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Comparison of ultrasonographic and morphological findings of paravertebral muscles at the apex of kyphoscoliosis in patients with neurofibromatosis type I

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Background With the extensive research literature on kyphoscoliosis diagnostic criteria remain clinical in patients with neurofibromatosis type I. Paraspinal muscles have been under-evaluated in the cohort of patients. **Objective** To compare ultrasonographic and morphological measurements of paraspinal muscles at the apex of kyphoscoliosis in patients with neurofibromatosis type I. **Material and methods** The study included 10 patients who underwent surgical correction of spine deformity. Ultrasound measurements of paraspinal muscles were produced preoperatively. Muscle biopsy specimen was obtained intraoperatively at the apex of the deformity and histologically evaluated with optical microscope. **Results** Ultrasonographic examination of paraspinal muscles showed typical hypertrophy at the kyphosis site, bilaterally impaired striation and high acoustic impedance in patients with neurofibromatosis. Morphological evaluation revealed fibrosis of endomysium and perimysium, extensive areas of fibrosis and adipocytes replacing muscular fibres that translated into a great number of hyperechogenic hypertrophic bundles of fibres on ultrasonographic images. **Conclusion** Ultrasonographic findings of paraspinal muscles (hypertrophy, impaired muscle striation, high acoustic impedance) are on par with pathomorphological manifestations (revealed fibrosis of endomysium and perimysium, extensive areas of fibrosis and adipocytes replacing muscular fibres). The findings indicate to degeneration of muscle tissue with malfunction to follow. Ultrasound imaging allows for examination of paraspinal muscles, measuring thickness of superficial and deep muscles, acoustic impedance to identify fibrous changes and replacement of muscles by adipose tissue.
Keywords: neurofibromatosis, kyphoscoliosis, paraspinal muscles, ultrasonography, optical microscopy

INTRODUCTION

Neurofibromatosis is the most frequent single-gene genetic disorder that affects 1:2000 to 1:4000 individuals [1, 2, 3]. Kyphoscoliosis is seen in 25 to 50 % of the patients with neurofibromatosis type I [3, 4, 5]. Patients with neurofibromatosis type I develop early clinical manifestations of the deformity with rapidly progressive curve [6, 7, 8]. Orthopaedic intervention is indicated for the curve correction to prevent irreversible cardiac, pulmonary and neurological disorders [7, 9]. As a multisystem disorder, neurofibromatosis requires multidisciplinary approach to the diagnosis and treatment [6, 7]. With the extensive research literature on kyphoscoliosis diagnostic criteria remain clinical in the patients [8]. Paraspinal muscles have been under-evaluated in the cohort. The condition can be addressed by posterior thoracoplasty and coordinated postoperative rehabilitation emphasizing morphofunctional conditions of soft tissues at the site of surgical intervention [10, 11].

Ultrasonographic examination of muscles has gained more popularity [12, 13, 14, 15, 16, 17] being a safe and noninvasive method without radiation exposure that can be reproduced at any phase of rehabilitation.

Objective. The aim of the study was to compare ultrasonographic and morphological measurements of paraspinal muscles at the apex of kyphoscoliosis in patients with neurofibromatosis type I.

MATERIAL AND METHODS

Ten 9 to 24 year-old patients (mean age 16.0 ± 1.9 years), 5 males and 5 females with kyphoscoliosis in neurofibromatosis type I were recruited for the study. Cob angle measured 50 to 140° (mean 98.0 ± 12.2°) [18]. Vertebral artery syndrome was presented as an arc in thoracolumbar spine (n = 8) and in the cervicothoracic spine (n = 2). The study was approved by Ethics Committee, Federal State Budgetary Institution Russian Ilizarov Scientific Centre “Restorative Traumatology and Orthopaedics” Ministry of Health of the Russian Federation and was in compliance with ethical standards of the Declaration of Helsinki. Written informed consent for diagnosis and publication of the findings was obtained from all participants including parents/legal representative.

Ultrasound images were generated using a US machine (AVISUS Hitachi, Japan) and a 7.5 Hz linear ar-

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ray transducer. Patients were lying in prone position at physical rest. For each US image of paraspinal muscles the transducer was placed obliquely and diagonally over the apex of the curve. Acoustic density (AD, conventional units (CU)) of muscles was measured using standard software with regularly adjusted machine. US findings of the patients were compared with those of 10 healthy males and females aged 9 to 24 years.

