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Rare nemaline myopathy (a case report)

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Abstract

Introduction Nemaline myopathies (NM) are a group of neuromuscular diseases, the distinctive histological feature of which are nemaline rods in myosymplasts. The purpose of this work is to describe the morphology of a rare form of primary nemaline myopathy that progresses in adulthood. **Material and methods** The surgical material of the paravertebral muscles of a 51-year-old patient with scoliotic deformity at the level of L4-S1, who was repeatedly operated on to correct spinal deformity due to neurological disorders, was studied. Paraffin sections were stained with hematoxylin-eosin, according to Masson, using the Ptah method, studied using a 3DHISTECH Pannoramic MIDI II BF scanning microscope to digitize preparations using Whole slide imaging technology in two modes: Single layer mode and Extended focus (3DHISTECH, Hungary). **Results** In the fragments of the altered muscle tissue, filamentous structures of nemaline bodies in myosymplasts were identified, which were located diffusely-dotted throughout the sarcoplasm or formed clusters of various configurations. There was an increased variability in the diameters of muscle fibers, internal nuclei, myosymplasts altered by contraction and with signs of myophagy, patterns of gradual replacement of muscle fibers by adipocytes, massive fatty degeneration of fibers, and fibrosis of the interstitial space. Intramuscular nerve trunks showed signs of complete involution; fibrous perineurium was preserved, and there were single nerve fibers; neuromuscular spindles were distinguished by an enlarged connective tissue capsule. The vessels of the arterial flow had signs of fibrosis and obliteration of the lumen; the vessels of the venous bed were tortuous. **Discussion** Due to a large number of genes responsible for NM, genetic search can be difficult and is effective only in 50 % of cases. It has been established that nemaline bodies can be distributed diffusely or form clusters of irregular shape, more often subsarcolemmal and characteristic of small fibers. In the presented clinical case, nemaline bodies were observed over the entire area of the fibers and were characteristic of myosymplasts of various sizes. **Conclusion** The histopathological study of the paravertebral muscles established the neuromuscular nature of the disease, being nemaline myopathy that progressed in adulthood and had not been diagnosed at previous stages of treatment.

Keywords: nemaline myopathy, paravertebral muscles, nemaline formations, fibrosis, fatty degeneration, involution of nerve conductors, vascular obliteration

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INTRODUCTION

Nemaline myopathy (NM) is a group of progressive neuromuscular diseases that are characterized by the presence of abnormal twisted or rod-shaped bodies in muscle fibers that are detected by histopathological study (*nema* (gr.) means thread). The disease belongs to the most common myopathies with the incidence of one per 50,000 newborns, equally men and women, usually inherited, but in single cases the disease is not observed in other family members [1, 2]. Its clinical manifestations vary significantly both in age of manifestation and severity of the course; in 90 % of cases, myopathy manifests itself at birth or in early childhood, and is very rarely found in adults [3]. The distinguished NM features are hypotonia, skeletal muscle weakness, spinal deformities, and problems by feeding [4].

There are six clinical groups of NMs in regard to the age of onset and severity. The relationship between the severity of the disease and a specific gene in which the mutation occurred has not been established; regular monitoring by a cardiologist is necessary [4]: 1) severe congenital form: severe muscle hypotonia, impaired respiratory function; 2) nemaline Amish myopathy at birth: muscle hypotonia, joint contractures, respiratory disorders; 3) intermediate congenital form: moderate severity, delayed motor development, loss of the ability to walk independently; 4) typical (mild) congenital form: hypotonia, lethargy in the muscles located closer to the body; 5) myopathy, manifested at 8 to 15 years of age: normal early motor development, symmetrical weakness of the ankle muscles; 6) myopathy, which manifests itself in adults aged 20-50 years: rapidly progressing general muscle weakness, muscle pain, spinal deformities. The mechanisms leading to the formation of nemaline myopathies are the subject of ongoing research [1, 3, 5, 6].

The clinical case we present here is interesting not only from the point of view of the features of the clinical picture of the disease (blurring of neurological manifestations), but also the features of the histostructure of muscle tissue detected by the pathohistological study. As part of the clinical work, a brief morphological description and discussion of this problem is presented [7].

