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Histochemistry and morphometry of Werdnig-Hoffmann disease

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Summary. We report a case of the Werdnig-Hoffmann disease in a 4-month-old male infant. The morphological study revealed perimysial fibrosis, variability in the size of muscle fibers, absence of target fibers, few central nuclei and normality in vessels, nerves and neuromuscular junctions. The morphometrical examination showed the existence of normal-sized and atrophic fibers in both fibrillar types, as well as in hypertrophic type I fibers. The percentage of fibrillar types and the data obtained from the form factor are normal. Random distribution of type I and II muscle fibers were observed.

Key words: Werdnig-Hoffmann disease, Muscle, Histochemistry, Morphometry

Introduction

The Werdnig-Hoffmann disease belongs to the group of spinal muscular atrophies (SMA). It is the most common of all denervations which occur in infancy (less than two years of age) (Carpenter and Karpati, 1984). Swash and Schwartz (1984), however, sustain that the Kugelberg-Welander disease is even more common. All authors consulted maintain that it is inherited with an autosomal recessive pattern and that its pathogenesis is currently unknown.

The heterogenicity of the lesions present in the muscle biopsy, as well as their lack of specificity and in particular that of the group of diseases belonging to the SMA, requires a detailed study of the lesions in order to establish which morphological data can assist diagnosis.

The object of our paper is to present a case of the Werdnig-Hoffmann disease with a histochemical and morphometrical study. We discuss the bibliography consulted, establishing important aspects which serve to arrive at a correct diagnosis.

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Materials and methods

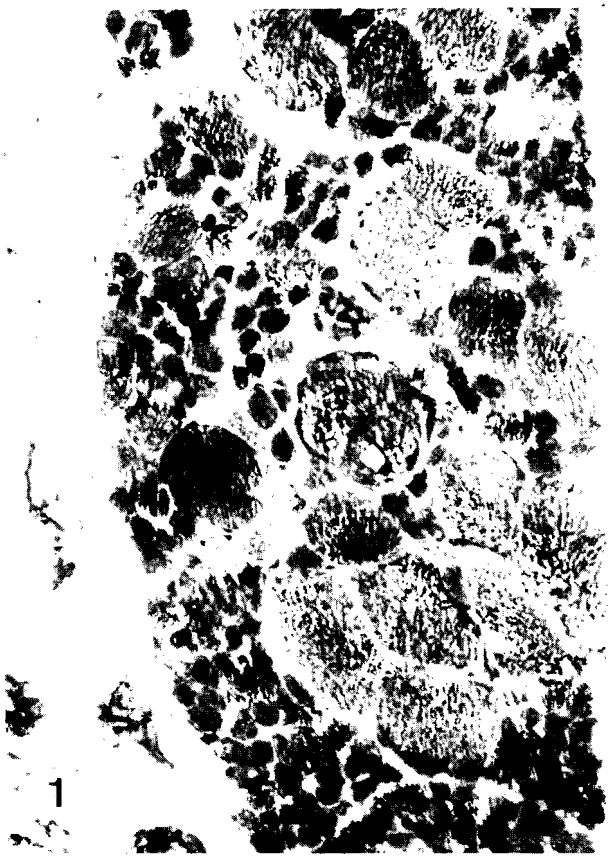
We have studied a case of the Werdnig-Hoffmann disease in a 4-month-old male infant. The muscle biopsy was frozen in isopentane cooled previously with liquid nitrogen at around -160°C. We obtained 6 μm cross sections in a cryostat to which histological techniques (hematoxylin-eosin and modified Gomori trichrome) and histochemical techniques (ATPase at pH 9.4, 4.6 and 4.3, lactic dehydrogenase, succinic dehydrogenase, NADH-tetrazolium reductase, phosphorylase, alkalyne phosphatase and periodic acid Schiff) were applied.

