Microanatomy of the lateral femoral cutaneous nerve in relation to inguinal ligament and its clinical importance

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BACKGROUND: A better knowledge of the composition and properties of connective tissue related to the Lateral Femoral Cutaneous Nerve (LFCN) and to the Inguinal Ligament may be important to understand the diagnosis and treatment applicable to injuries such as meralgia paresthetica.

OBJECTIVE: To determine the relative amounts of the non-fascicular components in the following areas: (i) proximal to the inguinal ligament [LFCN-1], (ii) deep to the inguinal ligament [LFCN-2], or (iii) distal to LFCN-2 [LFCN-3]. These amounts were discriminated as adipose [FAT] and non-adipose (connective) [NON-FAT] tissues.

METHOD: Samples of LFCN-1, LFCN-2 and LFCN-3 from 21 human cadaveric samples were used. Paraffin sections of these structures were processed by Masson’s trichrome stain for connective tissue. The number of fascicles was counted in each of these structures; FAT and NON-FAT areas were determined in the non-fascicular areas of the structures.

RESULTS: There were more fascicles in LFCN-3 vs. LFCN-1 or LFCN-2; there was more NON-FAT vs. FAT in LFCN-2 vs. LFCN-1 and LFCN-3; inversely, there was more FAT vs. NON-FAT in LFCN-3 vs. LFCN-1 and LFCN-2. All of these comparisons were statistically significant.

CONCLUSION: The presence of a higher content of NON-FAT in LFCN-2 and FAT in LFCN-3 may help to explain meralgia paresthetica resulting from compression or focal entrapment of the Lateral Femoral Cutaneous Nerve as it passes deep relative to the inguinal ligament.

KEYWORDS: Lateral femoral cutaneous nerve; Collagen fibers; Adipose tissue; Inguinal ligament.

INTRODUCTION

The Lateral Femoral Cutaneous Nerve (LFCN) is formed by the dorsal branches of the ventral rami of L2 and L3 spinal nerves. After emerging from the lateral border of the psoas major, the nerve reaches the notch, on the medial side of the anterior superior iliac spine. It passes deep to the Inguinal Ligament (IL) and enters the thigh. Compression or damage of the nerve in this osseofibrous or fibromuscular tunnel may cause pain or anesthesia or both on the lateral side of the thigh. In the thigh, the nerve divides into anterior and posterior branches, innervates the skin on the lateral surface of the thigh from the level of the greater trochanter to the mid-thigh and may also supply the gluteal region.

Meralgia paresthetica is a painful mononeuropathy resulting from compression or focal entrapment of the lateral femoral cutaneous nerve as it passes deep to the IL. Meralgia paresthetica is characterized by numbness, burning, tingling, or pricking sensation over the anterolateral side of thigh.

The factors protecting the peripheral nerves against stretching can be attributed to their anatomical structure and biomechanical characteristics. In the general population, an incidence of 43 per 100,000 people has been reported. In people with diabetes mellitus, a five times higher incidence has been reported, at 247 per 100,000 people. There is no gender preference. Meralgia paresthetica is most common during middle
age, but it has been reported in all age groups. Meralgia paresthetica is usually unilateral but may be bilateral in as many as 50% of cases. Various studies have shown that the amount of connective tissue varies in different peripheral nerves, even in different parts of the same nerve. In the available literature, there are only data for the changes in the non-fascicular area of the following nervous structures: (A) human: Sural, Facial, Sciatic and Radial nerves; Superficial Branch of the Radial Nerve, Lateral and Medial Antebrachial Cutaneous Nerves of the forearm; (B) rat: Tibial and Sciatic Nerves; (C) dog: Trochlear Nerve. Knowledge of the crosssectional microanatomy and of the arrangement of connective tissue pattern of the Lateral Femoral Cutaneous Nerve in relation to Inguinal Ligament may be important to understand the diagnosis and treatment following injury.

