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Quantitation of muscle pathology abnormalities in 18 patients with mitochondrial disorders

Quantificação das anormalidades patológicas musculares em 18 pacientes com desordens mitocondriais

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ABSTRACT

Introduction: Mitochondrial disorders can lead to the accumulation of mitochondria in muscle fibers, as indicated by ragged red (RRF) or ragged blue fibers when stained with modified Gomori trichrome or succinate dehydrogenase (SDH+), respectively, and, absence of activity of cytochrome c oxidase, COX negative fibers (COX-). The combined COX-SDH stain (COMBO+) can reveal even more COX-deficient fibers. **Objective:** To quantify RRFs, SDH+, COX-, and COMBO+ fibers in muscle biopsies with mitochondrial findings. **Material and methods:** We retrospectively selected 18 muscle biopsies with mitochondrial abnormalities based on the Walker criteria (percentage of RRFs/COX- fibers, and clinical picture), and/or the Sleigh criteria (percentage of RRFs, SDH+, and COX- fibers). **Results:** Females represented 83.3%, with a mean age of 38.6 years (5 months-70 years). Patients were diagnosed with chronic progressive external ophthalmoplegia (CPEO, 66.7%), proximal myopathy (22.2%), idiopathic hyperCKemia (11.1%), Kearns-Sayre syndrome (5.6%), mitochondrial encephalomyopathy with ragged red fibers and stroke-like episodes (5.6%), and a dystrophic pattern (5.6%). Some cases of CPEO were combined with proximal myopathy. The quantitative pathologic findings were: RRFs, 3.95% ± 3.17%; SDH+, 7.55% ± 6.1%; COX-, 10.9% ± 7.2%; COMBO+, 14.22% ± 12.79%. We found a slight variation in the diameter of muscle fibers, no necrosis or proliferative connective tissue, few fibers with internal nuclei, and some cases with fiber type grouping. **Conclusion:** Pathologic events, grouped in ascending order of frequency, were RRFs, SDH+ fibers, COX- fibers, and COMBO+ fibers. These data emphasize the importance of the COMBO technique in revealing occult COX deficiency in muscle fibers.

Key words: mitochondrial diseases; chronic progressive external ophthalmoplegia; cytochrome c oxidase deficiency; succinate dehydrogenase; mitochondrial myopathies.

INTRODUCTION

Mitochondrial disorders (MDs) arise through a primary dysfunction of the mitochondrial respiratory chain (MRC)[1]. The most affected organs and tissues are those that expend the most energy; these include skeletal muscle, brain, heart, and liver[2-4]. There is a wide clinical spectrum, with correlates between genotype and phenotype that may lack precision[2]. Mitochondria are primarily responsible for the provision of energy in the form of adenosine triphosphate (ATP)[5]. The first proven clinical report of a case of MD occurred in 1962[6], in a young woman with intense hypermetabolism.

MDs are due to dysfunctional oxidative phosphorylation in five enzymatic processes: I. nicotinamide adenine dinucleotide: ubiquinone oxidoreductase; II. succinate dehydrogenase; III. coenzyme-Q cytochrome c reductase; IV. cytochrome c oxidase; and complex V. ATP synthase, which uses energy generated by electron transport along the MRC to produce ATP[7, 8]. While complex II is encoded entirely by nuclear-deoxyribonucleic acid (nDNA) genes, the other complexes are encoded by both mitochondrial-DNA (mtDNA) and nDNA[1-3, 8]. Despite the contribution of mtDNA, the majority of the five complex subunits are encoded by nDNA, with just 13 proteins encoded by mtDNA[7].

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Mitochondrial DNA comprises 37 genes, with 13 structural genes encoding MRC subunits[9]. At cell division, hundreds or thousands of mtDNA copies are distributed randomly among daughter cells. In normal tissue, all of the mtDNAs are identical (homoplasmy). When this fails to occur (heteroplasmy), for example when mtDNA suffers mutations, a critical number of mutant mtDNAs (threshold effect) are required to trigger clinically detectable MD. The variation in number of mutated mtDNAs from one cellular generation to the next (mitotic segregation) can also alter the clinical phenotype of mtDNA-related disorders[5, 9].

