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ARTICLE

Muscle biopsy in Pompe disease

Biópsia muscular na doença de Pompe

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ABSTRACT

Pompe disease (PD) can be diagnosed by measuring alpha-glucosidase levels or by identifying mutations in the gene enzyme. Muscle biopsies can aid diagnosis in doubtful cases. **Methods:** A review of muscle biopsy from 19 cases of PD (infantile, 6 cases; childhood, 4 cases; and juvenile/adult, 9 cases). **Results:** Vacuoles with or without glycogen storage were found in 18 cases. All cases had increased acid phosphatase activity. The vacuole frequency varied (almost all fibers in the infantile form to only a few in the juvenile/adult form). Atrophy of type 1 and 2 fibers was frequent in all forms. Atrophic angular fibers in the NADH-tetrazolium reductase and nonspecific esterase activity were observed in 4/9 of the juvenile/adult cases. **Conclusion:** Increased acid phosphatase activity and vacuoles were the primary findings. Most vacuoles were filled with glycogen, and the adult form of the disease had fewer fibers with vacuoles than the infantile or childhood forms.

Key words: Glycogen storage disease type II, muscle biopsy, immunohistochemistry, acid phosphatase, vacuoles.

RESUMO

O diagnóstico da doença de Pompe (PD) pode ser feito pela dosagem da enzima alfa-glicosidase ou pela mutação do seu gene codificador. A biópsia muscular pode ajudar em casos duvidosos. **Métodos:** Revisão das biópsias musculares de 19 casos de PD (forma infantil, 6 casos; infantil tardia, 4; e juvenil/adulto, 9). **Resultados:** Encontrados vacúolos em 18 casos, com ou sem depósito de glicogênio. Todos mostraram aumento da fosfatase ácida. Os vacúolos estavam presentes na maioria das fibras nas formas infantis, menos frequentes nas formas juvenil e mais raros nas formas do adulto. A atrofia de fibras dos tipos 1 e 2 ocorreram em todas as formas. Fibras atróficas na NADH-tetrazolium redutase e esterase não específica foram observadas em 4/9 das formas infantil tardia/adulta. **Conclusões:** Os dados mais frequentes foram vacúolos, preenchidos por glicogênio com atividade aumentada da fosfatase ácida. A forma adulta apresenta menor número de vacúolos que as formas infantil e infantil tardia.

Palavras-Chave: Doença de depósito de glicogênio tipo II, biópsia muscular, imunohistoquímica, fosfatase ácida, vacúolos.

Pompe disease (PD), also known as glycogenosis type II or acid maltase deficiency, is a rare inherited disease caused by acid alpha-glucosidase (GAA) deficiency. This enzyme participates in the degradation of glycogen into glucose inside muscle fibers. Mutations in the GAA gene induce several degrees of enzyme deficiency, causing glycogen accumulation in muscle fibers lysosomes[1].

The prevalence of PD ranges from 1:40,000 to 1:60,000 and is dependent on ethnic and geographical factors. Compared with other diseases, it is rarely observed in centers for neuromuscular disorders[2,3]. The clinical manifestation varies with age and the degree of enzyme deficiency, and three clinical forms are recognized: infantile or "classic", clinical manifestation present in the first months of life; childhood or "non-classic", clinical manifestation present after the first or second year of life; and adult or late onset, no clear cutoff between these forms[1].

The diagnosis of PD can be confirmed by evaluating GAA activity in a dried blood spot assay or in leukocyte or skin fibroblast cultures in most cases[4]. A molecular analysis can also be used to identify GAA mutations in highly suspected cases[5]. However, for the childhood and adult forms, the clinical symptoms, physical findings and laboratory features may indicate another muscle disorder, such as muscular dystrophy, or a metabolic muscle disorder[1,5,6]. In cases with no family history of PD, muscle biopsy is performed routinely in the diagnostic work-up.

