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ORIGINAL ARTICLE

Morphology

Organization of the Fibrous Component of Connective Tissue Paraneural Structures in Different Periods of Ontogenesis

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Abstract

Background: The steady increase in road traffic accidents, seasonal injuries leading to damage to the peripheral nerves of the extremities, as well as the military conflicts that are becoming more frequent now actualize research aimed at improving the diagnosis, treatment, and prevention of post-traumatic changes in the nerve trunks of the extremities. The frequency of peripheral nerve injuries varies from 1.5% to 13% of all injuries in peacetime; and during hostilities, it reaches 20%, and disability is 60%. At the same time, specific destructive changes in the nerves of the extremities after their traumatic injury require extraordinary organizational, therapeutic, and functional approaches to their restoration. The purpose of this study was to evaluate the dynamics of changes in the fibrous component of the paraneurium connective tissue structures in different periods of ontogenesis.

Methods and Results: The study consisted of two stages. At the first stage, to study changes in the paraneurium connective tissue of the sciatic nerve in vivo, ultrasound was performed using an RS85 ultrasound scanner (Samsung Medison, South Korea, 2021) and two linear transducers, LA4-18B and LA2-9A. The thickness of the sciatic nerve and the surrounding paraneurium were measured, and their structural organization was evaluated. The inclusion criterion was the absence of pathology from the peripheral nervous system. All subjects were divided into four age groups (15 people in each group): Group 1 (0-11 years), Group 2 (12-25 years), Group 3 (26-40 years), and Group 4 (41-60 years).

The second stage was performed on cadaveric material of the paraneurium connective tissue of the sciatic nerve of persons of both sexes, of different ages, whose cause of death was not related to diseases or injuries of the nervous system. To determine the qualitative and quantitative ratio of collagen fibers of different degrees of maturity in the connective tissue structures of the paraneurium tissue of the sciatic nerve, polarization microscopy (MicMed-6, Lomo, Russia) was applied, and the basic principle of double refraction, which in combination with Sirius red staining, made it possible to differentiate types I and III collagen. The amount of each collagen type was determined by analyzing the color gamut after Sirius red staining in polarizing light. Fibers containing type I collagen had a red glow, while those containing type III collagen had a green glow. The ratio of collagen types was calculated using the Fiji program (USA, 2022).

US examination revealed the presence of a non-pronounced bilateral asymmetry in the structural organization of the paraneurium and a trend toward an increase in the thickness of the sciatic nerve with age, from 0-11 years to the age group of 41-60 years. Polarization microscopy of micro-preparations of the human sciatic nerve with paraneurium structures made it possible to analyze the density of the fibers of the paraneurium connective tissue and identify types I and III of collagen. At the age of 11, the amount of type I collagen fibers was maximum (69.74±0.41%), and type III collagen fibers amounted to 30.26±0.44%. Further, with increasing age, the amount of collagen III significantly diminished. The ratio of type I to type III collagen increased with patient age, with the highest ratio in the age group 41-60 years.

Conclusion: The revealed dynamics of changes are quite comparable with the age-related features of connective tissue since it is this tissue that makes up the morphological substrate of the paraneurium of the sciatic nerve.(**International Journal of Biomedicine. 2023;13(2):301-304.**)

Keywords: paraneurium coating • connective tissue • collagen fiber • paraneurium

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Introduction

It is necessary to consider the indisputable fact that the functional activity of the conductor component of the peripheral nerve is inextricably linked with its stromal component, which includes endo-, peri-, epineurium, and paraneural connective tissue structures, or "paraneurium."[1-3] The functional role of the paraneurium lies in its indissoluble morphological connection with the trunk of the peripheral nerve. One of the conditions determining the severity of damage to peripheral nerves and the subsequent rate of their morpho-functional recovery is the degree of traumatization of the surrounding soft tissues, including paraneurium connective tissue structures.[4-6] Currently, the legitimacy of using the term peripheral nerve paraneurium is being actively discussed in scientific circles. In 1995, Millesi et al. studied the mechanical properties of the peripheral nerve and convincingly demonstrated the role of paraneurium in the dynamics of changes in the length and diameter of the nerve trunk under conditions of limb flexion and extension.[7] In 2007, Millesi with colleagues explained the participation of paraneural tissue in the passive motility of the trunks of the peripheral nerves in conditions of active movement, for example, in a uniaxial joint.[8] In 2013, several researchers explained the need to use the term paraneurium in connection with its deepest role in introducing an anesthetic into the connective tissue membranes surrounding the nerve trunk.[9-11] At the same time, there were no data on the structural organization of paraneurium in different periods of ontogenesis in the available literary sources of domestic and foreign authors.