Mitochondrial DNA is inherited maternally and does not recombine, with both mtDNA and nDNA mutations found in approximately 1:4,300 of the population[10]. Examples of MDs include Leigh syndrome, the most frequent childhood MD; neuropathy ataxia and retinitis pigmentosa; Kearns-Sayre syndrome (KSS); mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS); myoclonic epilepsy with ragged red fibers (MERRF); mitochondrial neurogastrointestinal encephalopathy; Alpers syndrome; Leber hereditary optic neuropathy; and chronic progressive external ophthalmoplegia (CPEO)[11]. Note that the resulting skeletal muscle abnormalities are more frequent in adults, and are rarely seen in children[11].

MDs can be confirmed by molecular testing. However, a muscle biopsy can facilitate diagnoses, especially as muscle mitochondria are abundant in the subsarcolemmal and intermyofibrillar spaces, and are larger in muscle than in other tissues[2]. This diagnostic avenue is particularly useful when there is suspicion of multiple possible conditions, such as with the myopathic phenotype, ophthalmoplegia, myalgia, and exercise intolerance. The classic pathological finding in skeletal muscle, and the hallmark of MD, are ragged red fibers (RRFs), which are mitochondrial accumulations that are stained red by modified Gomori trichrome (MGT). The first description of this phenomenon (yet to be named RRF) was provided by Engel and Cunningham, in 1963[12]. The accumulation of mitochondria can also be detected by staining with succinate dehydrogenase (SDH), in which muscle fibers have a strong blue color and are termed hyper-reactive fibers, ragged blue fibers (RBFs), or simply SDH+ fibers. Cytochrome c oxidase (COX) negative fibers (COX-) are also frequent, and the number of COX- fibers can be increased using a combination of COX staining with SDH (COMBO+)[11].

The primary objective of the present study was to quantify RRFs, SDH+ fibers, COX- fibers, and COMBO+ fibers by retrospective analyses of muscle biopsies from 18 patients that had fulfilled pathological criteria for MD[13, 14]. The secondary non-quantitative objective was to collate our general findings for these cases.

MATERIAL AND METHODS

Patients

Between June 2006 and December 2016, we retrospectively analyzed 515 muscle biopsies performed at Hospital de Base, the teaching hospital of Faculdade de Medicina de São José do Rio Preto (Famerp), São Paulo, Brazil. In all patients, biopsy was done from the open field *Deltoideus* muscle (the majority), the *Biceps brachii*, or *Vastus lateralis*. We selected 33 of the 515 patients (6.4%) with a description of mitochondrial abnormalities due to the presence of RRFs, SDH+ fibers, COX- fibers or, SDH positivity (blue staining) when combined with COX (COMBO+). None of these patients was subject to molecular analyses.

Histochemistry and immunohistochemistry

The muscle specimens were immediately wrapped in neutral talc for cryoprotection, then mounted in Tissue-TEK O.C.T. Compound (Sakura®) and frozen by direct immersion in liquid nitrogen (-170°C) until bubbling ceased[15]. Five micron sections of each specimen were then cut using a cryostat (Leica®, Germany) at -30°C. The following histochemical stains were performed for each section: hematoxylin and eosin (HE), MGT, ATP-ase myofibrillar proteins with pre-incubation at pH 9.6 and 4.3, acid and alkaline phosphatases, nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), SDH, COX, COMBO (COX+SDH), periodic acid-Schiff, Oil Red O, and myophosphorylase. Immunohistochemistry was also performed for dystrophin, cluster of differentiation 4 (CD4), CD8, major histocompatibility complex-class I, developmental myosin, and neonatal myosin.

Pathological inclusion criteria: Walker and Sleigh

The Walker criteria[13], for "definite" MRC defects necessitates the presence of two major criteria (RRFs > 2%, and COX- fibers > 2% in patients < 50 years, or > 5% in patients > 50 years), or the presence of one major criterion, in combination with two minor criteria. The minor criteria were RRFs of 1%-2% from an age of 30-50 years, RRFs of any number at less than 30 years of age, or symptoms compatible with a MRC defect. The criteria for "probable" MRC were met when one major criterion was present, with a suitable clinical picture. Lastly, "possible" MRC was indicated by satisfying one of the major criteria only, or when one minor criterion was associated with a suitable clinical picture.