In this study, we analyzed the histological and histochemical findings of muscle biopsy specimens taken from Brazilian PD patients.

METHODS

A retrospective analysis of 4,500 muscle biopsies performed from 1979 to 2012 identified 19 patients with a PD diagnosis confirmed by the deficiency of GAA enzyme activity

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in a dried blood spot assay, leukocytes, muscle tissue or skin fibroblast cultures. In some cases, molecular analyses identified a pathogenic mutation[5]. Consent to analyze the muscle biopsy was obtained for each patient in the outpatient clinic or during patient admission to the hospital. The patients were classified according to the age of symptom onset (Table 1): infantile (6 cases), childhood (4 cases) and adult onset (9 cases). The reason for the muscle biopsy differed between the three groups: infantile form, PD manifestation with severe hypotonia, weakness and heart involvement; childhood form, differential diagnosis with Duchenne muscular dystrophy; and adult form, differential diagnosis with limb-girdle muscular dystrophy. Table 2 shows the results of the laboratory analyses (muscle enzyme levels) and electromyography for all cases prior to the muscle biopsy.

Muscle biopsy was performed at the quadriceps or biceps brachial muscles. In three cases, the muscle biopsy was performed twice due to technical problems. Muscle biopsies were frozen in liquid nitrogen, and fresh-frozen cryostat sections were stained for hematoxylin-eosin, modified Gomori trichrome, oil red O, periodic acid-schiff (PAS), cresyl violet and sirius red. The following histochemical reactions were performed according to standard procedures[7]: ATPase at pH 4.3, 4.6 and 9.4; NADH-tetrazolium reductase; nonspecific esterase; myophosphorylase; acid phosphatase; alkaline phosphatase; succinate dehydrogenase; cytochrome-c oxidase; and adenylate deaminase.

Table 1. Age of onset and disease duration in 19 cases of Pompe disease.

	Infantile	Childhood	Adult onset
Number of cases	6	4	9
Gender			
Males	5	3	5
Females	1	1	4
Age at the time of evaluation	0.54±0.28 (2–10) months	9.5±1.91 (7–11) years	28.22±9.74 (14–40) years
Age of onset	0.18±0.14 (0–4) months	3.0±2.0 (1–5) years	20.80±7.35 (13.8–35) years
Disease duration	0.35±0.23 (1–8) months	6.0±3.5 (2–8) years	7.41±6.56 (0.2–20) years

RESULTS

Table 3 shows the histological and histochemical abnormalities according to the different forms of PD.

The most common abnormalities found included vacuolated muscle fibers, PAS-positive vacuoles and increased acid phosphatase activity in muscle fibers.

Vacuoles were present in almost all cases, but were absent in one adult case. The vacuoles varied in size according to the clinical form and disease duration. In the infantile form, when the biopsy was performed in the first months of life, the vacuoles were small with a large sarcoplasm. In biopsies from several-month-old infants, the vacuoles were large and had replaced most of the sarcoplasm. In the childhood- and adult-onset clinical forms, the vacuoles were smaller than in the infantile form in some cases, allowing a large portion of the fiber to develop with an apparently normal structure. These vacuoles stained positive for PAS, although the vacuoles material were occasionally washed out due to the preparation techniques (alcohol and xylol). The acid phosphatase activity was abnormal in all cases, with focal increased activity in the sarcoplasm or vacuoles or diffuses activity (positive fibers) (Fig 1). Two cases had vacuoles without glycogen, and their GAA levels were below the normal range in the muscle tissue (2.60 and 3.33 nmoles/min/gm, normal 8.13±2.06 nmoles/min/gm). In some of the adult cases, only a few fibers had vacuoles (Figs 2–4).

