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Topographic anatomy of the tibial nerve bifurcation and its medial calcaneal branches. Macro and microscopic analysis.

Praca doktorska

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SPIS TREŚCI

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Rozprawa doktorska pt. „Zmienność odejścia gałęzi piętowej przyśrodkowej i podziału nerwu piszczelowego. Analiza makro i mikroskopowa." składa się z trzech prac oryginalnych:

1. **Ultrasound guided topographic anatomy of the medial calcaneal branches of the tibial nerve**

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

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3. **Comparison of the histological structure of the tibial nerve ant its terminal branches in the fresh and fresh-frozen cadavers.**

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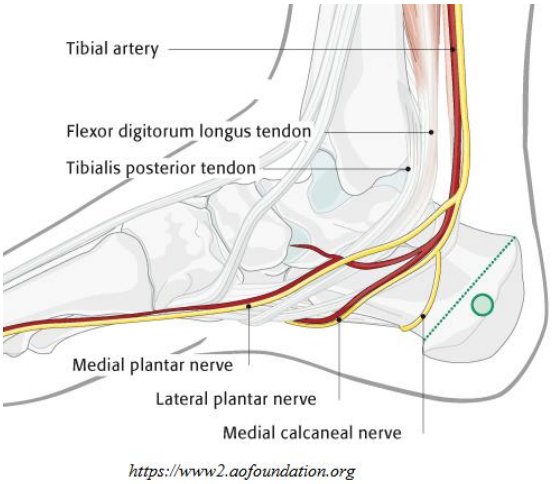
WSTĘP I UZASADNIENIE PODJĘTEJ TEMATYKI

Wprowadzenie

1) Anatomia nerwu piszczelowego

Nerw piszczelowy stanowi jedną z gałęzi końcowych (przedłużenie) nerwu kulszowego. Odchodzi od niego w dole podkolanowym. W przebiegu nerwu piszczelowego rozróżnia się dwa odcinki: podkolanowy oraz goleniowy. W pierwszym leży powierzchownie pod powięzią podkolanową, bocznie od tętnicy i żyły podkolanowych. Wraz z tymi naczyniami tworzy powrózek naczyniowo - nerwowy. W kącie dolnym dołu podkolanowego nerw piszczelowy wsuwa się między obie głowy mięśnia brzuchatego łydki. W odcinku goleniowym nerw piszczelowy przebiega pomiędzy powierzchowną a głęboką grupą zginaczy podudzia. Następnie w kanale kostki przyśrodkowej nerw ten przebiega mniej więcej w połowie odległości między brzegiem kostki przyśrodkowej i brzegiem ścięgna piętowego. Nerw i naczynia leżą między powierzchowną a głęboką blaszką troczka zginaczy, przy czym nerw przebiega przeważnie do tyłu od naczyń. W tym miejscu (lub na brzegu przyśrodkowym stopy) dzieli się na swoje gałęzie końcowe: nerw podeszwowy przyśrodkowy oraz nerw podeszwowy boczny.

W swoim przebiegu nerw piszczelowy oddaje: nerw skórny łydki przyśrodkowy, gałęzie mięśniowe bliższe, gałąź podkolanową, gałęzie stawowe bliższe, gałęzie mięśniowe dalsze, gałęzie stawowe dalsze, gałęzie piętowe przyśrodkowe oraz nerw podeszwowy przyśrodkowy i nerw podeszwowy boczny.



Gałęzie piętowe przyśrodkowe zaopatrują skórę przyśrodkowej powierzchni pięty oraz tylnej części podeszwy. Nerw podeszwowy przyśrodkowy zaopatruje czuciowo skórę przyśrodkowego brzegu stopy, zwróconych do siebie brzegów palców I-IV oraz ruchowo mięsień odwodziciel palucha, mięsień zginacz krótki palców, mięśnie glistowate I i II. Nerw podeszwowy boczny zaopatruje czuciowo skórę zwróconych do siebie brzegów palców IV-V oraz ruchowo mięsień zginacz krótki palca małego, przeciwstawiacz palca małego, czworoboczny podeszwy, mięśnie glistowate III-IV, międzykostne I-IV. Zakres unerwienia ruchowego nerwu piszczelowego obejmuje zginacze podudzia oraz wszystkie mięśnie podeszwy. Gałęzie skórne nerwu piszczelowego unerwiają czuciowo tylno-przyśrodkową powierzchnię łydki, obie okolice zakostkowe, piętę, brzeg boczny stopy i palca małego oraz skórę powierzchni podeszwowej palców i grzbietowych powierzchni dalszych i częściowo środkowych paliczków.

Porażenie nerwu piszczelowego uniemożliwia ruchy zgięcia podeszwowego stopy i palców. Niewykonalne jest wspięcie na palce oraz ich odwodzenie i przywodzenie. W wyniku porażenia występuje wtórny zanik mięśni międzykostnych ze szponiastym ustawieniem palców. Wskutek wtórnego przykurczu prostowników rozwija się stopa piętowa.

2) Praktyka chirurgiczna - powikłania

Artroskopia stawu skokowego górnego stanowi jedną z procedur diagnostyczno - terapeutycznych, podczas której dochodzi do jatrogennego uszkodzenia badanych struktur nerwowych. W publikowanych badaniach opisuje się, że podczas zakładania trokarów do artroskopii komplikacje neurologiczne występują u 1,9% pacjentów (w innych badaniach opisywane są nawet u 5,4% pacjentów) [Young BH, Flanigan RM, DiGiovanni BF.

Complications of ankle arthroscopy utilizing a contemporary noninvasive distraction technique. J Bone Jt Surg Am. 2011;93:963–968]. Pomimo spadającego odsetka powikłań artroskopii stawu skokowego (w roku 1989 - 17,4% powikłań, podczas gdy w roku 2012 - 8%) komplikacje neurologiczne wciąż opisywane są jako najczęstsze [Ferkel RD, Heath DD, Guhl JF. Neurological complications of the ankle arthroscopy. Arthroscopy. 1996 Apr;12(2):200-8].

3) Zmienność odejścia gałęzi piętowej przyśrodkowej i podziału nerwu piszczelowego

Szereg autorów nie jest zgodnych co do opisywanych wariantów przebiegu gałęzi piętowej przyśrodkowej, uwzględniając miejsce odejścia oraz ilość odgałęzień w odniesieniu do ogólnie przyjętych punktów topograficznych, podejmując próby utworzenia praktycznego systemu klasyfikacji [Kim BS, Choung PW, Kwon SW, Rhyu IJ, Kim DH. Branching patterns of medial and inferior calcaneal nerves around the tarsal tunnel. Ann Rehabil Med. 2015 Feb;39(1):52-5].

W badaniu ocena topografii i zmienności odejścia odgałęzień nerwu piszczelowego oceniana będzie makro i mikroskopowo oraz badaniem ultrasonograficznym in vivo. Autor zadaje sobie pytanie - czy możliwe jest określenie bezpiecznego dostępu chirurgicznego do okolicy części dalszej, tylno-przyśrodkowej powierzchni podudzia oraz stopy.

Istnieje zatem konieczność dokładniejszej analizy przebiegu dystalnego odcinka nerwu piszczelowego, jego podziału na gałęzie końcowe oraz gałęzie piętowe przyśrodkowe skutkująca uwzględnieniem jej w stosowanych dostępach chirurgicznych.

CELE PRACY

Celem pracy doktorskiej, opartej o 3 publikacje prac oryginalnych, było poznanie topografii podziału nerwu piszczelowego na nerw podeszwowy przyśrodkowy i nerw podeszwowy boczny oraz odejścia gałęzi piętowych przyśrodkowych. Badaniem ultrasonograficznym określono miejsce podziału nerwu piszczelowego na nerwy podeszwowe przyśrodkowy i boczy oraz ilość i miejsce odejścia gałęzi piętowych przyśrodkowych. Kolejnym celem była, wcześniej nie opisywana, ocena histologiczna (pole przekroju poprzecznego oraz liczba pęczków nerwowych) końcowego odcinka nerwu piszczelowego oraz proksymalnych części nerwów podeszwowego przyśrodkowego i bocznego. Następnie zaplanowano makroskopowe i mikroskopowe porównanie nerwów pobranych ze świeżych i świeżo mrożonych preparatów.

MATERIAŁ I METODY

Pierwsza części pracy polegała na przeprowadzeniu badania ultrasonograficznego okolicy kostki przyśrodkowej na 30 ochotnikach (60 kończyn dolnych). Badanie zostało wykonane przez specjalistę ortopedii z ponad 20-cio letnim doświadczeniem przy użyciu aparatu MyLabGold 25 głowica 18MHz. Podczas badania ultrasonograficznego określano topografię dystalnego odcinka nerwu piszczelowego, jego podziału, nerwu podeszwowego przyśrodkowego, nerwu podeszwowego bocznego oraz gałęzi piętowych przyśrodkowych. Określano również ilość i miejsce odejścia gałęzi piętowych przyśrodkowych. Następnie wyznaczono dwie poziomie linie referencyjne: jedną przebiegają przez szczyt kostki przyśrodkowej, drugą przebiegająca przez tylno-górny brzeg kości piętowej. Suwmiarką mierzono:

- odległość miejsca odejścia gałęzi piętowej przyśrodkowej od linii szczytu kostki przyśrodkowej;
- odległość miejsca odejścia gałęzi piętowej przyśrodkowej od linii tylno-górnego brzegu guza piętowego kości piętowej;
- odległość miejsca podziału nerwu piszczelowego na nerw podeszwowy przyśrodkowy i nerw podeszwowy boczny od linii szczytu kostki przyśrodkowej;
- odległość miejsca podziału nerwu piszczelowego na nerw podeszwowy przyśrodkowy i nerw podeszwowy boczny od linii tylno-górnego brzegu guza piętowego kości piętowej.

W drugiej części pracy z 30 nieutrwalonych zwłok (60 kończyn dolnych) oraz z 21 izolowanych, świeżo mrożonych kończyn dolnych wypreparowano i pobrano końcowy odcinek nerwu piszczelowego oraz proksymalne części nerwów podeszwowego

przyśrodkowego i bocznego. Z każdego z wyżej wymienionych nerwów pobrano wycinek, który utrwalono w 10% roztworze formaldehydu. Następnie każdy preparat przeszedł procedurę odwadniania, zatapiania w parafinie, cięcia na 2 μm skrawki oraz barwienia hematoksyliną i eozyną. Otrzymane preparaty badano pod mikroskopem świetlnym (Olympus BX53, powiększenie 20 x). Uzyskany obraz analizowano przy pomocy programu Olympus cellSens Standard 2.3, który określał pole przekroju poprzecznego badanego nerwu, a następnie ręcznie liczono ilość pęczków nerwowych.

Protokół badania zatwierdziła Komisja Bioetyczna Uniwersytetu Jagiellońskiego Collegium Medicum (nr 122.6120.315.2016).

ARTYKUŁ NR 1

Ultrasound guided topographic anatomy of the medial calcaneal branches of the tibial nerve

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ABSTRACT

Background: The purpose of this study was to evaluate the topographic anatomy of the tibial nerve and its medial calcaneal branches in relation to the tip of the medial malleolus and to the posterior superior tip of the calcaneal tuberosity using the ultrasound examination and to verify its preoperative usefulness in surgical treatment.