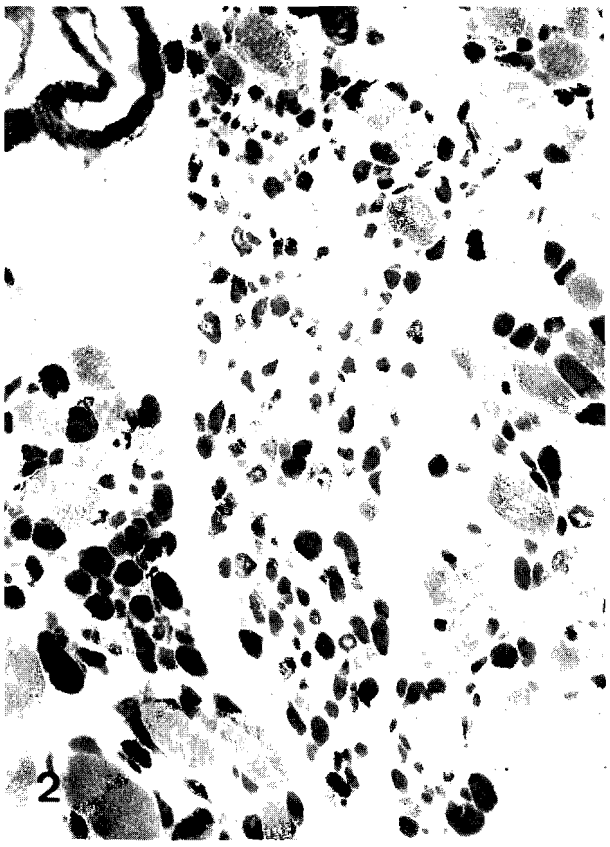
Cross-sections where ATPase activity (pH 9.4) was demonstrated were used for the morphometrical study utilizing at Leitz-A.S.M. semiautomatic image analyzer connected to a Hewlett-Packard 86-A computer. The morphometric parameters chosen were average area, perimeter, minimal diameter, maximal diameter, diameter equivalent circle, form factor ($4\ \pi$ area/perimeter^2) and percentage of fiber types. We also determined the centre of gravity of two muscle fascicles obtaining linking lines between them following the Venema method (1988); the calculation was carried out with the object of determining the presence of random distribution of muscle fibers, their segregation or else grouping of the same fibrillar type ($p < 0.05$).

Results

Microscopic observation of the cross-sections of the muscle biopsy revealed the presence of a fibrosis of interfascicular distribution which affected the whole section. This perimysial proliferation occasionally surrounded a complete fascicle of atrophic fibers which showed a rounding of their contours. The application of histochemical techniques confirmed that these atrophic fibers were both type I and type II (Fig. 1).

On other occasions, within a single fascicle, normal, atrophic and hypertrophic fibers appeared. These last fibers were sometimes two or even three times larger than





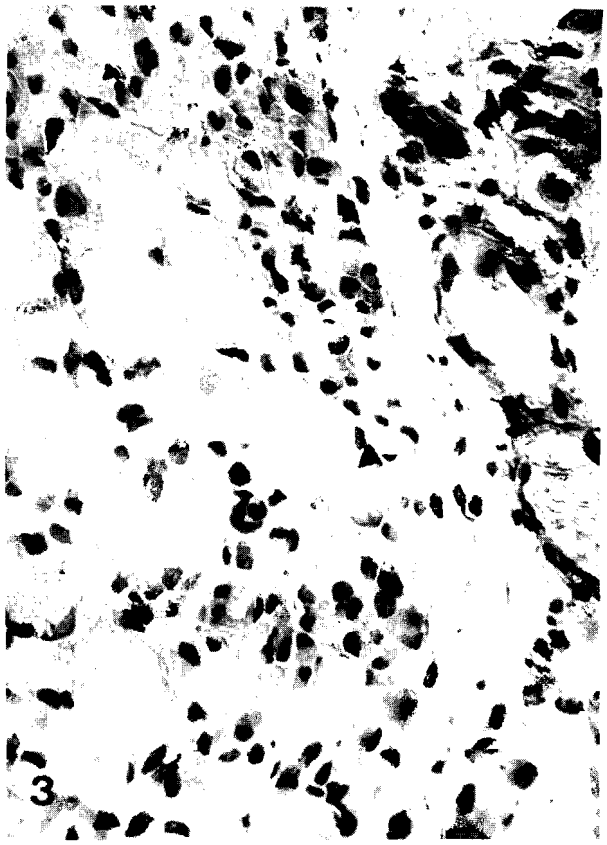


Fig. 1. Numerous atrophic type I and II fibers next to normal-sized fibers. NADH-tr. × 160

Fig. 2. Presence of hypertrophic type I fibers beside atrophic fibers of both types. ATPase at pH 9.4. × 100

Fig. 3. Atrophic fibers with excentric location of myonuclei. H & E. × 160

their normal size. With the application of ATPase, phosphorilase and oxidative enzymatic techniques it became manifest that hypertrophy affected type I fibers (Fig. 2). Other histochemical techniques revealed the existence of a decrease in both PAS positivity and alkaline phosphatase.