The **purpose** of this work was to describe the morphology of a rare form of nemaline myopathy that progresses in adulthood.

MATERIAL AND METHODS

The surgical material from the paraspinal muscles of a 51-year-old patient with scoliotic deformity of the spine at the level of L4-S1 was studied. Upon admission, neurological symptoms were painful spasms of the muscles of the face, neck, hypotonia and hypotrophy of the muscles of the thighs, shins, feet, restriction of movements in the limbs. The progressive neurological deficit did not fit into the clinical manifestations of secondary myopathy. Upon collecting an anamnesis, it was decided to conduct a histopathological study of the spinal muscles in order to clarify the etiology of the disease.

The muscle tissue material was fixed in 10 % neutral formalin solution, embedded in paraffin after histological processing; the sections were stained with hematoxylin and eosin, Masson's trichrome method, PTAH method. It was studied using a 3DHISTECH Pannoramic MIDI II BF scanning microscope to obtain digital micropreparations with Whole slide imaging technology in two modes: Single layer mode and Extended focus (3DHISTECH, Hungary).

The patient was examined following the requirements of the Helsinki Declaration of 1975, revised in 2013. An informed voluntary consent was signed for the implementation of diagnostics and medical intervention with the further use of data for scientific purposes.

RESULTS

The pathohistological study of the paravertebral muscles of the female patient, who was repeatedly operated on to correct spinal deformity due to neurological disorders, allowed us to clarify the etiology of the underlying disease and differentiate it as a genetically determined nemaline myopathy progressing in adulthood. Macroscopical observation found fragments of muscle tissue featuring severe fibrosis, atrophy, fat replacement. Microscopy of the altered muscle tissue detected myosymplasts, under the sarcolemma of which nemaline bodies were identified, that stained dark blue by Ptah (Fig. 1). Some of them were located diffusely in the form of dotted inclusions (Fig. 1 a), others were grouped into clusters of various configurations (Fig. 1 b, c). On longitudinal sections, nemaline formations were visualized as filaments (Fig. 1 d).

