non-polyneuropathy patients, nerve enlargement was more pronounced in the upper limbs. Pitarokoili et al. (2016) also described increased CSAs of peripheral nerves at compression and non-compression sites, and detected no correlation between sonographic and electrophysiological findings. They however analyzed only data of type 2 diabetic patients. We can only speculate as to why the nerve enlargement was more pronounced in the upper limb. One possible reason is that most common compression sites are in the upper limb, so the CSA changes are easier to detect. Further-

Table 3
P values of group comparisons stratified according to the presence/absence of polyneuropathy.

Nerve	Site	DM			DM1			DM2		
		No/Co (n = 34)	Yes/Co (n = 68)	No/Yes	No/Co1 (n = 11)	Yes/Co1 (n = 15)	No/Yes	No/Co2 (n = 23)	Yes/Co2 (n = 53)	No/Yes
Median	**Palm**	**<0.001**	**<0.001**	0.51	0.38	0.51	0.77	**<0.001**	**<0.001**	**0.04**
	Wrist	**0.001**	**<0.001**	0.55	**0.047**	**0.002**	0.53	**<0.001**	**<0.001**	0.16
	Forearm	0.07	**0.005**	0.28	0.98	0.24	0.36	**0.04**	**0.03**	0.24
	Arm	**0.007**	**0.01**	0.40	0.34	0.18	0.96	**0.004**	**0.01**	0.32
	AFR	**0.045**	0.77	**0.04**	0.36	0.81	0.48	**0.02**	0.57	0.06
Ulnar	**Wrist**	**<0.001**	**<0.001**	0.26	0.92	0.20	0.63	0.41	0.09	0.90
	Forearm	0.13	0.05	0.47	0.48	0.43	0.86	**0.004**	**0.02**	0.39
	Elbow	**0.005**	**<0.001**	0.06	0.59	0.13	0.30	**0.001**	**<0.001**	0.37
	Arm	**0.005**	**<0.001**	0.71	0.72	0.29	0.74	**0.009**	**0.004**	0.89
	AFR	0.92	**0.04**	0.05	0.71	0.40	0.20	0.70	0.09	0.07
Spf radial	**Forearm**	**0.002**	**0.023**	**0.02**	0.36	0.18	0.83	**<0.001**	**0.002**	**0.002**
Tibial	**Popl**	0.40	0.16	0.40	0.83	0.82	0.79	0.38	0.22	0.90
	Ankle	0.47	0.07	0.47	**0.03**	0.84	0.95	**0.02**	**0.02**	0.41
Fibular	**Popl**	0.207	0.09	0.85	0.25	0.53	0.19	**0.02**	**0.04**	0.49
	Fib	0.19	0.17	0.76	0.57	0.44	0.25	0.21	0.32	0.73
Sural	**Leg**	1.75	**0.005**	0.35	0.30	0.14	0.83	**0.001**	**0.001**	0.06

CSA: cross-sectional area; Yes: polyneuropathy present; No: no polyneuropathy; Co: Control; Spf: superficial; Popl: popliteal fossa; Fib: fibular head; AFR: arm-forearm ratio; p: Kruskal-Wallis test; Highlighted with bold: statistically significant difference.

