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MYOPATHY WITH ATROPHY OF TYPE 2 FIBERS: A MORPHOMETRICAL ANALYSIS OF THREE CASES

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ABSTRACT

Type 2 fiber atrophy is a non-specific phenomenon in several diseases, including rare cases of congenital myopathies. These myopathies manifest early in life, and are characterized by hypotonia and muscle weakness. In this report, we describe a morphometric analysis of type 1 (T1F) and type 2 (T2F) fibers in three children (0.8, 4 and 8 years old) with signs of myopathy and T2F atrophy and assess the extent of T2F involvement compared to T1F. We measured the lesser diameter of T2F and T1F processed for ATPase activity at pH 9.4, in order to determine the pattern of muscle involvement. A histogram of the frequency of muscle fiber lesser diameter was used to compare the results with other reports in the literature. Statistical analysis using the chi-square test for goodness of fit showed that the lesser diameter of T2F differed among the children ($p<0.05$). Hence, it was not possible to determine a pattern of T2F atrophy based on the frequency of fiber size distribution in these cases of myopathies.

Key words: Atrophy, morphometry, myopathy, skeletal muscle, type 2 fibers

INTRODUCTION

The morphological alterations of type 2 muscle fibers (T2F) has been observed as a secondary phenomenon in individuals with lesions of the superior motor neurons [1,7], myasthenia gravis [2,16], muscle disuse [8], Parkinson disease [7], Cushing syndrome, untreated collagen-vascular diseases [15], and inflammatory myopathies [11], or as an initial rare phenomenon in congenital myopathies. These myopathies are hereditary muscle disorders that are characterized clinically by generalized hypotonia and usually non-progressive muscle weakness. These conditions are accompanied by structural alterations in the muscle fibers [10]. Type 1 fiber (T1F) atrophy and/or a predominance of T1F are frequent findings in these myopathies [5,9,12], although T2F atrophy has been observed in rare cases. Selective T2F atrophy was reported by Matsuoka and collaborators in 1974 in patients with a clinical picture compatible with congenital myopathy [13], and other investigators later reported the involvement of this fiber type that in some cases was associated with T1F hypertrophy [4,14]. However, it is not known whether the involvement of T2F in congenital myopathies is uniform. Hence, the aim of this work was to quantify the degree of T2F involvement in congenital myopathies relative to T1F by measuring the lesser diameter (LD) of the two fiber types and, if possible, to determine the pattern of muscle fibers involvement in these cases.

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SUBJECTS AND METHODS

Three children (two boys 4 and 8 years old, and one girl 8 months old at the time of the muscle biopsy) with a diagnosis of myopathy associated with exclusive T2F atrophy were studied. A clinical examination revealed proximal muscle weakness in the boys and generalized weakness in the girl (Table 1). Biopsies of the brachial biceps muscle were collected in the Sector of Neuromuscular Diseases of the University Hospital, in the Faculty of Medicine at Ribeirão Preto (USP), and the anatomopathological exams were done in the Department of Pathology of the same institution. The tissue fragments were frozen in liquid nitrogen, sectioned with a cryostat, and processed for histoenzymological reactions that included the myofibrillar ATPase (mATPase) reaction with preincubation at acid and basic pH. The sections were also processed and stained for routine histological evaluation. The predominant anatomopathological finding in the biopsies was T2F atrophy.

Following the ATPase reaction at pH 9.4, morphometric analysis was done by considering the LD of 150 T2F and 50 T1F from each child using an Image-Pro Plus Olympus image analyzer (version 4.1 for Windows, Media Cybernetics). A histogram showing the frequency of muscle fiber size at 10% intervals was constructed for each child starting from the mean

LD established in the literature for the age of the child under study [6]. Using this approach, it was possible to compare the results, regardless of the age of the children. The results of fibers were analyzed statistically by the chi-square test for goodness of fit. The proportion of each type fibers was determined with the image analyzer for T1F and T2F from three random fields.

