

Computer-assisted measurements of the histological structure of the tibial nerve and its terminal branches

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Background: The aim of this study was to analyse the histological structure (cross-sectional area [CSA] and number of nerve bundles) of the distal part of the tibial nerve and its terminal branches (medial plantar nerve, lateral plantar nerve) using computer-assisted image analysis.

Materials and methods: The tibial nerve and its distal branches (medial and lateral plantar nerves) were dissected from the fresh cadavers. Each nerve was harvested 5 mm proximally and respectively 5 mm distally from the tibial nerve bifurcation, marked, dehydrated, embedded in paraffin, sectioned at 2 µm slices and stained with haematoxylin and eosin. Then photographed and analysed using Olympus cellSens software.

Results: The studied group comprised 28 female and 32 male feet (mean age 68.1 ± 15.2 years). The mean CSA and the number of nerve bundles were respectively 17.86 ± 4.57 mm², 33.88 ± 6.31 for the tibial nerve, 9.58 ± 1.95 mm², 23.41 ± 7.37 for the medial plantar nerve and 7.17 ± 2.36 mm², 15.06 ± 5.81 for the lateral plantar nerve in males and 12.27 ± 2.45 mm², 26.32 ± 8.87 for the tibial nerve, 7.81 ± 1.41 mm², 17.71 ± 5.28 for the medial plantar nerve and 5.83 ± 1.25 mm², 11.50 ± 3.72 for the lateral plantar nerve in females. Both CSA and number of nerve bundles of the tibial, medial plantar and lateral plantar nerves revealed no statistical differences when comparing foot side of the individual. The statistical difference was related to the gender, showing significantly bigger CSA and number of nerve bundles in males (CSA: $p = 0.000$, $p = 0.000$, $p = 0.016$; number of nerve bundles: $p = 0.01$, $p = 0.003$, $p = 0.004$, respectively). A positive correlation was found between the donor age and the tibial nerve CSA ($r = 0.44$, $p = 0.000$). A significant statistical difference was found between the medial and lateral plantar nerves both in CSA and number of nerve bundles ($p < 0.001$, $p < 0.001$, respectively).

Conclusions: The CSA and the number of nerve bundles in the distal part of the tibial nerve and its branches are significantly larger in males with no differences between right and left foot of the individual. The tibial nerve shows increasing CSA with advanced age. The medial plantar nerve has larger CSA and more nerve bundles than the lateral plantar nerve. (Folia Morphol 2021; 80, 2: 372–379)

Key words: tibial nerve, cross-sectional area, nerve bundles, medial and lateral plantar nerves, computer-assisted image analysis, histology

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INTRODUCTION

The tibial nerve is a peripheral sensorimotor nerve which is derived from the L4, L5 and S1–S3 spinal nerve roots [32]. It is the larger of the two terminal branches of the sciatic nerve arising in the popliteal fossa. It runs vertically on the tibialis posterior muscle together with the posterior tibial vessels. Postero-inferiorly to the medial malleolus it terminates emitting medial plantar nerve and lateral plantar nerve [26]. The tibial nerve bifurcation level shows a great variability with the most common occurrence below the tip of the medial malleolus, inside the tarsal tunnel [33].

Through its course the tibial nerve emits motor branches to the muscles of the posterior lower leg as well as sensory branches: medial sural cutaneous nerve and medial calcaneal nerve(s) innervating the skin of the posterolateral inferior third of the leg together with the lateral side of the foot and the skin of the heel, accordingly [10]. Medial calcaneal branches show diversity in terms of number (range from 1 to 4), location and nerve of origin [9, 17]. Both plantar nerves enter the sole of the foot supplying its muscles and skin. The medial plantar nerve innervates the skin medial to the line splitting fourth digit whilst the lateral plantar nerve the skin lateral to the line [20].

Tarsal tunnel syndrome is one of the entrapment conditions affecting the tibial nerve and its terminal branches in the medial ankle. It causes heel and sole burning pain and paraesthesia [2]. Such disorders together with other peripheral nerve pathologies may be examined by the ultrasound [24]. The cross-sectional area (CSA) is a parameter measured by the ultrasound which increasing value confirms the diagnosis [7].