Using the Sleigh criteria[14], mitochondrial abnormality was defined when fibers demonstrated the following event values: RRFs > 1.04%, COX- > 3.46%, and SDH+ > 0.89%. These cut-off

values were obtained for the *Deltoideus* muscle in 45 autopsy subjects with nonneuromuscular disorders, comparing these to muscle biopsies from MD patients. The values used here refer to the inferior limit [confidence interval (CI), 99%] from the biopsied patient group.

Exclusion criteria

After a detailed review of muscle biopsy slides, with photomicrographs and a clinical summary of the 33 selected cases, 15 patients were excluded: 10 cases failed to meet either pathological criteria, Walker[13] or Sleigh[14]; two cases manifested inflammatory myopathy; one case was biopsied twice, with one biopsy excluded; one case had inclusion-body myositis (IBM), with secondary mitochondrial abnormalities; one case involved myocarditis with plentiful RRF-like fiber necrosis.

Selected cases

After exclusion, 18 cases were selected for pathology review, with collation of the following variables/data: age, gender, and the clinical summary at the time of biopsy. This study was approved by the Research Ethics Committee of Famerp.

Quantitation of muscle fibers and statistics

Slides from muscle specimens were reviewed microscopically (Zeiss®, Germany) using the Zen Pro software 2012 (blue edition). Quantitative analyses were performed for RRFs, COX- fibers, SDH+ fibers, and COMBO+ fibers. RRFs were quantified on MGT staining (classic), but were also viewed by HE. Typical abnormalities ranged from small subsarcolemmal inclusions to almost complete sarcoplasmic disfigurement (''ragged'') with intense red staining. COX- fibers (counted) were either completely white or very pale (COX-deficient). The SDH+ fibers (either RBFs or hyper-reactive fibers) were similar to RRFs with subsarcolemmal deposits or intense blue staining. The muscle fibers that were COMBO+ stained blue (abnormal); COMBO+ fibers were particularly useful as the fiber pathology could be identified (by strong blue staining) even when COX activity appeared to be normal. The muscle fibers were counted by tracing a contour line in a fascicle to measure area. Similarly, we calculated the area for a single muscle fiber and used this value to determine the total number of fibers. The average muscle fiber counts were 446 (COMBO), 419 (COX), 326 (SDH), and 188 (MGT). The percentages of abnormal events were then calculated. To compare the four events, we used standard deviation (SD), one-way analysis of variance (Anova), multiple regression studies, and multiple comparisons with Bonferroni, with 95% significance levels. To test for normality, we used the Kolmogorov-Smirnov test.

RESULTS

In all 18 patients, the biopsy was performed on the *Deltoideus* muscle; 15 patients were female (83.3%), and the mean age of our cohort was 38.6 years (5 months to 70 years). Values for serum creatine kinase (CK) were reported in 13/18 cases (72.2%), with a mean value of 579 U/l, and median of 244 U/l, ranging from 32 to 1,800 U/l. CK was normal in 5/13 patients (38.5%). Electromyogram examinations were reported for 13/18 cases (72.2%); they were normal in 9/13 cases (69.2%), and myopathic in the remaining four cases (30.8%). Nerve conduction studies found one case (7.7%) of sensory axonal peripheral neuropathy. Using the Walker criteria[13], 77.8% were defined as definite MRC, 5.6% were probable, and 16.7% were possible. By the Sleigh criteria[14], 88.9% were defined by RRF percentage, and 83.3% by the percentage of their COX-fibers (**Table 1**). The positive cases by the Sleigh criteria[14] are shown in **Figure 1**. The clinical parameters taken from a summary at the time of the muscle biopsy are summarized in Table 1. The most frequent clinical phenotype was found to be CPEO (12 cases, 66.7%), either isolated (nine cases; 50%), or associated with proximal myopathy (three cases; 16.7%). Proximal myopathy, isolated or associated with CPEO, was the second most common phenotype (four cases; 22.2%). Idiopathic hyperCKemia was the reason for referral in two cases (11.1%), with one case associated with fatigue and consanguinity, and the other with statin use. A patient with a clinical picture suggestive of KSS, another of MELAS, and another with dystrophic pattern (5.6% each) completed the series. All CPEO cases were females, with a mean age of 41.9 years, an average duration of symptoms of 14.4 years (3 to 30 years), symmetric evolution in seven cases, asymmetric evolution in five cases, ophthalmoplegia/paresis in five cases, and family history in three cases. All four events, RRFs, COX-, SDH+, and COMBO+, passed the Kolmogorov-Smirnov normality test (p-value > 0.1). **Table 2** shows the four events using one-way Anova. Comparisons and significance for the percentages obtained for the four quantitative events, using multiple regression, are shown in **Table 3**. Specifically, in the 12 CPEO cases, each type of abnormal event was found: RRFs, mean 3.81% (0.42% to 10.24%); SDH+, mean 6.98% (1.53% to 18.45%); COX-, mean 11.06% (1.68% to 22.6%); COMBO+, mean 13.22% (1.59% to 35.86%). In all cases that were COX- and COMBO+, a mosaic pattern was observed. **Figure 2** shows significant differences for the means (Bonferroni) and SD when comparing RRFs vs. COMBO+, with non-overlapping intervals indicating that the mean and SD differed from each other. Other non-quantitative pathological findings are summarized in **Table 4**. RRFs, the hallmark of MDs, are shown in **Figure 3**. Ragged blue fibers or SDH hyper-reactive (SDH+ fibers) are shown in **Figure 4**.