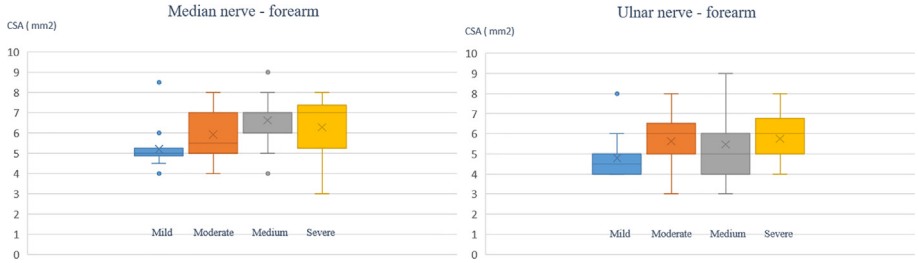

Fig. 4. Median and ulnar nerve-forearm CSA values, in the subgroups according to severity of polyneuropathy (mild: n = 14; moderate: n = 20; medium: n = 22; severe: n = 12). CSAs showed increase from mild to moderate/medium severity, with no further significant changes in severe polyneuropathy. Median nerve: mild v. moderate group: p = 0.054; moderate v. medium group: **p = 0.049;** medium v. severe group: p = 0.86**;** mild v. medium group: **p = 0.0005;** mild v. severe group: **p = 0.045;** Ulnar nerve: mild v. moderate group: **p = 0.049**; moderate v. medium group: p = 0.65; medium v. severe group: p = 0.51; mild v. medium group: p = 0.12; mild v. severe group: **p = 0.035**. Highlighted with bold: statistically significant difference.

more, CSA can be more accurately measured in the upper limb, as sonography of the lower limb nerves are less well differentiated from the surrounding structures on cross-section due to the echogenic properties of the surrounding tissues. It may also be that nerve enlargement in the upper limb develops at an earlier stage of polyneuropathy.

Increased nerve size at compression sites is congruent with the clinical finding of higher incidence of compression neuropathies in diabetes, who are sometimes asymptomatical *(*Brown et al. 1984*)*. It may be perhaps explained by a stronger tendency for edema in areas subject to compression *(*Prinz et al. 2005*)*. These results highlight that compression sites should always be included in the ultrasonography assessment of diabetic patients.

Besides anatomical location, the type of diabetes also had an effect on nerve size in our study with nerve enlargement demonstrated at only one compression site in type 1 diabetes, and mild diffuse nerve enlargement in type 2 diabetes. Data are scarce on the differential effect of the types of diabetes on nerve size. Similar to our results, Breiner et al. (2016) reported data of DM 1 and DM2 patients, and found larger CSAs in the DM 2 than in the DM 1 group at 4 of 11 measurement sites, both in the upper and lower limb compression sites and the upper limb non-compression sites. In our study, BMI was higher in the DM 2 than in the DM 1 group, and in the whole diabetic than in the control group, which may have affected our results. On the other hand, no significant difference in BMI was found between the patient subgroups and respective control groups. Böhm et al. (2014) also reported no consistent correlation with nerve CSA and BMI. Li et al. (2015) and Kelle et al. (2016) found no association between increased CSA and BMI. Concerning the differential effect of DM1 and DM2 on nerve size, it may reflect the different pathomechanism of polyneuropathy in type 1 and 2 diabetes. It has been shown that in addition to hyperglycemia, obesity, dyslipidemia and hypertension are also independent risk factors for polyneuropathy in type 2 diabetes (Smith et al. 2008). In line with this, the effect of intensive glycemic control is much more mitigated in type 2 than in type 1 diabetes (Callaghan et al. 2012). Furthermore, greater edema tendency was recently demonstrated in type 2 than in type 1 diabetes, indicating increased activity of aldolase reductase during glycolysis (Singh et al. 2021*)*.

Interestingly, the presence of an associated diabetic polyneuropathy had no significant effect on nerve size. However, when polyneuropathy severity was taken into account, some mild effect was demonstrated at the initial stages of polyneuropathy. In the study of Ishibashi et al. (2015), nerve size increase was also observed prior to the appearance of polyneuropathy, which then showed further increase with the development and worsening of neuropathy. Similar results were published by Breiner et al. (2016) and Tandon et al. (2021), who observed slightly larger CSAs in the polyneuropathy than in the non-polyneuropathy group, but

the difference was small and reached statistical significance only in the upper limbs. However, Breiner et al. (2017) did not create subgroups by severity of polyneuropathy in the polyneuropathy group, but divided the non-polyneuropathy group to two further subgroups based on presence or absence the clinical signs and symptoms suggestive of polyneuropathy. In these non-polyneuropathy subgroups, higher CSA values were found in those with more symptoms or signs suggestive of neuropathy. It has been shown in experimental studies (Powell and Myers, 1986) that axonal degeneration was associated with higher levels of endoneural oedema in the acute stages. Altogether, these data suggest that nerve size increase may precede the onset of electrophysiological/clinical signs of polyneuropathy in diabetic patients.

