Original Article

Ultrasonography Lower Extremity Nerve Cross-sectional Area Reference Values including Demographic and Electrophysiological Relationships in Healthy Thai Adults

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ABSTRACT

Objectives: To establish the average values of cross-sectional area (CSA) in the sural, fibular, and tibial nerves by ultrasonography and to investigate correlations with demographic data and nerve conduction studies (NCS)

Study design: A cross-sectional study

Setting: Lerdsin Hospital, Bangkok, Thailand

Subjects: Healthy Thai adults aged 19-80 years

Methods: Ninety participants were recruited and their CSA was measured at seven sites within the three nerves of the lower extremities. The NCS was performed and the mean was calculated for the values at each site. Demographic data or NCS parameters were identified as correlations with the CSA.

Results: The mean and standard deviation values of the CSA (mm^2) were: (1) sural: 2.4 (0.5), (2) fibular nerve - ankle 1.6 (0.5), fibular neck (FN) 8.3 (1.5), fibular head (FH) 9.5 (1.7), popliteal fossa (PF) 12.5 (1.5), (3) tibial nerve - ankle 11.5 (2.5), PF 16.4 (2.9), (4) the FH/PF and FH/FN ratios were 1.1 (0.2), and 1.2 (0.2). The CSAs of the sural ($p = 0.048$), fibular at FN ($p = 0.025$), fibular at PF ($p = 0.043$), and tibial at PF ($p = 0.043$) nerves were significantly greater in male than those in female. The CSA was statistically correlated with age, weight, and body mass index in the sural nerve, several sites of the fibular nerve, and the tibial nerve at the ankle. Height was associated with the CSA of the tibial nerve at the ankle ($r = 0.209$, $p = 0.048$). The CSA of the tibial nerve at PF was statistically significantly correlated with the proximal amplitude of the tibial motor NCS ($r = -0.233$, $p = 0.027$).

Conclusions: Establishing nerve CSA particular to the Thai population may offer normative values and differentiate aberrant neural structures. Some demographic characteristics and NCS can have an impact on the CSA.

Keywords: cross-sectional area, ultrasonography, peripheral nerves, electrodiagnosis

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Introduction

High-resolution ultrasonography can help to diagnose peripheral neurological disorders. It can locate nerve lesions and reveal nerve morphology.[1, 2] The benefits of using ultrasound imaging are that it is non-invasive, has dynamic capability, is affordable, and does not expose individuals to radiation. The nerve's cross-sectional area (CSA) is the most studied metric for diagnosing nerve disorders. That value is obtained by placing the probe perpendicular to the nerve. The CSA increases when the nerve is inflamed or compressed[2, 3] and decreases when the nerve fibers are lost, e.g., when there is motor neuron disease.[4] Previous studies have indicated that the CSA of the nerve is associated with race, sex, height, weight, and body mass index (BMI)[5-8], but is not related to the site on the body.[9] One study that investigated the upper extremities found that the ulnar nerve's CSA was affected by multiethnicity, even though the study group was predominantly Asian.[10] However, there have been few investigations of the CSA of the nerves of the lower extremities because the muscles, blood vessels, and nerves are deeper and more complex. Thus, the CSA in the lower extremities is more challenging to measure than in the upper extremities.[11] Seok et al.[12] reported that the CSA of the sciatic, fibular, and tibial nerves in the Korean population is lower than in the Caucasian population. However, the electrodiagnosis was not tested to exclude peripheral neuropathy in their study. Fisse et al.[13] conducted a systematic review of the CSA of the nerves of the lower limbs, with the majority of the subjects from Europe and North America and a minority from Asia. They concluded that the CSA of the fibular and sural nerves are not different in different populations.

According to a recent systematic review and meta-analysis, no statistically significant changes were seen in the tibial nerve's CSA at the ankle and popliteal fossa (PF) across different geographical regions.[8] However, there was a discrepancy in the tibial nerve regarding the level of the PF, which could be attributed to the testing approach.[13] The findings and conclusions of prior studies have been varied and not consistent with each other.

A nerve conduction study (NCS) is a physiological nerve test to diagnose peripheral nervous system issues. Use of NCS combined with ultrasonography is becoming increasingly

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widespread, improving differential diagnostic accuracy. Ultrasonography helps to identify peripheral nerve diseases, e.g., soft tissue edema, in individuals with NCS limitations.[14] However, few studies have investigated the relationship between the CSA and NCS parameters, which may potentially be beneficial for adjunctive diagnosis. According to one previous study, an increase in the CSA of the ulnar nerve at the wrist results in statistically significantly prolonged distal motor latency.[5.] This association has not been investigated in the nerves of the lower extremities.

The purpose of this study was to establish normal reference values of the CSA of the sural, the fibular, and the tibial nerves in a healthy Thai population and to investigate the potential relationship between the CSA and demographic data, including sex, age, weight, height, and BMI and the NCS parameters. Each of these measures can provide the clinician with diverse diagnostic tools for assessing pathological changes in peripheral nerve conditions.