Less specific NADH-TR and Oil Red O are shown in Figure 4. Necrotic fibers, when scattered, can sometimes display some similarity with RRFs and are shown in **Figure 5**. Lobulated fibers, usually found in some dystrophies, can look like mitochondrial accumulations, and are shown on Figure 5. COX-negative fibers distributed in a mosaic pattern (heteroplasmy) are shown in **Figure 6**. Some missing COX-deficient fibers can be found using COX plus SDH stain (COMBO) and are shown in Figure 6.

TABLE 1 – Age, gender, clinical summary, RRF, COX- and COMBO+ fibers in 18 cases with mitochondrial abnormalities. Walker clinical-pathological criteria[13] and Sleigh pathological criteria[14] are shown

Age	G	Clinical suspicion	RRF	COX-	COMBO+	Walker	Sleigh RRF	Sleigh COX-
9	M	Kearns-Sayre syndrome	2.2%	9.9%	ND	Definite	Yes	Yes
28	F	CPEO	0.7%	7.5%	7.9%	Definite	No	Yes
41	F	CPEO	6.8%	10.2%	35.9%	Definite	Yes	Yes
68	F	CPEO	0.4%	6.9%	1.6%	Probable	No	Yes
21	F	MELAS. Myalgia. Epilepsy	7%	11.9%	10.7%	Definite	Yes	Yes
43	F	CPEO	1.2%	10%	5.4%	Definite	Yes	Yes
39	F	CPEO + proximal myopathy	1.4%	4.7%	23.2%	Definite	Yes	Yes
60	F	Idiopathic hyperCKemia (statin use)	3.3%	2.7%	0.4%	Possible	Yes	No
36	F	CPEO + proximal lower limbs myopathy	1.2%	1.7%	1.9%	Possible	Yes	No
46	M	Proximal myopathy	1.2%	0%	2%	Possible	Yes	No
19	F	CPEO	1.7%	12.2%	16.6%	Definite	Yes	Yes
5 m	F	Dystrophic pattern	8.5%	29.1%	46%	Definite	Yes	Yes
30	F	CPEO. Dysarthria	3.7%	11.2%	8.1%	Definite	Yes	Yes
70	F	CPEO	8.6%	17.9%	24.1%	Definite	Yes	Yes
60	F	CPEO	3%	12.6%	8.4%	Definite	Yes	Yes
54	M	Idiopathic hyperCKemia. Fatigue	3.4%	10.1%	24.1%	Definite	Yes	Yes
42	F	CPEO	6.9%	22.6%	12.1%	Definite	Yes	Yes
27	F	CPEO. Chronic proximal myopathy	10.2%	15.3%	13.5%	Definite	Yes	Yes
38.6			3.95%	10.9%	14.22%	77.8%	88.9%	83.3%

RRF: ragged red fiber; COX-: cytochrome c oxidase negative fibers; COMBO+: COX staining with SDH; SDH: succinate dehydrogenase; G: gender; M: male; F: female; CPEO: chronic progressive external ophthalmoplegia.

