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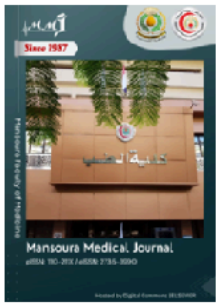

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MUSCLE INVOLVEMENT IN WERDING-HOFFMAN DISEASE: LIGHT AND ELECTRON MICRODCOPIC STUDY

Ibrahim Hassan Alzahrani

Consultant Pathologist & Assistant Professor Department of Pathology Faculty of Medicine King Abdulaziz University Jeddah, Kingdom of Saudi Arabia

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By

Ibrahim Hassan Alzahrani, Frcpath (UK)

From

Consultant Pathologist & Assistant Professor

Department of Pathology Faculty of Medicine

King Abdulaziz University

Jeddah, Kingdom of Saudi Arabia

ABSTRACT

The present study included muscle biopsies from 12 infants (5 females and 7 males) with Werding-Hoffman's disease (WHD). The mean age of presentation was 11.8 months. History of consanguinity was detected in 58.3%(7/12) of cases. The infants were presented with typical floppy baby pattern since birth or early in first few months of life. All had normal serum creatine phosphokinase level. The presence of large groups of atrophied small fibres and large hypertrophied muscle fibres is the classical microscopic picture. The histochemistry showed that the atrophied and the hypertrophied muscle fibres are of type I and type II. The electron microscopy showed disorganized Z bands in the atrophied muscle fibres. the diagnosis of WHD needs correlation of the clinical picture, serum creatine phosphokinase, the elecromyography, the light microscopy, enzyme histochemistry and the electron microscopy.

INTRODUCTION

Werding-Hoffman disease (WHD), though rare, is the most commonly lethal congenital recessive muscular dystrophy (CMD). It is the acute infantile form of spinal muscular dystrophy or atrophy (SMA type 1) [1,2]. It may presents in utero, at birth or in the early few months of life. It is one of the causes of the floppy baby syndrome. Its prognosis is very poor as 95% of infants are dead by the age of 18 to 24 months [3; 4]. In addition to progressive hypotonia and weakness

of the limbs, there may be arthrogryposis multiplex (deformed rigid joints associated with muscular weakness), bulbar signs and respiratory problems due to affection of the intercostal muscles with terminal respiratory infection[5]. Walker-Warburg syndrome is similar in pathology and pathogenesis to WHD but its onset is late and henc, its prognosis is better [6, 7].

Some authores [8] confirmed by autopsy studies on infants died from WHD that the basic etiology is the loss of the anterior horn cells with gliosis and neuronophagia at all levels of the spinal cord.

Recent opinions concluded that CMD proved by muscle biopsy is a valuable clinical clue necessary for the diagnosis of WHD along with the clinical and electromyographic changes [5; 9].

The muscle biopsy reveals large groups of atrophic rounded muscle fibers due to wide spread denervation (10). However there was a disagreement concerning the type of muscle fibers affected by the disease [11 & 12].

The aim of the present work is to highlight some of the morphological features of this serious disease by light and electron microscopic examination to alert the attention to its existence and to search for the type of muscle fibres affected by the disease.

MATERIAL AND METHODS

The present study included 12 cases of muscle biopsies of infants . Eight of them were collected from the files of the department of Pathology, King Abdul Aziz University Hospital in the period of 1983 to 1991 and 4 cases were prospectively selected during the period of 1991-1993. All the slides(transverse and longitudinal sections) and paraffin blocks of the eight cases were retrieved. The following stains were used routinely for muscle biopsy: heamtoxylin and eosin, Masson's trichrome, phospotungistic acid hematoxylin(PTAH) and periodic acid Schiff with and without diastase pretreatment for glycogen.

All the biopsies were taken from the quadriceps muscle. For the prospectively selected cases, part of the fresh stretched muscle biopsy was fixed in buffered formalin for light microscopy, part was taken for enzyme histochemistry and a smaller part for

electron microscopy. For the 8 retrospective cases, the paraffin embedded tissue was used for E/M by deparaffinization in xylol, rehydration in descending grades of alcohol up to water and processed as a fresh specimen for E/M. Both the fresh and the deparaffinnized tissue was fixed in 3% gluteraldehyde, postfixed with 2% osmium tetroxide and embedded in epon LX-112 resin(Ladd, Burlington, VT) for electron microscopy. Ultrasections were cut and stained with uranyl acetate and lead citrate and examined with Phillipse electron microscope.