Other abnormalities were also observed. Most cases had variations of fiber diameter with diffuse fiber atrophy, but it was more common in the infantile and childhood forms. Scattered atrophic fibers were common in the adult form; atrophy of fiber types 1 and 2 was present in all forms, but hypertrophy of

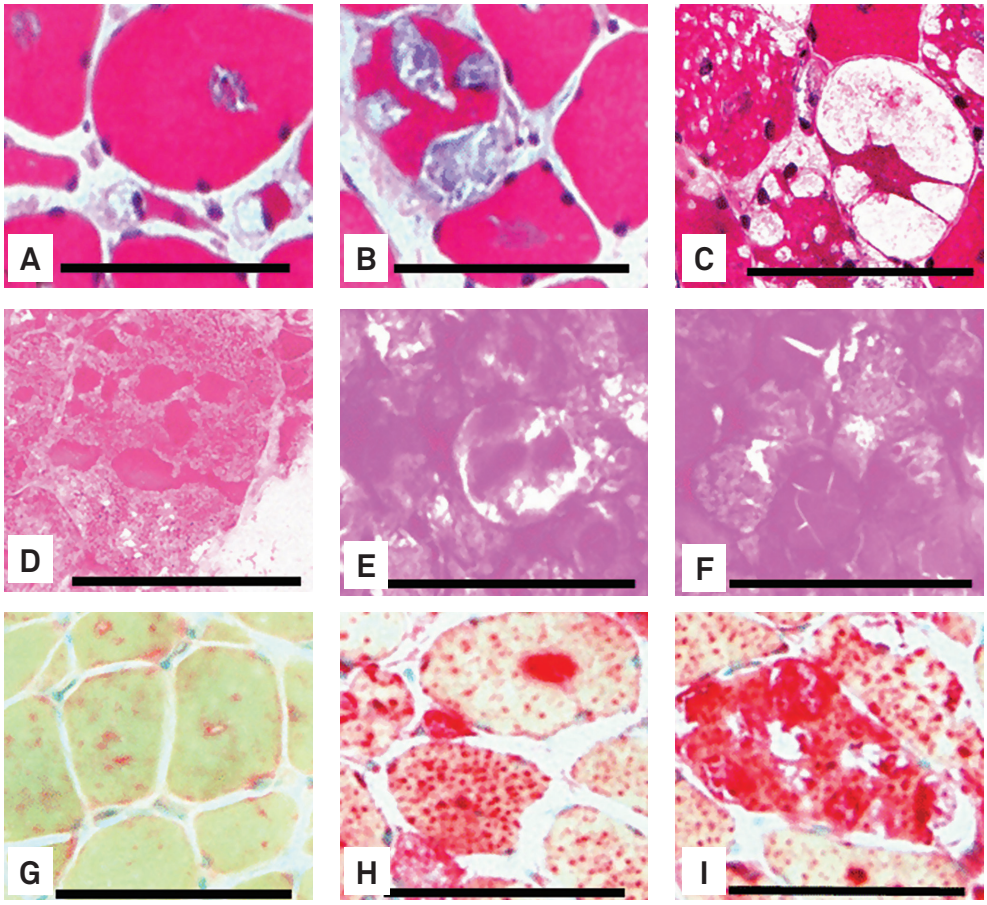
Table 2. Serum muscle enzyme levels and electromyography in 19 cases of Pompe disease.

	Infantile	Childhood	Adult onset
Number of cases	6	4	9
Creatine kinase (Fold increase above normal range)	3.12±2.27 (1–7)	2.50±3.0 (0–6)	13.44±22.39 (0–72)
Lactic dehydrogenase (Fold increase above normal range)	1.54±2.10 (0–5)	0.25±0.50 (0-1)	2.79±3.90 (0–9)
Aldolase (Fold increase above normal range)	3.11±3.12 (0–6)	0.21±0.42 (0–0.83)	2.53±4.67 (0–12)
Aspartate aminotransferase (Fold increase above normal range)	3.05±1.00 (2–4)	2.67±4.61 (0–8)	5.14±8.63 (0–18)
Alanine aminotransferase (Fold increase above normal range)	1.41±0.75 (1–2)	2.67±4.61 (0–8)	0.75±1.06 (0–2)
Electromyography			
Myopathic	2	2	7
Denervation	1	1	1
Mixed	0	0	1
Not performed	3	1	0