TABLE 2 – One-way Anova for the four quantitative events (%)

Sample	Sample size	Mean	SD	Min	Max	Individual 95% CI for mean
RRF	18	3.95	3.17	0.42	10.24	2.3808 to 5.5292
SDH+	18	7.55	6.1	1.38	20.97	4.5183 to 10.586
COX-	18	10.9	7.2	0	29.07	7.3286 to 14.486
COMBO+	17	14.22	12.79	0.44	46	7.6537 to 20.805

RRF: ragged red fiber; SDH+: succinate dehydrogenase; COX-: cytochrome c oxidase negative fibers; COMBO+: COX staining with SDH; SD: standard deviation; CI: confidence interval.

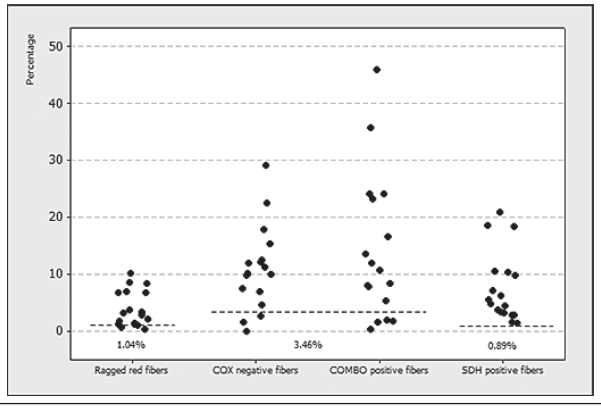

FIGURE 1 – *Comparison among the four quantitative events from 18 muscle biopsies with mitochondrial abnormalities. Limit of normality from Sleigh* et al. *(2011)*[14]

TABLE 3 – Multiple regression for four events (%) from 18 muscle biopsies with mitochondrial abnormalities

Variable	Independent variable	*r* squared	*p*-value	Significancy
RRF	COX-	51.99	0.0007	Extremely significant
COX-	COMBO+	39.8	0.0066	Very significant
RRF	COMBO+	31.93	0.0181	Significant
COMBO+	SDH+	20.84	0.0655	Not quite significant
COX-	SDH+	19.12	0.0699	Not quite significant
RRF	SDH+	7.46	0.2728	Not significant

RRF: ragged red fiber; COX-: cytochrome c oxidase negative fibers; COMBO+: COX staining with SDH; SDH+: succinate dehydrogenase.

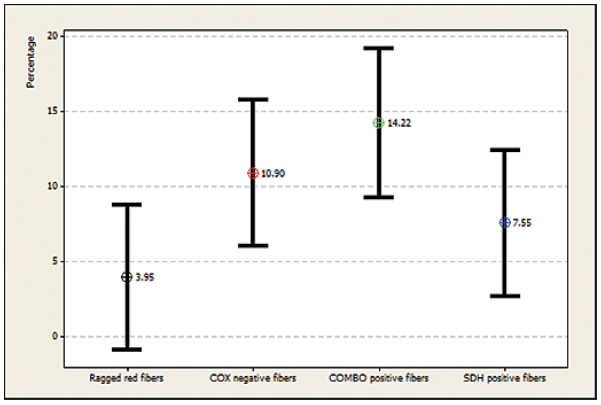

FIGURE 2 – *Bonferroni comparison (95%) among the four quantitative events from 18 muscle biopsies with mitochondrial abnormalities*

TABLE 4 – Non-quantitative findings on muscle biopsy from 18 cases with mitochondrial abnormalities

Variable	Findings	*n*	%
Changes in fiber size	Moderate: many hypertrophy/ few atrophy	11/18	61.1
	Slight: most hypertrophy/few atrophy	3/18	16.7
	Normal or slight variation	3/18	16.7
	Marked	1/18	5.6
Internal nuclei	> 5%	1/18	5.6
Positive acid phosphatase	Mild and scattered	5/18	27.8
	Diffuse	2/18	11.1
Positive alkaline phosphatase	Mild and scattered	6/18	33.3
Fibrosis and adipose tissue	Marked	1/18	5.6
Fiber type distribution	Muscle fiber type 2 atrophy	9/18	50
	Muscle fiber type 1 predominance	8/18	44.4
	Normal checkboard	4/18	22.2
	Mild muscle fiber type 1 grouping	4/18	22.2
	Muscle fiber type 2 predominance	1/18	5.6
	Muscle fiber type 1 and 2 atrophy	1/18	5.6
Oil Red O stain for lipid	Increased number of droplets in RRFs	5/18	27.8
	Diffuse increased number of droplets	1/18	5.6