Methods

Study design

This study was a prospective cross-sectional study and was approved by The Ethics Committee at Lerdsin Hospital. The certification number is LH651010. The Thai Clinical Trials Registry number is TCTR20220204003.

The study was conducted at Lerdsin Hospital from May 2022 to May 2023. Prior to assessment, signed informed consent was obtained from the participants. The inclusion criteria were Thai ethnicity, good health, and age between 19 and 80. Participants who had relevant neuromuscular symptoms, including numbness, neuropathic pain or weakness, a history of peripheral polyneuropathy, neuromuscular diseases, degenerative spinal stenosis, intervertebral disc disease, other medical conditions that affect peripheral nerves (dia betic mellitus, chronic renal failure, liver disease, thyroid disease, immunocompromised, autoimmune disease, alcoholism), were pregnant, had a history of internal fixation in the legs, used a pacemaker or had abnormal NCS by screening were excluded.

Following a study by Seok[12], the sample size was calculated using an 'infinite population mean formula'. The mean CSA and standard deviation (SD) of the sural, fibular, and tibial nerves were calculated for each site. We used an error (d) of 7% and an alpha value of 0.05 to calculate values with the maximum sample size (tibial nerve) used in the study. The sample size was 78 participants. With an estimated potential dropout rate of 10%, 90 participants were included. We divided the participants into three groups by age (19-30, 31-50, and 51-80) following the previous studies and due to the fact that the NCS parameters change as the age increases.[5, 15] Each age group included an equal number of men and women.

Intervention

A brief history of the patients was taken and clinical examinations were performed. Age, sex, height, weight, and BMI were determined. Before the ultrasonography examination, all participants were screened for NCS to rule out peripheral polyneuropathy using a reference from the American Association of Neuromuscular & Electrodiagnostic Medicine (AANEM) 2020.[16]

NCS

The tibial, fibular, ulnar, and sural nerves were tested bilaterally by a physiatrist using a Nicolet Synergy equipment (Natus Medical Inc., San Carlos, California, USA). Participants lay on a bed in the supine and lateral decubitus positions. Skin temperature was maintained at 32-34 degrees Celsius.

Compound motor action potential (CMAP) NCS

- Tibial motor study: the recording electrodes were placed on the abductor hallucis (AH) muscle. The stimulation sites were at the posterior medial malleolus 8 cm from the AH muscle and the PF.

- Fibular motor study: the recording electrodes were placed on the extensor digitorum brevis (EDB) muscle. The stimulation sites were at the anterior ankle 8 cm from the EDB muscle and posteroinferior to the fibular head (FH).

- Ulnar motor study: the recording electrodes were placed on the abductor digiti minimi (ADM) muscle. The stimulation sites were at the volar wrist 8 cm from the ADM muscle and the olecranon fossa.

Sensory nerve action potential (SNAP) NCS

- Sural sensory study: the stimulation site was at the lateral calf 14 cm from the electrode at the lateral malleolus.

- Ulnar sensory study: the stimulation site at the volar wrist was lateral to the flexor carpi ulnaris tendon 8 cm from the ring electrode on the little finger.

Ultrasonography

All individuals were tested using a multifrequency linear transducer 4-18 MHz (Konica Minolta, SONIMAGE® HS1, Tokyo, Japan) in B mode. The ultrasound examination was performed by a physician with six years of musculoskeletal ultrasound experience. Using ultrasonography, each nerve was detected, the transducer angle adjusted until perpendicular to the nerve to obtain the images with the smallest CSA. The color Doppler test was used to check the vascular component. The distance between the skin and the target location determined focus and depth. The CSA was measured at each location using the elliptical function to trace inside the nerve's hyperechoic border. Each location, including the body's left and right sides, was examined three times. Inter-rater reliability was evaluated one week after the first visit by a physician with one year of experience in neuromuscular ultrasonography who was blinded to the previous outcomes. Thirty participants were chosen at random for this measurement. Before beginning data collection, both investigators received specialized training specific to this study.

Sural Nerve. With the participant in a lateral decubitus position, the CSA was measured at 14 cm from the lateral malleolus, which runs lateral to the small saphenous vein (Figure 1).

