ULTRASTRUCTURE OF THE LIVER IN WILSON'S DISEASE

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SUMMARY: Ultrastrustural changes in the liver parenchymal cells were observed in the liver biopsy specimen taken from a child patient suffering from Wilson's disease which was diagnosed on the basis of laboratory and clinical grounds. A variety of peculiar degenerative changes at the level of organelles, in particular, the mitochondria have been observed in the electron microscobical examination of liver tissue. In addition, the presence of lipofuscin granules have been described in the hepatocytes as a characteristic feature.

Key Words: Wilson's disease, ultrastructural findings, mitochondria, lipofuscin granules.

INTRODUCTION

Wilson's disease or hepatolenticular degeneration is an inherited abnormality of copper metabolism, with copper retention attributable to inadequate hepatic excretion in childhood or early adult life (2). The most common mode of presentation is a neuropsychiatric disorder; hepatic disorders are second in frequency. Copper is deposited in many organs; in the liver and brain, these deposits cause damage that, if untreated, become severe enough to cause death (4,5,7).

The present paper deals with the electron microscopical examination of liver in Wilson's disease which is a worthwhile additional tool especially for the early diagnosis of the disease.

MATERIALS AND METHODS

An eight year old girl was sick with epistaxis, night blindness, icterus, pallor, loss of weight and dark urine and light colored (Hacettpe Children's Hospital, protocol number: 1035577). Physical examination revealed a mild hepatosplenomegaly. The neurological examination was normal and no Kayser-Fleischer rings were visible.

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The patient underwent a liver biopsy. On the basis of physical examination, laboratory findings and light microscopic findings of the liver parenchyma, the diagnosis of Wilson's disease was made.

Liver biopsy specimen was immediately fixed in 2.5% gluteradldehyde in phosphate buffer, 0.1 M. pH 7.4, at 4 contigrade for

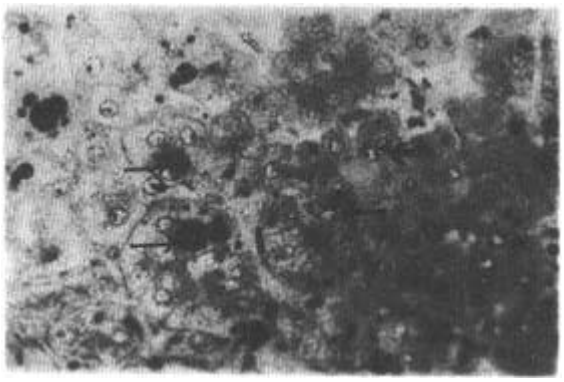

Figure 1: Photomicrograph of Wilson's diseased liver stained toluidin blue. Deeply stained asmiophilic bodies (arrows) in the cytoplasm correspond to the large lipid droplets. A few of the cells are binuclei (large arrows). Liver cells are swollen, but cord structure is preserved. X1250

1.5 hours, washed in the same buffer, then post-fixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol and embedded in araldite (CY 212) (15). Semi thin section (1 or 2 micrometre thick) were out on a LKB piramitome and stained with 1% toluidin blue.

Ultrathin sections, (50-60 nm thick) were out on a LKB ultramicrotome and stained with lead citrate and uranyl acetate and examined in a Zeiss EM 9S electron microscope.

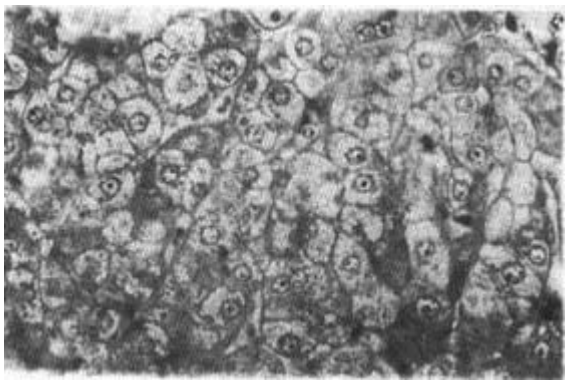

Figure 2: Light micrograph obtained from the thick sections of the biopsy specimen of the liver. Liver cord structure is normal. Black lipid droplets are not found in the cytoplasm of the liver cells as Figure 1. There are seen numerous empty spaces in the cytoplasm. X1250