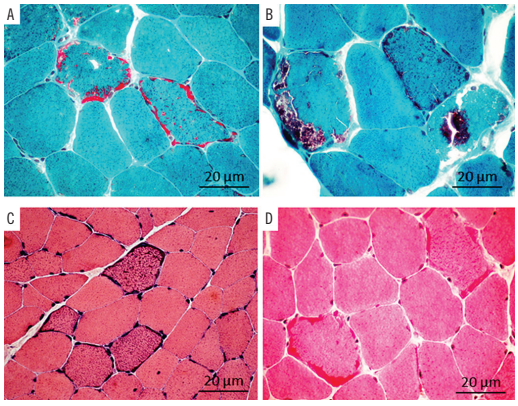

FIGURE 3 – *Ragged red fibers, hallmark of mitochondrial disorders, in modified Gomori trichrome (A and B) and HE (C and D). Note the red subsarcolemmal mitochondrial accumulation (A, B, and C) and the intermyofibrillar disruption ("*ragged*") in B*

HE: hematoxylin and eosin.

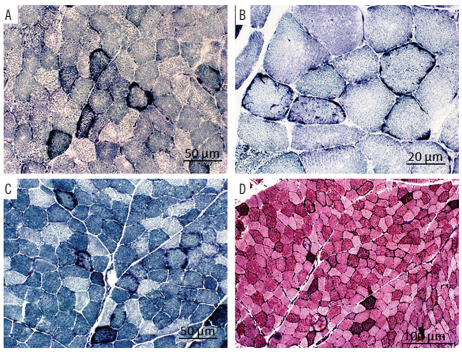

FIGURE 4 – *Ragged blue fibers, SDH hyper-reactive or simply SDH+ fibers are shown in A and B. NADH-TR hyper-reactive fibers are shown in C, but they are less specific due to mitochondria and sarcoplasmic reticulum stain activity. ORO hyper-reactive fibers with intense lipid droplets are shown in D*

SDH: succinate dehydrogenase; NADH-TR: nicotinamide adenine dinucleotide tetrazolium reductase; ORO: Oil Red O.

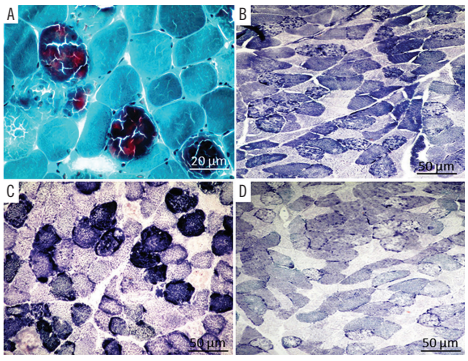

FIGURE 5 – *Necrotic fibers in modified Gomori trichrome (A) and NADH-TR (C). Note some similarity with ragged red fibers but with macrophages and acid phosphatase (not shown) positivity instead; NADH-TR can far resemble mitochondrial accumulation. In B and D lobulated fibers usually found in some dystrophies are shown; they can look like mitochondrial accumulations but they presented with the same aspect in all oxidative stains (NADH-TR, SDH and COX)*

SDH: succinate dehydrogenase; NADH-TR: nicotinamide adenine dinucleotide tetrazolium reductase; COX: cytochrome c oxidase.

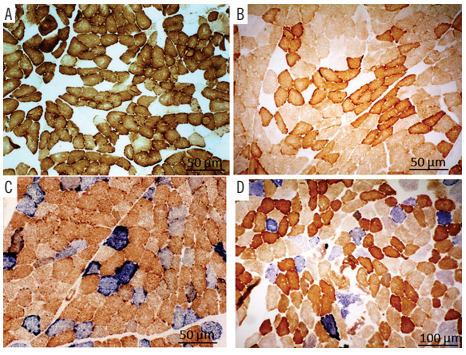

FIGURE 6 – *COX-negative fibers (A and B) distributed in a mosaic pattern (beteroplasmy) are the most specific and frequent event found in mitochondrial disorders (mtDNA mutations). Some missing COX-deficient fibers can be found using COX plus SDH stain (COMBO) and are shown in C-D. The strong blue stain from SDH (complex II, only nDNA) overpasses COX brown stain from COX-deficient fibers (complex IV, mtDNA and nDNA). COMBO+ fibers are even more frequent in those cases*

COX: cytochrome c oxidase; mtDNA: mitochondrial deoxyribonucleic acid; SDH: succinate dehydrogenase; COMBO: COX staining with SDH; nDNA: nuclear deoxyribonucleic acid.