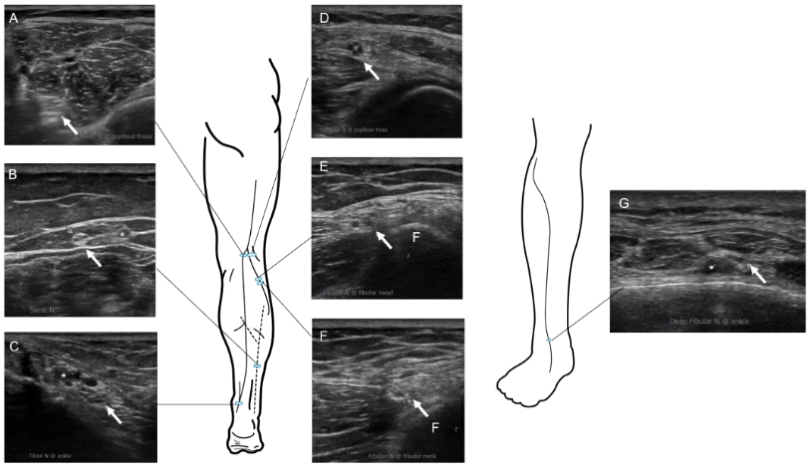

Figure 1. Sites of nerve ultrasonography to determine the cross-sectional area (CSA). (A) tibial nerve at popliteal fossa, (B) sural nerve, (C) tibial nerve at the ankle, (D) fibular nerve at the popliteal fossa, (E) fibular nerve at the fibular head, (F) fibular nerve at the fibular neck, (G) fibular nerve at the ankle. Arrows are the CSA of the nerve. The vessels are marked with an asterisk (*).

Fibular Nerve. With the participant in a lateral decubitus position, the first measurement was done at the PF, where the fibular nerve is lateral to the short head of the biceps femoris tendon and just superior to the lateral tibial condyle. The second was made at the FH. The third was obtained at the fibular neck (FN) before bifurcation into the deep fibular and superficial fibular nerves. The participant was then shifted to a supine position and the last measurement was made at the anterior ankle lateral to the dorsal pedis artery (Figure 1).

Tibial Nerve. Participants lay down in a prone position. Following the path of the sciatic nerve, the tibial nerve is located posterior to the popliteal vein. The initial measurement was taken at the PF. The second measurement was obtained at the medial ankle (Figure 1).

These sites were chosen based on various criteria, including anatomical landmarks, clinically significant points, and proximity to the point of NCS stimulation.

Outcome measurements

The CSA of each nerve in each of the selected locations was recorded. The FH/PF fibular nerve CSA ratio was calculated by dividing the FH fibular nerve CSA by the PF fibular nerve CSA. By dividing the fibular nerve CSA at the FH by the fibular nerve CSA at the FN, the FH/FN fibular nerve CSA ratio was obtained. The NCS were measured, including the distal and proximal CMAP latency, amplitude, and fibular and tibial nerve area. The latency and amplitude of the sural SNAP were also noted.

Statistical methods

Statistical analysis was performed using the PASW Statistics version 18.0 program. (SPSS Inc., Chicaco, IL, USA). Continuous data is presented as mean and standard deviation (SD). Categorical data is presented as frequencies and percentages. The mean ± 2SD was used to calculate the reference ranges of the nerve CSAs, the FH/PF, and the FH/FN ratios of the fibular nerve. The unpaired t-test was used for comparison between the sexes. The paired t-test was used for side-by-side comparison. The one-way ANOVA test was used to compare the mean CSA among age groups. The correlations between the CSA at each site of each nerve and demographic factors (age, weight, height, BMI) or NCS parameters were evaluated using Pearson's correlation coefficient (r). Intraclass correlation coefficient (ICC) was used to measure the inter-rater reliability of ultrasonographic testing. An ICC of 0.61-0.80 was regarded as a good agreement, and an ICC greater than 0.80 was considered excellent.[17] A p-value of < 0.05 was considered statistically significant.

Results

This study included a total of 90 participants. The CSA of bilateral fibular nerves at the FN and right tibial nerve at the PF could not be accurately measured in one male participant due to the vascular mixing in the nerve fascicle, so the study included 89 participants. Of the 89 participants, 14 were male and in the 31-50 age group (Figure 2). The mean age, weight, height, and BMI of the participants were 41.4±14.5 years, 64.1±13.8 kg, 163.2±8.5 cm, and 24.5±4.3 kg/m^2, respectively (Table 1). The inter-rater reliability was good to very good as indicated by the ICC, ranging from 0.80 to 0.98.

Comparison of demographic data and side-to-side

Statistically significant differences between sexes were found in the sural nerve ($p = 0.048$), the fibular nerve at the FN ($p = 0.025$), the fibular nerve at the PF ($p = 0.043$), and the tibial nerve at the PF ($p = 0.043$). The CSA of male participants was larger than that of female participants. The CSA of the fibular nerve at the FN ($p = 0.022$), the FH ($p < 0.001$), and the PF ($p = 0.040$) were significant different among age groups. The CSA of the 51-80 age group was greater than that of the others. There was no statistically significant difference in the CSA between the left and right sides at all measurement sites (Table 2).