Biopsy was collected at the apex of the curve during posterior approach to the spine for morphological evaluation of paraspinal muscles. The tissue sample was fixed in 10% neutral buffered formalin solution and paraffin embedded in histological cassettes. Sectioned with Bromma 2218 microtome (LKB, Sweden) tissues were hematoxylin and eosin stained using Van-Gizone and Masson's trichrome staining protocol and examined with Opton light microscope (Germany), built-in camera and DiaMorph hardware and software system (Russia) with Color programme.

Statistical analysis was performed using Microsoft Excel 2010 and AtteStat software [19]. Non-parametric statistical tests were applied used. The Student's t-test was used to confirm differences in cases with normal distribution. Non-parametric Mann-Whitney test was used when distribution was different from the norm. We set the significance threshold at $p < 0.05$.

RESULTS

Ultrasound imaging of thoracic paraspinal muscles in healthy individuals showed normal striated muscle structure with bundles of muscle fibres oriented with the tension vector. Echogenic perimysium and thin endomysium striae could be clearly visualised against a hypoechoic background of muscle fibres (**Fig. 1 a**).

Acoustic density measured in muscles of interests is presented in **Table**.

Ultrasound imaging of paraspinal muscle structures showed evidently asymmetric superficial and deep muscles, and characteristic hypertrophy at the kyphosis level in patients with neurofibromatosis. The superficial and deep muscle thicknesses measured 9.62 ± 3.2 mm and 7.12 ± 2.2mm at the kyphosis level, and 7.58 ± 3.4 mm and 6.52 ± 2.8 mm on the contralateral side, correspondingly, showing the tendency to greater muscle thickness at the kyphosis site. Impaired striated muscle structure was visualised on both sides (**Fig. 1 b**). Acoustic density of superficial and deep muscles was 11 % ($p < 0.05$) and 98 % ($p < 0.05$) greater than that in the control group, correspondingly (Table). US imaging exhibited a great number of extensive hyperechogenic structures, 0.4 to 1.1 cm long, with high acoustic density of 198 to 200 CU and thickness of 0.04 to 0.08 cm corresponding to the bundles of muscle fibres. Thus, a fair amount of hyperechogenic hypertrophic bundles of muscle fibres with high acoustic density indicated to expressed fibrous changes in the muscle tissue. The results of non-invasive ultrasound imaging of muscles at kyphoscoliosis site were in line with morphological findings.

All patients with kyphoscoliosis in neurofibromatosis type I showed evident fibrosis of endomysium, perimysium in the majority of muscle bundles (**Fig. 2 a**), extensive areas of adipocytes replacing muscle fibres (**Fig. 2 b**) as major pathomorphological manifestations. Biopsies exhibited different diameters of round muscle fibres with rare small nuclei and degenerating, atrophic muscle fibres (**Fig. 2 c**). Signs of axonal degeneration were seen in intramuscular nerve trunks nerve fibres separated by thickened connective tissue septums (**Fig. 2 d**).