Materials and Methods

The study consisted of two stages. At the first stage, to study changes in the paraneurium connective tissue of the sciatic nerve in vivo, ultrasound was performed using an RS85 ultrasound scanner (Samsung Medison, South Korea, 2021) and two linear transducers, LA4-18B (a frequency range of 4.0 MHz - 18.0 MHz) and LA2-9A (a frequency range of 2.0 MHz - 9.0 MHz); the use of two transducers is due to the different mass of patients (a total of 60 patients were examined). The thickness of the sciatic nerve and the surrounding paraneurium were measured, and their structural organization was evaluated. The ultrasound images were obtained by experienced operators. The cross-sectional areas of the sciatic nerve were measured at the lower edge of the gluteus maximus in the posterior midline of the thigh (the gluteal sulcus). The cross-sectional area was measured by tracing the nerve just inside its hyperechoic rim, and three measurements were obtained with the probe repositioned.

The inclusion criterion was the absence of pathology from the peripheral nervous system. Informed consent was obtained from patients before collecting the data. All subjects were divided into four age groups (15 people in each group): Group 1 (0-11 years), Group 2 (12-25 years), Group 3 (26-40 years), and Group 4 (41-60 years).

The second stage was performed on cadaveric material of the paraneurium connective tissue of the sciatic nerve of persons of both sexes, of different ages, whose cause of death was not related to diseases or injuries of the nervous system (REC approval No. 7 of 10/14/19). Dissection of the sciatic nerve and the surrounding paraneurium connective tissue was carried out in layers throughout – from the point 5cm above the gluteal fold and up to the upper corner of the popliteal fossa. The resulting biomaterial, a total of 94 complexes, consisting of a segment of the sciatic nerve with surrounding connective tissue structures and skeletal muscles, was placed in a 10% solution of neutral formalin, enclosed in paraffin according to the standard procedure and histological sections were made, which were stained with hematoxylin and eosin for overview light microscopy. To determine the qualitative and quantitative ratio of collagen fibers of different degrees of maturity in the connective tissue structures of the paraneurium of the sciatic nerve, polarization microscopy (MicMed-6, Lomo, Russia) was applied, and the basic principle of double refraction, which in combination with Sirius red coloration, made it possible to differentiate types I and III collagen. The amount of each collagen type was determined by analyzing the color gamut after Sirius red coloring in polarizing light. Fibers containing type I collagen had a red glow, while those containing type III collagen had a green glow. The ratio of collagen types was calculated using the Fiji program (USA, 2022).

Statistical analysis was performed using the Statistica 10.0 software package (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. The mean (M) and standard error of the mean (SEM) were calculated. Mann-Whitney U test and Kruskal-Wallis test were used, respectively, to compare means of 2 and 3 or more groups of variables not normally distributed. A probability value of $P \leq 0.05$ was considered statistically significant.

Results

Considering the relevance of ultrasound (US) as one of the additional methods of diagnosing peripheral nerve lesions, this method was used in this work. US examination revealed the presence of a non-pronounced bilateral bilateral asymmetry in the structural organization of the paraneurium and a trend toward an increase in the thickness of the sciatic nerve with age, from 0-11 years to the age group of 41-60 years (Table 1).

When working with cadaveric material, it was revealed that the nerve in the upper third of the thigh was surrounded by its own fascial sheath (OFS), the thickness of which varied from 12 to 30 microns and was directly proportional to age. In cross-sections, the OFS was visualized at different distances from the nerve trunk and separated by a paraneurium fascial-cellular space (PFCP), the thickness of which varies from 1 to 1.5 mm and has no pronounced dependence on age. At the same time, it should be noted that the shape of such a paraneural space from the lateral and medial sides had the form of a crescent (up to 40-45 years of age) or the shape of a triangle, at the age of 50-60 years (in some cases, a polygonal shape was observed). The paraneurium fiber was divided by connective cords extending from the common fascial sheath toward the epineurium and merging with its connective tissue fibers. Thin connective tissue, vaginal fascial sheets, branch outwards, toward the skeletal muscles from the OFS, at different angles, which then merge with the fascial cases of the surrounding muscles. The space between the fascial leaves is filled with white adipose tissue forming lobules of adipocytes. The shape, number, and size of such lobules have age-specific features. The identified morphological features are shown in Figure 1.