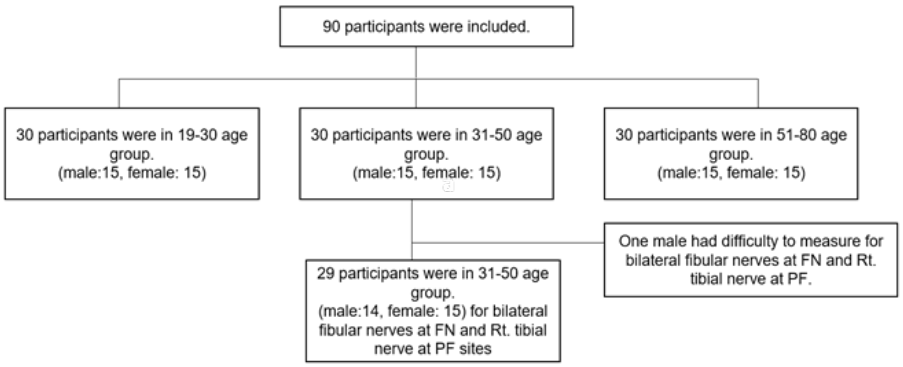

Figure 2. Flow chart of participants: FN, fibular neck; PF, popliteal fossa

Table 1. Demographic data of participants

Items	All[1] (n = 90)	Men[1] (n = 45)	Women[1] (n = 45)
Age (year)	41.4 (14.5) [19-72]	40.6 (14.4) [19-72]	42.2 (14.8) [23-70]
Weight (kg)	64.1 (13.8) [42-100]	69.4 (12.5) [45-100]	58.9 (13.1) [42-90]
Height (cm)	163.2 (8.5) [144-189]	169.2 (6.4) [157-189]	157.3 (5.8) [144-168]
BMI (kg/m^2)	25.0 (4.3) [15.2-34.1]	24.2 (4.1) [15.2-33.9]	23.7 (4.5) [17.5-34.1]

[1]Mean (SD) [range]; n, number; SD, standard deviation; kg, kilogram; cm, centimeter; BMI, body mass index; m^2, square meters

Table 2. Ultrasonography cross-sectional area (mm^2) of lower limb peripheral nerves in healthy participants

Nerve site	n	All[1]	Min, max	Ref range (2SD)	Side[1,a]			Sex[1,b]			Age group[1,c]			
					Left	Right	*p*-value	Male	Female	*p*-value	19-30	31-50	51-80	*p*-value
Sural nerve	180	2.4 (0.5)	1.0,4.2	1.3,3.4	2.4 (0.6)	2.3 (0.6)	0.714	2.5 (0.5)	2.2 (0.5)	0.048*	2.3 (0.5)	2.3 (0.4)	2.5 (0.6)	0.134
Fibular nerve														
Ankle	180	1.6 (0.5)	1.0,3.0	0.6,2.6	1.6 (0.6)	1.6 (0.5)	0.685	1.6 (0.5)	1.7 (0.5)	0.089	1.6 (0.5)	1.6 (0.5)	1.7 (0.5)	0.688
FN	178	8.3 (1.5)	4.0,12.3	5.2,11.4	8.4 (1.8)	8.2 (1.6)	0.404	8.7 (1.6)	8.0 (1.4)	0.025*	7.9 (1.1)	8.1 (1.4)	8.9 (1.8)	0.022*
FH	180	9.5 (1.7)	6.5,14.0	6.1,12.9	9.5 (2.0)	9.5 (1.8)	0.834	9.7 (1.8)	9.2 (1.6)	0.162	8.9 (1.4)	9.0 (1.3)	10.6 (1.9)	<0.001*
PF	180	12.5 (1.8)	9.2,16.7	8.9,16.1	8.5 (1.6)	8.6 (1.6)	0.184	12.9 (1.5)	12.1 (2.0)	0.043*	12.2 (1.6)	12.1 (1.6)	13.2 (2.0)	0.040*
FH/PF	180	1.1 (0.2)	0.8,1.6	0.8,1.4	1.1 (0.2)	1.1 (0.2)	0.149	1.1 (0.2)	1.1 (0.1)	0.387	1.2 (0.1)	1.2 (0.1)	1.2 (0.2)	0.077
FH/FN	178	1.2 (0.2)	0.9,2.4	0.8,1.6	1.2 (0.3)	1.2 (0.2)	0.629	1.1 (0.1)	1.2 (0.3)	0.207	1.1 (0.1)	1.2 (0.2)	1.2 (0.3)	0.139
Tibial nerve														
Ankle	180	11.5 (2.5)	7.0,20.5	6.6,16.4	11.6 (2.5)	11.4 (2.5)	0.226	11.9 (2.6)	11.1 (2.3)	0.159	11.4 (2.6)	10.9 (2.0)	12.2 (2.5)	0.098
PF	179	16.4 (2.9)	10.5,22.0	10.6,22.2	16.3 (2.9)	16.5 (3.0)	0.328	17.0 (2.6)	15.8 (3.1)	0.043*	15.8 (2.8)	16.3 (2.7)	17.1 (3.1)	0.230

[1]Mean (SD) [range], [a]Paired t-test, [b]Unpaired t-test, [c]One-way ANOVA test, *statistically significant; mm^2; square millimeter; SD, standard deviation; n, number; Min, minimum; Max, maximum; Ref, reference; FN, fibular neck; FH, fibular head; PF, popliteal fossa

The CSA measurements.