Ray et al. reported the fascicular area and total cross sectional area of the LFCN as it passes deep to the inguinal ligament in 12 nerves using hematoxylin and eosin stain. There are no studies on the cross sectional microanatomy nor to changes in the non-fascicular components (adipose (FAT) and non-adipose (NON-FAT) tissues) of LFCN in relation to the IL.

The aim of the present study was to find out and compare the cross sectional microanatomy and to determine the amounts of the non-fascicular components (adipose (FAT) and non-adipose (NON-FAT) tissues) of the Lateral Femoral Cutaneous Nerve proximal to IL (LFCN-1), deep to IL (LFCN-2) and distal to LFCN-2 (LFCN-3) which may help to explain meralgia paresthetica resulting from compression or focal entrapment of the LFCN as it passes deep to the inguinal ligament.

## METHODS

### Anatomical dissection and materials

Twenty one samples of LFCN-1 (proximal to IL), LFCN-2 (deep to IL) and LFCN-3 (distal to LFCN-2) were collected from 13 (ten male and three female) formalin embalmed cadavers for histological study shown in Figure 1. These cadavers were not affected by meralgia paresthetica. These LFCN was observed to pass superomedial to the anterior superior iliac spine and deep to the IL. Out of 21 samples, 3 samples were collected from the right and two from the left side of 5 cadavers; the contralateral LFCNs of these cadavers were not used for histologic study because of damaged surroundings. Sixteen samples were collected from both sides of 8 cadavers.

The age of the 13 cadavers at the time of death ranged between 45 and 84 (mean ± std error of mean: 69.6 ± 3.1). These cadavers were donated to the Department of Anatomy, Kasturba Medical College, Manipal, India.

Tissue sampling and processing

Samples of the Lateral Femoral Cutaneous Nerve (LFCN-1, LFCN-2, and LFCN-3) measuring 1 cm each were collected, processed and embedded in paraffin for histological study. Serial 6-micron thick paraffin sections were made at approximately the mid region of each nerve by using a Rotary microtome (Leica RM2125RT, Leica Biosystems Nussloch GmBH, Deutschland). In each nerve sample, every 10th section was selected in such a way that the three consecutive sections were selected and stained using Masson’s trichrome. Morphometric analysis was performed under a light microscope on these stained sections and the obtained mean values were used.

### Histological Masson’s trichrome staining for connective tissue (collagen fibers)

Chemicals used were purchased from Sigma-Aldrich Chemicals Private Limited, Bangalore, India. They included 1% Ponceau-Fuchsin in 1% acetic acid, 1% Phosphomolybdic acid in distilled water and 2% light green in 2% acetic acid.

### Staining protocol

Six micron thick paraffin sections were placed on gelatin coated slides. These nerve sections were hydrated in a series of graded alcohol, brought to distilled water, and stained in a Celestine blue Hemalum sequence followed by decolorization with 1% acid alcohol. Then the sections were washed with distilled water, stained
with Ponceau-Fuchsin solution. Sections were quickly rinsed in distilled water and placed in phosphomolybdic acid. Sections were dried and stained with light green, and treated with 1% acetic acid to remove excess green covering. Sections were dehydrated in a graded series of alcohol, cleared with xylene and mounted with coverslips. These Masson’s trichrome stained sections were observed under the binocular light microscope and photographed with the Motic live image programme (Version 2.0, Motic China Group Co., Ltd.) for morphometric analysis.

Morphometric analysis

Morphometric analyses of the nerve sections were performed under a light microscope with a projection screen at a magnification of 50x. The images were analyzed using the in-house developed software named “Tissue Quant” (TQ, Version 1.0), which is designed for color quantification. This software provides the facility to select a color for selectively measuring the areas in the image containing that particular color.

For evaluating fascicle areas, circles were drawn manually around each of the fascicles in all the images. The circles were segmented out of the image by appropriately adjusting the color settings. The same setting was used for all the images. The area covered by the circle was then calculated by the software. In the same way, total cross section, Masson’s trichrome connective tissue (green and red color) areas were also selectively segmented out of the images by appropriately adjusting the color settings. The areas occupied by these tissues were then obtained in terms of number of pixels.