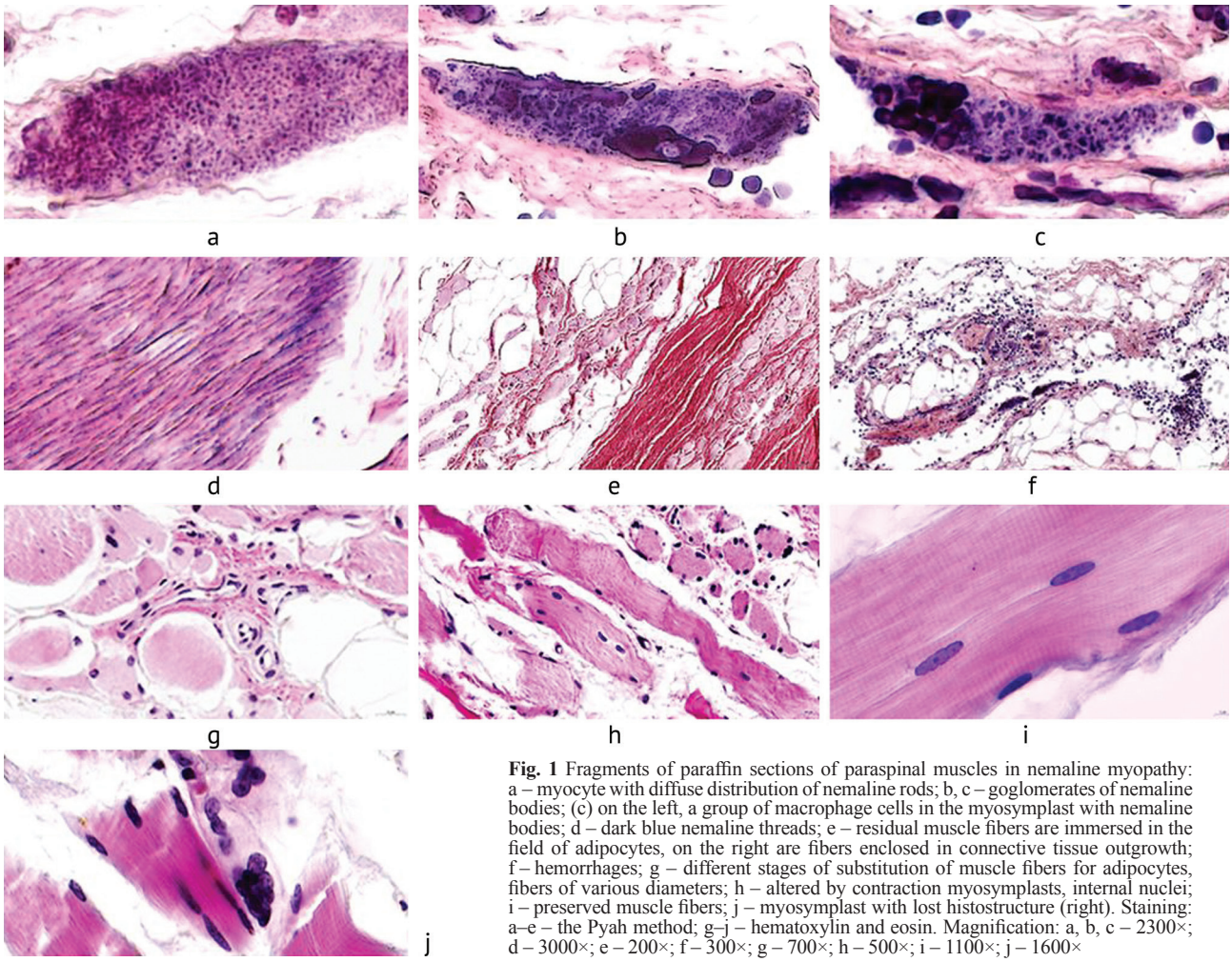

Fig. 1 Fragments of paraffin sections of paraspinal muscles in nemaline myopathy: a – myocyte with diffuse distribution of nemaline rods; b, c – goglomerates of nemaline bodies; (c) on the left, a group of macrophage cells in the myosymplast with nemaline bodies; d – dark blue nemaline threads; e – residual muscle fibers are immersed in the field of adipocytes, on the right are fibers enclosed in connective tissue outgrowth; f – hemorrhages; g – different stages of substitution of muscle fibers for adipocytes, fibers of various diameters; h – altered by contraction myosymplasts, internal nuclei; i – preserved muscle fibers; j – myosymplast with lost histostructure (right). Staining: a–e – the Pyah method; g–j – hematoxylin and eosin. Magnification: a, b, c – 2300×; d – 3000×; e – 200×; f – 300×; g – 700×; h – 500×; i – 1100×; j – 1600×

Fields of adipocytes that replaced muscle fibers, various stages of transformation of myosymplasts into fat cells were observed in the surgical material; residual small myocytes were immersed in connective tissue conglomerates (Fig. 1 e); hemorrhages were often encountered (Fig. 1 f). Fibers of various diameters were characteristic, patterns of gradual replacement of myosymplasts by fat cells were visualized (Fig. 1 g), internal nuclei and contracture-modified fibers were noted (Fig. 1 h). In symplasts with distinct transverse striation, elongated proper nuclei were identified along the periphery and in the center (Fig. 1 i); along with preserved fragments of muscle tissue, muscle fibers with a lost histostructure were observed (Fig. 1 j).

Vessels were frequently immersed in adipocyte conglomerates; the lumens were obliterated, membranes had signs of fibrosis, smooth muscle cells (SMC) t. media had disturbed circular orientation (Fig. 2 a, b). Vessels of the venous flow were thin-walled and tortuous (Fig. 2 c). Neuromuscular spindles were characterized by an enlarged connective tissue capsule (Fig. 2 d), intramuscular nerve conductors underwent complete involution, fibrosed perineurium and single nerve fibers were preserved (Fig. 2 e).