DISCUSSION

We found that identification of pathological findings for typical skeletal muscle in MD (i.e. RRFs, SDH+, COX- fibers and COMBO+)[5] could be of value in reaching a diagnosis. MD might be suspected in patients with proximal myopathy, MERRF, CPEO and MELAS, or in patients presenting with associated exercise intolerance, fatigue, neurosensory hearing loss, increased lactic acid, pigmentary retinitis, axonal neuropathy, ptosis without fluctuation, hypertrophic cardiomyopathy, short stature, diabetes *mellitus*, and renal tubular acidosis[16]. RRFs (the classic MD abnormality), as well as SDH positivity, indicate pathologic subsarcolemmal accumulation (deposits) of mitochondria (perhaps as a compensatory response; Figure 3) and are considered to be a hallmark of MD[16, 17]. The technique of NADH-TR also revealed fibers with subsarcolemmal mitochondrial accumulation, occasionally appearing as hyper-reactive fibers, although this stain lacked specificity as staining enhanced both the mitochondria and the sarcoplasmic reticulum (Figure 4)[2]. In spite of the typical and unmistakable aspects of RRFs, care should be taken in rare cases where necrotic, scattered fibers, could be misdiagnosed (Figure 5).

COX-negative or -deficient fibers appear as a mosaic pattern because of heteroplasmy[1, 5, 17-19] and are secondary to mutations of mtDNA. They arise from abnormalities of MRC complex IV, encoded by both mtDNA and nDNA (Figure 6). Occasionally, lobulated fibers, which are atypical in MD, can mimic mitochondrial accumulation. In those cases, muscle fibers will stain in a similar fashion with each of the three oxidative histochemical stains (Figure 5). Occasional COX-deficient fibers are detected only when combining COX with SDH staining (COMBO), and generate a strong blue stain with SDH, which clearly demonstrates the COX negativity of those fibers (Figure 6)[5, 19]. Defects involving complexes I, III, or V will demonstrate normal COX and SDH reactions, with no histochemical methods with which to assess the activity of these defects[3].

In each case the muscle biopsy was of the *Deltoideus*. This muscle is especially useful when MD is suspected[20]. The percentage of abnormal events in our series, in ascending order, was RRFs: mean 3.95% (0.42-10.24); SDH+: 7.55% (1.38-20.97); COX-: 10.9% (0-29.07); and COMBO+: 14.22% (0.44-46). The significant difference between the percentage of RRFs and COMBO+ fibers indicates that the latter stain is absolutely necessary when MD is suspected. We identified one case with a severe dystrophic pattern, although this abnormality is rare in MD[21]. As a rule, we noted only a slight variation in fiber diameter, and in some cases, fiber diameter was increased without a strong eosinophilic reaction. In MD, the RRFs were almost always COX- or COX-deficient. Reduced COX staining can occur in RRFs and in apparently normal fibers, and is very specific to MD[17]. Mitochondrial accumulation as seen in RRFs and SDH+ fibers demonstrates less sensitivity in terms of a diagnosis for MD compared to the COX- and COMBO+ stains[22]. Koenig (2008)[16] and Reichmann *et al.* (1996)[23] also found that an increased number of COX- fibers was the most common finding in MD. Combining the COX-SDH stain identified 2-2.5-fold more abnormal fibers (12%) by biopsy than using RRFs alone (5%)[24]. In our series, this difference was almost four-fold (14.22% versus 3.95%). Tanji[7] identified SDH as the most sensitive and precise method with which to demonstrate the segmental accumulations of mitochondria compared to classic RRFs. We found more difficulty in quantifying SDH positivity compared to COX- or COMBO+ staining because of subjectivity when interpreting slight SDH hyper-reactivity. Pfeffer and Chinnery[1] stressed that COX- and SDH+ muscle fibers manifest the best sensitivity and specificity for MD.