We have observed neither fiber type grouping nor target fibers. Nuclear centralizations did not surpass 3% of the total of fibers. Atrophic fibers showed an excentric nucleus (Fig. 3). Vessels, nerves and neuromuscular junctions were normal. Necrosis of muscle fibers appeared on very few occasions.

The morphometrical analysis revealed and confirmed the existence of normal-sized and atrophic fibers of both fiber types, while hypertrophy was apparent only in type I fibers. The minimal diameter of the fibers oscilated with an amplitude of 2.1 μm to 42.3 μm and of 1 μm to 22.5 μm for the type I and II fibers respectively. Similar data were found in the calculation of the maximal diameter and diameter equivalent circle. This last measurement, however, showed a coefficient of variation lower than the minimal and maximal diameter.

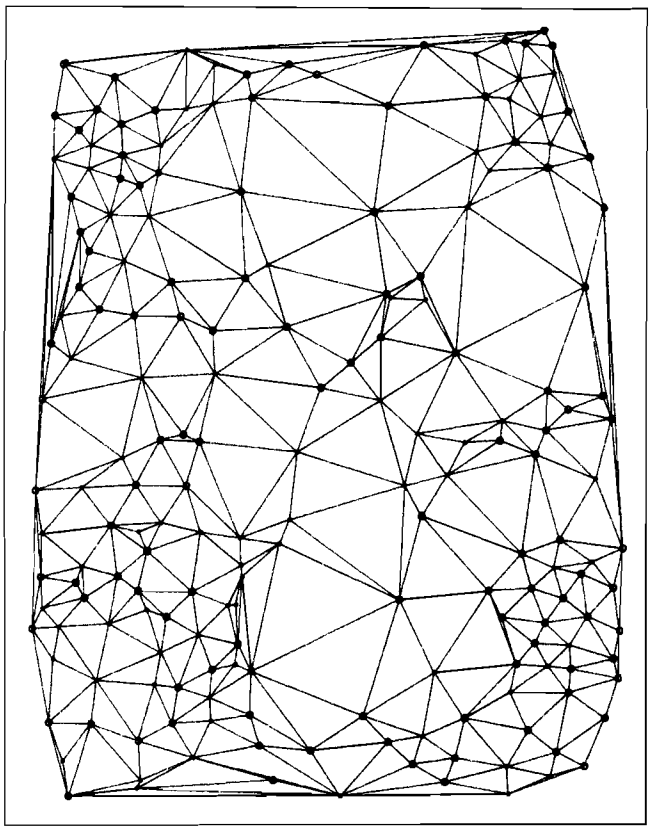


Fig. 4. Schematic representation of the centers of gravity of type I (.) and II (o) fibers. Neighbours are connected by joints.

