Hepatocyte ultrastructure in non-hemolytic hyperbilirubinemias

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Abstract

The non-hemolytic hyperbilirubinemias comprise a group of syndromes that are clinically and some in cases biochemically and genetically well characterized, and ultrastructural investigations of biopsy samples have still diagnostic applications. In this study ultrastructural analysis of biopsy tissues in order to identify pattern of features characteristic for different non-hemolytic hyperbilirubinemias among group of patients with clinical presentation of hyperbilirubinemia and normal histopathology were done. A group of 62 patients was selected on the basis of mild unconjugated hyperbilirubinemia and, in some cases, on the basis of a mild unconjugated or conjugated hyperbilirubinemia in the presence of repeatedly normal liver function tests and absence of hyperhemolysis. Electron microscopic investigation distinguished between patients in three groups of primary hyperbilirubinemia: Gilbert syndrome (21 pts), Dubin-Johnson syndrome (5 pts), Rotor syndrome (3 pts) and a group with non specific changes (33 pts). The ultrastructural features of hepatocytes that univocally distinguish primary hyperbilirubinemias from secondary diseases were presented. Electron microscopic methods permit on recognition of non-specific changes in the ultrastructure of liver tissue among samples obtained from patients with non-hemolytic hyperbilirubinemias and can be still usefull in diagnosis of jaundince.

Key words: congenital hyperbilirubinemia, Dubin-Johnson disease, Gilbert disease, Rotor disease, ultrastructure

Introduction

Idiopathic hyperbilirubinemias may encompass several primary and secondary diseases of bilirubin metabolism [2]. Bilirubin, an oxidative product of the hem group of hemoglobin, myoglobin and cytochrome P-450, in the liver is conjugated with glucuronic acid and than excreted in bile via ATP-dependent transporter [5]. Currently, injuries of molecular bases of this metabolic pathway are known [5]. Although, this knowledge allow for precision diagnosis of non-hemolytic hyperbilirubinemias in most cases, but still ultrastructural investigation of biopsy samples have diagnostic application. Clinically, conjugated and unconjugated hyperbilirubinemia can be distinguished on the basis of blood bilirubin level, normal liver tests and exclusion of hemolytic disease. In conjugated hyperbilirubinemias i.e Gilbert, Crigler-Najjar syndrome, transport of bilirubin from plasma into liver cells is impaired, while in conjugated hyperbilirubinemias, i.e Dubin-Johnson, Rotor syndrome, microsomal enzymes transferase, bilirubin transport from hepatic cells into bile canalicu system are disturbed [2, 5]. In non-hemolytic hyperbilirubinemias, liver histopathology may present normal feature or cholestasis. These investigations were aimed to identify ultrastructural pattern of characteristic features for different non-hemolytic hyperbilirubinemias among group of patients with clinical presentation of hyperbilirubinemia and normal histopathology.

Material and methods

Material

62 biopsy samples from patients (age 6-17 years, mean 10.2 ± 4.6) with marked liver disfunction and clinical symptoms of non-hemolytic hyperbilirubinemia were selected for electron microscopic studies among samples collected in the registry of Pathology Department of CMHI. Tissue samples were routinely preserved for histological, histochemical and ultrastructural examination. All of the examined patients had total bilirubin in the range of 1.3-5.1 mg%. (Table 1).
Table 1

Total and direct bilirubin level in serum in distinguished groups of primary hyperbilirubinemia and with non-specific changes

<table>
<thead>
<tr>
<th>Disease</th>
<th>Number of patients</th>
<th>Age (years)</th>
<th>Total Bilirubin (mg%)</th>
<th>Average</th>
<th>Direct</th>
<th>Av.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gilbert</td>
<td>10</td>
<td>10-17</td>
<td>1,5-4,6</td>
<td>2,56</td>
<td>0,5-0,9</td>
<td>0,62</td>
</tr>
<tr>
<td>Compl.</td>
<td>11</td>
<td>6-7</td>
<td>1,2-5,1</td>
<td>2,48</td>
<td>0,3-1,1</td>
<td>0,95</td>
</tr>
<tr>
<td>Incompl.</td>
<td>5</td>
<td>9-17</td>
<td>1,3-4,7</td>
<td>2,54</td>
<td>0,6-2,0</td>
<td>1,05</td>
</tr>
<tr>
<td>Dubin-Johnson</td>
<td>3</td>
<td>16-17</td>
<td>1,6-4,5</td>
<td>2,10</td>
<td>0,4-0,8</td>
<td>0,55</td>
</tr>
<tr>
<td>Rotor</td>
<td>5</td>
<td>3</td>
<td>1,2-5,7</td>
<td>2,62</td>
<td>0,2-2,8</td>
<td>1,45</td>
</tr>
<tr>
<td>Non specific</td>
<td>33</td>
<td>7-17</td>
<td>1,2-5,7</td>
<td>2,62</td>
<td>0,2-2,8</td>
<td>1,45</td>
</tr>
</tbody>
</table>

Histology

Five mm thick paraffin sections were stained in a routine manner with hematoxylin-eosin, azan, silver, PAS, PAS with diastaze digestion and than reviewed to exclude other disease than hyperbilirubinemia.

Electron microscopy

Samples were fixed in 2.5% glutaraldehyde in cacodylate buffer at pH 7.3, postfixed in 2% OsO₄, dehydrated in alcohols and embedded in Epon 812. Thick sections stained with toluidine blue were analysed under light microscope. Ultrathin sections stained with uranyl acetate and lead citrate were examined by electron microscopy.