Most of our cases were CPEO (66.7%), with a sensitivity from a limb muscle biopsy for diagnosis of approximately 75%[25]. The percentage of RRFs in CPEO have been reported to range from 1% to 39%[26], and from < 1% to 7%[22]. In the latter publication, authors also reported that COX- fiber rates ranged from 1% to 23%, so they were much more sensitive for reaching a diagnosis. Mitochondrial pathology abnormalities in childhood are uncommon, with muscle biopsy proving to be more useful in the adult population[11]. Both RRFs and COX- fibers can appear with aging, as has been pointed out previously[14, 16, 18, 27, 28]. Necrosis, increased variation in fiber diameter, internal nuclei, and the proliferation of connective tissue were uncommon findings. Fiber type grouping can, however, occur as a consequence of peripheral neuropathy, and is described in as many as 25% of cases of MD[24]. Increased lipid is common in KSS and CPEO, in some genetic forms of Leigh encephalopahy, but not in either MELAS or MERRF[2].

Mitochondrial abnormalities can occur as secondary effects of other disorders, particularly IBM, dysferlinopathy, polymyositis, dermatomyositis, and drug toxicity[29], but as a rule, they are accompanied by other myopathic features that typify each condition. Santorelli *et al.* (1996)[27] found a remarkable increase in RRFs (71%), COX- fibers (52%), and SDH-hyper-reactive fibers (55%) in IBM patients. The average frequencies of RRF, SDH+, and COX- fibers in polymyositis were 0.6%, 1.2%, and 4.7%, respectively, with values of 2.3%, 1.21%, and 1.75%, respectively, in dysferlinopathy[28].

CONCLUSION

In conclusion, general findings for the *Deltoideus* muscle biopsy in 18 cases with MD (mostly CPEO) revealed a slight variation in muscle fiber diameter (some substantially increased), an absence of necrosis or proliferation of connective tissue, some fibers with internal nuclei, and some cases with fiber type grouping. More specifically, quantitative findings in ascending order of frequency involved RRFs (mean 3.95%, 0.42-10.24), SDH+ fibers (7.55%, 1.38-20.97), COX- (10.9%, 0-29.07), and COMBO+ (14.22%, 0.44-46). These data stress the importance of the COMBO technique with which to detect unapparent COX- fibers.

RESUMO

Introdução: *Desordens mitocondriais são usualmente caracterizadas por: 1. acúmulo de mitocôndria nas fibras musculares que aparecem como fibras vermelhas rasgadas (FVR) ou azuis rasgadas quando coradas, respectivamente, pelo tricrômio modificado de Gomori ou pelo succinato desidrogenase (SDH+); 2. ausência de atividade da citocromo c oxidase (COX), originando fibras COX negativa (COX-). A combinação de colorações COX e SDH pode revelar ainda mais fibras COX deficiente (COMBO+).* ***Objetivos:*** *Quantificar FVR, SDH+, COX- e COMBO+ em biópsias musculares com anormalidades mitocondriais.* ***Material e métodos:*** *Foram analisadas retrospectivamente 18 biópsias com anormalidades mitocondriais com base no critério de Walker (percentagem de FVR/COX- e quadro clínico) e/ou critério de Sleigh (percentagem de FVR, SDH+ e COX-).* ***Resultados:*** *Sexo feminino representou 83,3% e média de idade 38,6 anos (5 meses a 70 anos). Oftalmoplegia externa progressiva crônica (OEPC) representou 66,7%; miopatia proximal, 22,2%; hiperCKemia idiopática, 11,1%; síndrome de Kearns-Sayre, 5,6%; encefalopatia mitocondrial com FVR e episódios semelhantes a acidente vascular cerebral, 5,6%; e padrão distrófico, 5,6%. Alguns casos de OEPC estavam associados à miopatia proximal. Achados patológicos quantitativos: FVR, 3,95% ± 3,17%; SDH+, 7,55% ± 6,1%; COX-, 10,9% ± 7,2%; COMBO+, 14,22% ± 12,79%. Encontramos leve variação de calibre das fibras musculares sem necrose ou proliferação de tecido conjuntivo, poucas fibras com núcleos internos e alguns casos com agrupamento de fibras.* ***Conclusão:*** *As anormalidades patológicas nas fibras musculares em ordem ascendente de frequência foram: FVR, SDH+, COX- e COMBO+. Nossos achados enfatizam a importância da técnica COMBO (COX + SDH) para aumento na frequência de fibras musculares COX deficiente ocultas.*

Unitermos: *doenças mitocondriais; oftalmoplegia externa progressiva crônica; deficiência de citocromo c oxidase; succinato desidrogenase; miopatias mitocondriais.*

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