The captured (imaged) object was compared to a micrometer in the microscope at a magnification of 50x for calibration purpose. The number of pixels representing a length of 1 mm was calculated for both horizontal and vertical arrangements. This provides the calibration for the number of pixels representing one square millimeter of area. The non-fascicular area was obtained by difference between the total cross section area and fascicular area.

The first part of present study included the estimation of the total number of fascicles (total Nf) present in the total LFCN-1, LFCN-2 and LFCN-3 cross sectional areas, the measurement of total LFCN-1, LFCN-2 and LFCN-3 cross sectional area (Asc), individual and total fascicular area (Af) and non-fascicular area (Anonf).

The second part of the study included the measurement of the non-adipose area (NON-FAT) in the non-fascicular areas of LFCN-1, LFCN-2 and LFCN-3 (green color) by Masson’s trichrome stain. The adipose tissue areas (FAT) in the LFCN-1, LFCN-2 and LFCN-3 non-fascicular areas were calculated by taking the difference between non-fascicular area and non-adipose area. (FAT = non-fascicular area - NON-FAT).

Statistical analysis

The data were analyzed using “SPSS” (Version 11.5, The Predictive Analytics Company) Statistical packages. Each set of data was analyzed for range, mean and standard error of mean (SEM).

For comparison of non-fascicular area, adipose tissue and non-adipose tissue at the three sites, One-Way Analysis of Variance (ANOVA) followed by Tukey’s “post hoc test” was used.

RESULTS

The study of the LFCN-1, LFCN-2 and LFCN-3 cross sections showed differences in number, size, shape, and distribution of the fascicles. The cross sections of the LFCN-2 were flattened due to their deep course to the IL. The cross sections of LFCN-1 and LFCN-3 were oval. In all cases, the fascicular patterns were of the polyfascicular type. The sites at which the 1 cm segments of nerve were collected had no branches. We found no statistically significant differences between right and left side nerve parameters. So, we treated them as independent samples.

In the cross sections of these nerves, non-fascicular and fascicular areas were identified for morphometric analysis. Cross sections of LFCN-1, LFCN-2 and LFCN-3 are shown in Figures 2, 3, 4, respectively. Measurements of cross sectional areas, nerve fascicles, fascicular and non-fascicular areas, NON-FAT and FAT areas for LFCN-1, LFCN-2 and LFCN-3 were obtained during morphometric analysis and range, mean, and SEM were calculated. Results are displayed in Table 1.

The LFCN-3 (range and mean; 5-11, 8.5) has a higher number of nerve fascicles as well as larger fascicular and cross sectional areas, when compared to LFCN-1 (range and mean; 3-8, 5.2), or to LFCN-2 (range and mean; 3-9, 6.3). This was due to division of the fascicle with inclusion of additional amounts of connective or adipose tissue for the newly divided fascicle. No morphometric differences were found between LFCN-1 and LFCN-2 number of fascicles (Nf), fascicular area (Af) and cross sectional area (Asc).

In the cross sections of LFCN-1, LFCN-2 and LFCN-3, the non fascicular areas were larger when compared to the fascicular area. The mean non fascicular area in LFCN-3 was larger when compared to LFCN-1 and LFCN-2.