RESULTS

The morphological analysis revealed polyhedral muscle fibers with the nucleus in a subsarcolemmal position. The fiber types were distributed in a mosaic pattern and the fibers were heterogenous in size, with T2F being smaller than T1F.

Figure 1 (A, B and C) shows that the three children had T1F hypertrophy with a unimodal frequency distribution, whereas the frequency of the T2F was bimodal, with the fiber diameters ranging mainly from 0.8 to 1.2 intervals for the boys and from 1.0 to 1.4 intervals for the girl, i.e., T2F size was shifted to the right in the graph, between the values for normal and hypertrophic fibers. A comparison between T2F and T1F in the biopsies showed significant differences in the mean LD values for T1F compared to T2F (the range of variation detected was 37%, 27% and 31% for the 8-month-old and 4- and 8-year-old children, respectively). The mean LD for each child is shown in Table 2. Statistical comparison of the distribution of fiber types in each child with the distribution for similar myopathies reported in the literature revealed considerable variability among individual measurements; the histograms for the LD of T2F for the three children differed significantly from each other ($p<0.05$). There was a predominance of T2F only in the girl, with a homogeneous distribution for the boys (Table 2).

DISCUSSION

The mean LD showed that all of the children had T1F hypertrophy and two boys had T2F atrophy when compared with international reference values [6]. The mean LD for T2F in the girl was similar to the reference values for her age and showed a predominance of T2F. Literature reference values were used here for comparison because of a lack of similar data for Brazilian subjects. The results cited by Dubowitz [6] were from a study by Brooke and Engel [3] for a series of children of both sexes subjected to a biopsy for clarification of their diagnosis. The children considered normal (n=16) were those whose anatomopathological and clinical correlations did not indicate a specific disease.

Table 1. Summary of clinical cases studied

Case	Gender	Age at biopsy	Neonatal hypotonia	Developmental milestones	Best motor achievement	Weakness face	Additional features	Family history	CK
						prox distal			
1	F	8 months	Yes	Delayed	Change in decubitus	+ + +	Arthrogryposis Multiple joint contractures	-	43
2	M	4 years	No	Delayed	Ambulant	- + -	Short neck Retrognathia	-	242
3	M	8 years	No	Delayed	Ambulant	- + -	Ptosis of eyelids	-	134

prox = proximal; CK = creatine kinase (normal values up to 204 U/L); (+) = present; (-) = absent.

Table 2. Summary of the morphometric data for the cases studied

Parameters	Subjects		
	C1 (8 months)	C2 (4 years)	C3 (8 years)
T1F : T2F proportion	1 : 3.5	1 : 1.1	1 : 1.4
Mean lesser diameter of T2F (μm) ± (SD) [Reference value for the age]*	15.4 ± 2.5 [<16]	19.2 ± 4.1 [22]	26.9 ± 5.3 [33.3]
Mean lesser diameter of T1F (μm) ± (SD) [Reference value for the age]*	24.4 ± 2.8 [<16]	26.3 ± 2.9 [22]	38.4 ± 4.9 [33.3]

*Dubowitz [6].
C = child; SD = standard deviation.

However, the values obtained may not represent the general population since the children that these authors studied showed signs or symptoms of myopathy (otherwise they would not undergone a biopsy) that might not have been identified because of limitations inherent to the knowledge and technology available at the time. In addition, these values were obtained for children from a population different from that studied here. The children studied here showed significant differences in the mean lesser diameter for T1F compared to T2F, a finding that led to the establishment of a diagnosis of myopathy with difference in the size fibers.