The normal CSA values of each nerve, the FH/PF ratio, and the FH/FN ratio of the fibular nerve in all participants are shown in Table 2.

Correlation of nerve cross-sectional area with demographics

Age, weight, and BMI showed a significant positive correlation with the CSA in the following nerves: the sural, the fibular at the FN, FH, PF, and the tibial at the ankle. There was also a significant association between age and the FH/FN ratio of the fibular nerve. Height was associated with the CSA of the tibial nerve at the ankle (Table 3) (Figures 3, 4).

Correlation of nerve cross-sectional area with NCS

The CSA of the tibial nerve at the PF correlated significantly with the proximal amplitude of the tibial motor NCS (r = -0.233, *p* = 0.027). The CSA of the tibial nerve at the PF rises as the proximal tibial amplitude motor NCS decreases (Table 4).

Discussion

This study was the first in Thailand to establish normal lower extremity nerve CSA reference values. We presented the CSA of seven sites of three common nerves. When just the mean values were compared in each nerve at each site (sural nerve, fibular nerve; ankle, FN, FH, PF, tibial nerve; ankle, PF), our findings were consistent with reported

Table 3. Correlation of cross-sectional area (mm^2) with age, weight, height, and body mass index

Nerve sites	Age[a]		Weight[a]		Height[a]		BMI[a]	
	r	*p*- value	r	*p*- value	r	*p*- value	r	*p*- value
Sural nerve	0.253	0.016*	0.540	<0.001*	0.167	0.116	0.554	<0.001*
Fibular nerve								
Ankle	0.176	0.098	0.026	0.805	-0.107	0.315	0.289	0.317
FN	0.282	0.007*	0.344	<0.001*	0.100	0.351	0.359	<0.001*
FH	0.453	<0.001*	0.293	0.005*	0.069	0.519	0.317	0.002*
PF	0.249	0.018*	0.250	0.017*	0.112	0.294	0.233	0.028*
FH/PF	0.178	0.093	-0.030	0.780	-0.066	0.539	0.002	0.984
FH/FN	0.245	0.020*	-0.130	0.224	-0.064	0.554	-0.116	0.277
Tibial nerve								
Ankle	0.237	0.024*	0.365	<0.001*	0.209	0.048*	0.311	0.004*
PF	0.185	0.082	0.144	0.176	0.073	0.496	0.127	0.232

[a]Pearson's correlation coefficient (r); * statistically significant; mm^2, square millimeter; BMI, body mass index; FN, fibular neck; FH, fibular head; PF, popliteal fossa

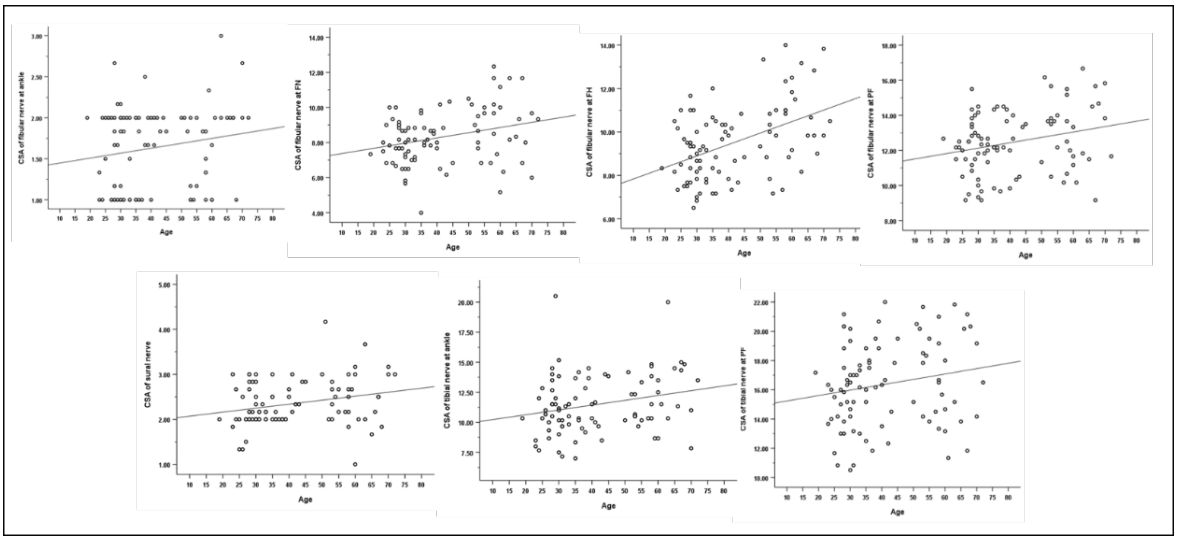

Figure 3. Correlation of cross-sectional area (mm^2) with age at each site of the lower limbs' nerves; CSA, cross-sectional area; FN, fibular neck; FH, fibular head; PF, popliteal fossa