For enzyme histochemistry, the small fresh specimen was taken and kept in liquid nitrogen and cryostate sections were stained for the following enzymes (13):

nicotin adenine dinucleotide tetrazolium reductase (NADH-TR) as a mitochondrial stain (Type I rich mitochondria is dark and type II is pale) and myosin adenosine triphosphatase (ATPase) at pH 9.4 where fibre type I is light and type II is dark.

Serum sample was taken for creatine phosphokinase enzyme for the twelve cases.

RESULTS

Out of the twelve infants involved in this study, 7 were males and 5 were females. The age of presentation ranged between 6 and 19 months with a mean age of 11.8 months. History of consanguinity was positive in the parents of 4/7 males and 3/5 females with a total percentage of 58.3%

All the babies were presented with a typical clinical picture of WHD specially the floppy baby appearance with frog posture and internal rotation of the arms with spared facial muscles and occasional muscle fibrillation. This clinical picture was confirmed by the EMG which showed features of denervation of central origin (i.e. anterior horn cell degeneration). The serum creatine phosphokinase was normal in all specimens.

The muscle biopsy showed the classical pathology of WHD where the histological changes were similar in all cases(Fig. 1). The internal architecture of the muscle fibres including glycogen contents (Fig. 2) and cross striations were preserved(Fig. 3). The relation of epi- and perimysium was undisturbed. Regarding the size of muscle fibres, there were three sizes

of muscle fibres, small(atrophic), normal (in relation to age) and large (hypertrophied) muscle fibres(Fig 1). The atrophic fibres were rounded having circular outline and rarely to be angulated. All the biopsies showed these atrophic fibres arranged in large groups. These fibres may be small and narrow as to be little more than chains of sarcolemmal nuclei in thin tortous threads of sarcoplasm. These was interspersed by fascicles containing markedly hypertrophied muscle fibres which were up to three to four times the normal size for the patient's age. The muscle spindle was prominent(Fig. 4). The enzyme histochemistry of both small and large fibres proved to be of type I and type II by(ATP-ase) and (NADH-TR) in 8/12 of cases(75%). The type I was identified by its dark colour due its richness in mitochondria by NADH-TR(Fig. 5) and it plae colour by ATP-aseon pH 9.4(Fig 6). The type II was identified by its pale colour by NADH-TR (Fig. 7) due to its poor content of mitochondria and its dark colour by ATP-ase (Fig. 8)due to its high content of ATP.

The nuclear changes are relatively rare in these biopsies. These changes were limited to the occurrence of occasional pyknotic nuclei in the atrophic fibres. The nuclei in the normal and hypertrophied muscle fibres are usually normal in appearance and location. Occasional internal nuclei were seen in two cases.There was increased amount of premysial and endomysial connective tissue. Fibrosis was present in 10/12 of cases. It was mostly present between the fascicles(Fig. 9).

The electron microscopy showed that the small muscle cell diameter varied in size between 8 to 22 um and the large fibres between 45 to 125 um. In many muscle fibres, the sarcomere was disorganized and the Z line was disrupted or twisted or zigzag in appearance(Fig.10). The plasma membrane was mostly preserved (10/12 of cases) but it was occasionally discontinuous or absent for a short segment in two cases. In 7/12 of cases, there was areas contained small pads of glycogen granules and small or large fat vacuoles(Fig.11) and dilated vesicles of rough endoplasmic reticulum. The mitochondria in the atrophic fibres in 8/12 of the cases were normal while in 4/12 of cases, the mitochondria were swollen. The mitochondria in the hypertrophic fibres were in-

creased and enlarged (Fig. 12). The outstanding feature is the increased amount of collagen fibrils between the muscle fibres(Fig10). The collagen fibres were sometimes packed in bundles and other times randomly dispersed or applied upon the sarcolemma. In two cases, the collagen fibres were closley related to unmylentaed nerve processes under the sarcolemmal membrane(Fig. 13).