The aim of this study was to assess the histological structure of the tibial nerve, medial plantar nerve and lateral plantar nerve as well as to determine the distribution of the nerve bundles of the distal tibial nerve to its terminal branches.

MATERIALS AND METHODS

The study was conducted on 60 lower limbs of the fresh cadavers in the Department of Anatomy of the Jagiellonian University Medical College between December 2016 and December 2019. The exclusion criteria were any deformation of the lower limb or the lower limb trauma, surgical or radiotherapeutic procedures of the lower limb, chronic disease of the lower limb in the medical record of the donor.

The research protocol was approved by the local Ethics Committee (registry no. 122.6120.315.2016).

The study has been performed in accordance with the ethical standards established in the 1964 Declaration of Helsinki and its later amendments.

Dissection technique

The incision was made in the midline between the tip of the medial malleolus and the Achilles tendon. It was continued 10 cm proximally along the Achilles tendon and 10 cm distally curving anteriorly 2 cm below the tip of the medial malleolus. Upon dissecting the skin and the subcutaneous tissue, the tibial nerve was visualised together with the posterior tibial artery and two posterior tibial veins. After meticulous dissection, the tibial nerve, its bifurcation and lateral and medial plantar nerves were exposed. The plantar nerves were marked 2 cm distally from the tibial nerve bifurcation point with the following pattern: blue thread — lateral plantar nerve, white thread — medial plantar nerve. The tibial nerve was left without any marking. Then 3 cm proximally to the bifurcation the tibial nerve was cut out from the main nerve trunk. Accordingly, 3 cm distally the medial and lateral plantar nerves were cut out. The excised tibial nerve and its terminal branches were removed *en bloc* from the cadaver. The incision was closed with the running subcuticular suture. The harvesting was carried out by the same surgeon.

Preparation of histological slide

The excised block of nerves was fixed in a 10% solution of the formaldehyde (pH 7.4). After 2–5 days it was removed from the formaldehyde. The tibial nerve was cut transverse to the nerve axis 5 mm and 10 mm proximally to the tibial nerve bifurcation point as were the medial and lateral plantar nerves 5 mm and 10 mm distally to the tibial nerve bifurcation point. Obtained 5 mm long nerve fragments were dehydrated separately and embedded in paraffin according to its initial marking. Each paraffin cube was transverse sectioned with the microtome providing one 2 μ m thick slice. Subsequently each slice was stained with haematoxylin and eosin (Fig. 1).

Micromorphometry

The CSA and the number of nerve bundles of the tibial nerve, the medial plantar nerve and the lateral plantar nerve were assessed using a light microscope (Olympus BX53, 20 \times magnification). Each cross-section was photographed (20 \times magnification). Afterwards, the CSA was measured semi-automatically using Olympus cellSens Standard 2.3 software with the producer's