Methods: Bilateral ultrasound examination was performed on 30 volunteers and the location of the tibial nerve bifurcation and medial calcaneal branches origin were measured. Medial calcaneal branches were analyzed in reference to the amount and their respective nerves of origin.

Results: In 77% cases tibial nerve bifurcation occurred below the tip of the medial malleolus with the average distance of 5.9 mm and in 48% cases above the attachment of the posterior superior tip of the calcaneal tuberosity with the average distance of 2.7 mm. In 73% cases medial calcaneal branches occurred as a single branch originating from the tibial nerve (60%). The average distance of the first, second and third medial calcaneal branch was accordingly 9.3 mm above, 9.5 mm below and 11.6 mm below the tip of the medial malleolus and 17.7 mm above, 1.6 mm below and 4 mm below the posterior superior tip of the calcaneal tuberosity.

Conclusion: As the tibial nerve and its branches presents a huge variability in the medial ankle area in order to prevent the iatrogenic injuries the preoperative or intraoperative ultrasound assessment (sonosurgery) of its localization should be introduced into the clinic.

KEYWORDS: medial calcaneal nerve, ultrasound-guided nerve examination, tarsal tunnel syndrome, medial plantar nerve, lateral plantar nerve, sonosurgery

INTRODUCTION

The tibial nerve arises as a branch of sciatic nerves bifurcation in the popliteal fossa. It runs distally on the tibialis posterior muscle together with the posterior tibial vessels. Usually at the level of flexor retinaculum it terminally divides into lateral and medial plantar nerve. During distal course the tibial nerve emits medial calcaneal branch(es) which is variable in number and origin. Tibial nerve and its branches provides innervation to the posterior lower leg, foot and sole muscles and the skin of medial foot and sole[28].

The knowledge of topographic anatomy of peripheral neurovascular bundles is important in surgical procedures, especially in the medial ankle surgery. It helps to understand the pathophysiology of the tarsal tunnel syndrome and its symptoms such as heel and sole burning pain, paresthesia and numbness radiating to the toes and proximally on the medial side of the calf with often nocturnal presentation [2, 5, 22, 24, 25, 39]. Tibial nerve and its branches may be entrapped in the tarsal tunnel by various internal and external mechanisms [8]. Additionally other medical conditions with body fluid retention and chronic inflammatory processes may lead to nerve compression [13]. Rising prevalence of diabetes mellitus contributes to a large number of compression syndromes [36]. Recently popular outdoor activities (e.g. jogging) also brings new cases of foot pain [23].

One of the utmost treatment options for the foot pain syndromes is a surgery [1, 20]. It must be performed with the highest awareness of tibial nerve anatomy without inflicting iatrogenic damage. According to anatomy books, atlases and cadaveric dissection studies tibial nerve presents a various pattern of its bifurcation as well as origin and number of medial calcaneal branches [6-7, 9-11, 14-17, 21, 27, 30-31, 33]. In relation to the anatomical landmarks we tried to establish the most common topographic localization of the tibial nerve and its final branches and to encourage to the preoperative ultrasound examination prior to medial ankle surgeries.

MATERIALS AND METHODS

Patients: The study was conducted on the 30 volunteers (n=60 lower limbs). There were 16 females and 14 males. The average age of the volunteers was 25.7 years (range 19-50 years). The inclusion criteria were as follows: age 18 years or older, written informed consent for the examination. The exclusion criteria's were any lower limb trauma, surgical or radiotherapeutic procedures of the lower limb, deformation of the lower limb, chronic disease of the lower limb.

The ultrasound examination was performed on the Mylab Gold 25 ultrasound scanner with a 18MHz linear probe (penetration depth 3.0 cm) in the Department of Anatomy between December 2016 and April 2017. The examination and measurements were performed by an orthopedic surgeon with more than 20 years of experience in ultrasound examination.

The ultrasound examination was performed with the volunteer lying prone with the foot in neutral position (ankle fixed in the foot stabilizing device and adjusted to the right angle). Each procedure was initiated 40 cm proximally to the tip of the medial malleolus, continuing distally along the tibial nerve course up to its bifurcation and further looking for medial malleolus branches. Following points were marked on the skin with the fine tip skin marker: the tibial nerve bifurcation point, medial calcaneal branches origin, the tip of the medial malleolus and the posterior superior tip of the calcaneal tuberosity (attachment point of the Achilles tendon to the calcaneal tuberosity). Lines crossing the marked points were drawn parallel to the foot plane (Figure 1). Distances from the reference lines (the tip of the medial malleolus line and the posterior superior tip of the calcaneal tuberosity) to the tibial nerve bifurcation line and to the medial calcaneal branches origin lines were measured with the caliper. If the measured point was below the reference line the value is in negative numbers, if above the reference line the value is in positive number. Medial calcaneal branches were

analyzed with regards to the number of branches, nerve of origin and relation to the reference lines. The results were transformed into rates and tabulated.

Statistics: Obtained data was statistically processed using descriptive statistics such as percentage, mean, standard deviation. A p-value of < 0.05 was considered as statistically significant. Two groups were compared using the Mann-Whitney test or t-test depending normal distribution. All analyses were performed using MedCalc version 16.8.

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. The volunteers were informed about the study protocol and gave both informed and written consent to participate in the study.

RESULTS

There were thirty volunteers (n = 60 lower limbs) with an average age of 25.7 ± 7 amongst which 32 were female (53.3%) and 28 male (46.7%) feet.

The bifurcation of the tibial nerve into to medial and lateral plantar nerve most frequently occurred below the tip of the medial malleolus (76.7%) with the mean distance of -5.93 ± 19.59 mm and above the posterior superior tip of the calcaneal tuberosity (48.3%) with the mean distance of 2.67 ± 19.79 mm (Tables I, II). There were no significant difference between the sexes ($p > 0.05$). The correlation between the reference lines of the tip of the medial malleolus and the posterior superior tip of the calcaneal tuberosity has been proven to be statistically significant ($r = 0.9874$, $p < 0.05$) (Figure 2).

The medial calcaneal branches were identified in the range from one to three ramifications. A total of 80 medial calcaneal branches were visualized. In 44 patients (73.3%) only one medial calcaneal branch was identified with no significant differences between the sexes ($p > 0.05$).

Two medial calcaneal branches were presented in 12 patients, and three branches in 4 patients (Table III). Most commonly medial calcaneal branch originated from the tibial nerve as a single branch (60%). As there were two medial calcaneal branches it emerged from the tibial and lateral plantar nerve most frequently (Table IV). Most of the medial calcaneal branches were located below the tip of the medial malleolus with the mean distance of 3.97 mm and above the posterior superior tip of the calcaneal tuberosity with the mean distance of 4.36 mm. It may be assumed that majority of branches is located were between the tip of the medial malleolus and the posterior superior tip of the calcaneal tuberosity.

First medial calcaneal branch (n = 60) originated 9.27 ± 61.73 mm above the tip of the medial malleolus and 17.67 ± 61.18 mm above the posterior superior tip of the calcaneal tuberosity with no statistically significant differences between the sexes ($p > 0.05$) (Table VI). Second medial calcaneal branch (n = 16) originated 9.50 ± 10.09 mm below the tip of the medial malleolus and 1.63 ± 11.28 mm below the posterior superior tip of the calcaneal tuberosity. Third medial calcaneal branch (n = 4) originated 11.75 ± 9.03 mm below the tip of the medial malleolus and 4.00 ± 8.45 mm below the posterior superior tip of the calcaneal tuberosity (Table II, Figures 3, 4). In one volunteer (n = 2 feet) an exceptionally long distance of the first medial calcaneal branch was measured: 330 mm on the left lower limb and 337 mm on the right lower limb above the tip of the medial malleolus and respectively 335 mm and 343 mm above the posterior superior tip of the calcaneal tuberosity.

DISCUSSION

Up to 15% of adult population suffers from the plantar heel pain[3]. According to Oztuna et al.[32] nerve entrapment is one of the reasons for this condition. As operative decompression of the tarsal tunnel is one of the most effective treatment options it is essential to perform the

surgery in concordance with the anatomical structures in order to avoid its iatrogenic injury [12].

First description of the tibial nerve and its branches variable anatomy was published by Horwitz[17] in 1938. Dissecting 100 lower extremities author states that the tibial nerve bifurcation occurs 1.3cm above the tip of the medial malleolus. As to the medial calcaneal branch(es) underlines its difference in number, location and origin. Dellon et al.[10] in 1984 examined 31 cadaver feet. For the first time the malleolar-calcaneal axis (MCA) was proposed as the reference line for the measurements. In 90% of the cases the tibial nerve bifurcation occurred within 1cm of the MCA. Medial calcaneal branch originating above the flexor retinaculum in 65% cases. The author emphasize variability between left and right feet bifurcation location as well as to the number and location of the medial calcaneal branch(es). For the first time a substantial differences between mentioned studies are pointed out. Only 15% of Dellon cases presented the tibial nerve bifurcation at the level of Horwitz results.

Comparing with the other published studies the tibial nerve bifurcation was located inside the tarsal tunnel in 99.9% (Joshi et al.[21]), 93% (Havel et al.[15]), 88% (Torres et al.[37]) and 73% (Louisa et al.[27]) cases. Heimkes et al.[16] defined the tarsal tunnel as the oval osteofibrous canal between talus, calcaneus and flexor retinaculum which stretches from the medial malleolus to the calcaneus. As so it corresponds with the results of the present study in which 76.7% cases presented the bifurcation below the tip of the medial malleolus with the mean distance of 5.93 ± 19.59 mm. According to the tarsal tunnel definition it may be assumed that majority of the nerves (tibial nerve, lateral and medial plantar nerve) localized by the authors runs and divides in the tarsal tunnel where it may by compressed.

Location of the tibial nerve bifurcation was the subject of many cadaveric studies [37]. Most of them were conducted according to the malleolar-calcaneal axis (MCA) reference line which was fixed between the center of the medial malleolus and the medial calcaneal

tuberosity [21, 27]. Some authors suggest the tip of the lateral malleolus as the reference point which is localized below the tip of the medial malleolus [26, 38]. Nevertheless in the present study the authors introduced different, parallel to the foot plane reference lines: line crossing the tip of the medial malleolus and line crossing the posterior superior tip of the calcaneal tuberosity. Measurement according to those two reference lines proved to have a high correlation (r = 0.9874). The authors believe that those bony, easy palpable through skin orientation points may appear of better use in the clinic environment.

Many authors published various results in relation to the number, location and origin of the medial calcaneal branch(es). Havel et al[15], Louisa et al.[27] reported the occurrence of the range of one to two branches of the medial calcaneal nerves. Other published studies states the occurrence of the range of one to three (Torres et al.[37]) and even four (Joshi et al.[21]) branches of the medial calcaneal nerves. Single medial calcaneal branch is the most often finding in reports from Havel et al.[15] and Torres et al.[37] whilst two branches are most commonly registered by Louisa et al.[27] and Dellon et al.[10]. Joshi et.al[21] finds single medial calcaneal branch in the same number of dissected lower limbs as double. In the present study the authors visualized a range of one to three medial calcaneal branches with the most common single branch (73.3% of the lower limbs) which is similar to Torres et al and Havel et al reports [37, 15].