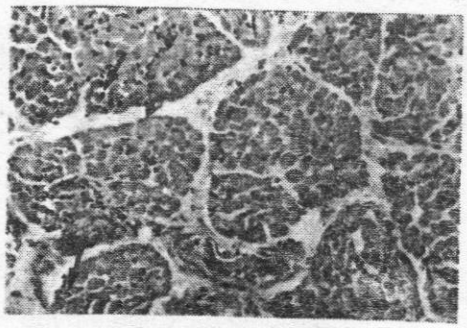

Figure 1. Muscle involvement of WHD showing group atrophy of muscle fibres with atrophic (arrow) and occasional hypertrophic (arrow head) fibres. H&E stain, original magnification X 10).

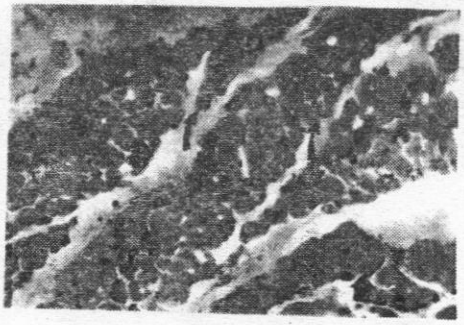

Figure 2. Atrophic and hypertrophic muscle fibres in WHD having good amount of glycogen and eccentric nuclei. (PAS stain, original magnification X 20).

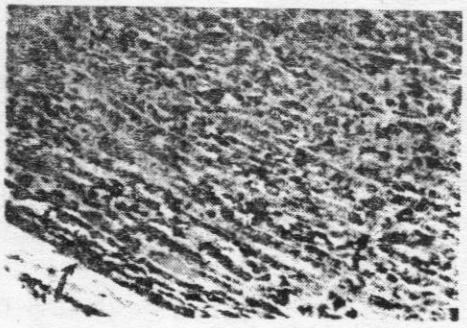

Figure 3. Longitudinal section of muscle fibres in WHD showing thick(arrow) and thin (thick arrow) muscle fibres with preserved cross striations and small and large nuclei. (PTAH stain, original magnification X 10)

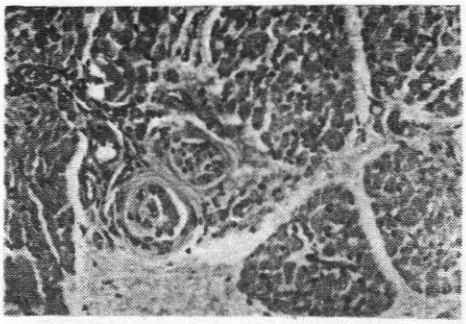

Figure 4. Prominent muscle spindle (arrow) surrounded by atrophied and hypertrophied muscle fibres . (H&E stain, original magnifiFigure 6. Atrophied muscle fibres are mostly dark (type I) with occasinal pale fibres (type II) and the hypertrophic fibres are mostly pale (type II) with occasional dark fibres (type I). (ATP-ase stain, original magnification X 10).

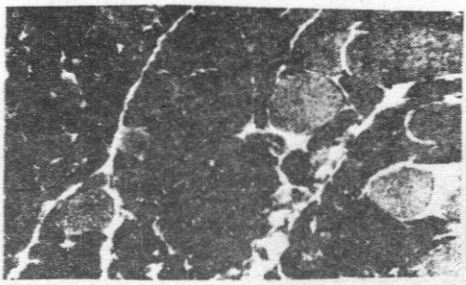

Figure 5. The atrophied muscle fibres are dark (type I) with occasional pale hypertrophied fibres (type II). (NADH-TR stain, original magnification X 20)

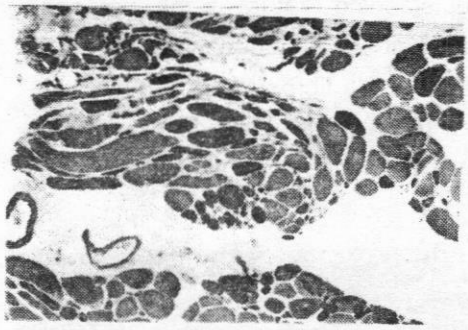

Figure 6. Atrophied muscle fibres are mostly dark (type I) with occasinal pale fibres (type II) and the hypertrophic fibres are mostly pale (type II) with occasional dark fibres (type I). (ATP-ase stain, original magnification X 10)