Table 3. Histological and histochemical abnormalities identified in muscle biopsies from 19 cases of Pompe disease.*

	Infantile	Childhood	Adult onset
Number of cases	6	4	9
Muscle studied			
Quadriceps	6	3	6
Biceps	0	1	3
HE-Gomori			
Proliferation connective tissue	0	1	2
Adipose tissue infiltration	0	1	2
Variation in fiber diameter	5	4	9
Small group atrophy	0	1	0
Diffuse fiber atrophy	5	4	0
Scattered fiber atrophy	0	0	6
Angulated fiber atrophy	0	0	2
Round atrophic fibers	5	4	7
Fiber hypertrophy	0	0	3
Internal nuclei	2	1	5
Nuclear clumps	0	0	1
Necrosis	1	0	4
Phagocytosis	1	0	2
Fiber splitting	0	0	1
Sarcoplasmic masses	0	1	0
Basophilic fibers	0	0	1
Vacuolated fibers	6	4	8
PAS			
Vacuoles positive	6	2	7
Oil Red O			
Increased lipid drops (type 1 fibers)	0	0	1
Deposit in vacuoles	0	1	0
External deposition	1	0	0
Sirius Red			
Increased endomysial tissue	0	1	3
ATPases			
Type 1 predominance	0	1	2
Type 2 predominance	1	0	0
Types 1 and 2 hypertrophy	0	0	3
Type 1 atrophy	2	2	6
Type 2 atrophy	2	4	7
NADH-Tetrazolium reductase			
Angular atrophic fibers	0	0	3
Focal increase	1	3	2
Moth-eaten	0	1	3
Core like structure	0	1	0
Whirling	0	0	1
Snake coils	0	0	1
Ring fibers	0	0	1
Nonspecific esterase			
Angular atrophic fibers	0	1	4
Interstitial positive mononuclear cells	1	0	0
Acid Phosphatase			
Increase fiber focal activity	6	4	9
Positive fibers	5	1	7
Interstitial mononuclear cells	0	0	1
Macrophages in necrotic fibers	0	0	1
Alkaline Phosphatase			
Positive fibers	0	0	1
Increased interstitial activity	0	0	1
Succinic Dehydrogenase			
Increased in periphery	1	0	1
Focal increase	0	1	1
Cytochrome c-oxidase			
Increased in periphery	1	0	0
Negative fibers	0	1	0

*Only abnormalities that occurred at least once were listed. HE: hematoxylin-eosin; PAS: periodic acid-schiff.



(A–C) Hematoxylin-eosin; (D–F) Periodic acid-schiff; (G–I) Acid phosphatase. Bar 100 micra.

Fig 1. Vacuoles of several size and location, variable amount of glycogen storage and acid phosphatase activity in adult-onset clinical forms of Pompe disease.

fiber types 1 and 2 occurred only in two adult cases. Internal nuclei and necrosis were found in half of the adult cases.

Seventeen of the 19 cases had a histological diagnosis of vacuolar myopathy, 1 with hypertrophy of type 1 and 2 fibers with focal increased acid phosphatase activity and another with chronic and active myopathy with focal increased acid phosphatase activity. These last two cases had a low blood GAA level, one of whom had no deoxyribonucleic acid (DNA) mutation detected.