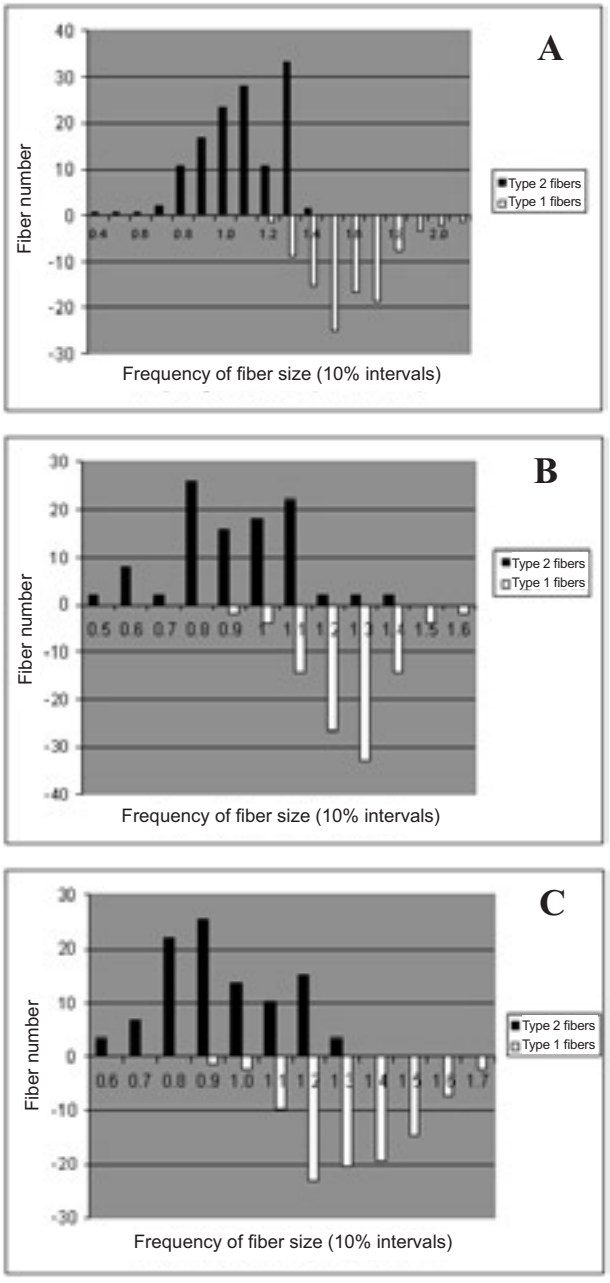

Figure 1. Size frequency distribution for type 1 and type 2 fibers of children aged 8 month-years-old (**A**), 4 (**B**) years-old and 8 (**C**) years-old.

T1F hypertrophy was also observed by Muranaka *et al.* [14] in studies on congenital myopathy. Other authors have observed T2F atrophy in association with normal T1F size [17] and a predominance of T1F [4,14]. The data reported by Brooke and Engel [3] for the proportion of fibers in the brachial biceps muscle of children considered normal, i.e., without a clinical diagnosis confirmed by anatomopathological examination, did not indicate the predominance of a specific fiber type. The results obtained here based on proportion analysis for this myopathy yielded similar proportions of oxidative and glycolytic fibers for boys and a predominance of T2F for the girl.

The analysis of the individual distribution of the fibers in order to determine the frequency and intensity of T2F involvement in this disease was not directly comparable to the findings of other investigators who studied congenital myopathy based on clinical and descriptive case studies [4,14,16]. The approach used here fulfilled the initial objective of characterizing the degree of T1F and T2F involvement and made it possible to establish a correlation between cases, regardless of patient age. Our results demonstrated that the intensity of atrophy and the distribution of the proportional T2F sizes (compared to T1F) differed among individuals, with no clear pattern that could identify the T2F atrophy occurring in this disease. The fibers subtypes determined by mATPase incubated at pH 9.4, 4.6 and 4.3 was not highlighted or quantified here because there was no uniformity in its expression among the children. Indeed, morphological analysis of sections from the three children showed that the fiber 2 subtypes had variable dimensions, with no preferential damage for a specific subtype. Rather, the size of all subtypes was affected. For this reason, we have generally presented our results as T2F.

The rarity of this specific type of myopathy precluded the use of a larger number of cases. Future studies are needed to definitively determine the pattern of atrophy in the T2F in myopathy with T2F atrophy.

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