In the range of differences the authors of all other publications indicated the tibial nerve as the most frequent nerve of origin for the medial calcaneal branch(es) [15, 21, 27, 37]. The present study states that despite there is a single, double or triple branching pattern the tibial nerve is the most often nerve of origin (87% cases). As it goes to the further ramification models the lateral plantar nerve gives off medial calcaneal branch(es) in 25% cases followed by medial plantar nerve present in 8% cases. Some authors finds medial calcaneal branch(es) originating only from the tibial nerve [35], others claim it goes off only form the tibial or lateral plantar

nerve [14, 17, 21, 23], yet another reports it originates only from the tibial or medial plantar nerve [4].

Medial calcaneal branch(es) location also appears to be a matter of variance among published studies. Some authors observes majority of the medial calcaneal ramifications proximally to the tarsal tunnel [7, 27, 37], others locate it distally [9 ,14]. In the present study the authors registered 60% of medial calcaneal branches located below the tip of the medial malleolus at the same time 70% of them is located above posterior superior tip of the calcaneal tuberosity. It allows to assume that most of the ramifications are located between the two reference lines.

As a single case of an exceptionally distant location of the medial calcaneal branch of 330 mm and 337 mm above the tip of the medial malleolus may appear odd or suggest examiners mistake. It finds confirmation with Torres et al.[37] study where the authors also report a maximal ramification occurring 346.6 mm above the malleolar-calcaneal axis.

Iborra et al and Mullick et al. confirms that the ultrasound usage in the tarsal tunnel syndrome operative treatment leads to respectively 90.12 % and 93 % excellent and good results. In the cadaveric study the authors proved that a high-resolution ultrasonography can visualize the entire course of the tibial nerve as well as its tiny branches which may be applied in the decompression surgeries [18, 19, 29]. Also a sonosurgery, which is a "minimally invasive surgical technique performed with the continuous ultrasound imagining and the use of endoscopic tools" seems promising in improving the surgical result by reducing the risk of iatrogenic injuries [34].

Limitations

The fact that tibial nerve and its branches run together with vessels between muscles and other anatomical structures may mean that its localization changes during the lower limb movement. Therefore for the sake of this study a standardized positioning set up was arranged with all patients lying prone with the foot in neutral position (ankle fixed in the foot

stabilizing device and adjusted to the right angle). Second limitation is the localization of the of the posterior superior tip of the calcaneal tuberosity which depends on the foot arch. The angle between calcaneal inclination line and the horizontal line (heel pitch angle) varies in cavus, neutral and flat foot. Therefore in cavus foot the measured distance may be longer whilst shorter in the flat foot. Another restriction is a limited ultrasound resolution. Although authors were able to track the tibial nerve till its final bifurcation the high frequency ultrasound might miss some tiny terminal nerves, such as medial calcaneal branches. To reduce this factor ultrasound examination was conducted by an experienced orthopedic surgeon.

CONCLUSIONS

To conclude the authors of the present study together with the other analyzed publications proved that the anatomy of the tibial nerve and its distal branches observed in the medial ankle area is different between left and right limbs, gender and amongst individuals. As is the origin, location and division pattern on the medial calcaneal branch(es). Because of this anatomical variations it is difficult to suggest any safe zone area for the medial ankle surgical treatment, as so the preoperative or intraoperative ultrasound examination is highly recommended.

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Table I. Tibial nerve bifurcation location according to the reference line

Table I. Tibial nerve bifurcation location according to the reference line				
location	tip of the medial malleolus		posterior superior tip of the calcaneal tuberosity	
	n	%	n	%
above the reference line	14	23,3%	23	38,3%
at the level of the reference line	0	0,0%	8	13,3%
below the reference line	46	76,7%	29	48,3%

Table II. Results of measurements (mm) and statistics

Table II. Results of measurements (mm) and statistics										
Reference line	Measured point		n	Mean±SD	Median	Max.	Min.	Lower quartile (Q1)	Upper quartile (Q3)	Sex differences
tip of the medial malleolus	tibial nerve bifurcation		60	-5,93±19,59	-8,00	60	-33	-19,50	-1,50	p=0,6929
	medial calcaneal branch origin	1 st branch	60	9,27±61,73	-1,00	337	-27	-7,00	6,50	p=0,9409
		2 nd branch	16	-9,50±10,09	-8,50	2	-35	-14,00	-0,50	
		3 rd branch	4	-11,75±9,03	-11,00	-4	-21	-19,50	-4,00	
posterior superior tip of the calcaneal tuberosity	tibial nerve bifurcation		60	2,67±19,79	0,00	72	-26	-10,00	7,50	p=0,6776
	medial calcaneal branch origin	1 st branch	60	17,67±61,18	7,00	343	-17	3,00	13,50	p=0,8939
		2 nd branch	16	-1,63±11,28	-1,00	12	-29	-6,50	8,00	
		3 rd branch	4	-4,00±8,45	-2,50	3	-14	-11,00	3,00	

Table III. Division of the medial calcaneal nerve according to the number of branches

Table III. Division of the medial calcaneal nerve according to the number of branches				
Number of branches	Male feet (n=28)	Female feet (n=32)	Pooled sexes feet (n=60)	Percentage
One	16 (57,1%)	28 (87,5%)	44	73,3%
Two	10 (35,7%)	2 (6,3%)	12	20%
Three	2 (7,1%)	2 (6,3%)	4	6,7%

Table IV. Pattern of the medial calcaneal branches presentation according to the nerve of origin

Table IV. Pattern of the medial calcaneal branches presentation according to the nerve of origin				
Nerve of origin	Male feet (n=28)	Female feet (n=32)	Pooled sexes feet (n=60)	Percentage
single branch of TN	14 (50,0%)	22 (68,8%)	36	60%
single branch of LPN	2 (7,1%)	5 (15,6%)	7	11,7%
one branch of TN and one of LPN	6 (21,4%)	0 (0,0%)	6	10%
two branches of TN	3 (10,7%)	1 (3,1%)	4	6,7%
two branches of TN and one of MPN	0 (0,0%)	2 (6,3%)	2	3,3%
two branches of TN and one of LPN	2 (7,1%)	0 (0,0%)	2	3,3%
one branch of TN and one of MPN	1 (3,6%)	1 (3,1%)	2	3,3%
single branch of MPN	0 (0,0%)	1 (3,1%)	1	1,7%
TN - tibial nerve; LPN - lateral plantar nerve; MPN - medial plantar nerve				

Table V. Location of all medial calcaneal branches according to the reference lines (mm)

Table V. Location of all medial calcaneal branches according to the reference lines (mm)								
	Reference line	Location	n=78	%	Mean	Median	Max.	Min.
all medial calcaneal branches	tip of the medial malleolus	above the reference line	25	31,25%	-3,97	-3	23	-35
		at the level of the reference line	7	8,75%				
		below the reference line	48	60,0%				
	posterior superior tip of the calcaneal tuberosity	above the reference line	56	70,0%	4,36	5	34	-29
		at the level of the reference line	2	2,5%				
		below the reference line	22	27,5%				

*2 max. distal locations were excluded from the statistics (330/335mm, 337/343mm)

Table VI. First medial calcaneal branch location in relation to reference according to the sex

Table VI. First medial calcaneal branch location in relation to reference according to the sex											
	Reference line	Sex	n	Mean±SD	Median	Max.	Min.	Lower quartile (Q1)	Upper quartile (Q3)	p-value	correlation
1 st medial calcaneal branch	tip of the medial malleolus	F	32	-0,91±11,51	-0,5	23,0	-22	-8,5	6,0	0,9409	0,9463
		M	28	20,89±88,95	-1,5	337,0	-27	-6,5	6,5		
	posterior superior tip of the calcaneal tuberosity	F	32	7,56±12,03	6,5	34,0	-16	3,5	14,5	0,8939	
		M	28	29,21±88,05	7,0	343,0	-17	2,0	13,0		

Figure 1. Foot scheme with the measured points and the reference lines

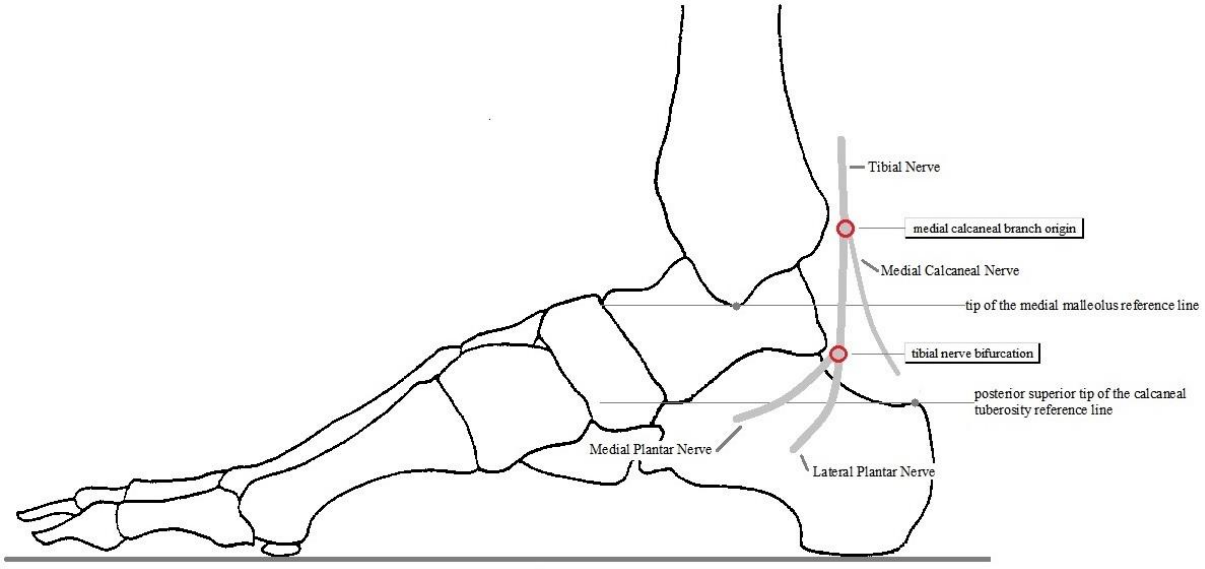


Figure 2. Correlation of the reference lines according to the tibial nerve bifurcation