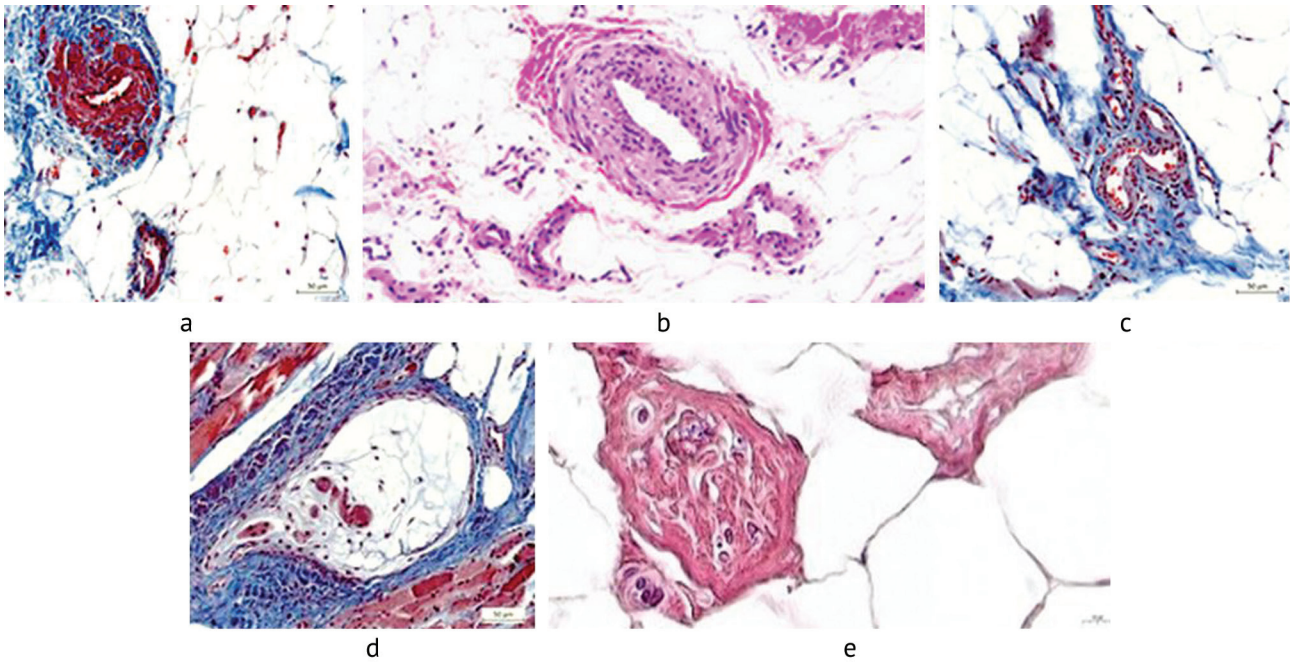

Fig. 2 Fragments of paraffin sections of paravertebral muscles in nemaline myopathy, features of the histostructure of vessels, neuromuscular spindles and nerve conductors: a – vessels are immersed in accumulations of adipocytes, lumen occlusion; b – pronounced fibrosis t. media, MMCs are devoid of circular orientation; c – convoluted contours of a venous vessel; d – enlarged capsule of the neuromuscular spindle in the connective tissue conglomerate; e – involution of the intramuscular nerve trunk with massive degeneration of nerve fibers, fibrosed perineurium is preserved. Staining: a, c, d – Masson method; b – with hematoxylin and eosin, e Ptah method. Magnification: a, c, d – 500×; b – 270×; d – 1600×

DISCUSSION

The pathology that was studied manifested itself in adolescence and was steadily progressing in adulthood. As literature states, due to the large number of genes responsible for NM, genetic search can be difficult and is effective only in 50 % of cases [1]. Therefore, additional studies are needed, in particular, histopathological analysis.