There was no overall significant difference between non-adipose vs. adipose tissue in the non-fascicular areas as shown in Figure 2. This similarity also applied to LFCN-1. However, it did not hold for LFCN-2 and LFCN-3. In LFCN-2 sections the non-fascicular areas consisted mainly of non-adipose tissue as seen in Figure 3. Concentrically arranged thick peri- and epineurium were observed around the LFCN-2 fascicles. This may have developed in order to protect the LFCN fibers while passing deep to the IL. In contrast, LFCN-3 sections showed larger amounts of adipose and less non-adipose tissue in the non-fascicular area as can be seen in Figure 4.
Table 1 - Descriptive statistics of 21 evaluated cases LFCN-1, LFCN-2 and LFCN-3 mean morphometric parameters

<table>
<thead>
<tr>
<th>Name</th>
<th>Range</th>
<th>Nf Mean (SEM)</th>
<th>Asc Range</th>
<th>Af Mean (SEM)</th>
<th>Anonf Range</th>
<th>NON-FAT Mean (SEM)</th>
<th>FAT Mean (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFCN-1</td>
<td>3 to 8</td>
<td>5.2</td>
<td>1.508 - 2.705</td>
<td>2.085</td>
<td>0.617 - 1.009</td>
<td>0.841</td>
<td>0.680 - 1.719</td>
</tr>
<tr>
<td>LFCN-2</td>
<td>3 to 9</td>
<td>6.3</td>
<td>1.507 - 2.694</td>
<td>2.054</td>
<td>0.588 - 0.983</td>
<td>0.811</td>
<td>0.725 - 1.735</td>
</tr>
<tr>
<td>LFCN-3</td>
<td>5 to 11</td>
<td>8.5</td>
<td>1.634 - 2.847</td>
<td>2.238</td>
<td>0.628 - 1.099</td>
<td>0.892</td>
<td>0.721 - 1.848</td>
</tr>
</tbody>
</table>

LFCN-1 - lateral femoral cutaneous nerve of thigh above inguinal ligament; LFCN-2 - lateral femoral cutaneous nerve of thigh at inguinal ligament; LFCN-3 - lateral femoral cutaneous nerve of thigh below inguinal ligament; Nf - number of fascicles; Asc - cross sectional area; Af - total fascicular area; Anonf – non-fascicular area, NON-FAT – non-adipose (collagen) tissue area; FAT - adipose tissue area.

Figure 2 - Photomicrograph of the LFCN-1 above the inguinal ligament stained with Masson’s trichrome shows fascicular and non-fascicular tissue pattern. (A) LFCN-1 shows equal amount of adipose tissue (fat) and non-adipose tissue (collagen fibers) in the epifascicular and interfascicular connective tissue region (green area shows the more collagen fibers) (50 x); (B) The results of the automated measurement of the total cross section area (Asc) shown as white, individual fascicular area (Af) shown as green and the non-fascicular area (Anonf) (collagen fibers) shown blue of a single LFCN-1 were calculated by the image analysis software in (50 x). In (50 x) magnification, 1 mm² fascicular or non-fascicular area = approx. 490 x 490 pixels. (C), (D) shows the area of figure (A) in (100 x) and (200 x) respectively. Figures 2(A)–(D) confirms clearly equal amount of adipose and non-adipose tissue in non-fascicular region. Arrows indicate the adipose tissue (fat) in non-fascicular area. Scale bar = 100 mm valid for all the images.

Figure 3 - Photomicrograph of the LFCN-2 at the inguinal ligament stained with Masson’s trichrome shows fascicular and non-fascicular tissue pattern. (A) LFCN-2 shows more amount of non-adipose tissue (collagen fibers) in the epifascicular and interfascicular connective tissue region (green area shows the collagen fibers) (50 x); (B) The results of the automated measurement of the total cross section area (Asc) shown as white, individual fascicular area (Af) shown as green and the non-fascicular area (Anonf) (collagen fibers) shown blue of a single LFCN-1 were calculated by the image analysis software in (50 x). In (50 x) magnification, 1 mm² fascicular or non-fascicular area = approx. 490 x 490 pixels. (C), (D) shows the area of figure (A) in (100 x) and (200 x) respectively. Figures 3(A)–(D) confirms clearly more amount of non-adipose tissue in non-fascicular region. Arrows indicate the non-adipose tissue (collagen fibers) in non-fascicular area. Scale bar = 100 mm valid for all the images.