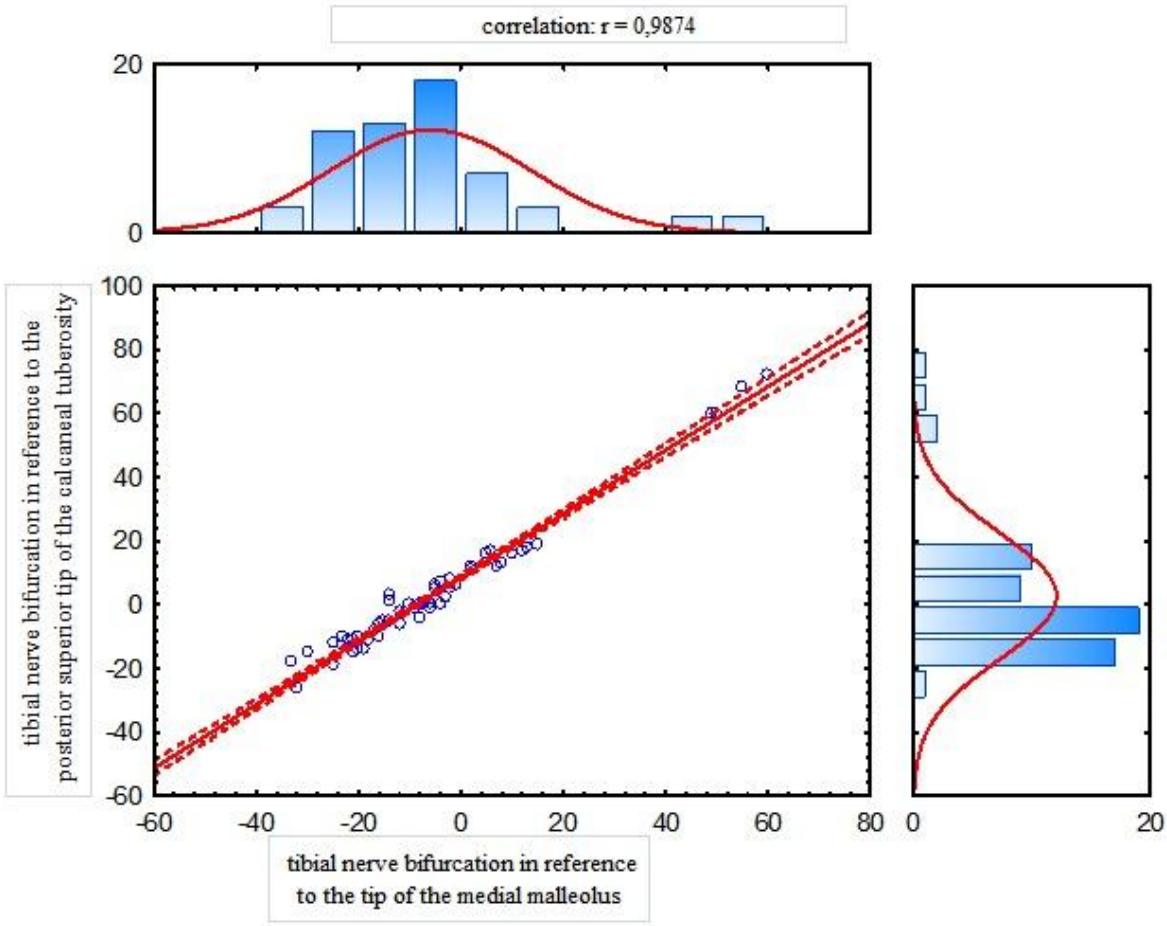
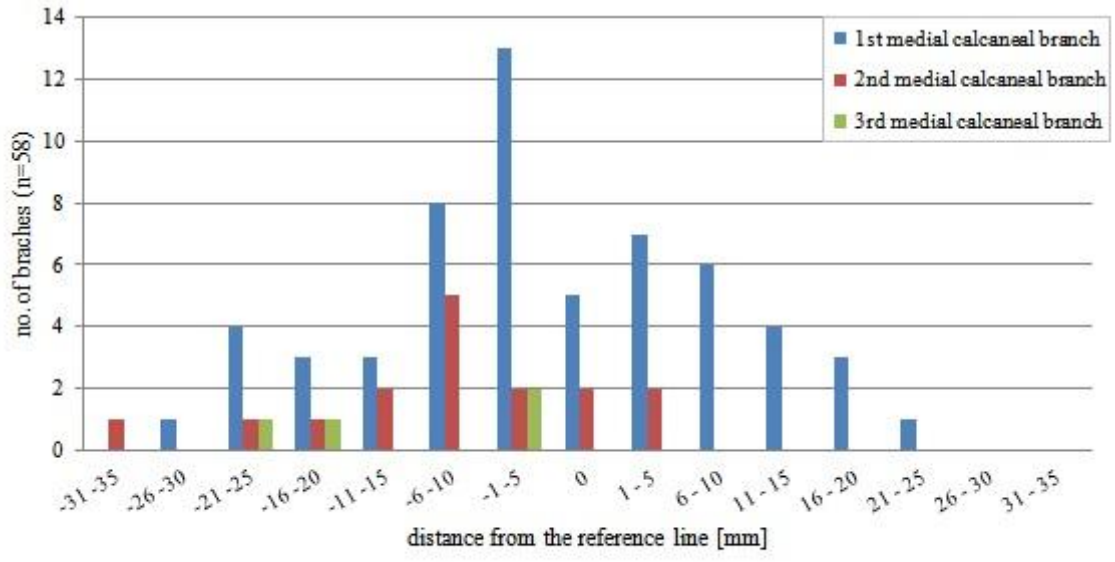


Figure 3. Medial calcaneal branches location in relation to the medial malleolus



*2 max. distal locations were not included in the table (330mm, 337mm)

ARTYKUŁ NR 2

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

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ABSTRACT

Background: The aim of this study was to analyze 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 with 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 analyzed 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 mm^2, 33.88 ± 6.31 for the tibial nerve, 9.58 ± 1.95 mm^2, 23.41 ± 7.37 for the medial plantar nerve and 7.17 ± 2.36 mm^2, 15.06 ± 5.81 for the lateral plantar nerve in males and 12.27 ± 2.45 mm^2, 26.32 ± 8.87 for the tibial nerve, 7.81 ± 1.41 mm^2, 17.71 ± 5.28 for the medial plantar nerve and 5.83 ± 1.25 mm^2, 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 significant 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 donors 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 bigger 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.

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

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 branch(es) shows diversity in terms of number (range from one to four), 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 paresthesia [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 visualized 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 nerves 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 μm thick slice. Subsequently each slice was stained with haematoxylin and eosin (Figure 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 x magnification). Each cross-section was photographed (20 x magnification), afterwards the CSA was measured semi-automatically using Olympus cellSens Standard 2.3 software with the producers 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 or t-test depending on normal distribution. Leven test was checked 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 distributed. Correlation coefficients were calculated to establish any statistical dependence between parameters. All analyses were performed using MedCalc version 16.8.

RESULTS

There were thirty fresh cadavers dissected (n = 60 lower limbs) with an mean age of 68.1 ± 15.2 (range from 27 to 91 years). 28 feet were female (46.7 %) and 32 were male (53.3 %). 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 I. Differences between the gender and foot side are shown in Table II and Table III 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 is 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 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 ($r = 0.44$, $p = 0.000$) (Figure 2). No statistically significant correlation was found between the age of donors and CSA 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 old were 15.37 ± 0.80 mm^2 and 20.35 ± 0.80 mm^2 for those above 70 years old, whilst in females 10.83 ± 0.92 mm^2 and 13.35 ± 0.80 mm^2 respectively. There was no statistically significant sex and age interactions in 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 were 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 authors knowledge this is the first publication analyzing 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 based on the ultrasound or magnetic resonance imagining 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 nerved 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 IV. 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. As so, it proves the reliability and usefulness of those imagining methods.

Analyzing results summarized in Table IV the authors found that the average cross-sectional area of the tibial nerve (15.25 mm^2) is almost identical with Riazi et al. [27] - 17.7 mm^2 and Cartwright et al. [6] - 13.7 mm^2. At the same time it is more than two times larger than the

values provided by Singh et al. [30] - 6.0 mm^2, Yiu et al. [35] - 6.3 mm^2 and Kerasnoudis et al. [19] - 6.3 mm^2. The differences may be the result of the average age of the examined patients. As so Yiu et al. [35] examined children with the mean age of 11.3 mm^2 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 IV 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 authors knowledge no previous studies for 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 in 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 et al. [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 ($\pi * r^2$). 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 mm^2 and the average number of nerve bundles to be 20.75 in the medial plantar nerve and 6.54 mm^2 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 et al. [17] together with Bilge et al.[4] states that in the majority of cases the tibial nerve bifurcation is located inside the tarsal tunnel. As Heimkes et al. [14] points 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 paresthesia among older people is higher.

Lothet et al. [24] together with Cartwright et at. [7] prove 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 what is consistent with Grimm et al. [12] and Cartwright et al. [6] findings. 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 age related decreasing of 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 of 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 is 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

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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Table I. The tibial nerve and its terminal branches measurements

Measurement		n	Mean ± SD	Median	Min.	Max.	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		8.76 ± 1.93	8.45	5.53	14.22	7.19	9.90
	lateral plantar nerve		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		20.75 ± 7.04	20.00	5.00	38.00	16.00	25.00
	lateral plantar nerve		13.40 ± 5.22	14.00	3.00	38.00	10.75	15.00

Table II. The tibial nerve and its terminal branches measurements - comparison by gender

		Women					Men					
Measurement		n	Mean ± SD	Median	Lower quartile (Q1)	Upper quartile (Q3)	n	Mean ± SD	Median	Lower quartile (Q1)	Upper quartile (Q3)	p
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		7.81 ± 1.41	7.37	6.70	9.10		9.58 ± 1.95	9.16	8.40	10.66	**0.000**
	lateral plantar nerve		5.83 ± 1.25	5.77	4.61	6.86		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		17.71 ± 5.28	18.00	14.50	20.50		23.41 ± 7.37	23.00	17.50	29.50	**0.003**
	lateral plantar nerve		11.50 ± 3.72	12.00	9.00	14.00		15.06 ± 5.81	15.00	12.50	16.50	**0.004**

Footnotes: numbers in bold indicate statistically significant differences between males and females ($p < 0.05$).

Table III. The tibial nerve and its terminal branches measurements - comparison by foot side

		Left foot					Right foot					
Measurement		n	Mean ± SD	Median	Lower quartile (Q1)	Upper quartile (Q3)	n	Mean ± SD	Median	Lower quartile (Q1)	Upper quartile (Q3)	p
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		8.80 ± 1.98	8.32	7.21	9.88		8.71 ± 1.90	8.78	7.08	9.92	0.805
	lateral plantar nerve		7.05 ± 2.48	6.99	5.05	8.30		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		20.37 ± 6.77	20.00	17.00	23.00		21.13 ± 7.39	21.00	15.00	26.00	0.412
	lateral plantar nerve		14.20 ± 6.37	14.00	11.00	16.00		12.60 ± 3.68	13.00	10.00	15.00	0.296

Footnotes: statistically significant differences between left and right foot when $p < 0.05$.