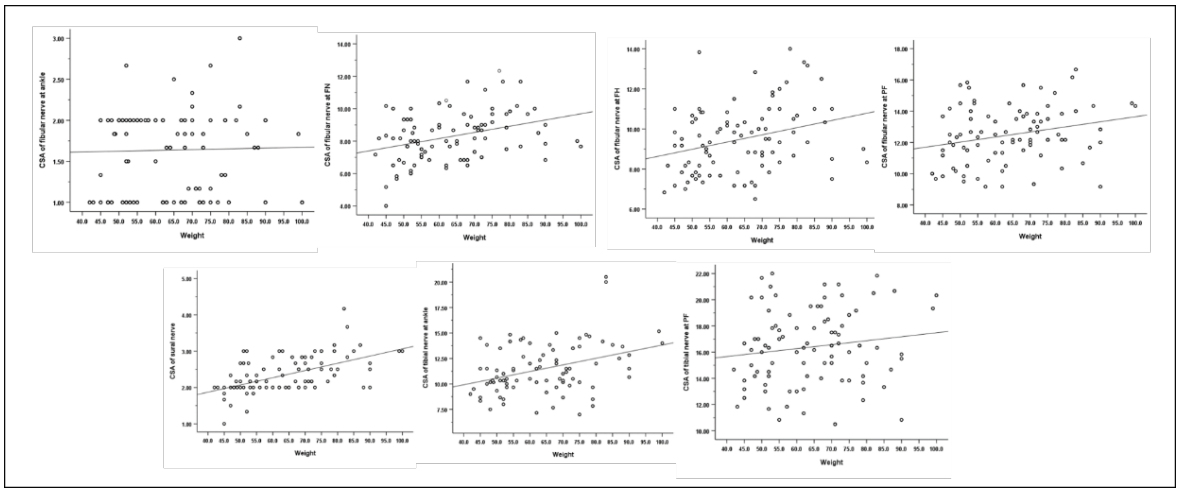

Figure 4. Correlation of the cross-sectional area (mm^2) with weight at each site of the lower limbs' nerves; CSA, cross-sectional area; FN, fibular neck; FH, fibular head; PF, popliteal fossa

Table 4. Correlation of cross-sectional area (mm^2) with nerve conduction study

Deep fibular motor study	CSA of the fibular nerve at ankle[a]		Deep fibular motor study	CSA of fibular nerve at FN[a]	
	r	*p*- value		r	*p*- value
Distal latency	-0.090	0.407	Proximal latency	-0.025	0.813
Distal amplitude	0.123	0.258	Proximal amplitude	-0.039	0.716
Distal area	0.088	0.420	Proximal area	-0.059	0.583
Tibial motor study	CSA of tibial nerve at ankle[a]		Tibial motor study	CSA of tibial nerve at PF[a]	
	r	*p*- value		R	*p*- value
Distal latency	0.044	0.680	Proximal latency	0.183	0.084
Distal amplitude	-0.148	0.163	Proximal amplitude	-0.233	0.027*
Distal area	-0.146	0.169	Proximal area	-0.150	0.160

[a]Pearson's correlation coefficient (r); *statistically significant; mm^2, square millimeters; NCS, nerve conduction study; CSA, cross-sectional area; FN, fibular neck; PF, popliteal fossa

Table 5. Comparison of demographic data with the previous studies

Study	Ethnicity	Age[1]	Sex (M:F)	Weight[1]	Height[1]	BMI[1]
Present study	Thai	41.4 (14.5)	45:45	64.1 (13.8)	163.2 (8.5)	24.9 (4.3)
Tan[10]	Malaysian	40.0 (14.4)	38:46	64.7 (14.3)	160 (10.1)	24.2 (4.8)
Hsieh[21]	Taiwanese	42.1 (14.0)	30:36	63.6 (11.3)	163.2 (8.1)	23.9 (3.3)
Seok[12]	Korean	43.9 (14.4)	44:50	62.8 (12.2)	165.2 (9.0)	22.9 (3.1)
Bae[18]	Korean	46.3 (14.2)	51:56	64.4 (11.4)	165 (8.7)	23.3 (2.7)
Bedewi[7]	Asian	38.3 (12.1)	N/A	77.1 (18.4)	161.5 (9.8)	29.3 (6.6)
Qrimli[19]	Canadian	44.1 (18.4)	30:70	N/A	N/A	25.3 (5.3)
Kerasnoudis[20]	German	53.5 (14.8)	45:30	N/A	175 (9.0)	77.9 (10.7)
Grimm[22]	German	51.2 (18.2)	55:45	74.5 (24.1)	174.5 (11.4)	N/A
Boehm[23]	European	N/A	26:30	N/A	N/A	N/A
Cartwright[24]	American	45.9	22:38	74.5	168	26.5
Lothet[6]	American	N/A	N/A	N/A	N/A	N/A