Polarization microscopy of micro-preparations of the human SN with paraneurium structures made it possible to analyze the density of the fibers of the paraneurium connective tissue and identify types I and III of collagen. Collagen fibers in the paraneurium had different diameters, densities, and compactness of location, and the vector of their orientation had not only topographical, but also age dependence. Collagen fibers formed a fibrous-parallel (a common fascial case with outgoing slings) and fibrous-mesh architectonics (areas of connective tissue located in the paraneurium space). Mature connective tissue fibers containing type I collagen had a red glow when passing polarization light, and immature (type III collagen) had a green glow. At the age of 11, the amount of type I collagen fibers was maximum (69.74±0.41%), and type III collagen fibers amounted to 30.26±0.44%. Further, with increasing age, the amount of collagen III significantly diminished. The ratio of type I to type III collagen increased with patient age, with the highest ratio in the age group 41-60 years (Figure 2).

Discussion

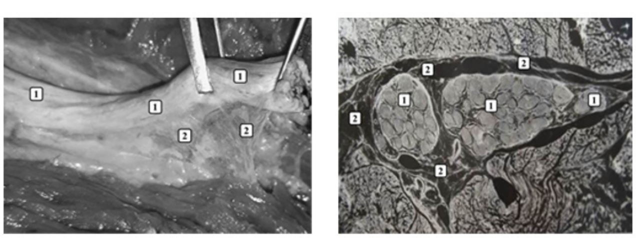
Thus, our data are not only consistent with the conclusions of foreign colleagues but also complement the knowledge about the role of the paraneurium, of the sciatic nerve, in the practice of regional anesthesia. The study revealed qualitative and quantitative transformations in the structural organization of the paraneurium connective tissue. US revealed the presence of slight bilateral asymmetry and, with age, a thickening of the sciatic nerve and a thickening of the common fascial sheath, as well as an increase in its cross-sectional area.

The analysis of changes in the paraneurium structures of the sciatic nerve revealed a gradual complication in its structural organization, which depended on age. At the same time, the observed organization of paraneurium structures was inextricably linked with the complication of the structure of not only the conduction apparatus of the sciatic nerve but also the entire stromal apparatus. The so-called “morphological improvement” was manifested both in quantitative transformation – the appearance of new structures in the paraneurium; and in qualitative transformation – a change in the ratio of types I and III collagen toward a significant decrease in the latter to its complete absence by the age of 60. The revealed dynamics of changes are quite comparable with the age-related features of connective tissue since it is this tissue that makes up the morphological substrate of the paraneurium of the sciatic nerve. In our opinion, the data

Table 1.

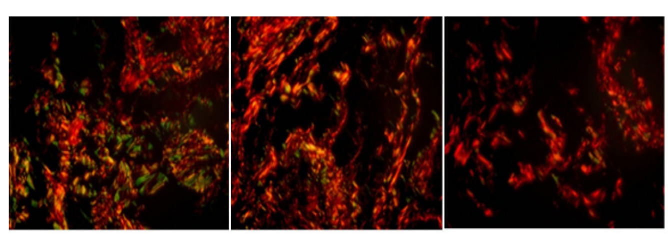
Changes in the thickness of the sciatic nerve and the surrounding paraneurium in different age periods according to US data