Table IV. Studies of the tibial nerve CSA measured at the level of medial malleolus

	Group (n)	Mean age	CSA of the tibial nerve at the level of medial malleolus [mm^2]	Reference range [mm^2]	Type of study
He et al., 2019 [12]	n = 40	55.2	11.6 ± 1.6	-	US 4 - 15 MHz
Lothet et. al., 2019 [23]	n = 15	21.7	12.3	-	US 18 MHz
Singh et al., 2019 [29]	n = 45	30 - 68	6.0 ± 1.8	-	US 5 - 18 MHz
Bedewi et al., 2018 [3]	n = 138	38.3	12.7 ± 4.5	2.0 - 30.0	US 18.5 MHz
Grimm et al., 2018 [11]	n = 100	51.2	10.2 ± 2.0	-	US 14 MHz
Kronlage et al., 2017 [20]	n = 60	30.5	* 8.1 ± 2.0	4.0 - 12.1	MRI
Singh et al., 2017 [28]	n = 75	39.5	12.4 ± 1.1	10.0 - 14.0	US 7 - 18 MHz
Kang et al., 2016 [17]	n = 20	65.0	12.4 ± 2.9	-	US 7 - 12 MHz
Yiu et al., 2015 [34]	n = 29	11.3	6.3 ± 1.9	8.6 - 14.1	US 7 - 13 MHz
Boehm et al., 2014 [5]	n = 56	50.2	9.6 ± 2.2	9.0 - 10.2	US 12 - 15 MHz
Seok et al., 2014 [27]	n = 94	43.9	12.1 ± 3.1	8.5 – 22.8	US 5 - 12 MHz
Kerasnoudis et al., 2013 [18]	n = 75	53.5	6.3 ± 1.5	3.5 - 9.3	US 18 MHz
Riazi et al., 2012 [26]	n = 43	46.8	17.7 ± 6.5	-	US 6 - 13 MHz
Tagliafico et al., 2012 [30]	n = 58	47.0	9.6 ± 4.0	7.2 - 13.7	US 17.5 MHz
Cartwright et al., 2008 [7]	n =60	45.9	13.7 ± 4.3	5.1 - 22.3	US 15 MHz
Ito et al., 2007 [15]	n = 35	52.8	7.9 ± 1.5	5.0 - 10.7	US 7.5 MHz
Lee et al., 2005 [21]	n = 24	57.4	12.0	-	US 10 - 12 MHz

Footnotes:
* measured at the proximal third of the calf
CSA - cross-sectional area; US - ultrasonography; MRI - magnetic resonance imaging.

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 x magnification.
A: 58-year-old male, right food - cross-sectional area (CSA): TN - 12.76 mm^2, MPN - 6.79 mm^2, LPN - 4.37 mm^2; number of nerve bundles: TN - 35, MPN - 18, LPN - 12
B: 58-year-old male (same individual as in A), left food CSA: TN - 12.83 mm^2, MPN - 9.92 mm^2, LPN - 5.47 mm^2; number of nerve bundles: TN - 29, MPN - 26, LPN - 8
C: 63-year-old male, right foot - CSA: TN - 13.92 mm^2, MPN - 5.63 mm^2, LPN - 5.05 mm^2; number of nerve bundles: TN - 34, MPN - 14, LPN - 12
D: 84-year-old male, left food - CSA: TN - 18.39 mm^2, MPN - 10.32 mm^2, LPN - 7.09 mm^2; number of nerve bundles: TN - 44, MPN - 34, LPN - 13

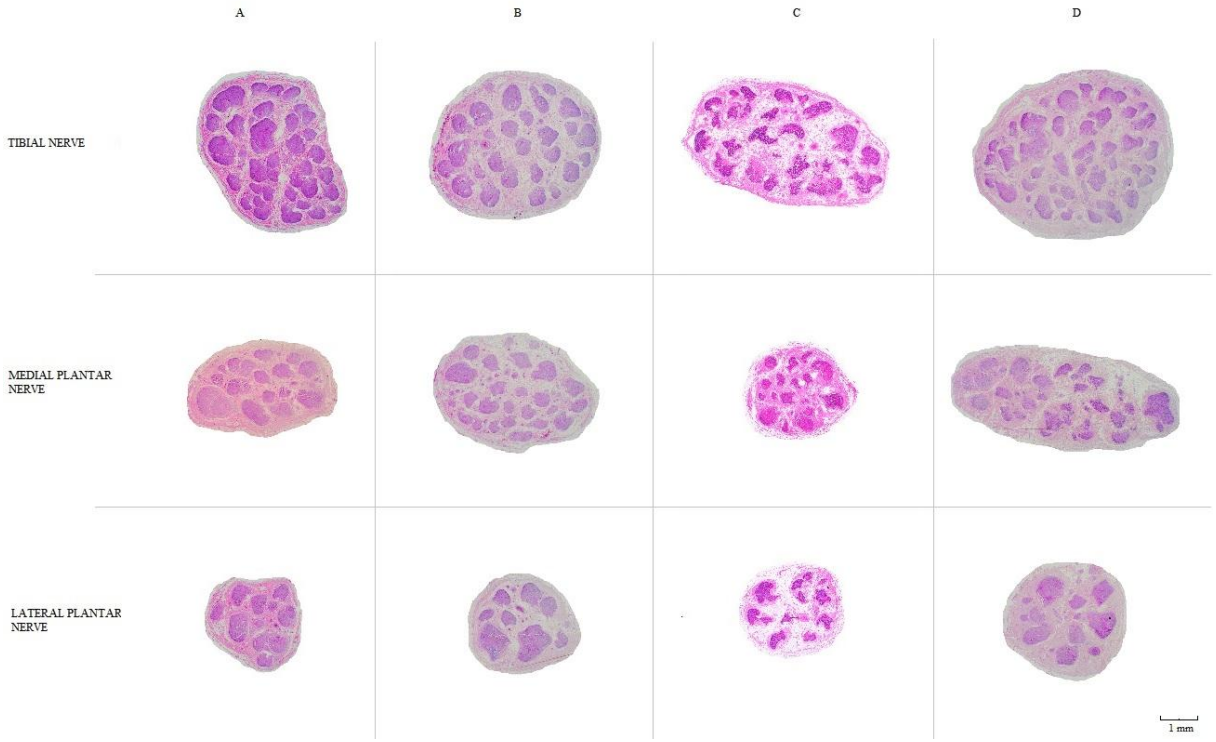
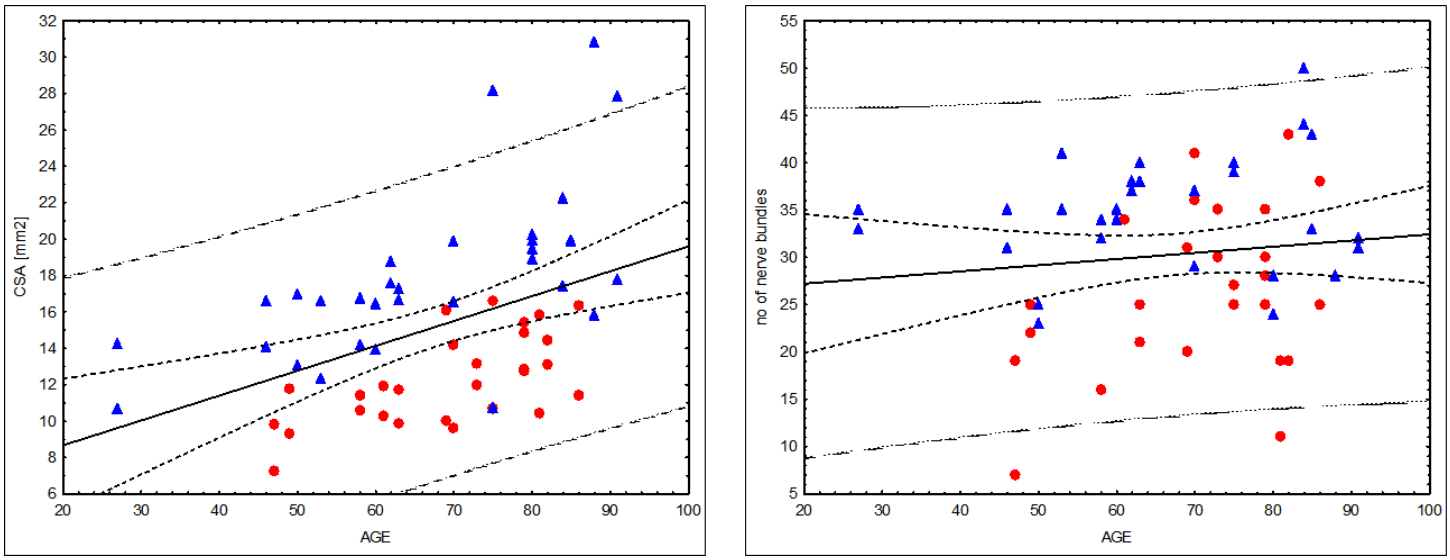


Figure 2. On the left: a scatter plot of donors age correlation with tibial nerve cross-sectional area (CSA). Blue triangles represent males (r = 0.69, p = 0.000), red dots represent females (r = 0.60, p = 0.001). On the right: a scatter plot of donors age correlation with number of tibial nerve bundles. Blue triangles represent males (r = -0.04, p = 0.846), red 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.



ARTYKUŁ NR 3

Comparison of the histological structure of the tibial nerve and its terminal branches in the fresh and fresh-frozen cadavers

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ABSTRACT

Background: The aim of this study was to compare the histological structure (cross-sectional area (CSA) and number of nerve fascicles) of the distal part of the tibial nerve (TN) and its terminal branches (medial plantar nerve - MPN, lateral plantar nerve - LPN) in the fresh and fresh-frozen cadavers using computer assisted image analysis.

Materials and methods: The tibial nerve with terminal branches (medial and lateral plantar nerves) were dissected from the fresh and fresh-frozen 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 the specimens were photographed and analyzed using Olympus cellSens software.

Results: The fresh cadavers group comprised 60 feet (mean age 68.1 ± 15.2 years). The mean CSA and the number of nerve fascicles were respectively 15.25 ± 4.6 mm^2, 30.35 ± 8.45 for the tibial nerve, 8.76 ± 1.93 mm^2, 20.75 ± 7.04 for the medial plantar nerve and 6.54 ± 2.02 mm^2, 13.40 ± 5.22 for the lateral plantar nerve. The fresh-frozen cadavers group comprised 21 feet (mean age 75.1 ± 9.0 years). The mean CSA and the number of nerve fascicles were respectively 13.71 ± 5.66 mm^2, 28.57 ± 8.00 for the tibial nerve, 7.55 ± 3.25 mm^2, 18.00 ± 6.72 for the medial plantar nerve and 4.29 ± 1.93 mm^2, 11.33 ± 1.93 for the lateral plantar nerve. Only lateral plantar nerves showed statistical differences in the CSA and the number of nerve fascicles between examined groups ($p = 0.000$, $p = 0.037$ respectively). A positive correlation was found between donors age and tibial nerve CSA in the fresh cadavers group ($r = 0.44$, $p = 0.000$). A statistical difference was found between the medial and lateral plantar nerves both in the CSA and the number of nerve fascicles ($p < 0.001$, $p < 0.001$ respectively).

Conclusions: The CSA and the number of nerve fascicles of the tibial and medial plantar nerves were similar in the fresh and fresh-frozen cadavers whilst different in the lateral plantar nerve. The tibial nerve showed increasing CSA with the advanced age in the fresh cadavers. The medial plantar nerve had larger CSA and more nerve fascicles than the lateral plantar nerve.

KEYWORDS: tibial nerve, cross-sectional area, medial and lateral plantar nerves, fresh cadavers, fresh-frozen cadavers

INTRODUCTION

The tibial nerve is a peripheral sensorimotor nerve arising as a branch of sciatic nerve bifurcation in the popliteal fossa [40]. 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 smaller lateral plantar nerve [28]. The tibial nerve bifurcation level shows a great variability as so depending on the study its localization is referred to the medial or lower located lateral malleolus [24, 43]. Most commonly it is described below the tip of the medial malleolus, inside the tarsal tunnel [42]. Tibial nerve and its branches provides innervation to the posterior lower leg, the muscles and skin of the sole of the foot [21].