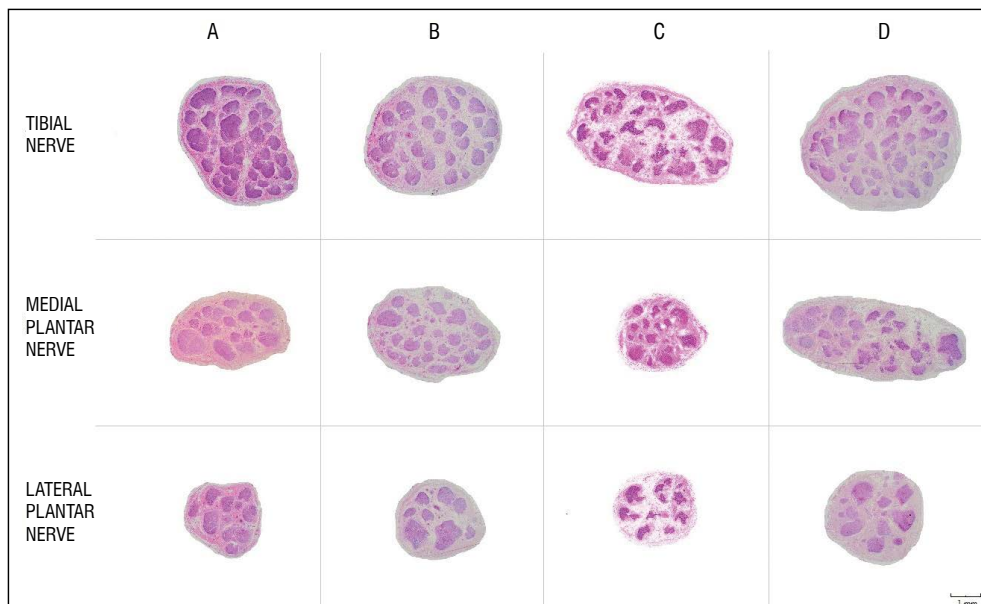


Figure 1. Cross-section of the tibial nerves (TN), the medial plantar nerves (MPN) and the lateral plantar nerves (LPN). Haematoxylin and eosin staining. 20 × magnification; **A.** 58-year-old male, right foot — cross-sectional area (CSA): TN — 12.76 mm², MPN — 6.79 mm², LPN — 4.37 mm²; number of nerve bundles: TN — 35, MPN — 18, LPN — 12; **B.** 58-year-old male (same individual as in panel A), left foot CSA: TN — 12.83 mm², MPN — 9.92 mm², LPN — 5.47 mm²; number of nerve bundles: TN — 29, MPN — 26, LPN — 8; **C.** 63-year-old male, right foot — CSA: TN — 13.92 mm², MPN — 5.63 mm², LPN — 5.05 mm²; number of nerve bundles: TN — 34, MPN — 14, LPN — 12; **D.** 84-year-old male, left foot — CSA: TN — 18.39 mm², MPN — 10.32 mm², LPN — 7.09 mm²; number of nerve bundles: TN — 44, MPN — 34, LPN — 13.

Table 1. The tibial nerve and its terminal branches measurements

Measurement		N	Mean ± standard deviation	Median	Minimum	Maximum	Lower quartile (Q1)	Upper quartile (Q3)
Cross-sectional area [mm ²]	Tibial nerve	60	15.25 ± 4.65	14.66	7.22	30.82	11.77	17.29
	Medial plantar nerve	60	8.76 ± 1.93	8.45	5.53	14.22	7.19	9.90
	Lateral plantar nerve	60	6.54 ± 2.02	6.44	3.90	16.06	5.12	7.41
Number of nerve bundles	Tibial nerve	60	30.35 ± 8.45	31.00	7.00	50.00	25.00	35.25
	Medial plantar nerve	60	20.75 ± 7.04	20.00	5.00	38.00	16.00	25.00
	Lateral plantar nerve	60	13.40 ± 5.22	14.00	3.00	38.00	10.75	15.00

precision of 10 μm, whilst the number of nerve bundles was calculated manually. Each slice was assessed once by the same pathologist. Then the values of the CSA and the number of nerve bundles were tabulated.

Statistics

Obtained data were statistically processed using descriptive statistics such as percentage, mean, median, standard deviation, upper and lower quartiles. A p-value of < 0.05 was considered as statistically significant. Two groups were compared using the Mann-Whitney test and t-test for non-normally and normally distributed data, accordingly. Levene’s test was used to check for homogeneity of variance. Two-way analysis of variance and possible interactions

between the sex and age (> 70/< 70 years old) were checked in selected nerve parameters. Post-hoc analysis was performed using HSD test. To compare the nerve features between the left and right foot, the paired t-test or Wilcoxon rang test was used depending on whether data were normally or non-normally distributed. Correlation coefficients were calculated to establish any statistical dependence between parameters. All analyses were performed using MedCalc version 16.8.

