MORPHOLOGICAL AND MORPHOMETRIC ANALYSIS OF FASCICULAR STRUCTURE OF TIBIAL AND COMMON PERONEAL NERVES

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Abstract. In clinical practice, the common peroneal nerve palsy is more frequent compared to the tibial nerve, although both are part of the sciatic nerve. The aim of our study was to analyze and compare the fascicular structure of the tibial and common peroneal nerve. For the study we used tissue samples of the sciatic nerve, 19 subjects aged 8–86 years. The samples were processed by standard histological procedures, after which were made transverse sections of 5 microns and stained with hematoxylin-eosin staining. First, we analyzed the number, size and arrangement of fascicle. After that we made photographs of each common peroneal and tibial nerve fascicles and to each the area and maximum diameter were determined. In all studied cases, a connective tissue septum that separates the fascicular groups, tibial and common peroneal nerve was detected. The tibial nerve fascicular group was numerous and medially localized while the common peroneal nerve fascicular group was less numerous and placed laterally. Morphometric analysis showed that the common peroneal nerve has statistically significantly lower number of fascicles (16.28 ± 4.39) and the total fascicular area (2.13 × 106 ± 0.91 × 106 μm²) compared to the tibial nerve (average 33 fascicles ± 13.29 and mean fascicular area 5.05 × 106 ± 2.46 × 106 μm²). The average value of the maximum diameter and the average area of the investigated fascicle, tibial and common peroneal nerves showed no statistically significant differences. Correlation analysis of the studied parameters with the age of subjects also did not reach statistical significance. Fascicular structure with a smaller number of larger fascicles and sparse epineurial connective tissue sheath may increase susceptibility of the common peroneal nerve injury.

Key words: Common peroneal nerve, fascicular pattern, morphometry, aging

Introduction

Normal conducting of electric impulses through the nerve provides connective tissue sheaths which prevent interference of electrical impulses. They are also important for the process of regeneration of fibers and provide strength and elasticity nerve. Endoneurium is a connective tissue that separates the individual myelinated fibers and groups of amyelinated nerve fibers. In addition to blood vessels in the endoneurium collagen fibers, fibronectin and laminin are present [1,2]. Perineurium is specific layer built of the concentrically arranged cell lamella (up to 15 in humans). This one connective sheath is considered to be a significant diffusion barrier which preserves constant endoneurial content [1,3]. Endoneurial content surrounded by perineurium makes the fascicle, which is a histological nerve unit. Nerves are composed of different number of fascicles, which are also of different sizes. According to the number and size of the fascicle, nerves can be divided into monofascicular nerve with a single fascicle, oligofascicular nerve with 2–10, and polifascicular nerve with more than 10 fascicles, which can be arranged in groups or diffusely [4]. Fascicles within the nerve stem are interconnected, which contributes to resistance of the nerve stretching. Diameter of majority of fascicles ranged from 0.04 to 2 mm, although there were fascicles up to 4 mm in diameter [1]. Epineurium is the most superficial connective tissue layer that unites all fascicles in a single nerve stem. It is composed of a network of collagen fibers, loose connective tissue with blood vessels and varying amounts of adipose tissue designed to provide a loose matrix for nerve fascicles.

The sciatic nerve is the largest nerve of the human body which consists of two parts, tibial and common peroneal nerve, covered by a common connective nerve sheath. Tibial nerve arises from the anterior branches of L4–L5 and S1–S3 nerves and its motor fibers innervate the posterior compartment of muscles of the thigh (except the short head of the biceps femoris) and leg, and also the muscles of the soles. The common peroneal nerve arises from the anterior branches of L4–L5 and S1–S2 nerves and its motor fibers innervate the short head of the biceps femoris in the thigh, the muscles of anterior and lateral compartment of leg, and also the
Morphological and Morphometric Analysis of Fascicular Structure of Tibial Nerves

Materials and Methods

The study was performed on sciatic nerve tissue samples of 19 individuals (10 males and 9 females) aged from 8 to 86 years. During lifetime they had not been diagnosed with neurologic or metabolic disorder or any other kind of SNs damage. Nerve tissue was obtained during the routine autopsies performed at the Institute for Forensic Medicine in Niš. The time period from death to the autopsy was not longer than 24h. Firstly, the cut of the skin, 5 cm long, was made at the middle part of posterior femoral region. Afterwards, the muscles of posterior femoral region were separated with blunt dissection. Then, 3 cm long SNs part was cut and afterwards fixed with 10% buffered formalin. The tissue was further embedded in the paraffin, cut into the slices 5 µm thick and then, routinely processed for the histochemical staining. Afterwards, slices were stained by hematoxylin-eosin.