For many years ankle arthroscopy has proved to be a useful diagnostic and therapeutic procedure for ankle and foot disorders. Although it is a minimally invasive surgery neurological complications are most frequently reported referring to the tibial, sural, superficial peroneal and deep peroneal nerves [1, 45, 47]. According to Freedman et.al [13] all neurovascular impairments are caused by distractor pin or portal placement. In order to avoid iatrogenic injuries and to perform safe and reproducible arthroscopy constant training is highly recommended.

Nowadays necessity of constant practicing of surgical skills is emphasized by professionals [2]. They clearly highlight the superiority of fresh cadavers among any frozen or anatomically preserved. However, due to ethical and technical problems as well as limited access to the fresh bodies, fresh-frozen cadavers proved to be convenient surgical training model [35]. Because of their most lifelike features they are used by surgeons, orthopedics, radiologists and anesthesiologist to practice and improve operating skills [12, 17]. Fresh-frozen bodies also found application in the research and bioengineering, allowing development of new instruments and procedures.

The aim of the this study was to compare the histological structure of the tibial nerve and its terminal branches in the fresh and fresh-frozen cadavers.

MATERIALS AND METHODES

The study was conducted on 60 lower limbs of the fresh cadavers and on 21 lower limbs of the fresh-frozen cadavers in the Department of Anatomy between December 2016 and March 2019. The group of fresh-frozen cadavers composed of already amputated lower limbs at the level of the knee originating from mixed donors with known medical record. 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 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 visualized 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. In the group of fresh-frozen cadavers the dissection was performed after thawing of the specimens overnight at room temperature. 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 μm thick slice. Subsequently each slice was stained with haematoxylin and eosin (Figure 1).

Micromorphometry

The CSA and the number of nerve fascicles of the tibial nerve, medial plantar nerve and lateral plantar nerve were assessed using a light microscope (Olympus BX53, 20 x magnification). Each cross-section was photographed (20 x magnification), afterwards the CSA was measured semi-automatically using Olympus cellSens Standard 2.3 software with the producers precision of 10 μm, whilst the number of nerve fascicles was calculated manually. Each slice was assessed once by the same pathologist. Then the values of the CSA and the number of nerve fascicles were tabulated according to the group (fresh or fresh-frozen cadavers).

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 or t-test depending on normal distribution. To compare CSA and number of nerve fascicles between TN, MPN and LPN paired t-test or Wilcoxon rang test were used depending on whether data was 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 thirty fresh cadavers dissected (n = 60 lower limbs) with a mean age of 68.1 ± 15.2 (range from 27 to 91 years). 28 feet were female (46.7%) and 32 were male (53.3%). In the group of fresh-frozen cadavers twenty one lower limbs were dissected with a mean age of 75.1 ± 9.0 (range from 60 to 92 years). 12 feet were female (57.1%) and 9 were male (42.9%). The mean CSA and number of nerve fascicles of the tibial, medial plantar and lateral plantar nerves in the fresh and fresh-frozen groups are presented in Table I. Gender differences between examined groups are presented in Table II. In both examined groups males' tibial nerves showed larger CSA and more nerve fascicles than females'. Only lateral plantar nerves showed statistical differences in the CSA and number of nerve fascicles between examined groups. The lateral plantar nerve also proved statistical difference among males (CSA and number of nerve fascicles) and females (CSA) in fresh and fresh-frozen cadavers. In the fresh cadavers no statistically significant differences between right and left foot of the individual were found ($p > 0.05$). Such comparison was not possible to perform in the fresh-frozen cadavers as the examined lower limbs originated from different individuals. There is statistically significant difference between medial and lateral plantar nerve in CSA and number of nerve fascicles in both groups ($p < 0.001$). CSA of the medial plantar nerve confirmed to be 1.3 times and 1.8 times larger than the lateral plantar nerves' in the fresh and

fresh-frozen specimens respectively. The medial plantar nerve also proved to have more nerve fascicles than the lateral plantar nerve in both examined groups. A positive correlation was noted between the age of donors and the CSA of the tibial nerve in the fresh cadavers group ($r = 0.44$, $p = 0.000$) (Table III).

DISCUSSION

The present study compares histological structure (CSA and number of nerve fascicles) of the distal part of the tibial nerve and its terminal branches (medial and lateral plantar nerves) in the fresh and fresh-frozen cadavers assessed using computer-assisted measurements. Literature analysis shows that in the previous studies the CSA of the tibial nerve was evaluated by ultrasound or magnetic resonance imagining on the living patients or volunteers [5, 15, 22]. To the best of authors knowledge this is the first publication analyzing histological differences in peripheral nerves obtained from the fresh and fresh-frozen cadavers. It is also the first study revealing tibial, medial and lateral plantar nerves CSA measured directly on the nerves harvested from the fresh cadavers. Furthermore no reference values for the CSA of the medial and lateral plantar are available in the literature.

In the present study the tibial, medial plantar and lateral plantar nerves harvested from the 60 fresh cadavers were compared to 21 collected from the fresh-frozen cadavers. The fresh cadavers group composed of younger donors (mean age: 68.1 vs 75.1) and presented slightly higher values of CSA (tibial nerve: 15.25 vs. 13.71; medial plantar nerve 8.76 vs 7.55; lateral plantar nerve: 6.54 vs. 4.29) and more nerve fascicles (tibial nerve: 30.35 vs. 28.57; medial plantar nerve 20.75 vs 18.00; lateral plantar nerve: 13.40 vs. 11.33). Nevertheless tibial nerve CSA measured in both groups is in line with results of ultrasound and magnetic resonance imaging performed on living patients (Table IV). The statistical analysis proved that the tibial and medial plantar nerves are similar in the fresh and fresh-frozen groups. On the other hand

the lateral plantar nerves appeared to be statistically different. Such discrepancy may be the result of anatomical differences of the examined nerves. The lateral plantar nerve is the smaller terminal branch of the tibial nerve bifurcation [21]. Because of that it may be suggested that freezing process does not alter larger nerves (TN, MPN) whilst impacts smaller ones (LPN). Although the differences proved to be statistically insignificant (except for LPN) their slightly decreased values in fresh-frozen cadavers is worth noticing. Besides micromorphometric assessment some differences between two examined groups appeared during its histological preparation. Fresh-frozen specimens showed grater stiffness and hardness of the nerve trunks, poorly stained with haematoxylin and eosin and revealed more artifacts in the microscopic analysis.

Decreased CSA of the assessed nerves may be explained by Bakhach [4] who described changes occurring in biological tissues during freezing using thermodynamic and biophysical laws. Emphasizing that water may reach up to 70% of tissues volume he examined its transfer between intra and extracellular compartments throughout crystallization process. Intracellular formation and aggregation of ice crystals destroy its structures and cause mechanical stress on the cell walls resulting in deformation and fragmentation. Moreover water transition into a solid state leads to changes in extracellular chemical composition with the increased ion accumulation. Such concentration gradient between cell membrane makes water run out of the intracellular space causing its dehydration. These may elucidate rigidness of the nerve samples, artifacts in the microscopic assessment and slightly decreased CSA of the fresh-frozen cadavers registered in the present study.

Although fresh cadavers retain biomechanical features and are most suitable for the surgical training, they putrefy and are available only for the short time [3]. Searching for the best fresh body equivalent brought to many studies on its preservation [9, 12]. Along proved advantages each method revealed some limitations, as so: formalin fixation makes the specimens stiff and

discolored, Thiel embalming requires infrastructure for the process and is not suitable for all tissues, fresh-freezing brings the risk of infection and needs time for thawing [39]. Nevertheless fresh-frozen cadavers seems to be the most flexible and realistic [19]. They appeared to be even better than the virtual reality stimulator [34].

While literature provides comparative analysis of the fresh and fresh-frozen tendons [18, 6], bones [10, 26, 41], osteochondral allografts [29] there is lack of such comparison for the human peripheral nerves. Hohmann et al. [18] revealed that the long head of biceps tendons showed higher loads to failure and lower elasticity in the fresh-frozen samples when compared to the fresh specimens. At the same time fresh tendons were wider and presented larger CSA. On the contrary Bitar et al. [6] state that fresh-frozen tendons of the semitendinosus muscle show no histological differences referring to the fresh ones. Similarly Panjabi et al. [30] deny any physical or histological changes in the fresh-frozen specimens. Opposite to that Giannini et al. [14] noted an increased CSA in the fresh-frozen tendons of the posterior tibial muscles as well as increased stiffness and decreased ultimate load. An interesting study was performed by Zarb et al. [46] who analyzed the quality of the Magnetic Resonance (MR) images of a live patients', fresh-frozen and Thiel embalmed bones, ligaments, tendons and muscles of the ankle. The image quality of the fresh-frozen specimen appeared to be higher when compared to the live patient. Unfortunately no nerves of the ankle were included in the research which might have been beneficial for the present study reference.

Fresh-frozen peripheral nerves were examined mostly in relation to their biomechanical properties [8, 44]. Stouthandel et al. [37] compared Thiel embalmed and fresh-frozen median nerves showing slight increase of CSA in the embalmed group, no significant difference in elasticity and similar biomechanical patterns. Enlarged CSA of the nerves preserved with the Thiel method is interpreted to be the result of the embalming fluid uptake. Sargon et al. [32]

counted the myelinated nerve fibers of the fresh-frozen facial nerve terminal branches concluding that both fresh and fresh-frozen human specimens are better than formalin fixed in order to perform the anatomic dissection and find tiny nerves.

To the best of authors knowledge there has not been any publication which compared histological structure of the fresh-frozen human nerves to the fresh ones. As so, such analysis of the peripheral nerves together with biomechanical experiments may constitute a valuable subject for the future studies.

Albeit there were relatively high number of lower limbs examined in the present study (81 feet) their uneven distribution among the compared groups (60 vs. 21) and low number of fresh-frozen cadavers might have influenced the results. Only nine males in the fresh-frozen cadavers group would have significantly hindered the gender comparison. Second limitation is the fact that lower limbs included in the group of fresh-frozen cadavers originated from different donors which impeded the intra-individual left-right comparison. Another restriction is the various age of the analyzed groups which is proved to correlate with peripheral nerves CSA [15, 27]. Narrow range of age in the fresh-frozen cadavers (from 60 to 92 years) might have also biased the age correlation which was confirmed for the tibial nerve CSA in the fresh cadavers (range of age from 27 to 91 years). Therefore, for the sake of future studies, the authors would recommend to collect and compare specimens from the contralateral sides of the individual (followed by the left-right difference exclusion).

CONCLUSION

To conclude, the authors of the present study proved that freezing process alters tissue properties of the smaller nerves on top of impacting biomechanical features of the peripheral nerves. Histological structure of the larger nerves remains uninfluenced by the freezing process.