Fibrosis of the walls of blood vessels, caused by excessive deposition of extracellular matrix proteins, is associated with the diseases such as atherosclerosis, hyperglycemia, dyslipidemia, hyperhomocysteinemia; as well as with the renin-angiotensin-aldosterone system, oxidative stress, growth factors, and an imbalance in the secretion of endothelial-derived cytokines [8]. The imbalance of collagens leads to fibrosis of the wall and, as a result, to obliteration of the lumen. The role of collagens in providing hemostasis has been established, with an emphasis on collagen types I–IV, VI, XV, and XVIII [9]. The role of SMC in the development of arterial wall stiffning has also been shown; this is the second important component after extracellular matrix proteins, which not only regulates actomyosin interactions during contraction, but also mediates mechanotransduction in cells and hemostasis of extracellular matrix proteins. Arterial stiffning, the inverse of stretching, is involved in the etiology of chronic cardiovascular disease and is a major cause of morbidity and mortality worldwide [10].

The data presented in this study have been reflected in other studies. It is known that the features of muscle tissue in NM are abnormal variability in the

size of muscle fibers, which determines the bimodal distribution of dimeters on the histogram; internal nuclei, local necrosis of muscle fibers, pronounced fibrosis of the endo- and perimysium [11]. It has also been established that nemaline bodies can be distributed diffusely or form clusters of irregular shape, more often subsarcolemmal, and are characteristic of small fibers [12]. The last two facts were not confirmed in the presented work. Nemaline formations were observed not only under the sarcolemma, but over the entire area of the fibers, and were equally characteristic of myosymplasts of various sizes.

Electron microscopy revealed that NM can be identified by the presence of rod-shaped or ovoid structures; nemaline rods have a lattice structure similar to Z-disks; their shape in patients with mutations in *KLHL40* and *LMOD3* is different and can be useful for diagnostics [4].

It has been established that disorders in the contractility and organization of the cytoskeleton in NM may cause such defects in the nucleus as a destroyed membrane, an altered arrangement of chromatin, which, in turn, given the important role of the nucleus in the regulation of gene expression, as well as the cytoskeleton in maintaining the integrity of myosymplasts, explains the violation of contractility of filaments [12].

The thin filament of actin is the main component of the sarcomere. It is at this level of contraction that muscle weakness occurs in NM patients [3]. Ten of the 12 known genes in NM encode proteins that are components of the thin filament or contribute to actin stability and turnover. Proteins encoded by the *NEM2* and *ACTA1* genes are involved in the formation of muscle tone and muscle contraction. *NEM2* encodes the nebulin protein; mutations here are the main cause of myopathy and were found in 66.7 % of cases [5]. It has been shown how nebulin-deficient mice exhibited severe muscle atrophy and weakness in vivo associated with low *NEM2* [13]. The morphological analysis of muscles with a mutation in *NEM2* found that the number of fibers occupied by nemaline bodies correlated inversely with the severity of the disease [14]. The abnormal folding, altered polymerization, and aggregation of mutant α-actin isoforms are common properties of NM mutants, some of these effects are specific and likely lead to variations in the degree of muscle weakness [6].

Understanding the contribution of sarcomere dysfunction to muscle weakness through the genes involved will help develop targeted therapeutic strategies [3]. Accurate diagnosis of the disease will assist in choosing the tactics of treatment and avoiding adverse outcomes of the surgical methods used [15, 16].

CONCLUSION

The pathomorphological study of the paravertebral muscles confirmed the neuromuscular nature of the disease that was defined as nemaline myopathy, progressing in adult age and not diagnosed at previous stages of treatment. The affected muscles are characterized by nemaline bodies of two types, localized diffusely-dotted in the sarcoplasm or forming conglomerates of various shapes, both under the sarcolemma and over the entire area of the fibers; increased variability of myosymplast diameters, internal nuclei, contracture-modified fibers, myophagy patterns. Fields of fatty degeneration, involution of nerve conductors with fibrosed perineurium, fibrosis of the interstitial space and vessel walls, and their obliteration were observed.

In complex spinal deformities, it is necessary to assess the neurological status and collect an anamnesis using the maximum range of tests; histopathological study of the surgical material is one of the objective diagnostic criteria.

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