RESULTS

There were 30 fresh cadavers dissected (n = 60 lower limbs) with a mean age of 68.1 ± 15.2 (range from 27 to 91 years). 28 (46.7%) feet were female and

Table 2. The tibial nerve and its terminal branches measurements — comparison by gender

Measurement		Women					Men					P
		N	Mean ± SD	Median	Q1	Q3	N	Mean ± SD	Median	Q1	Q3	
Cross-sectional area [mm ²]	Tibial nerve	28	12.27 ± 2.45	11.85	10.35	14.31	32	17.86 ± 4.57	17.10	15.02	19.90	0.000
	Medial plantar nerve	28	7.81 ± 1.41	7.37	6.70	9.10	32	9.58 ± 1.95	9.16	8.40	10.66	0.000
	Lateral plantar nerve	28	5.83 ± 1.25	5.77	4.61	6.86	32	7.17 ± 2.36	7.08	5.18	8.35	0.016
Number of nerve bundles	Tibial nerve	28	26.32 ± 8.87	25.00	19.50	34.00	32	33.88 ± 6.31	34.00	28.50	38.00	0.001
	Medial plantar nerve	28	17.71 ± 5.28	18.00	14.50	20.50	32	23.41 ± 7.37	23.00	17.50	29.50	0.003
	Lateral plantar nerve	28	11.50 ± 3.72	12.00	9.00	14.00	32	15.06 ± 5.81	15.00	12.50	16.50	0.004

Numbers in bold indicate statistically significant differences between males and females ($p < 0.05$); SD — standard deviation; Q1 — lower quartile; Q3 — upper quartile

Table 3. The tibial nerve and its terminal branches measurements — comparison by foot side

Measurement		Left foot					Right foot					P
		N	Mean ± SD	Median	Q1	Q3	N	Mean ± SD	Median	Q1	Q3	
Cross-sectional area [mm ²]	Tibial nerve	30	15.82 ± 5.08	15.64	11.73	17.79	30	14.67 ± 4.19	14.14	11.91	16.64	0.229
	Medial plantar nerve	30	8.80 ± 1.98	8.32	7.21	9.88	30	8.71 ± 1.90	8.78	7.08	9.92	0.805
	Lateral plantar nerve	30	7.05 ± 2.48	6.99	5.05	8.30	30	6.03 ± 1.28	5.91	5.12	7.08	0.075
Number of nerve bundles	Tibial nerve	30	30.43 ± 8.36	31.00	25.00	35.00	30	30.27 ± 8.67	32.00	24.00	37.00	0.989
	Medial plantar nerve	30	20.37 ± 6.77	20.00	17.00	23.00	30	21.13 ± 7.39	21.00	15.00	26.00	0.412
	Lateral plantar nerve	30	14.20 ± 6.37	14.00	11.00	16.00	30	12.60 ± 3.68	13.00	10.00	15.00	0.296

Statistically significant differences between left and right foot when $p < 0.05$; SD — standard deviation; Q1 — lower quartile; Q3 — upper quartile

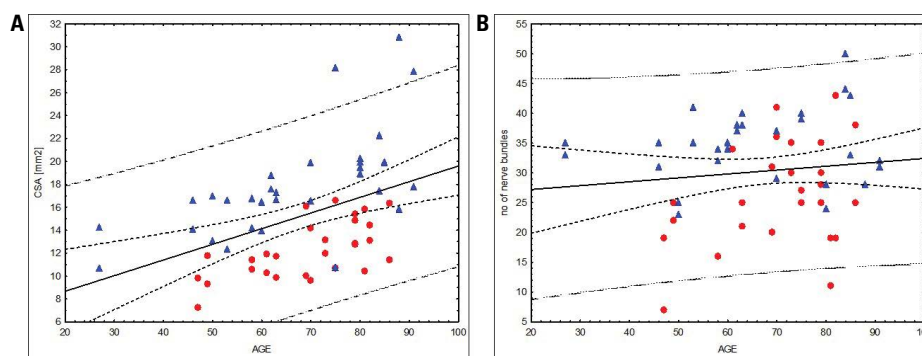


Figure 2. **A.** A scatter plot of donors age correlation with tibial nerve cross-sectional area (CSA). Triangles represent males ($r = 0.69$, $p = 0.000$), dots represent females ($r = 0.60$, $p = 0.001$); **B.** A scatter plot of donors age correlation with number of tibial nerve bundles. Triangles represent males ($r = -0.04$, $p = 0.846$), dots represent females ($r = 0.31$, $p = 0.110$). The continuous line represents progression. The dash lines represent the 95% confidence intervals of the progression. The dash-dot lines represent the 95% prediction intervals.