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Table I. Measured nerve parameters for TN, MPN and LPN - comparison between fresh and fresh-frozen cadavers

Measurement		Fresh cadavers n	Fresh cadavers Mean ± SD	Fresh cadavers Median	Fresh cadavers Lower quartile (Q1)	Fresh cadavers Upper quartile (Q3)	Fresh-frozen cadavers n	Fresh-frozen cadavers Mean ± SD	Fresh-frozen cadavers Median	Fresh-frozen cadavers Lower quartile (Q1)	Fresh-frozen cadavers Upper quartile (Q3)	p
Cross-sectional area [mm^2]	tibial nerve	60	15.25 ± 4.65	14.66	11.77	17.29	21	13.71 ± 5.66	12.84	9.50	16.15	0.094
	medial plantar nerve		8.76 ± 1.93	8.45	7.19	9.90		7.55 ± 3.25	7.53	4.61	10.36	0.156
	lateral plantar nerve		6.54 ± 2.02	6.44	5.12	7.41		4.29 ± 1.93	4.31	2.52	5.76	**0.000**
Number of nerve fascicles	tibial nerve	60	30.35 ± 8.45	31.00	25.00	35.25	21	28.57 ± 8.00	31.00	22.00	35.00	0.403
	medial plantar nerve		20.75 ± 7.04	20.00	16.00	25.00		18.00 ± 6.72	18.00	12.00	22.00	0.123
	lateral plantar nerve		13.40 ± 5.22	13.50	10.75	15.00		11.33 ± 1.93	11.00	7.00	14.00	**0.037**

Footnotes: numbers in bold indicate statistically significant differences between fresh and fresh-frozen cadavers ($p < 0.05$).

Table II. Measured nerve parameters for TN, MPN and LPN - comparison by gender between fresh and fresh-frozen cadavers

Gender	Measurement		Fresh cadavers n	Fresh cadavers Mean ± SD	Fresh cadavers Median	Fresh cadavers Lower quartile (Q1)	Fresh cadavers Upper quartile (Q3)	Fresh-frozen cadavers n	Fresh-frozen cadavers Mean ± SD	Fresh-frozen cadavers Median	Fresh-frozen cadavers Lower quartile (Q1)	Fresh-frozen cadavers Upper quartile (Q3)	p
Women	Cross-sectional area [mm^2]	tibial nerve	28	12.27 ± 2.45	11.85	10.35	14.31	12	12.70 ± 3.90	13.46	9.28	15.27	0.802
		medial plantar nerve		7.81 ± 1.41	7.37	6.70	9.10		7.77 ± 3.38	7.41	5.88	10.78	0.988
		lateral plantar nerve		5.83 ± 1.25	5.77	4.61	6.86		4.47 ± 2.05	4.56	2.70	5.79	**0.030**
	Number of nerve fascicles	tibial nerve	28	26.32 ± 8.87	25.00	19.50	34.00	12	28.08 ± 9.13	31.50	20.50	34.25	0.555
		medial plantar nerve		17.71 ± 5.28	18.00	14.50	20.50		16.50 ± 7.23	17.00	12.00	19.75	0.426
		lateral plantar nerve		11.50 ± 3.72	12.00	9.00	14.00		11.42 ± 7.23	9.00	6.00	14.25	0.417
Men	Cross-sectional area [mm^2]	tibial nerve	32	17.86 ± 4.57	17.10	15.02	19.90	9	15.06 ± 7.45	12.57	10.09	16.29	0.053
		medial plantar nerve		9.58 ± 1.95	9.16	8.40	10.66		7.26 ± 3.25	7.64	4.61	9.83	0.092
		lateral plantar nerve		7.17 ± 2.36	7.08	5.18	8.35		4.05 ± 1.86	3.35	2.28	5.66	**0.001**
	Number of nerve fascicles	tibial nerve	32	33.88 ± 6.31	34.00	28.50	38.00	9	29.22 ± 6.67	30.00	25.00	35.00	0.119
		medial plantar nerve		23.41 ± 7.37	22.50	17.50	29.50		20.00 ± 5.77	20.00	17.00	24.00	0.270
		lateral plantar nerve		15.06 ± 5.81	14.50	12.50	16.50		11.22 ± 2.73	12.00	9.00	13.00	**0.020**

Footnotes: numbers in bold indicate statistically significant differences between males and females ($p < 0.05$).

Table III. Association between age and measured nerve parameters for TN, MPN and LPN in fresh and fresh-frozen cadavers

Measurement		Fresh cadavers			Fresh-frozen cadavers		
		n	R	p	n	R	p
Cross-sectional area [mm^2]	tibial nerve	60	**0.439**	**0.000**	21	0.112	0.629
	medial plantar nerve		0.083	0.531		0.040	0.862
	lateral plantar nerve		0.110	0.401		-0.045	0.847
Number of nerve fascicles	tibial nerve	60	0.086	0.512	21	-0.161	0.485
	medial plantar nerve		-0.224	0.085		-0.140	0.545
	lateral plantar nerve		-0.104	0.428		-0.204	0.376

Footnotes: numbers in bold indicate statistically significant age correlation ($p < 0.05$).

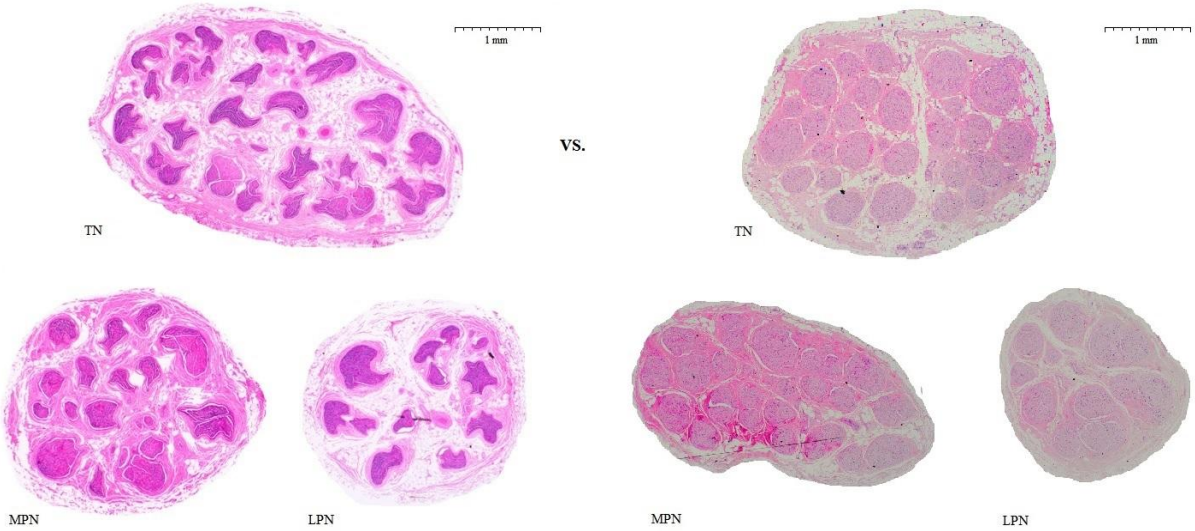
Table IV. Studies of the tibial nerve CSA measured at the level of medial malleolus

	Group (n)	Mean age	CSA of the tibial nerve at the level of medial malleolus [mm^2]	Reference range [mm^2]	Type of study
He et al., 2019 [16]	n = 40	55.2	11.6 ± 1.6	-	US 4 - 15 MHz
Lothet et. al., 2019 [25]	n = 15	21.7	12.3	-	US 18 MHz
Bedewi et al., 2018 [5]	n = 138	38.3	12.7 ± 4.5	2.0 - 30.0	US 18.5 MHz
Grimm et al., 2018 [15]	n = 100	51.2	10.2 ± 2.0	-	US 14 MHz
Kronlage et al., 2017 [22]	n = 60	30.5	* 8.1 ± 2.0	4.0 - 12.1	MRI
Singh et al., 2017 [36]	n = 75	39.5	12.4 ± 1.1	10.0 - 14.0	US 7 - 18 MHz
Kang et al., 2016 [20]	n = 20	65.0	12.4 ± 2.9	-	US 7 - 12 MHz
Boehm et al., 2014 [7]	n = 56	50.2	9.6 ± 2.2	9.0 - 10.2	US 12 - 15 MHz
Seok et al., 2014 [33]	n = 94	43.9	12.1 ± 3.1	8.5 – 22.8	US 5 - 12 MHz
Riazi et al., 2012 [31]	n = 43	46.8	17.7 ± 6.5	-	US 6 - 13 MHz
Tagliafico et al., 2012 [38]	n = 58	47.0	9.6 ± 4.0	7.2 - 13.7	US 17.5 MHz
Cartwright et al., 2008 [11]	n =60	45.9	13.7 ± 4.3	5.1 - 22.3	US 15 MHz
Lee et al., 2005 [23]	n = 24	57.4	12.0	-	US 10 - 12 MHz

* measured at the proximal third of the calf

CSA - cross-sectional area; US - ultrasonography; MRI - magnetic resonance imaging

Figure 1. Cross-section of tibial nerve (TN), medial plantar nerve (MPN) and lateral plantar nerve (LPN) of the fresh cadaver (on the left) and fresh-frozen cadaver (on the right). Haematoxylin and eosin staining.



PODSUMOWANIE WYNIKÓW I WNIOSKI

W badaniach potwierdzono zmienność topografii dystalnego odcinka nerwu piszczelowego, podeszwowego przyśrodkowego, podeszwowego bocznego oraz gałęzi piętowych przyśrodkowych. Wykazano również zmienną ilość oraz miejsce odejścia gałęzi piętowych przyśrodkowych. W związku z opisaną różnorodnością w lokalizacji struktur nerwowych okolicy kostki przyśrodkowej zalecono przed lub śródoperacyjne badanie ultrasonograficzne celem określenia ich przebiegu i uniknięcia jatrogennych powikłań.

Mikroskopowa analiza badanych nerwów potwierdziła, iż nerw podeszwowy przyśrodkowy jest większy od nerwu podeszwowego bocznego. Jednocześnie stwierdzono większe pole przekroju i ilość pęczków nerwowych u mężczyzn. Wartym zwrócenia uwagi jest dostrzeżony w badaniu wzrost pola przekroju i liczby pęczków nerwowych nerwu piszczelowego wraz z wzrastającym wiekiem pacjenta. Ponadto bezpośrednie pomiary pola przekroju nerwów podeszwowych przyśrodkowego i bocznego określiły ich wartości referencyjne, które dotychczas określane były jedynie badaniem ultrasonograficznym. Potwierdzono również, iż proces mrożenia nie wpływa na pole przekroju dużych nerwów obwodowych.

STRESZCZENIE W JĘZYKU POLSKIM

Zmienność odejścia gałęzi piętowej przyśrodkowej i podziału nerwu piszczelowego. Analiza makro i mikroskopowa.

Wstęp

Nerw piszczelowy powstaje z podziału nerwu kulszowego w dole podkolanowym. Na podudziu przebiega wzdłuż mięśnia piszczelowego tylnego wraz z naczyniami piszczelowymi tylnymi. Następnie w kanale kostki przyśrodkowej dzieli się na gałęzie końcowe: nerw podeszwowy przyśrodkowy i nerw podeszwowy boczny. W dystalnej części oddaje różniące się liczbą i miejscem odejścia gałęzie piętowe przyśrodkowe. Wraz z odgałęzieniami unerwia mięśnie grupy tylnej podudzia i stopy oraz skórę przyśrodkowej powierzchni stopy i podeszwę.