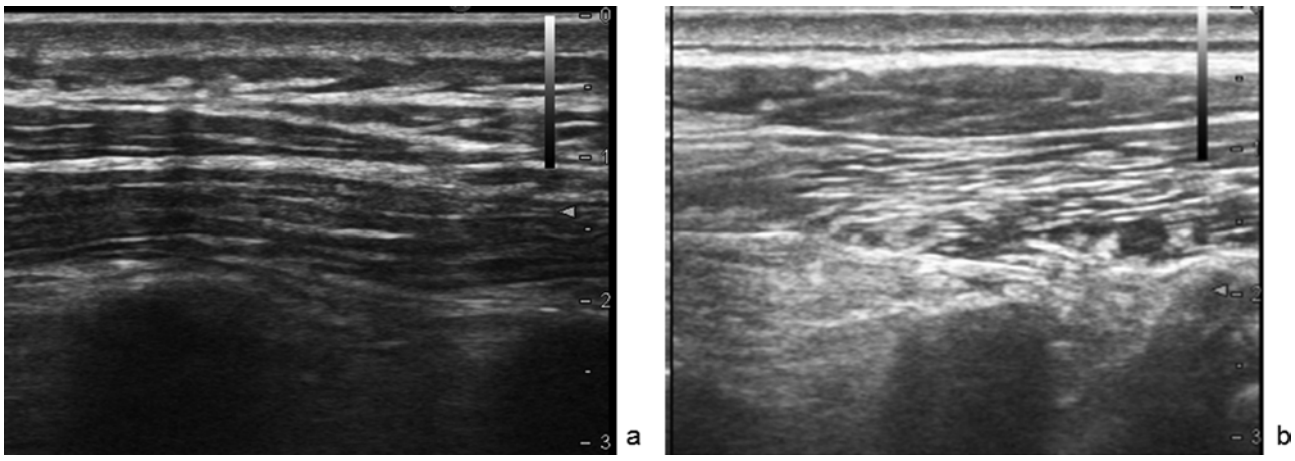

Fig. 1 Ultrasound images of paraspinal muscles of (**a**) 24-year-old healthy individual and (**b**) a 24-year-old patient G. With kyphoscoliosis in neurofibromatosis type I. Imaging is produced at the level of kyphosis. The superficial and deep paraspinal muscle thickness of the healthy person measured 4.4 mm and 7.3 mm, correspondingly with acoustic impedance of the superficial and deep paraspinal muscles being 127 conventional units (CU) and 91 CU, correspondingly. The superficial and deep paraspinal muscle thickness of patient G. measured 5.2 mm and 7.5 mm, correspondingly with acoustic impedance of the superficial and deep paraspinal muscles being 152 conventional units (CU) and 178 CU, correspondingly

Table

Paraspinal muscle acoustic density (M ± m) measured in patients with kyphoscoliosis in neurofibromatosis type I

Groups of examinees	Acoustic density (conventional units)	
	Superficial muscles	Deep muscles
Healthy individuals (n = 10)	137 ± 6	91 ± 11
Patients with neurofibromatosis type I (n = 10)	152 ± 4*	180 ± 5*

Note: * – statistically significant differences from normal values, $p < 0.05$.