[1]Mean (SD); M, male; F, female; BMI, body mass index; N/A, not applicable

Table 6. Comparison with cross-sectional area (mm^2) of the lower extremities' nerves in the previous studies

Study	Ethnicity	Sural nerve[1]	Fibular nerve[1]				Tibial nerve[1]	
			Ankle	FN	FH	PF	Ankle	PF
Present study	Thai	2.3 (0.5)	1.5 (0.5)	8.3 (1.5)	9.5 (1.7)	12.5 (1.8)	11.5 (2.5)	16.4 (2.9)
Tan[10]	Malaysian	1.5 (0.6)	N/A	N/A	8.9 (2.0)	7.5 (1.8)	10.1 (2.0)	11.8 (2.2)
Hsieh[21]	Taiwanese	2.3 (0.7)	N/A	N/A	N/A	12.1 (2.2)	8.5 (2.2)	20.8 (4.8)
Seok[12]	Korean	2.6 (0.6) (D)	N/A	N/A	9.2 (2.9)	10.4 (2.7)	12.1 (3.1)	24.4 (4.4)
Bae[18]	Korean	3.3 (1.0) (D)	2.1 (1.0)	N/A	12.5 (3.5)	11.4 (3.7)	15.6 (3.8)	24.7 (6.1)
Bedewi[7]	Asian	3.5 (1.4)	N/A	N/A	8.9 (3.2)	9.7 (4.1)	12.7 (4.5)	19.1 (6.9)
Qrimli[19]	Canadian	2.1 (1.0)	N/A	N/A	11.1 (3.9)	11.7 (3.8)	12.7 (3.4)	N/A
Kerasnoudis[20]	German	1.8 (0.6)	N/A	N/A	7.1 (2.3)	8.6 (1.8)	6.4 (1.5)	8.4 (2.7)
Grimm[22]	German	2.2 (0.6)	N/A	N/A	N/A	8.4 (1.6)	10.2 (2.0)	23.2 (4.9)
Boehm[23]	European	1.8 (0.6) (P)	N/A	8.9 (2.0)	N/A	N/A	9.6 (2.2)	N/A
Cartwright[24]	American	5.3 (1.8) (D)	N/A	N/A	11.2 (3.3)	11.7 (4.6)	13.3 (4.3)	35.3 (10.3)
Lothet[6]	American	3.8 (1.4)	N/A	N/A	12.4 (3.3)	N/A	12.3 (3.5)	N/A

[1]Mean (SD); mm^2, square millimeter; D, distal; P, proximal FN, fibular neck; FH, fibular head; PF, popliteal fossa; N/A, not applicable

values in most of the previous studies, although some findings were different. Our results and those of prior studies are summarized in Tables 5 and 6. Despite a finding that Asians did not differ in age, weight, height, and BMI, the CSAs at the same location varied. Bae et al.[18] In that study, reported CSAs were greater than those in the present study at multiple locations. In Qrimli's study in Canada, the CSA at several locations appears comparable to our findings[19], but specific CSA sites investigated in a study in Germany were smaller than ours.[20] This finding suggests that ethnicity may have an impact on CSA values.

The CSA of the fibular nerve at the FH has shown variation across many investigations (7.1-12.5 mm^2). This variation may be due to differences in ultrasound measurement methods. The common fibular nerve curves around the fibular neck, close to the bone. It goes from posterolateral to anterolateral beneath the fibular neck before splitting into its branches.[25] The fibular nerve assumes an average angle of 28.2±7.3 degrees relative to the longitudinal axis of the fibula bone in Thais, but this angle varies across populations.[26] Positioning the ultrasound probe in the short axis perpendicular to the nerve for the smallest CSA measurement was not easy. It was also challenging to determine the CSA of the tibial nerve at the PF. There have been measurement discrepancies in many previous studies ranging from 8.4 to 35.3 mm^2. The standard deviation has also showed a wide variation in different studies. The frequency of the ultrasound may affect the trace of the inner border of the nerve site due to the deep structure and poor visualization.

The CSA values of the sural nerve, fibular nerve (FN, FH), and tibial nerve (PF) were significantly higher in males. This finding is similar to a previous study.[20] The explanation for the difference might be that on average men have a higher weight, height, and BMI than women which might influence the CSA of nerves. The side-to-side difference was not statistically significant, a finding comparable to several studies.[7, 10, 12, 21] Based on this observation, the side-to-side difference might be used as an internal control, e.g., detecting nerve disease in extremely thin or obese individuals or patients of different ethnicities with a unilateral lesion. We discovered that the CSA of nerves at numerous sites (sural nerve, fibular nerve at FN and FH, and PF tibial nerve at the ankle) had a statistically significant positive association with advanced age. These results correspond with previous studies.[10, 12, 19, 21, 24] Research findings on the morphometric effects of aging on the tibial nerves in mice may explain this. Aging affects the diverse elements of the tibial nerve differently. In mice, outfolded myelin loops with irregular shapes, macrophages, and mast cells increase significantly with advanced age in the endoneurium, resulting in the CSA becoming larger.[27]