32 (53.3%) were male. The mean CSA and number of nerve bundles of the tibial nerve, the medial plantar nerve and the lateral plantar nerve are presented in Table 1. Differences between the gender and foot side are shown in Tables 2 and 3, respectively. Males showed larger CSA and more nerve bundles than females. No statistically significant differences between the right and left foot of the individual were found ($p > 0.05$). There was a statistically significant

difference between medial and lateral plantar nerve both in CSA and number of nerve bundles ($p < 0.001$, $p < 0.001$, respectively). The medial plantar nerve was confirmed to have 1.3 times larger CSA and 1.5 times more nerve bundles than the lateral plantar nerve. A positive correlation was noted between the age of donors and CSA of the tibial nerve ($r = 0.44$, $p = 0.000$) (Fig. 2). No statistically significant correlation was found between the age of donors and CSA

Table 4. Studies of the tibial nerve cross-sectional area (CSA) measured at the level of medial malleolus

	Group (n)	Mean age [years]	CSA of the tibial nerve at the level of medial malleolus [mm ²]	Reference range [mm ²]	Type of study
He et al., 2019 [13]	40	55.2	11.6 ± 1.6	–	US 4–15 MHz
Lothet et al., 2019 [24]	15	21.7	12.3	–	US 18 MHz
Singh et al., 2019 [30]	45	30–68	6.0 ± 1.8	–	US 5–18 MHz
Bedewi et al., 2018 [3]	138	38.3	12.7 ± 4.5	2.0–30.0	US 18.5 MHz
Grimm et al., 2018 [12]	100	51.2	10.2 ± 2.0	–	US 14 MHz
Kronlage et al., 2017 [21]	60	30.5	8.1 ± 2.0*	4.0–12.1	MRI
Singh et al., 2017 [29]	75	39.5	12.4 ± 1.1	10.0–14.0	US 7–18 MHz
Kang et al., 2016 [18]	20	65.0	12.4 ± 2.9	–	US 7–12 MHz
Yiu et al., 2015 [35]	29	11.3	6.3 ± 1.9	8.6–14.1	US 7–13 MHz
Boehm et al., 2014 [5]	56	50.2	9.6 ± 2.2	9.0–10.2	US 12–15 MHz
Seok et al., 2014 [28]	94	43.9	12.1 ± 3.1	8.5–22.8	US 5–12 MHz
Kerasnoudis et al., 2013 [19]	75	53.5	6.3 ± 1.5	3.5–9.3	US 18 MHz
Riazi et al., 2012 [27]	43	46.8	17.7 ± 6.5	–	US 6–13 MHz
Tagliafico et al., 2012 [31]	58	47.0	9.6 ± 4.0	7.2–13.7	US 17.5 MHz
Cartwright et al., 2008 [7]	60	45.9	13.7 ± 4.3	5.1–22.3	US 15 MHz
Ito et al., 2007 [16]	35	52.8	7.9 ± 1.5	5.0–10.7	US 7.5 MHz
Lee et al., 2005 [22]	24	57.4	12.0	–	US 10–12 MHz

*Measured at the proximal third of the calf; US — ultrasonography; MRI — magnetic resonance imaging

of medial or lateral plantar nerves as well as number of nerve bundles. In the two-way analysis of variance, the mean CSA of the tibial nerve in males below 70 years of age was $15.37 \pm 0.80 \text{ mm}^2$ and $20.35 \pm 0.80 \text{ mm}^2$ for those above 70 years old, whilst in females $10.83 \pm 0.92 \text{ mm}^2$ and $13.35 \pm 0.80 \text{ mm}^2$, respectively. There was no statistically significant sex and age interactions with the CSA of the tibial nerve ($p = 0.14$). Post-hoc analysis revealed significant differences between younger and older males as well as between younger males and younger females ($p < 0.05$). Older males' CSA was significantly higher when compared to the younger and older females ($p < 0.05$).