Therefore, the mean adipose tissue area in LFCN-3 was greater vs. LFCN-1 and LFCN-2; conversely, the mean non adipose tissue area in LFCN-2 was greater vs. LFCN-1 and LFCN-3. These data and their statistical analysis are displayed in Table 2.

Discussion

This study was undertaken to determine differences in the non-fascicular components (adipose and non-adipose connective tissue) of the Lateral Femoral Cutaneous Nerve in areas (i) proximal to inguinal ligament (LFCN-1), (ii) deep to the inguinal ligament (LFCN-2) and distal to LFCN-2 (LFCN-3).

Neurosurgeons and orthopedicians, who usually perform microsurgical interventions on peripheral nerves, are familiar with the nerve anatomy and its significance in the diagnosis and management of nerve lesions.23

Meralgia paresthetica is a painful mononeuropathy resulting from compression or focal entrapment of the LFCN as it passes deep to the IL.
The factors protecting the peripheral nerves against stretching can be attributed to their anatomical structure and biomechanical characteristics. In the present study, LFCN-2 has a higher NON-FAT content, which may protect the LFCN as it passes deep to IL. The structural organization of peripheral nerves enables them to function while resisting and adapting to stresses placed on them by varying postures and movements. The nerve fascicles are loosely positioned within the connective tissue covering. The fascicles are exposed to combinations of tensile, shear, and compressive stresses that may result in nerve excursion, strain, and transverse contraction. In the present study, there may have been compressive stresses on the LFCN-2 at IL which flattened the total nerve cross section and compressed the fascicles in contrast to what was found in LFCN-1 and LFCN-3.

The LFCN-3 (range and mean; 5-11, 8.5) was observed to have a higher number of nerve fascicles as well as larger fascicular and cross sectional areas, when compared to LFCN-1 (range and mean; 3-8, 5.2) or LFCN-2 (range and mean; 3-9, 6.3) due to the branching of the fascicle with inclusion of additional amount of connective tissue in each newly formed branch. The number of reported fascicles in LFCN-2 ranges from 3 to 6 (mean, 4.5). The number of LFCN-2 (mean, 6.3) fascicles in the present study is higher when compared to previous work. As far as LFCN-1 & LFCN-3 are concerned, there is no available data in the literature. McCormick et al. compared the non-fascicular area of the antebraclial cutaneous nerve of the forearm with the sural nerve by using Masson's trichrome method and concluded that the sural nerve has more adipose tissue than the antebraclial forearm nerve. Various studies have shown that the amount of connective tissue varies in different peripheral nerves, even in different parts of the same nerve. In the present study also, the amount of connective tissue varied in different parts (LFCN-1, LFCN-2, and LFCN-3) of LFCN in relation to IL.

Ray et al. used the Hematoxylin-Eosin stain in 12 nerves and identified the larger non-fascicular relative to the fascicular areas and stated that this is due to the increased amount of collagen fibers covering the fascicles at the inguinal ligament. The above study did not include the identification of adipose vs. non-adipose tissue area of LFCN in relation to IL. The present investigation used Masson's trichrome method as described in by McCormick et al.

The chances of injury to nerve are greater near the anterior superior iliac spine structure. Excessive traction or direct compression of the nerve is thought to be the main causative factor for blood flow reduction. Mackinnon et al. observed an increase in the thickness of the epineurium and perineurium confined to the region of nerve compression. Previous studies indicated that the increased amount of collagen fibers is a consequence of compression rather than a protective mechanism. Nerves with more connective tissue and fewer fascicles may be better protected and undergo slower neural changes than nerves with less connective tissue. Morphometric studies on peripheral nerves have documented the presence of an age related increase in the amount of perineural connective

**Table 2** - LFCN-1, LFCN-2 and LFCN-3 morphometric parameters comparison by using one way analysis of variance (ANOVA) - Tukey Test