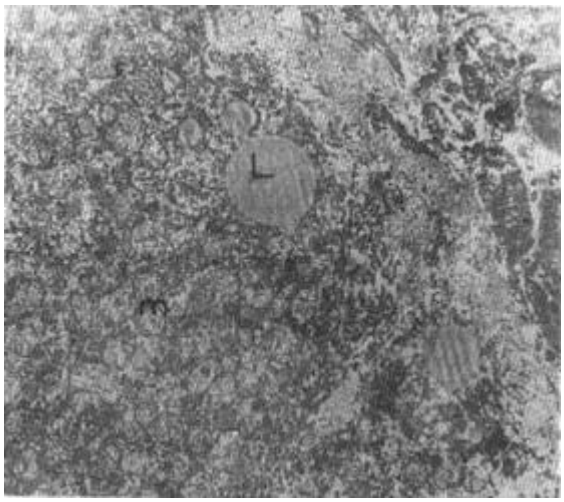

Figure 3: Liver biopsy specimen from affected Wilson's disease. Electron micrograph of hepatocyte showing degerating mitochondria (m). There are some lipid droplests (L) in the cytoplasm of this hepatocyte. Uranyl acetate and lead citrate. X12000

RESULTS

Histologic examination of thick sections of liver specimens showed that liver cells were swollen, but cord structure was preserved. Large lipid droplets were increased (Figure 1) in some while lipid droplets were not found in some other liver cell groups (Figure 2).

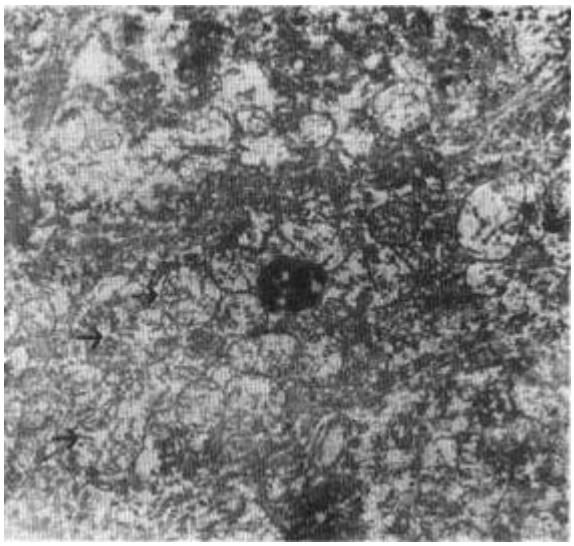

Figure 4: Electron micrograph of liver biopsy specimen from Wilson's diseased liver. Toxic changes of copper are apparent in diated smooth reticulum (arrows) and degeneration of mitochondria. A few lipofuscin granules (Li) are also seen in the center of the figure. X18 800

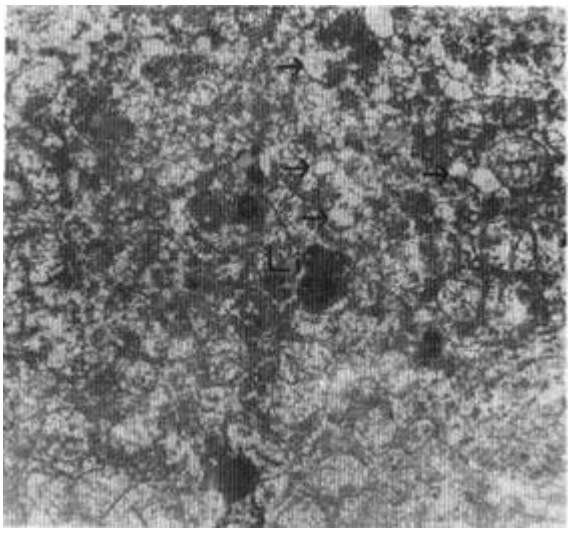

Figure 5: Electron micrograph of liver biopsy specimen obtained from liver. Completely degerated and swollen mitochondria are observed in the cytoplasm (arrows). Only one lipofuscin granule is also seen in the center of the micrograph. Cytoplasma has a fibros character. X18 800