DISCUSSION

The present study reveals data obtained using computer-assisted analysis of the histological structure of the distal tibial nerve and its terminal branches: medial and lateral plantar nerves. Literature analysis shows that the previous studies focused mostly on the variations of the topographic anatomy of the tibial nerve, its bifurcation, branching pattern and the cross-sectional area measured by the ultrasound imaging [33]. A limited number of studies concentrated on the histological structure of the tibial nerve

and its distal branches. To the best of the authors' knowledge this is the first publication analysing histological structure of the medial and lateral plantar nerves as well as tibial nerve bundles distribution to its terminal branches. As the previous publications of the cross-sectional area were based on the ultrasound or magnetic resonance imaging, the present study is the first to reveal the CSA measured directly on the nerves harvested from the fresh cadavers, which shows greater accuracy.

In the present study, 60 lower limbs of the fresh cadavers were dissected. The majority of donors presented advanced age (mean age 68.1 years) and relatively equal gender distribution (53% males). The mean CSA of the tibial nerve measured with the computer-assisted image analysis is 15.25 mm^2 , which is comparable with the results obtained in the previous studies collected in Table 4. Nonetheless, it needs to be noticed that majority of those measurements are slightly below 15.25 mm^2 as well as the mean age is lower than 68.1 years. It confirms the positive correlation between the age of the donors and the CSA observed in the present study. Despite different methodology (micromorphometry vs. ultrasound vs. magnetic resonance imaging) the obtained results showed insignificant differences in CSA of the tibial

nerve. Thus, it proves the reliability and usefulness of those imagining methods.

Analysing the results summarised in Table 4, the authors found that the average cross-sectional area of the tibial nerve (15.25 mm²) is almost identical with Riazi et al. [27] — 17.7 mm² and Cartwright et al. [6] — 13.7 mm². At the same time it is more than two times larger than the values provided by Singh et al. [30] — 6.0 mm², Yiu et al. [35] — 6.3 mm² and Kerasnoudis et al. [19] — 6.3 mm². The differences may be the result of the average age of the examined patients. Namely, Yiu et al. [35] examined children with the mean age of 11.3 mm², which probably is the reason for the small CSA. The other cause of slight variation may be related to a different level of measurements. Lothet et al. [24] together with Kang et al. [18], Kerasnoudis et al. [19], Cartwright et al. [7], Bedewi et al. [3], Boehm et al. [5] and Grimm et al. [12] performed the examination at the level of the ankle whilst He et al. [13] and Singh et al. [30] measured the CSA 3 cm above the medial malleolus and Riazi et al. [27] 1 cm, 3 cm and 5 cm above the medial malleolus. As the tibial nerve bifurcation level shows great topographic variability, such inaccuracy may bring different results. Its location was subject of many studies and frequently referred to the medial or lower located lateral malleolus [23, 34]. For the sake of comparison in the present study, all publications mentioned in Table 4 were qualified as if the measurements were at the level of medial malleolus. The other sources of the differences may be the reliability and accuracy of the researchers as well as the ultrasound resolution or the ethnical groups which was not comprised in the study.

To the best of the authors' knowledge, no previous studies of the cross-sectional area of the tibial, medial and lateral plantar nerves harvested from the fresh cadavers have been reported. The first report of the tibial nerve measurements dates back to 1938 when Horwitz [15] performed a dissection on 100 cadavers reporting the average diameter of tibial nerve to be between 6 and 10 mm. Unfortunately, there is no information about the level of assessment. In 2006 Joshi and Joshi [17] examined 112 cadavers describing an average width of the tibial nerve above its bifurcation to be 8.23 mm. The authors also measured the width of the medial plantar nerve to be 5.32 mm and the lateral plantar nerve to be 4.61 mm. Since the tibial nerve shows clear flattening at the level of its bifurcation it would be wrong to assess the CSA

using the circle area formula ($\pi \times r^2$). These are the only measurements of the medial and lateral plantar nerves found in the literature. Complementing the available data with the mean CSA of 8.76 mm² and the average number of nerve bundles to be 20.75 in the medial plantar nerve and 6.54 mm² and 13.40 in the lateral plantar respectively should serve as a starting point for future researchers.