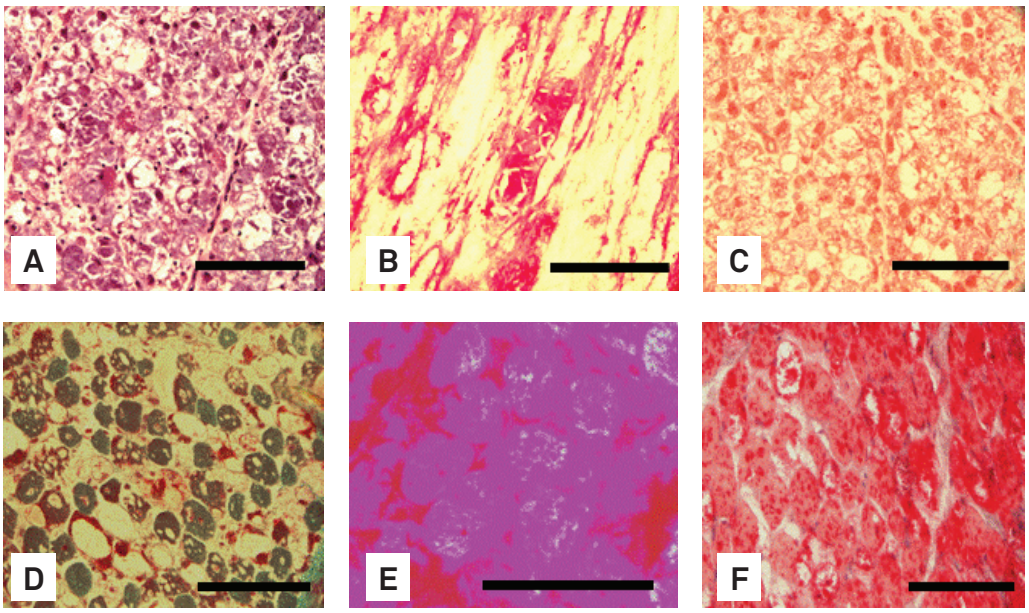
DISCUSSION

In PD, GAA is unable to breakdown glycogen, leading to its accumulation in lysosomes. As the amount of glycogen increases in the lysosomes, they become larger and occasionally rupture their membranes, releasing and dispersing the free glycogen into the sarcoplasm, which displaces and disrupts the contractile apparatus. The glycogen accumulates in the sarcoplasm and causes secondary damage to the muscle cell[8].

Muscle fiber damage of muscle cell is not only by glycogen deposition. The accumulation of glycogen in vacuoles induces an autophagy (self-eating) process of the muscle fiber, with formation of autophagic vacuoles originating from lysosomal degradation. These autophagic vacuoles acts in the sarcoplasm, damaging the muscle fibers, and contains heterogeneous material from the cytoplasm, organelles degradation products, myeloid structures and lipofuccin detected by electron microscopy. These large autophagic vacuoles can occupy more than half of the fiber diameter and interrupt the fiber contractile apparatus[8-10]. Experimentally, in type 1 fibers, the lysosomes are lined up and appear connected and enlarged, which is typical of Pompe disease. However, in type 2 fibers, they are randomly distributed, do not touch and become very large, with the appearance of large autophagic vacuoles[11].