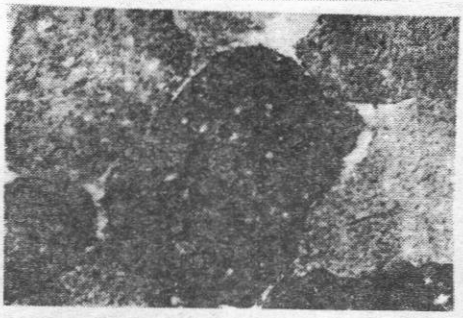

Figure 7. Hypertrophied muscle fibres of both dark (Type I) and pale(type II) staining. (NADH-TR stain, original magnification X 20)

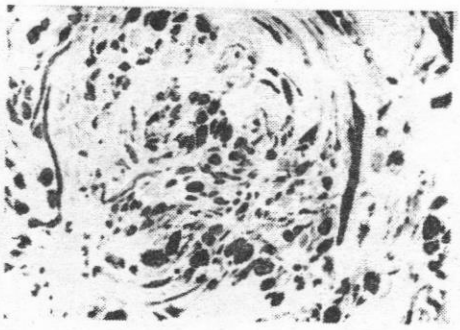

Figure 8. Muscle involvement by WHD showing dark (typeII) atrophied and hypertrophied muscle fbres as well as pale (type I) atrophied and hypertrophied fibres. (ATP-ase stain, original magnification X 10)

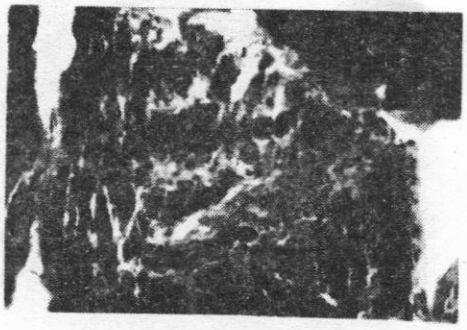

Figure 9. Increased fibrous tissue seperating the atrophied group of muscle fibres with few hypertrophied fibres. (Masson's tricrome stain, original magnification X 10)

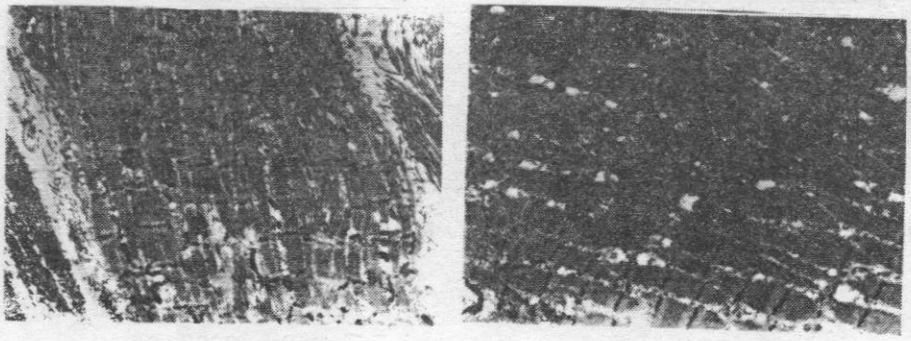

Figure 10. Atrophied muscle fibres showing zigzag like appearance of the Z line. A normal muscle for comparison. (E/M, original magnification X8900).

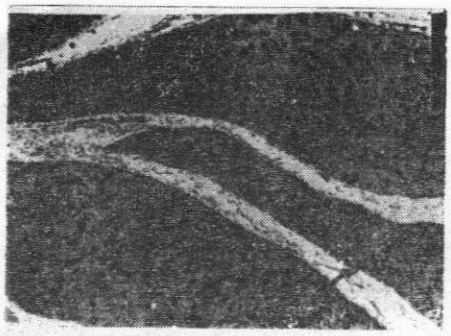

Figure 11. Atrophied muscle fibres with focally interrupted sarcolemmal membrane(arrow) (E/M, original magnification X8900).