According to Alshami et al. [1], one of the causes of the foot pain is the tarsal tunnel syndrome. As the tibial nerve and its divisional branches pass through tarsal tunnel it may be entrapped or compressed. Joshi and Joshi [17] together with Bilge et al. [4] state that in the majority of cases the tibial nerve bifurcation is located inside the tarsal tunnel. As Heimkes et al. [14] point out, it is a tight, stretch resistant osteofibrous canal between talus, calcaneus and flexor retinaculum. It may be suggested that the larger the size (CSA) of the nerves (tibial, medial and lateral plantar), the higher the risk of its entrapment. Therefore the prevalence of the foot pain and paraesthesia among older people is higher.

Lothet et al. [24] together with Cartwright et al. [7] proved that in the medial ankle ultrasound examination the tibial nerve CSA remains uninfluenced by the patient's height and weight. In the present study the authors confirm that CSA of the tibial nerve increases with the advanced age, which is consistent with the findings of Grimm et al. [12] and Cartwright et al. [6]. It needs to be mentioned that according to Kerasnoudis et al. [19] and Mizia et al. [25] other peripheral nerves such as median nerve, radial nerve or sural nerve present an age-related decrease in the CSA values. This exceptional finding was explained by Ceballos et al. [8] in 1999 on the mouse model. The authors observed the age-related increase in mastocytes and macrophages depositing in the endoneurium as well as collagen accumulation in the perineurium, causing the enlargement of the cross-sectional area. Tibial nerve age-related thickening was also described by Grimm et al. [12] as a higher fibrous tissue deposition in the nerve.

In the present study, the number of nerve bundles in the tibial, medial plantar and lateral plantar nerves was also counted, finding respectively 30.35 ± 8.45 , 20.75 ± 7.04 , 13.40 ± 5.22 nerve bundles. Interestingly the number of nerve bundles of the tibial nerve is lower than the summative number of its two terminal branches (medial and lateral plantar nerves). The similar finding was reported by Delgado-Martinez

et al. [11] who counted the number of nerve bundles of the median nerve. Despite the muscle and cutaneous branches sprouting from the main trunk of the median nerve along its course, the authors found the increasing number of nerve bundles in distal part of the forearm (11.81 ± 0.32 in the proximal upper arm, 12.81 ± 0.73 in the distal upper arm, 21.87 ± 0.58 in the forearm). Although there is no study explaining this finding available in the literature, the authors suggest that the increased summative number of nerve bundles in the medial and lateral plantar nerves might result from the split of (some) nerve bundles of the tibial nerve at the bifurcation level. Therefore, finding out the branching pattern of the tibial nerve bundles and its distribution to the medial and lateral plantar nerves (by measuring the CSA of each nerve bundle or by counting the number of axons) at the bifurcation level might be an interesting subject for the future studies.

Limitations of the study

The fact that the cross-sectional area and number of nerve bundles were assessed on the nerves harvested from the fresh cadavers donated to the Department of the Anatomy results in the high average age of the examined group. Because of the technical difficulties no weight and height of the donors were obtained which might have been beneficial for this study. The other limitation of the present study is the fact that only one slice of each nerve was prepared for the micromorphometric assessment. Single pathologist, performing all measurements only once also biased the possibility to ascertain the inter-observer and intra-observer variabilities. Another restriction is a diverse level of the tibial nerve CSA measurements presented in available studies as well as lack of medial and lateral plantar nerve assessments which handicapped the comparison possibility.

CONCLUSIONS

To conclude, the authors of the present study proved that CSA and number of nerve bundles of the tibial nerve, medial plantar nerve and lateral plantar nerve are larger among males whilst shows no differences comparing to the side of the lower limb. This study also confirms that the CSA and number of nerve bundles of the medial plantar nerve is higher than the lateral plantar nerve. The authors proved the increasing CSA and number of nerve bundles among older donors. This work also contributes to

the establishment of reference values for the medial and lateral plantar nerves.

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