Results

Examinations of paraffin and semi-thin sections showed in all cases an apparently normal liver, except for Dubin-Johnson that shown the existence of dark pigment predominantly in centrilobular areas.

On the basis of EM analysis morphological equivalents of primary congenital hyperbilirubinemias were confirmed in 29 cases and secondary reactive symptomatic hyperbilirubinemias were recognized in 33 cases.

Gilbert syndrome

Gilbert syndrome was diagnosed in 21 cases. There were observed shortening and mal-developed vascular pole microvilli or loss of microvilli, and increase of collagen in the Disse space in various intensity. In all patients an increase of the endoplasmic reticulum profiles with coexisting dilatation of rough endoplasmic reticulum with degranulation and pigment granules variable in size containing both electron dense material which alternate with less dense material and lipid droplets was observed (Fig. 1A). In 10 biopsy samples enlarged or giant, oval or polymorphic mitochondria with paracrystalline inclusions (Fig. 1B) were present. In all cases the biliary pole of hepatocytes shown normal caniculi and microvilli, but with numerous pigment granules around. Lipofuscin granules were common finding. The remaining group of 11 patients showed uncompleted ultrastructural features of Gilbert disease, i.e. lack of giant mitochondria and no paracrystalline in mitochondrial matrix.

Dubin-Johnson syndrome

Dubin-Johnson syndrome was defined in 5 cases. There were not seen alterations in the vascular pole of hepatocytes, which exhibit normal microvilli. Slightly increased number of smooth endoplasmic reticulum profiles that formed small vesicles, normal mitochondria with some variation in size, well developed Golgi profiles located around bile canicules were common features. Dilation of bile caniculi with loss of microvilli and characteristic pigment granules around were seen (Fig. 2). Pigment granules were composed mainly of granular material, however vesicle-like or lipid components were also seen. They were polygonal in shape and delineated by a single membrane. The lack of lipofuscin in hepatocytes was common feature.
**Rotor Syndrome**

Rotor syndrome was recognised in 3 cases. Only slight changes in hepatocytes were observed: some of the bile canicules were dilated and exhibited reduced number of microvilli, the interhepatocytic junctional interspaces were dilated and cell membranes along the lateral cell surface formed microvilli (Fig. 3a), cell membranes facing the Disse space showed reduced number of microvilli. In side of hepatocytes a few pleomorphic mitochondria or megamitochondria with paracrystalline were seen. The most characteristic feature was lack of lipid component in pigment granule that composed of electron dense and less dense granular material. They were distributed mainly around bile canicules (Fig. 3b).

**Non characteristic lesions**

Non characteristic ultrastructural fine lesions accompanying secondary hyperbilirubinemia were found in 33 cases. Reactive changes were recognized in 9 cases. There were increased number and elongation of microvilli on the vascular pool (Fig. 4), polymorphic mitochondria (sometimes with paracrystalline inclusions), increased number of smooth endoplasmic reticulum profiles, the presence of single lipid droplets and pigment or lipofuscin granules in cytoplasm, and collagen fibrils in Disse spaces.

**Discussion**

In about 50% of the patients with clinical symptoms of hyperbilirubinemia electron microscopy confirmed primary congenital hyperbilirubinemia. Among of them, complete ultrastructural features of Gilbert syndrome were presented in 10 cases, incomplete Gilbert in 11 cases, Dubin-Johnson in 5 cases and Rotor in 3 cases.

In seventies the ultrastructural observations were helpful to establish the morphological equivalent of primary hyperbilirubinemia and distinguish combination of defects impairing primary hyperbilirubinemas, particularly in Gilbert syndrome when complete and incomplete features coexisted [1, 4, 6]. Our patients with Gilbert disease clinically were characterized by relatively low-grade direct bilirubin and they reached a higher proportion of unconjugated bilirubin. These abnormalities were correlated with decrease or the lack of microvilli at sinusoidal pole.

It is known that the liver in Dubin-Johnson syndrome might be characterized by slight and inconsistent changes. In our patients, the canicular pole exhibited normal microvilli or disappearing microvilli coexisting with a slightly dilated lumen. These results suggest that disturbances of bile flow were caused by different disfunctions of the canicular area. These are in agreement of with distinct mechanism of bilirubin glucuronide transport involving ATP-dependent and ATP-independent system that have been proposed [7].
Patients with Rotor syndrome ultrastructurally presented fairly similar features to Gilbert and to Dubin-Johnson simultaneously: some injury of vascular and canicular pole concomitant with an increase of smooth endoplasmic reticulum and pleomorphic mitochondria in hepatocytes. These similarities between some ultrastructural features of Gilbert and Dubin-Johnson syndrome were signaled in literature before [8]. The presence of microvilli along the lateral surface of hepatocytes was a particular feature, which distinguished patients with Rotor syndrome from other patients. Patients with Rotor syndrome exhibited lower level of direct bilirubin than unconjugated, and this observation does not agree with main idea of liver defect [5]. However, similar data on a direct bilirubin in serum have been mentioned in literature [3].

Electron microscopic methods permit on recognition of non-specific changes in the ultrastructure of the liver tissue among samples obtained from patients with non-hemolytic hyperbilirubinemias and can be still useful in diagnosis of jaundice.

References