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
<th>NF Mean diff. (A-B)</th>
<th>Sig.</th>
<th>Anonf Mean diff. (A-B)</th>
<th>p</th>
<th>NON-FAT Mean diff. (A-B)</th>
<th>p</th>
<th>FAT Mean diff. (A-B)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFCN-1</td>
<td>LFCN-2</td>
<td>1.05</td>
<td>0.67</td>
<td>0.001</td>
<td>&gt;0.99</td>
<td>0.509 (*)</td>
<td>&lt;0.001</td>
<td>0.511 (*)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LFCN-1</td>
<td>LFCN-3</td>
<td>3.19 (*)</td>
<td>&lt;0.001</td>
<td>0.102</td>
<td>0.451</td>
<td>0.464 (*)</td>
<td>&lt;0.001</td>
<td>0.566 (*)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LFCN-2</td>
<td>LFCN-3</td>
<td>2.14 (*)</td>
<td>&lt;0.001</td>
<td>0.103</td>
<td>0.441</td>
<td>0.973 (*)</td>
<td>&lt;0.001</td>
<td>1.076 (*)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

LFCN-1 - lateral femoral cutaneous nerve of thigh above inguinal ligament; LFCN-2 - lateral femoral cutaneous nerve of thigh at inguinal ligament; LFCN-3 - lateral femoral cutaneous nerve of thigh below inguinal ligament; NF - number of fascicles; Anonf – non-fascicular area, NON-FAT - non-adipose (collagen) tissue area; FAT - adipose tissue area.
tissue. Consequently, the presence of increased amounts of collagen fibers in the LFCN at the level of IL could also be related to aging.\(^{12,13,16,20}\) Our morphometric study on the adipose and non-adipose tissue was limited to older individuals because of unavailability of young cadaver specimens. The cadaveric specimens which had a history of meralgia paresthetica during their life were excluded from our study.

The results of the present study showed that the non-fascicular area of LFCN-2 sections was mainly occupied by non-adipose tissue (collagen fibers) when compared with LFCN-1 and LFCN-3. Concentrically arranged thick peri and epineurium was observed around the LFCN-2 fascicles. This may have evolved for the protection of the LFCN nerve fibers while passing deep under the inguinal ligament. As LFCN-2 with increased amount of collagen fibers pass deep under the IL, the risk of compression or focal entrapment may be higher when compared to LFCN-1 and LFCN-3. Therefore, our findings were consistent with those of Mackinnon et al.\(^{26,27}\)

It has been shown that in conditions such as entrapment, hereditary neuropathies, acquired neuropathies, trauma, partial or full nerve transactions, and nerve tumors, there is an increase in cross sectional, fascicular, and/or non-fascicular areas. Carai et al.\(^{30}\) suggested surgical decompression of the nerve as an option when the conservative treatments fail.\(^{30}\) Knowledge regarding the anatomy of the Lateral Femoral Cutaneous Nerve is essential for decompression and neurolysis of the nerve in the treatment of meralgia paresthetica.\(^{31}\) Variable courses and relationships of the LFCN to surface landmarks became significant in pulse radiofrequency neuro-modulation or ultrasound guided LFCN blockade.\(^{32,33}\) There are no available values for cross sectional, fascicular, and/or non-fascicular areas even though they have been studied using various methods. This study includes only samples collected from normal individuals, but fails to compare with samples affected by meralgia paresthetica. This study is an attempt to build a normal database for the Lateral Femoral Cutaneous Nerve in relation to IL (LFCN-1, LFCN-2, and LFCN-3). Such data might be relevant for clinical use. Any deviation from this reference value captured on image analysis may help the clinician to diagnose problems related to LFCN.

### Conclusion

More non adipose tissue as compared to adipose tissue was found in LFCN-2. The presence of higher NON-FAT in LFCN-2 and FAT in LFCN-3 might help to understand meralgia paresthetica resulting from compression or focal entrapment of the Lateral Femoral Cutaneous nerve as it passes deep under the inguinal ligament.

### References