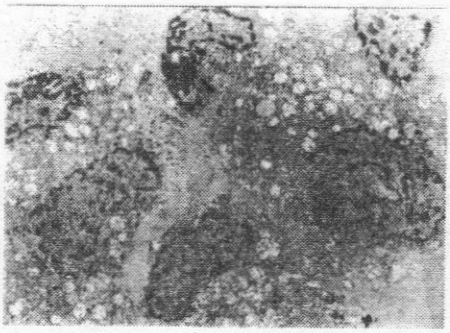

Figure 13. Prominent mitochondria in an hyperophied mucle fibre of WHD. (E/M, original magnification X8900)

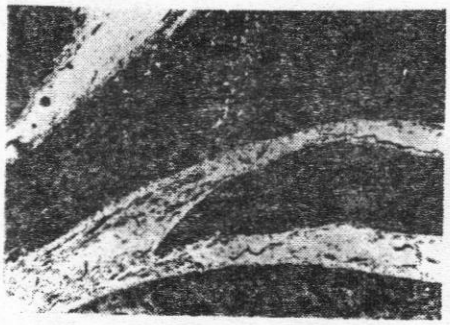

Figure 12. Small darke glycogen granules and fat vacuoles in atrophied muscle fibres of WHD. (E/M, original magnification X8900).

Figure 14. Coarse collagen fibres seperating the muscle fibres. (E/M, original magnification 8900).

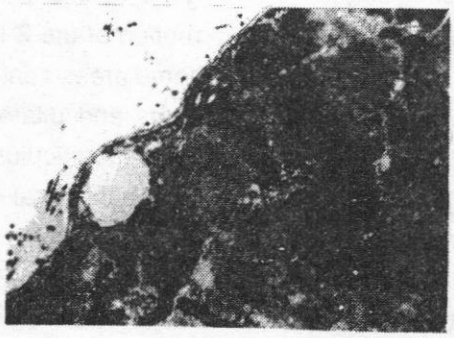

Figure 15. Unmylenated figures in atrophic muscle fibres with interrupted Z lines. (E/M, original magnification 8900) cation X 10).

DISCUSSION.

The importance of muscle biopsy in the diagnosis of Werding-Hoffman's disease or spinal muscular atrophy type 1 (SMA 1) was recommended by many authors [9,14,15].

The positive history of consanguinity of parents in 58.3% of cases supports the view of others [9] that WHD and its mild form (Walker-Werburg syndrome) are of autosomal recessive inheritance. The histological features as described in the present work does not differ very much from that described by others [10,12] who reported that the presence of large groups of atrophic fibres due to wide spread denervation and hypertrophy of the remaining innervated fibres. Also, there was bands of fibrous tissue separating the groups of muscle fibres and not around the muscle fibres itself. The muscle spindle was prominent and relatively increased in number. It was similar to the observation of others [8] who observed up to 9 muscle spindle in one section. They added that the intrafusal fibres of the spindles appear normal on histological and histochemical assesment.

The typing of atrophied and hypertrophied fibres was controversial. Although others (16) reported that the extremely atrophic fibres were

type I. and all the normal fibres were type II. Other author [13, 17] disagreed with them on that. He reported that the small fibres were of both types. From this he concluded that the onset of the disease in utero is usually after the twenty sixth week of fetal life when fiber type differentiation occurs. The histochemical study in the present work is in support of the view of that the small fibres were mostly of both types(I & II) [13, 17].

Since the abnormally hypertrophied fibres tended to be either dark or light by ATP-ase, some considered them as to be of type I origin [16] whereas others reported that those muscle fibres were of type II origin [8,13]. Still others reported them to be of both types [17]. The uniformity of the histochemical staining in these large fibres was the clue for the suggestion that these large muscle fibers are not normal fibers but they were reinnervated fibres by the sprouting of the surrounding nerve fibers [8]. The hypertrophy may be due to the overfunction of these fibres to compensate for the function of the weak atrophic fibres. The results in the present work is in full agreement with others [8,13,17, 18].

The E/M findings were similar to those reported by others [2,9,19, 20] They reported atrophic fibres, disorganization of the sarcomere and disruption of the Z line. The presence of some areas cont aining glycogen granules and dilated vesicles of endoplasmic reticulum was described in other congenital muscular dystrophies as Duchenne muscular dystrophy(DMD) and Fukuyama's perimysial fibrosis [4,21,22]. The histological similarities include endomysial and perimysial fibrosis, increased adipose tissue and variability in the size of the muscle fibres. So it is evident that the diagnosis of WHD needs correlation of the clinical picture, serum creatine phosphokinase, the elecromyography, the light microscopy, enzyme histochemistry and the electron microscopy.

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