The characteristic changes of copper toxicity in the liver were best seen in the electron micrographs of the liver (Figure 3,4). The smooth endoplasmic reticulum appeared vesicular and irregularly dilated (Figure 4). Degenerative changes were pronounced in mitochondria, most of which were found to be swollen and irregular. Large, faintly osmiophilic lipid droplets were present, as was observed by light microscopy (Figures 1,3).

Lysosomes were increased in number and mitochondria could sometimes be seen within phagolysosomes (Figures 4,5). There were prominent phagolysosomal residuel bodies.

DISCUSSION

Wilson described the disease that bears his name, hepatolenticular degeneration, in 1912 (20). He established the familial nature of the disease; its typical clinical characteristics of a movement disorder, often with mental symptoms, associated with liver disease its progressive and fatal outcome; and its characteristic pathology of bilateral symmetrical softening in the lenticuilar nucleus (putamen and ghlobus pallidus) with cirrhosis of the liver (1). Since than much more has been uncovered about this remarkable condition (8,9,12).

The disease is hereditary and many family members are asymptomatic (13,14). Its immediate cause is copper poisoning, due to failure to excrete copper in bile, so it accumulates in liver (18). In untreated patients with Wilson's disease the hepatic copper concentration is generally markedly increased. It is generally in excess of 250 microgram/g dry tissue (normal less than 50 microgram /g), or greater than 50 microgram/g wet weight (normal less than 10 microgram/g) (12, 21).

With time, liver damage occurs due to copper intoxication. The characteristic changes of copper toxicity in the liver cells are best seen in mitochondria, lysosomes (3,4,10,16).

Where there is doubt after physical examination and biochemical investigation, that liver biopsy is necessary. Histological liver abnormalities in Wilson's disease are fatty metamorphosis, fibrosis and cirrhosis (6, 19). In our case, histological examination of liver specimens showed mild fatty infiltration of hepatocytes. This is a common findings during the early stages of Wilson's disease (7, 19). Therefore, our case might be the early stages of Wilson's disease.

The morphological changes associated with accumulation of excess liver copper are consisted of (1) variation in size and shape of mitochondria, many of which appeared degenerate; (2) dilation of smooth endoplasmic reticulum; and (3) greatly enhanced autophagolysosomal activity with digestion of mitochondria and accelerated production of lipofuscin residual bodies (10). Lipid deposition was prominent only in the untreated affected patients.

In our case, the swollen and degenerated abnormal mitochondira were numerous in the cytoplasm of the liver cells. It was also seen that mitochondria in some cells were completely degenerated and emptied. In the present study, phagosomal activity and residual pigment deposition were also increased. Steatosis were frequently seen in hepatocytes.

It has been suggested that Wilson's disease might be the result of a lysosomal defect (18). Liver fractionation studies have shown copper in increasing concentration in cytosol, in the microsomal fraction (which corresponds to the endoplasmic reticulum and Golgi apparatus) and in the mitochondria and the lysosomes. The total fractional copper content in a patient with Wilson's disease when compared with a normal control was found to be increased by fourfold in the cytosol, by fivefold in the microsomes, by 15 times in the mitochondria, and by 43 times in the lysosomal fraction (10). These determinations indicate that the copper is incorporated and retained in the residual bodies with membrane phospholipid.

Our case demonstrate that the accepted ultrastructural characteristic changes of Wilson's disease may be present in the hepatocytes while the liver cells appear normal contours on light microscope. Therefore, it is worthwhile to add electron microscopic examination of liver tissue as a tool for the early diagnosis of Wilson's disease, since characteristic signs of the disease are evident when the liver appears normal on light microscopy.

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