Age group	Right limb			Left limb			P_{1-4}	P_{2-5}	P_{3-6}
	Thickness of the sciatic nerve (mm)		Paraneurium thickness (mm) (3)	Thickness of the sciatic nerve (mm)		Paraneurium thickness (mm) (6)			
	D (p-s) (1)	D (m-d) (2)		D (p-s) (4)	D (m-d) (5)				
0-11, years	2.38±0.1	5.7±0.21	0.46±0.61	2.22±0.21	5.32±0.22	0.54±0.14	0.01	0.005	0.005
12-25, years	2.61±0.3	7.01±0.12	0.62±0.11	2.54±0.13	7.84±0.11	0.62±0.61	0.01	0.001	0.10
26-40, years	2.42±0.22	6.94±0.11	0.68±0.13	2.41±0.11	7.25±0.11	0.65±0.63	0.10	0.01	0.10
41-75, years	2.53±0.20	7.12±0.22	0.74±0.12	2.32±0.33	7.22±0.21	0.74±0.11	0.005	0.01	0.12
P-value	0.1145	0.1215	0.1174	0.1182	0.1205	0.1194			



A B

Fig. 1. Macro (A) and microphotography (B) of the sciatic nerve (1) and the surrounding paraneural connective tissue (2) (“paraneurium”).



A B C

Fig. 2. Micrography of paraneurium at the age of 0-11 years (A), 12-25 years (B), and 41-60 years (C). Polarization microscopy data. Sirius red staining. Magnification x400.

obtained can be used in practical healthcare, particularly in such areas of medicine as neurosurgery, traumatology, oncology, and neurology.

Competing Interests

The authors declare that they have no competing interests.

References

1. Klinge U, Zheng H, Si Z, Schumpelick V, Bhardwaj RS, Muys L, Klosterhalfen B. Expression of the extracellular matrix proteins collagen I, collagen III and fibronectin and matrix metalloproteinase-1 and -13 in the skin of patients with inguinal hernia. Eur Surg Res. 1999;31(6):480-90. doi: 10.1159/000008728.
2. Borodina KM, Zatolokina MA, Kharchenko VV, Tenkov AA, Zatolokina ES. [Features of the structural organization of the paraneural connective tissue sheath of the sciatic nerve of rats in different periods of postnatal development]. Bulletin of the Volgograd State Medical University. 2020;4(76):152-155. [Article in Russian].
3. Ghazanfari S, Khademhosseini A, Smit TH. Mechanisms of lamellar collagen formation in connective tissues. Biomaterials. 2016 Aug;97:74-84. doi: 10.1016/j.biomaterials.2016.04.028.

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4. Wu K, Li G. Investigation of the Lag Phase of Collagen Fibrillogenesis Using Fluorescence Anisotropy. Appl Spectrosc. 2015 Oct;69(10):1121-8. doi: 10.1366/14-07780.
5. Polivanova TV, Manchuk VT. Morphofunctional parameters of collagen in norm and pathology. Successes of Modern Natural Science. 2007;2:225-30.
6. Borodina KM, Zatolokina MA, Sozykin A.A, Tsymbalyuk VV, Shmatko IA, Zatolokina ES, Banchenko DA. [On the question of the features of the topography and morphometry of the sciatic nerve of a rat]. Bulletin of the Volgograd State Medical University. 2021; 3 (79):159-163. [Article in Russian].
7. Millesi H, Zöch G, Reihsner R. Mechanical properties of peripheral nerves. Clin Orthop Relat Res. 1995 May;(314):76-83. PMID: 7634654.
8. Millesi H, Hausner T, Schmidhammer R, Trattnig S, Tschabitscher M. Anatomical structures to provide passive motility of peripheral nerve trunks and fascicles. Acta Neurochir Suppl. 2007;100:133-5. doi: 10.1007/978-3-211-72958-8_28.
9. Sala-Blanch X, Reina MA, Ribalta T, Prats-Galino A. Sciatic nerve structure and nomenclature: epineurium to paraneurium: is this a new paradigm? Reg Anesth Pain Med. 2013 Sep-Oct;38(5):463-5. doi: 10.1097/AAP.0b013e3182a1b6c5.
10. Ip V, Tsui B. Injection through the paraneural sheath rather than circumferential spread facilitates safe, effective sciatic nerve block. Reg Anesth Pain Med. 2013 Jul-Aug;38(4):373. doi: 10.1097/AAP.0b013e318298671b.
11. Reina MA, De Andrés JA, Hadzic A, Prats-Galino A, Sala-Blanch X, van Zundert AAJ (editors). Atlas of Functional Anatomy for Regional Anesthesia and Pain Medicine: Human Structure, Ultrastructure and 3D Reconstruction Images. Springer, 2015.