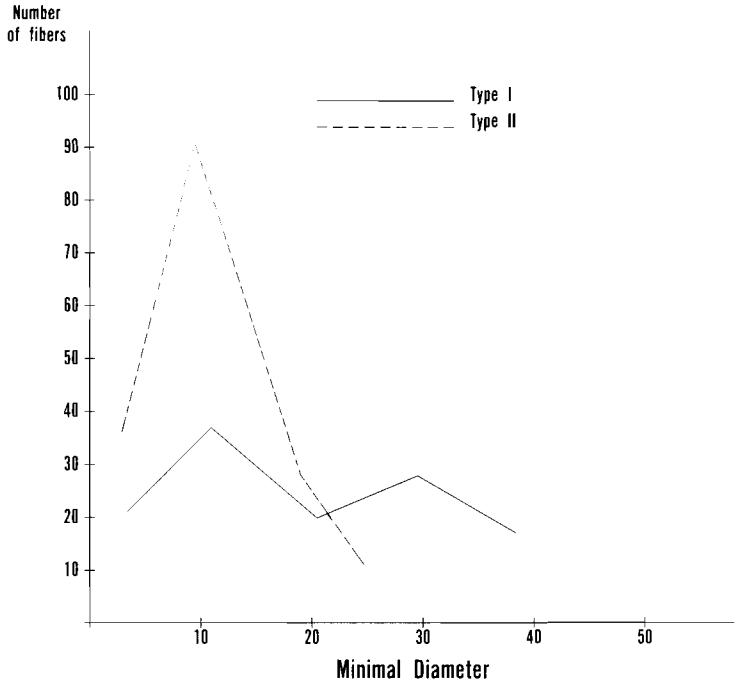


Fig. 5. Histogram obtained in relation to minimal diameter of type I and II fibers.

Table 1. Mean ± standard deviation of mean, coefficient of variation and range of the morphometrical parameters obtained in both fibrillary types.

		Fibers	
		Type I	Type II
Perimeter	$\bar{X}$ ± s.d. (c.v.) Range	81.2 ± 25.1 (30.9) 10.2 — 200	51.0 ± 16.1 (31.6) 8.9 — 104
Area	$\bar{X}$ ± s.d. (c.v.) Range	479.2 ± 180.4 (37.6) 51.9 — 2296	252.1 ± 78.0 (30.9) 23.0 — 662
Form factor	$\bar{X}$ ± s.d. (c.v.) Range	0.86 ± 0.11 (12.8) 0.46 – 0.98	0.83 ± 0.14 (16.8) 0.41 – 0.99
Diameter equivalent circle	$\bar{X}$ ± s.d. (c.v.) Range	23.4 ± 6.1 (26.0) 2.5 — 54	14.8 ± 3.5 (23.6) 1.7 — 29
Maximal diameter	$\bar{X}$ ± s.d. (c.v.) Range	30.5 ± 11.1 (36.4) 4.5 – 47.2	19.2 ± 6.1 (31.8) 4.2 — 41
Minimal diameter	$\bar{X}$ ± s.d. (c.v.) Range	18.0 ± 5.2 (28.9) 2.1 – 42.3	12.8 ± 3.9 (30.5) 1.0 — 22.5
Percentage		41.83	58.17

Table 2. Results of the test to ascertain the distribution of types of muscle fibers.

	Fascicle 1	Fascicle 2	Total
n	80	192	272
n_1	37	77	114
n_2	43	115	158
n_n	220	543	763
n_{11}	53	92	145
n_{12}	126	260	386
n_{22}	41	191	232
A_1 (μ^2)	645	106.4	375.7
A_2 (μ^2)	247	101.7	174.3
A_1/A_2	2.61	1.04	2.15
F_1	0.462	0.401	0.419
En_{12}	110.76	262.23	372.88
$sd(n_{12})$	7.47	11.25	13.50
Z	2.10 / 1.97	–0.15 / –0.24	1.00 / 0.93
$n_{12}(cor)$	124.38	259.62	377.09
Z (cor)	1.88 / 1.75	–0.18 / –0.27	0.34 / 0.27

Table 3. Summary of the findings of various authors in relation to Werdnig-Hoffmann disease.

	SARNAT	SWASH and SCHWARTZ	CARPENTER and KARPATI	CANCILLA	DUBOWITZ and BROOKE	HUGHES	JENNEKENS	Our Results
Fibrosis	Perimysial	Perimysial	Perimysial	Perimysial	Perimysial	Perimysial	Perimysial	Perimysial
Central Nuclei	–	–	Yes	–	No	No	No	No
Pyknotic N.	–	–	–	–	Yes	–	–	No
Atrophy	I and II	I and II	I and II	I and II	I and II	I and II	I and II	I and II
Hypertrophy	I and II	I	I	I and II	I and II	–	I	I
Normal	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Grouping	Possible	Possible	No	–	–	–	–	No
Angulated F.	No	No	No/Yes	No	Occasional	No	No	Occasional
Target F.	–	–	–	–	No	–	No	No
Muscle Spindle	–	Normal	Normal	–	–	–	–	–
Motor end-plates	–	Atrophy	–	–	–	Normal	Normal	–
Nerves	–	Normal	Myelin deg.	–	–	–	–	–
Necrosis	–	–	–	–	No	–	No	No

The morphometrical data are shown in Table I. We may observe that type I fibers are more rounded than those of type II although there are no significant differences when comparing form factor results of both. With relation to the percentage of muscle fiber types we observed that this was found to be within the normal values.