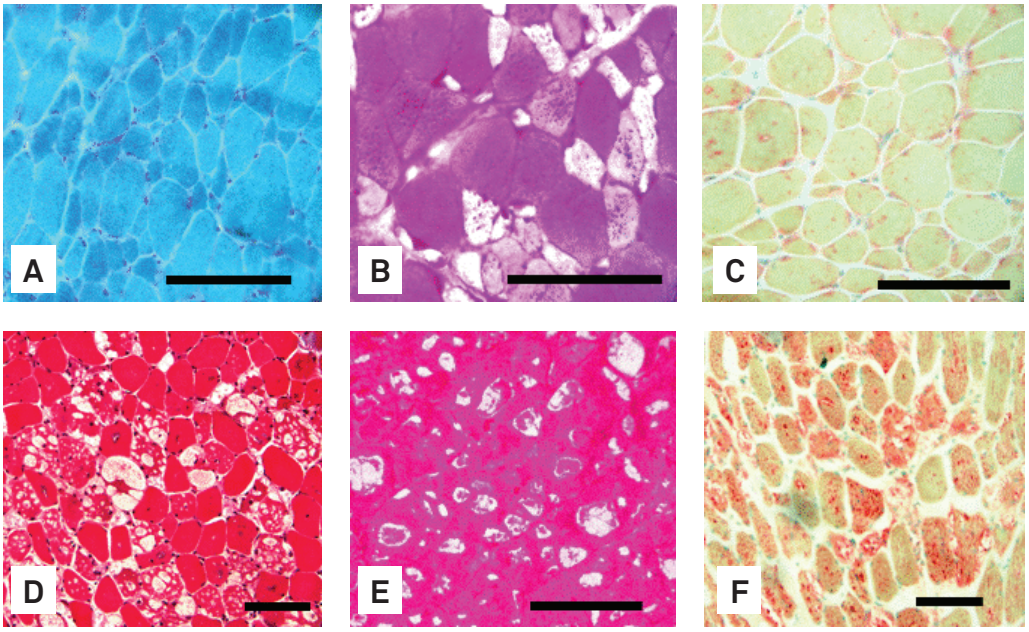
In our patients, the most common abnormalities in the muscle biopsies were vacuolated fibers filled with PAS-positive material (glycogen) and increased acid phosphatase activity, that was focal or diffuse (positive fibers), similar to previous studies[12].

Acid phosphatase is an intralysosomal enzyme that is apparent in muscle biopsy histochemistry reactions due to lysosomal activation. The activated lysosomes in the PD biopsies in our study were identified in the positive fibers (small and numerous), with focal increases (large and giant) and inside the vacuoles or in the vacuole wall (giant). Additionally, the glycogen stored in the vacuoles was detected by the PAS stain, but, in some cases, the vacuoles appeared empty due to artifact fixation and wash out during the histological preparation.



(A) Hematoxylin-eosin; (B and E) Periodic acid-schiff; (C and F) Acid phosphatase; (D) Modified Gomori trichrome. Bar 100 micra.

Fig 2. Pompe disease (infantile form). Most of fibers sarcoplasm replaced by vacuoles filled with glycogen and with high activity of acid phosphatase. (A to C) two months-old; (D to F) ten months-old.



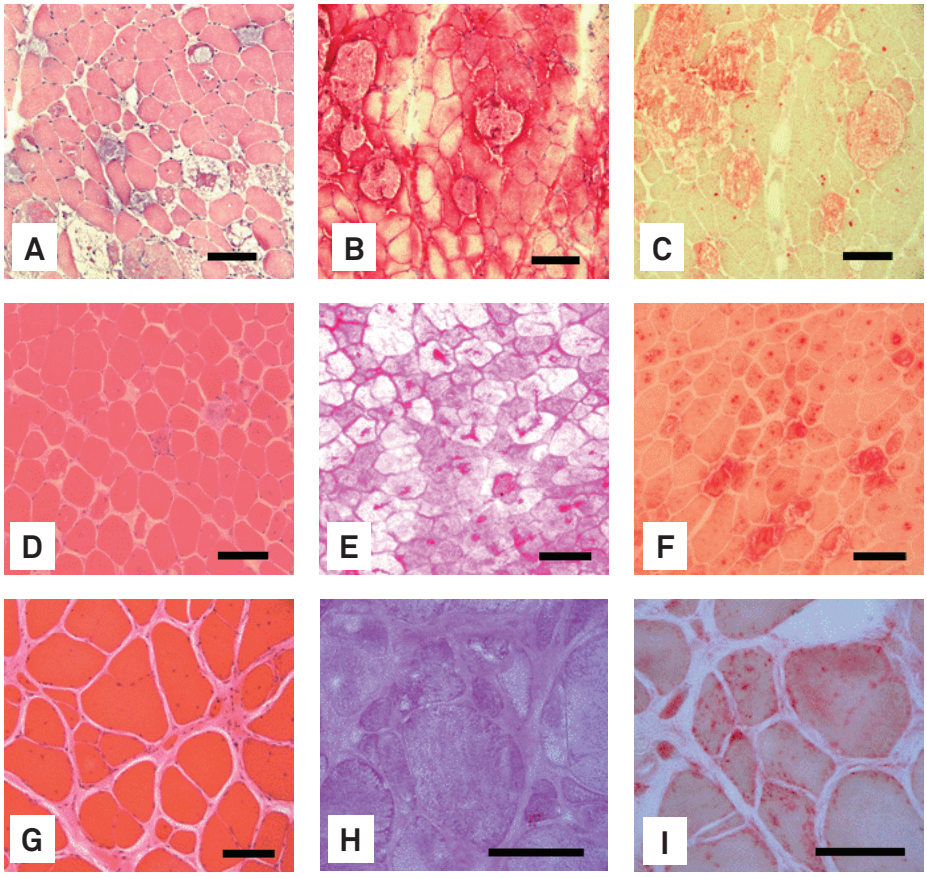
(A) Modified Gomori trichrome; (B and E) Periodic acid-schiff; (C and F) Acid phosphatase; (D) Hematoxylin-eosin. Bar 100 micra.