Diabetic polyneuropathy is typically a length-dependent axonal polyneuropathy, however in some patients electrophysiological studies also suggest the presence of demyelination as well. In diabetes, Dunnigan et al. (2013) distinguished axonal, demyelinating and mixed types of polyneuropathy, based on electrophysiological abnormalities. During electrophysiological studies, some of our patients had some degree of overall mild/moderate temporal dispersion or diffuse conduction velocity decrease in the lower limb motor nerves, with no conduction blocks or upper limb involvement. There was no direct association between the presence of demyelinating features in a particular nerve and the nerve specific ultrasound CSA values. Moreover, no significant difference in nerve size was found between the subgroup with some degree of demyelination and the purely axonal polyneuropathy subgroup. A large body of evidence shows that acquired or inherited demyelinating polyneuropathies are associated with a more robust nerve swelling than axonal polyneuropathies (Zaidman et al. 2009; Grimm et al. 2014). Narayan et al. (2021) reported a more marked nerve swelling in diabetic polyneuropathy with a demyelinating pattern, but these finding were observed in patients with mixed EMG abnormalities and mainly at the compression sites. Thus, the possibility that compression neuropathy is the cause of this difference can be raised. In our study, no additional increase in nerve size was associated with demyelination. This may be related to methodological issues, e.g. low sample number, and the generally mild degree of demyelination and nerve swelling in diabetes, rendering the demonstration of group differences more difficult. In addition, the group of patients with mixed (demyelination and axon loss) electrophysiological abnormalities was not separated in our study, we classified these patients in the demyelinating group also. This could be affected our results, as axon loss causes minor nerve swelling.

To conclude, our prospective study has shown that the pattern of nerve enlargement in diabetes is different from that of other polyneuropathies. While acquired demyelinating polyneuropathies are characterized by robust nerve enlargement predominantly in proximal upper limb nerves (Scheidl et al. 2014; Padua et al. 2013) and other axonal neuropathies show diffuse nerve enlargement albeit of lesser degree (Zaidman et al. 2009), diffuse mild-to-moderate (in comparison to acquired demyelinating polyneuropathies) increase in nerve size is characteristic in diabetes, which is most pronounced at compression sites as opposed to non-compression sites, and in the upper limbs, as opposed to the lower limbs. Changes occur even before the onset of electrophysiological/clinical signs of polyneuropathy. Nerve enlargement at compression sites may indicate susceptibility to compression neuropathy, and changes at non-compression sites may suggest early morphological abnormalities of polyneuropathy. In addition to anatomical location, the degree of nerve enlargement was affected mainly by the type of diabetes, with type 2 diabetes having a more robust effect, and with some secondary effect of the severity of polyneuropathy. These results highlight that ultrasonography may show neuropathic changes early, even before the clinical/electrophysiological signs of polyneuropathy are detected, but it is yet to be determined whether it can be put to use in the diagnostic setting in an individual patient. Furthermore, our results may contribute to the delineation of an ultrasonography protocol for the assessment of nerves in patients with diabetes.

There are limitations to our study. Patients with subclinical small-fiber neuropathy, characteristic in diabetes and often preceding the development of thick-fiber neuropathy, were missed, as electrophysiological examination detects only thick-fiber polyneuropathy (Ishibashi et al. 2015). However, small-fiber neuropathy is difficult to quantify and involves skin biopsy, which hinders a large-scale study. Another limitation is that only CSA values were used for morphological analyses, other methods, such as echogenicity analysis were not performed. Finally, the physician performing the ultrasound was not 'blinded' to the electrophysiological results due to lack of staff.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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