In order to try to give a diagnostic value to these results we have calculated the coefficient of variation of type II fibers and the ratio between the average area of fiber types II and I, which, according to Cornelisse et al. (1980), are the best parameters for differentiating pathological biopsies from normal ones. In this respect our results indicate the pathological character of the biopsy.

In Table II we give the data obtained with relation to the distribution of muscle fiber types, represented schematically in Fig. 4. By way of parameter Z(c), we can affirm, morphometrically, that there is a random distribution of the two muscle fiber types.

Furthemore, in the histogram in Fig. 5 the bi-modal distribution of type I fibers was observed. We were unable to determine the atrophic and hypertrophic factors of both fiber types due to the age of the patient.

Discussion

The data obtained from the authors consulted on the histological characteristics of the Werdnig-Hoffmann disease has been collected in Table III. The histological and morphometrical changes observed in our study revealed a manifest similitude with those described by other authors (Jennekens, 1982; Swash and Schwartz, 1984).

The perimysial fibrosis and atrophy of fiber types I and II, the existence of normal-sized fibers, plus hypertrophy of type I fibers, as well as, according to some authors (Dubowitz and Brooke, 1973; Sarnat, 1983; Bennington and Krupp, 1984), hypertrophy of type II fibers are data of paramount relevance. The lack of fiber type grouping as well as the absence of both target fibers and angulated fibers does not justify labelling this lesion as a myopathic disease. We have encountered neither necrosis nor regeneration nor fiber splitting nor values above 3% in the number of nuclear centralizations. Nor have we observed muscle fibers that give a positive reaction to alkaline phosphatase which according to Carpenter and Karpati (1984), tend to be present in the deep region; apart from this, Sarnat (1983) indicates that this positive reaction represents the only data appearing in the Werdnig-Hoffmann disease that is rarely seen in other diseases of neurogenic-myopathic origin.

Our morphometrical data can be compared with the Fenichel and Engel data (1963), although the existing information is too scarce to be able to establish the degree of severity of the morphometrical values encountered.

Despite this, the application of the Cornelisse et al. method (1980) allows us to catalogue our biopsy as pathological. The value of morphometry is, therefore, assured.

From the morphometrical point of view, our case is characterized by hypertrophy of type I fibers, as well as atrophy of both fiber types. In spite of being unable to calculate the atrophy and hypertrophy factors established by Brooke and Engel (1969) due to the age of the patient these same authors collected the average standard values of fiber size in children from 3 months to 13 years. Comparing those values with our own allows us to apply the term hypertrophy even without the factorial calculation.

Our results indicate an abnormal variability in the size of muscle fibers as a result of the existence of the coefficient of variation above 250 (Dubowitz and Brooke, 1973). The histogram plotted in Fig. 5 shows that this is not due to a uniform reduction or increase in fibrillar size. For Bennington and Krupp (1984) the increase in variability is often encountered in myopathic diseases and helps to differentiate these diseases from the denervating ones which are generally characterized by two peaks on the histogram. In our case, the histogram of type I fibers revealed their bi-modal distribution.

We have encountered no predominating fiber type, nor have we found any fiber type grouping, using the latter the Jennekens et al. method (1971). At least 12 fibers of the same type are needed to form fiber type grouping. The disadvantage of this method appears when any fiber type is predominant. For this reason, we have also applied the Venema method (1988) in which not only the percentage of the fiber types is taken into account but also the average size of the muscle fibers. These two factors enable us to make an important correction in the definition of the said grouping. Furthemore, this method does not only discriminate between muscle fibers which present type grouping in random distribution amongst themselves, but can also result in a third type of fibrillar association following a pattern of segregation. Venema (1988) bases his findings on the number of neighbouring pairs of fibers; in the grouping pattern he finds a greater number of like neighbouring pairs of fiber in relation to the random distribution and, consequently, a smaller number of unlike neighbouring pairs of fiber.

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