Podczas przebiegu przez zatokę stępu nerw piszczelowy oraz jego końcowe odgałęzienia mogą ulec uwięźnięciu i uciśnięciu. Zespół zatoki stępu charakteryzuje pieczący ból okolicy pięty i podeszwy, parastezje oraz mrowienie promieniujące do palców stopy oraz na podudzie. Uzupełniającym badaniem potwierdzającym ucisk nerwu obwodowego jest ultrasonografia. Natomiast jedną z form leczenia jest dekompresja zatoki stępu wykonywana zwykle artroskopowo. Najczęstszym powikłaniem zabiegów okolicy kostki przyśrodkowej są jatrogenne uszkodzenia nerwów.

Materiał i metody

Pierwsza części pracy polegała na przeprowadzeniu badania ultrasonograficznego okolicy kostki przyśrodkowej na 30 ochotnikach (60 kończyn dolnych). Badanie zostało wykonane przez specjalistę ortopedii z ponad 20-sto letnim doświadczeniem przy użyciu aparatu

MyLabGold 25 głowica 18MHz. Podczas badania ultrasonograficznego określano topografię dystalnego odcinka nerwu piszczelowego, jego podziału, nerwu podeszwowego przyśrodkowego, nerwu podeszwowego bocznego oraz gałęzi piętowych przyśrodkowych. Określano również ilość i miejsce odejścia gałęzi piętowych przyśrodkowych. Następnie wyznaczono dwie poziomie linie referencyjne: jedną przebiegają przez szczyt kostki przyśrodkowej, drugą przebiegająca przez tylno - górny brzeg kości piętowej. Suwmiarką mierzono odległość podziału nerwu piszczelowego na nerw podeszwowy przyśrodkowy i nerw podeszwowy boczny oraz miejsca odejścia każdej gałęzi piętowej przyśrodkowej od każdej z linii referencyjnych.

W drugiej części pracy z 30 nieutrwalonych zwłok (60 kończyn dolnych) oraz z 21 izolowanych, świeżo mrożonych kończyn dolnych wypreparowano i pobrano końcowy odcinek nerwu piszczelowego oraz proksymalne części nerwów podeszwowego przyśrodkowego i bocznego. Z każdego z wyżej wymienionych nerwów pobrano wycinek, który utrwalono w 10% roztworze formaldehydu. Następnie każdy preparat przeszedł procedurę odwadniania, zatapiania w parafinie, cięcia na 2 μm skrawki oraz barwienia hematoksyliną i eozyną. Otrzymane preparaty badano pod mikroskopem świetlnym (Olympus BX53, powiększenie 20 x). Uzyskany obraz analizowano przy pomocy programu Olympus cellSens Standard 2.3, który określał pole przekroju poprzecznego badanego nerwu, a następnie ręcznie liczono ilość pęczków nerwowych.

Wyniki

W badaniu ultrasonograficznym stwierdzono, że podział nerwu piszczelowego na nerwy podeszwowy przyśrodkowy i podeszwowy boczny zlokalizowany jest 5.93 ± 19.59 mm poniżej szczytu kostki przyśrodkowej (77%). Gałęzie piętowe przyśrodkowe odchodzą w

liczbie od jednej do trzech z najczęściej występującą jedną gałęzią (73%) odchodzącą od nerwu piszczelowego (60%), 3.97 mm poniżej szczytu kostki przyśrodkowej.

W badaniu mikroskopowym w grupie nerwów pochodzących od nieutrwalonych donatorów określono średnie pole przekroju i ilość pęczków nerwowych odpowiednio 17.86 ± 4.57 mm^2, 33.88 ± 6.31 dla nerwu piszczelowego, 9.58 ± 1.95 mm^2, 23.41 ± 7.37 dla nerwu podeszwowego przyśrodkowego oraz 7.17 ± 2.36 mm^2, 15.06 ± 5.81 dla nerwu podeszwowego bocznego. Badane nerwy nie wykazały różnic pomiędzy lewą i prawą stopą. Stwierdzono większe pole przekroju oraz większą liczbę pęczków nerwowych u mężczyzn niż u kobiet. Stwierdzono korelację pomiędzy wiekiem donatora a polem przekroju nerwu piszczelowego ($r = 0.44$, $p = 0.000$). W grupie nerwów pobranych z preparatów mrożonych określono średnie pole przekroju i ilość pęczków nerwowych odpowiednio 13.71 ± 5.66 mm^2, 28.57 ± 8.00 dla nerwu piszczelowego, 7.55 ± 3.25 mm^2, 18.00 ± 6.72 dla nerwu podeszwowego przyśrodkowego oraz 4.29 ± 1.93 mm^2, 11.33 ± 1.93 dla nerwu podeszwowego bocznego. Statystyczną różnicę pomiędzy nerwami nieutrwalonymi a nerwami mrożonymi wykazano dla nerwu podeszwowego bocznego ($p = 0.000$, $p = 0.037$).

Wnioski

1. Topografia podziału nerwu piszczelowego na nerwy podeszwowe przyśrodkowy i boczny wykazuje zmienność anatomiczną.
2. Gałęzie piętowe przyśrodkowe wykazują zmienność w odniesieniu do liczby i miejsca odejścia.
3. Nerwy: piszczelowy, podeszwowy przyśrodkowy oraz podeszwowy boczny wykazują większe pole przekroju i więcej pęczków nerwowych u mężczyzn niż u kobiet.
4. Nerw podeszwowy przyśrodkowy wykazuje większe pole przekroju i więcej pęczków nerwowych niż nerw podeszwowy boczny.

5. Pole przekroju nerwu piszczelowego wzrasta wraz z wiekiem.
6. Pole przekroju oraz liczba pęczków nerwowych większych nerwów obwodowych nie zmienia się w wyniku mrożenia.

STRESZCZENIE W JĘZYKU ANGIELSKIM

Topographic anatomy of the tibial nerve bifurcation and its medial calcaneal branches. Macro and microscopic analysis.

Introduction

The tibial nerve arises as a branch of sciatic nerve bifurcation in the popliteal fossa. It runs distally on the tibialis posterior muscle together with the posterior tibial vessels. Usually at the level of flexor retinaculum it terminally divides into lateral and medial plantar nerve. During distal course the tibial nerve emits medial calcaneal branch(es) which is variable in number and origin. Tibial nerve and its branches provides innervation to the posterior lower leg, foot and sole muscles and the skin of medial foot and sole.

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 paresthesia. Such disorders may be examined by the ultrasound. For many years ankle arthroscopy has proved to be a useful diagnostic and therapeutic procedure for ankle and foot disorders. Although it is a minimally invasive surgery neurological complications are most frequently reported. In order to avoid iatrogenic injuries and to perform safe and reproducible arthroscopy knowledge of topographic anatomy is of vital importance.

Methods

In the first stage of the study the ultrasound examination was conducted on 30 volunteers (n=60 lower limbs). It was performed on the Mylab Gold 25 ultrasound scanner with a 18MHz linear probe by the orthopedic surgeon with more than 20 years of experience in ultrasound examination.

The tibial nerve, its bifurcation point, the medial plantar nerve, the lateral plantar nerve and the medial calcaneal branches origin were marked on the skin. Then two parallel reference lines were drawn: first crossing the tip of the medial malleolus, second crossing the posterior superior tip of the calcaneal tuberosity. Distances from the reference lines to the tibial nerve bifurcation line and to the medial calcaneal branches origin lines were measured with the caliper. Medial calcaneal branches were also analyzed with regards to the number of branches and nerve of origin.

Second part of the study was conducted on 60 lower limbs of the fresh cadavers and on 21 lower legs of the fresh-frozen cadavers. Meticulous dissection revealed the tibial nerve, its bifurcation and lateral and medial plantar nerves which were excised and fixed in a 10% solution of the formaldehyde (pH 7.4). Next each nerve were dehydrated, embedded in paraffin and transverse sectioned on a 2 μm thick slice. Subsequently each slice was stained with haematoxylin and eosin. The cross-sectional area (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 x magnification). Each cross-section was measured semi-automatically using Olympus cellSens Standard 2.3 software whilst the number of nerve bundles was calculated manually.

Results

In the ultrasound examination the tibial nerve bifurcation occurred below the tip of the medial malleolus (77% cases) with the average distance of 5.9 mm ± 19.59 mm. The medial calcaneal branches were identified in the range from one to three ramifications with the most frequent presentation of a single branch (73% cases) originating from the tibial nerve (60% cases) with the mean distance of 3.97 mm below the tip of the medial malleolus.

In the microscopic examination of the nerves collected from the fresh cadavers the mean CSA and the number of nerve bundles were respectively 15.25 ± 4.6 mm^2, 30.35 ± 8.45 for the tibial nerve, 8.76 ± 1.93 mm^2, 20.75 ± 7.04 for the medial plantar nerve and 6.54 ± 2.02 mm^2, 13.40 ± 5.22 for the lateral plantar nerve. In the fresh-frozen cadavers group the mean CSA and the number of nerve bundles were respectively 13.71 ± 5.66 mm^2, 28.57 ± 8.00 for the tibial nerve, 7.55 ± 3.25 mm^2, 18.00 ± 6.72 for the medial plantar nerve and 4.29 ± 1.93 mm^2, 11.33 ± 1.93 for the lateral plantar nerve. 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 significant bigger CSA and number of nerve bundles in males. A positive correlation was found between the donors 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). When comparing nerves collected from the fresh and fresh-frozen only lateral plantar nerves showed statistical differences in the CSA and the number of nerve bundles ($p = 0.000$, $p = 0.037$ respectively).

Conclusions

1. The tibial nerve and its terminal branches presents anatomical variability in the medial ankle area.
2. The origin, location and division pattern of the medial calcaneal branch(es) presents anatomical variability.
3. The CSA and the number of nerve bundles of the tibial, medial plantar and lateral plantar nerves are significantly bigger in males.
4. The medial plantar nerve has larger CSA and more nerve bundles than the lateral plantar nerve.

5. The tibial nerve shows increasing CSA with advanced age.
6. Histological structure of the larger nerves remains uninfluenced by the freezing process.

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OŚWIADCZENIE

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Kraków, 02.08.2020r

OŚWIADCZENIE

Jako współautor pracy: ***"Comparison of the histological structure of the tibial nerve ant its terminal branches in the fresh and fresh-frozen cadavers"***, *Folia Morphologica, Gdańsk, 2020; DOI: 10.5603/FM.a2020.0088* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji wynosi 10% i polegał na:

- wsparciu merytorycznym,
- opracowywaniu pomysłu badań,
- stworzeniu hipotezy badawczej.

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek Łukasza Warchoła jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część w/w. pracy wykazuje indywidualny wkład lek Łukasza Warchoła polegający na:

- opracowywaniu pomysłu badań,
- stworzeniu hipotezy badawczej,
- opracowaniu koncepcji badań,
- pobieraniu materiału,
- barwienie materiału,
- Analiza mikroskopowa oraz pomiary,
- interpretacji wyników,
- przygotowaniu manuskryptu.

Jerzy Walocha

dr. n. med. Ewa Mizia

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Ewa Mizia

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dr. n. med. Henryk Liszka

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Henryk Liszka

dr. n. med. Mariusz Bonczar

Centrum Medyczne Intermed

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Mariusz Bonczar