Fig 3. Pompe disease (childhood form). Variations in fiber diameter, fibers with and without vacuoles (small and large), vacuoles filled with glycogen, increased activity of acid phosphatase (focal or into vacuoles). (A to C) 7 years-old; (D to F) 11 years-old.

The number of vacuolated fibers varies with the clinical type and disease duration. One of our cases had less than 1% vacuolated fibers, and no vacuoles were found in another, which has previously been described. The vacuole frequency has been reported to range from present in nearly all fibers in the infantile form to 75% in the childhood form and 10–50% in adult patients. In infantile cases, the type 1 and 2 fibers are equally involved, while in the childhood and adult forms, the vacuoles are predominant in type 1 fibers in some cases and in type 2 fibers in others[12-14].

The majority of our childhood and adult cases had atrophy of type 1 and 2 fibers, while three of nine adult cases had hypertrophy of both fiber types. These data are similar to those reported in the literature[13].

Muscle biopsy may be requested in cases of suspected PD, but it is not routine. Typically, the histological diagnosis of childhood or adult PD is made during the investigation of Duchenne muscular dystrophy with a normal DNA analysis for dystrophin, limb girdle muscular dystrophy or adult-onset myopathies, as in our cases and previously reported by several authors[1,5,6]. In the past, most infantile cases already had a suspicion of PD because of the young age (months), hypotonia, heart disorder or respiratory insufficiency, but muscle biopsy



(A) Hematoxylin-eosin; (B) Periodic acid-schiff; (C) Acid Phosphatase. Bar 100 micra.

Fig 4. Pompe disease (adult form). Variations in fiber diameter, vacuolated fibers filled with glycogen, increase activity of acid phosphatase (focal, diffuse and positive fibers) and splitting fibers. (A to C) 22 years-old; (D to F) 32 years-old; (G to I) 41 years-old.

was important to confirm the diagnosis. However, muscle biopsy is now infrequently performed in infantile cases because it has been replaced by the determination of GAA activity using a dried blood spot assay that is confirmed in blood leukocytes[4].

Muscle biopsy abnormalities in PD vary with the level of GAA deficiency, the clinical type (which is related to the enzyme level) and the disease duration. The most important findings of our study are as follows: (1) vacuoles with or without glycogen deposition (PAS+); (2) increased acid phosphatase activity, either in vacuoles or focal areas; (3) the adult form has fewer vacuoles in the muscle fibers, and their histological findings resemble limb-girdle muscular dystrophy; (4) focal increases of acid phosphatase activity in the muscle fibers of patients with limb-girdle syndrome raise the possibility of Pompe disease.

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