Morphological and morphometric analysis of fascicular structure

First, we analyzed fascicular pattern of TN and CPN (number, size, shape and arrangement of fascicle) and the presence of fibrous-fatty Compton-Cruveilhier septum (“Co-Cu” septum). In the second part, with the help of digital cameras on the lowest magnification pictures were taken of all the fascicles of TN and CPN (1280 × 960 RGB format). Morphometric analysis was performed using the program for the analysis and processing of digital images “ImageJ”. Before each measurement system calibration was performed with the help of an objective micrometer (1:100) at the same magnification. In the section “set measurements” area and Feret diameter options are checked. With the help of polygonal selections a line along the outer edge of perineurium of each fascicle is marked and using the option “measure” the values of areas and Feret diameter for each test nerve fascicles were obtained. After completing the analysis of the number, diameter and areas of TN and CPN, their average values were calculated are then statistically analyzed.

Statistical analysis

The collected data were verified by the authors, coded and entered into a special monitoring database. Statistical analysis was performed by SPSS 16.0. The results were presented in tables and graphically with a text comment. We used standard statistical methods for qualitative and quantitative assessment of the results: the absolute numbers, relative numbers (%), the arithmetic mean value (X), standard deviation (SD). Normality of distribution of individual values was investigated using Shapiro-Wilk test. For the evaluation of the significance of the difference (p) between the measured values of the tested two samples used in the t-test for two independent samples, if the distribution of the parameters is normal, if the regularity is not satisfied, the comparison is performed by Mann-Whitney-U test. Correlation analysis between different variables was performed by Pearson simple linear correlation. The statistical significance of the frequency on the absolute difference between the samples was tested by χ² test. A statistical hypothesis was tested at the level of risk of significance α = 0.05, the difference between the samples was considered significant if p < 0.05.

Results

Connective-fatty (Co-Cu) septum was detected in all examined cases. This septum divided the sciatic nerve into the two major morphofunctional fascicular groups (Fig. 1, a–b). Common peroneal nerve fascicular group was less numerous, occupied a lateral part of entire nerve cross-section (Fig. 1, b). Tibial nerve fascicular group was more numerous and occupied a medial section of the nerve (Fig. 1, b). Both fascicular groups were polifascicular types of nerves (Fig. 1). Single fascicle and fascicular groups were detected in both nerve, TN (Fig 2, a–b) and CPN (Fig. 2, c–d). Epi- and interfascicular parts of the epineurium were well developed and with noticeably greater amount of adipose tissue in older cases (Figs. 1, b; 2, d).

Morphometric analysis included 298 CPN fascicles and 644 TN fascicles. The results of morphometric analysis are presented in Table 1. Statistically significant difference (p <0.001) was detected in the average number of fascicles, and the average value of the total fascicular area between TN and CPN. The common peroneal nerve had a significantly lower number of fascicles (16.28 ± 4.39) compared to TN (35.33 ± 13.29) and a lower total fascicular area (2.13 × 106 ± 8.91 × 106 µm²) in relation to the TN (5.05 × 106 ± 2.46 × 106 µm²). Other parameters (such as average value of fascicular area and diameter) were not significantly different between NPC and NT.
Fig. 1. The sciatic nerves cross-section of cases aged 8 (a) and 81 (b) years. In the lateral part of the cross section was observed CPN (common peroneal nerve) fascicular group; in the medial part was observed a fascicular group of TN (tibial nerve); fascicular groups were separated by Co-Cu septum. Staining with hematoxylin-eosin, photographed under magnifying glass.

Table 1. Average value of fascicular number, total fascicular area and average value of fascicular area and diameter of CPN (common peroneal nerve) and TN (tibial nerve)

<table>
<thead>
<tr>
<th></th>
<th>CPN</th>
<th>TN</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fascicular number</td>
<td>16.28±4.39</td>
<td>35.33±13.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total fascicular area (μm²)</td>
<td>2.13x10⁶±</td>
<td>5.05x10⁶±</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fascicular area (μm²)</td>
<td>142138.99±</td>
<td>141629.64±</td>
<td>0.503</td>
</tr>
<tr>
<td>Fascicular diameter (μm)</td>
<td>476.57±139.26</td>
<td>481.19±73.32</td>
<td>0.405</td>
</tr>
</tbody>
</table>

Based on the average values of fascicular diameter and area CPN and TN histogram of distribution was obtained (Figs. 1–2). Fascicles which is the average value of the diameter ranged between 350 and 450 microns where most common in both nerves (Chart 1). Also in both studied nerves, the most numerous fascicles are those whose area ranged from 50000–75000 μm² (Chart 2). Correlation analysis of all parameters with the age of the examined cases showed no statistic significance (Table 2).