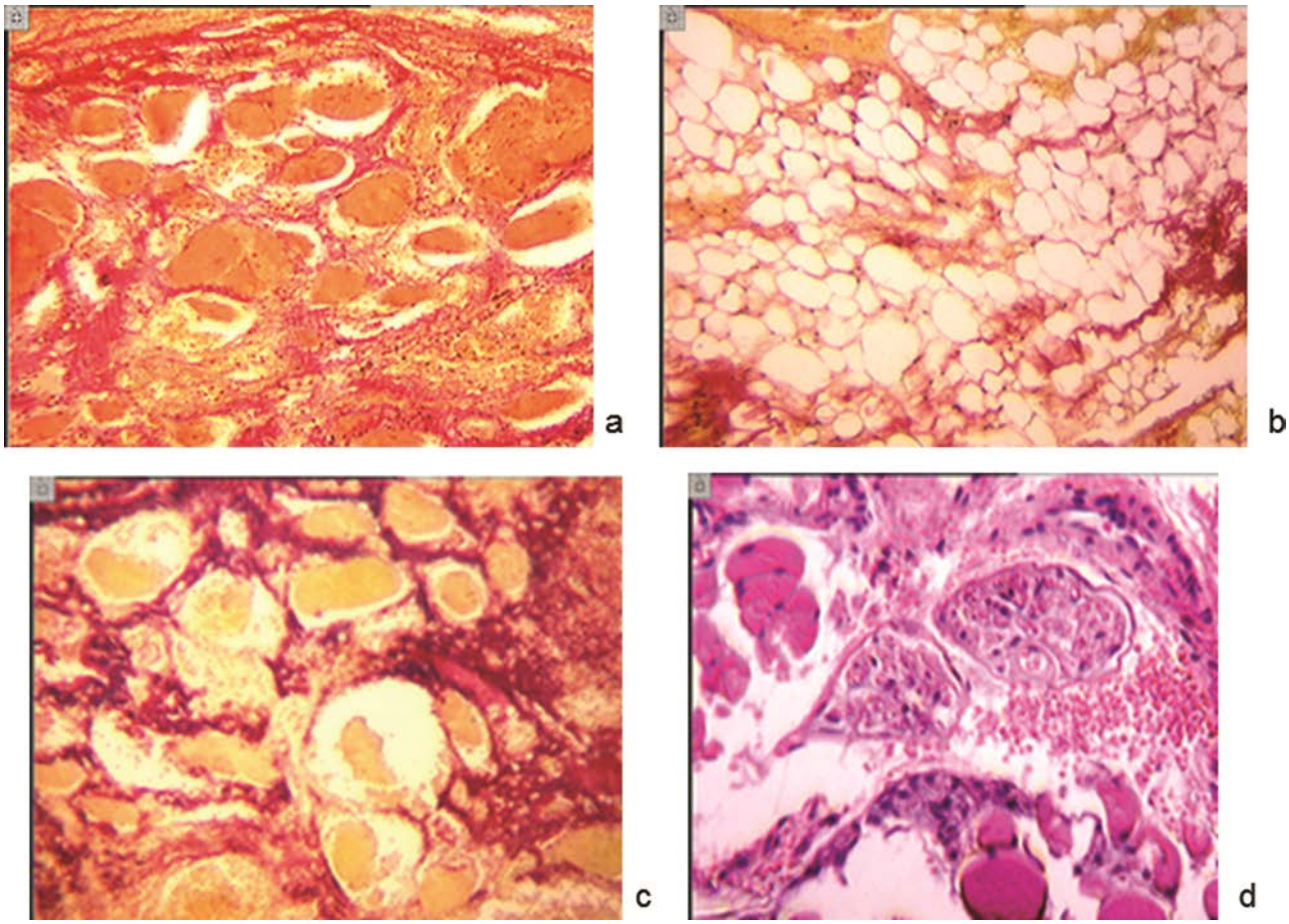

Fig. 2 Biopsy paraffin sections of paraspinal muscle of a patient with kyphoscoliosis in neurofibromatosis type I showing (**a**) fibrosis of endomysium, perimysium and interstitial space, and degenerating muscle fibres; (**b**) extensive areas of adipocytes replacing muscle fibres; (**c**) degenerating and atrophic muscle fibres, and interstitial fibrosis; (**d**) fibrosis of intramuscular nerve trunks, axonal degeneration in nerve fibres. Van Gizone (**a, b, c**) and hematoxylin and eosin (**d**) stain. Magnification: ob. – 16×, ok. – 12,5×

Therefore, morphological findings showed fibrosis, atrophy, degeneration and adipose replacement of paraspinal muscle fibres in patients with neurofibromatosis type I.

DISCUSSION

There is a paucity of scientific papers on neurofibromatosis type I (Recklinghausen disease or peripheral neurofibromatosis) that define specific structures of soft tissues including paraspinal muscles [10, 20, 21, 22]. The most common orthopaedic problems are hypotonia and poor coordination [2].

Ultrasound imaging technology is being used increasingly to study muscles in children and adults, males and females [13]. Muscles have low echogenicity. Echogenic perimysium and thin endomysium striae could be clearly visualised against a hypoechoic background of muscle fibres [13, 14, 15, 17].

Ultrasound imaging of paraspinal muscle structures showed characteristic hypertrophy at the kyphosis level, impaired muscle striation on both sides and high acoustic density in patients with neurofibromatosis. The study exhibited a great number of extensive hyperechogenic structures with high acoustic density and thickness corresponding to the bundles of muscle fibres indicating to evident fibrosis of the tissue.

The normal muscle appearance includes muscle fibres of polygonal shape with normally located nuclei, layers of perimysium and endomysium, arteriolar vessels with circular smooth muscle cells and normal lumens.

Our morphological findings showed fibrosis, atrophy, degeneration and adipose replacement of paraspinal muscle fibres resulting in functional failure of patients with neurofibromatosis type I. Morphologically revealed fibrosis of perimysium and endomysium, extensive areas of fibrosis and adipocytes replacing muscle fibres could account for a great number of hyperechogenic hypertrophic bundles of fibres visualised with ultrasound imaging.

According to S. Pillen et al. [16] muscle atrophy, fat infiltration and intramuscular fibrosis can be detected with ultrasound. Ultrasound imaging allows for the study of paraspinal muscles in patients with neurofibromatosis type I at any stage of treatment and rehabilitation, measuring thickness of superficial and deep muscles, acoustic impedance to identify fibrous changes and replacement of muscles by adipose tissue.

CONCLUSIONS

Ultrasonographic findings of paraspinal muscles (hypertrophy, impaired muscle striation, high acoustic impedance) are on par with pathomorphological manifestations (revealed fibrosis of endomysium and perimysium, extensive areas of fibrosis and adipocytes replacing muscular fibres). The findings indicate to degeneration of muscle tissue with malfunction to follow. Ultrasound imaging allows for examination of paraspinal muscles, measuring thickness of superficial and deep muscles, acoustic impedance to identify fibrous changes and replacement of muscles by adipose tissue.

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