Statistically significant differences in the CSA and weight were seen in the sural, fibular (FN, FH, PF), and tibial (ankle) nerves. However, our investigation found only the CSA of the tibial nerve at the ankle to be correlated with height, a finding which differs from previous research.[10, 12, 22-24] The CSA should be used primarily in tall and obese people. The intra-nerve variability (calculated as maximal CSA/minimal CSA) and the inter-nerve CSA variability have recently been determined, allowing quantification of heterogeneous nerve involvement in immune-mediated neuropathies.[28] The fibular nerve ratio has also been calculated using specific landmarks, including the FH and the PF. These novel measurements are less affected by height and weight.

Our investigation found that as the CSA increases, there is a corresponding drop in the proximal amplitude in the tibial motor NCS. Similar results were found in a prior study conducted in an general population.[5] Studies of focal neuropathy have shown that the CSA is enlarged when neuropathy is more severe. Motor NCS amplitude is usually significantly reduced.[2] Furthermore, the amplitude of the tibial CMAP and the CSA of the tibial nerve exhibit a negative correlation in patients with diabetic polyneuropathy,[29] a finding notable for its parallels with nerve pathology.

Moreover, the histology of the tibial nerve shows a greater CSA as the nerve fascicle increases.[30] However, the amplitude of the CMAP, which indicates the number of motor nerve fibers that responded to stimulus and which could transmit impulses to the recorded muscle, decreased. This finding seems to imply that the morphology of a nerve may not always reflect its physiological characteristics.

Inter-rater reliability in our study ranged from good to very good. The findings of this study are consistent with an earlier report[9], indicating that high-resolution ultrasonography may be used as a reliable method for measuring nerve CSA in the lower extremities. However, technical expertise, knowledge of anatomical landmarks, and development of study protocols are necessary to accurate ultrasound examinations.

Currently, there has been an increasing amount of research focused on using ultrasonography to diagnose focal neuropathy and polyneuropathy. The common fibular nerve is the most common form of compressive neuropathy of the lower limbs. Previous research has found that cut-off values of the CSA at the FH and a ratio of the FH/PF higher than 11.7 mm^2 and 1.1, respectively, indicate high sensitivity and specificity.[2, 31] Likewise, the CSA of the tibial nerve at the ankle which identifies posterior tarsal tunnel syndrome is more than 15 mm^2.[2] However, our research found that the upper normal limit of the fibular CSA at the FH was 12.9 mm^2, and that of the tibial CSA at the ankle was 16.4 mm^2. The normal CSA values in healthy individuals were discovered to be slightly higher than the in patients with diseases. Telleman et al.[32] presented an overview of a scoring system for assessing the pattern of nerve abnormality in hereditary and acquired polyneuropathies. With that system, the degree of nerve CSA enlargement in the upper and lower limbs at each landmark can be used to differentiate peripheral neuropathy diagnoses. Thus, the CSA reference values are essential.

The optimal CSA cut-off values in the lower limb nerves for diagnosing diabetic polyneuropathy were 11.6 mm^2 of the fibular nerve at the FH and 4.2 mm^2 of the sural nerve.[29] The present CSA findings may serve as reference values in investigations or can be utilized as supplementary evidence when electrodiagnosis is limited. Additionally, when subgroup analysis was conducted, significant differences in nerve CSAs were observed between sexes and among age groups. Consequently, normal CSA values should be determined with consideration given to subject characteristics. However, when the CSA values are outside the normal range, it is essential to utilize clinical and physical examinations as well as

additional investigations to identify possible diseases. A potential approach for further research would be to investigate the accuracy of specific cut-off values for each condition.

A limitation of this study was that we used the ellipsoid function to measure the CSA of nerves; some nerves were not round or oval which could have resulted in a measurement error. It may be more accurate to trace inside the nerve if it is difficult to visualize the deep structure of the popliteal fossa. Thus, the use of ultra-high-frequency ultrasound will be necessary. In clinical and electrophysiologic studies, the normal nerve CSA should be compared to the CSA of patients with peripheral nerve disorders. In addition, in future research repeat reliability of the same rater should be obtained to help ensure the accuracy of the results.

Conclusions

Our study was a large-scale prospective study that used ultrasonography to assess the CSA of several nerves in the lower limbs of a healthy Thai population. The CSA of the several nerve locations differed by sex and age. A statistically significant correlation was observed between the values of CSA and demographic characteristics including age, weight, and BMI, as well as electrodiagnostic parameters. Specifically, an increase in nerve CSA was shown to be associated with a reduction in amplitude. This study's results might serve as standard reference values for the Thai population to differentiate nerve diseases.

Disclosure

The authors have no conflicts of interest to report.

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