Table 2  Correlation analysis between the studied parameters: mean fascicular area and fascicular diameter of CPN (common peroneal nerve) and TN (tibial nerve) with the age of the examined cases

<table>
<thead>
<tr>
<th>Age (r)</th>
<th>Fascicular area of CPN</th>
<th>Fascicular area of TN</th>
<th>Fascicular diameter of CPN</th>
<th>Fascicular diameter of TN</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>Age (r)</td>
<td>p</td>
<td>p</td>
<td>p</td>
</tr>
<tr>
<td>-0.398</td>
<td>0.102</td>
<td>0.094</td>
<td>0.719</td>
<td></td>
</tr>
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</table>

r, simple linear correlation coefficient

Fig. 2. Fascicular structure of the TN with individual macro fascicle inside which can be seen intrafascicular connective septum (2a) and one of the fascicular groups (2b). Fascicular structure of the CPN (common peroneal nerve) with one large fascicle (2c) and fascicular group (2d). Ep, epineurium; En, endoneurium; arrows marked perineurium. Staining with hematoxylin-eosin; magnification × 4.
**Discussion**

Peroneal palsy often happens in clinical practice and leads to a foot drop due to paralysis of the anterior and lateral muscles compartment of the lower leg. In order to explain this problem, researchers are looking into CPN anatomical position, its relationship to neighboring structures, sparse vascularization and fascicular morphology. At the level of the gluteal region CPN makes lateral sciatic nerve division and can be compressed by the piriformis. This often

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**Chart 1.** Histogram of CPN (common peroneal nerve) and TN (tibial nerve) fascicular distribution according to the values of their diameters.

**Chart 2.** Histogram of CPN (common peroneal nerve) and TN (tibial nerve) fascicular distribution according to the values of their area.
happens if CPN passes through suprapiriform foramen or through piriformis. Such anatomic variations can lead to the so-called piriformis syndrome with the appearance of ischialgia [10,11]. In the posterior femoral region TN continues in the direction of sciatic nerve vertically downwards to the popliteal fossa. Opposed to it, in the top corner of the popliteal fossa CPN changes direction and descends laterally to the neck of the fibula. Because of such direction it is believed that CPN tolerates greater tensile strength during the leg movements than NT. Fractures of the fibular neck or long-press on the nerve in this area (eg. long press of military boots) can damage the CPN [9,12]. In addition to anatomical features, it is considered that the blood supply contributes to the greater vulnerability of CPN to injury and damage. Namely, the TN receives several nutritive arteries from perforating, popliteal and posterior tibial arteries in the region of popliteal fossa. These branches form a richly Anastomotic arterial chain inside the NT so it is relatively resistant to ischemic damage. On the other hand, CPN usually supplies a solitary blood vessel, most often a branch of popliteal artery in the region of popliteal fossa [13]. When the arterial nerve chain forms solitary blood vessel, establishing collateral circulation during the compression is more difficult and therefore more easily resulting of ischemic nerve damage [14]. In addition to the clinical and forensic significance, peripheral nerve fascicular pattern has a fundamental, biological significance, since it provides the necessary biomechanical resistance of peripheral nerves, especially limb nerve [4].

Analyzing the fascicular structure, we found that both investigated nerves TN and CPN were of polifascicular type with the group fascicular arrangements. Fascicles were of various sizes, so that they are described as micro-, meso-, and macro fascicles. However, a significant difference was detected in a number of fascicles. Common peroneal nerve had smaller fascicular number (approximately 16) compared to the TN (approximately 35). Also, the total CPN fascicular area was significantly lower compared to TN. There are literature data on the number of whole sciatic nerve fascicles, which ranged from 27 to 70 [13] and from 11 to 93 [1]. Đorđević-Čamba et al. [4] stated that the TN fascicular number ranged from 5–52, while the CPN fascicular number ranged from 1–8, which also confirms a significant difference in the fascicular number between two nerves. There are no literature data for total fascicular area, in particular NPC and NT. The other analyzed parameters, such as average values of fascicular area and fascicular diameter, showed no significant differences between the nerves. Diameter of the majority of fascicles ranged from 350–450 microns of both nerves. Obtained results suggest that CPN fascicular structure is characterized by a smaller number of fascicles, whose diameter and area did not differ from TN fascicles. Baima and Krivickas [9] in their findings suggest that the nerve which has a smaller number of larger fascicles and poor epineurial sheath is more sensitive to pressure and stretching. They also claim that in such fascicular structure, blood vessels are more superficially placed (in epifascicular part of epineurium) and consequently the risk of ischemia is higher (due to the compression of the blood vessels).

Conclusion

In the analysis of fascicular structure of the CPN and TN we have found that CPN has a significantly smaller number of larger diameter fascicles, which may increase its susceptibility to compression, stretching and ischemia.

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References