INTRODUCTION

Congenital disorders of the mitochondrial respiratory chain enzymes are the most common group of inborn errors of metabolism, affecting at least 1 in 5,000 individuals (Skladal et al., 2003; Thorburn, 2004; Munnich and Rustin, 2001). It can affect most organ systems, alone or in combination, so it is not surprising that these disorders could be associated with liver disease presenting as liver failure.

A considerable number of patients with liver failure remain undiagnosed in Japan. They often show liver steatosis, suggesting the presence of mitochondrial damage. On the other hand, mitochondrial damage secondary to metabolic disease, which should modify the liver physiology, has never been investigated sufficiently.

We present here mitochondrial respiratory disorder and liver disease, especially mitochondrial DNA depletion syndrome and secondary effect to metabolic liver disease.

MATERIALS AND METHODS

Subject

We investigated the mitochondrial respiratory chain enzymes in liver samples obtained from 8 patients with liver failure due to unknown etiology (Table 1) and from 15 patients with metabolic disease: ornithine transcarbamylase deficiency 6 cases; Wilson disease, 3 cases; methylmalonic aciduria (MMA) 3 cases, neonatal hemochromatosis 2 cases (Table 2).
<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset age</td>
<td>Day 8</td>
<td>Day 2</td>
<td>8 months</td>
</tr>
<tr>
<td>Family history</td>
<td>Elder brother (+)</td>
<td>Elder sister (+)</td>
<td>Elder brother (+)</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Low birth weight</td>
<td>Nystagmus</td>
<td>Tachypnea, Hypoglycemia</td>
</tr>
<tr>
<td></td>
<td>Feeding difficulty</td>
<td>MR</td>
<td>MR</td>
</tr>
<tr>
<td>Liver function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GOT / GPT (IU/l)</td>
<td>120 / 46</td>
<td>49 / 215</td>
<td>109 / 42</td>
</tr>
<tr>
<td>T-Bil / D-Bil (mg/dl)</td>
<td>2.5 / 1.7</td>
<td>9.6 / 1.4</td>
<td>0.7 / -</td>
</tr>
<tr>
<td>ChE (U/l)</td>
<td>179</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PT (%)</td>
<td>16</td>
<td>2.4 (INR)</td>
<td>63</td>
</tr>
<tr>
<td>Lactate / pyruvate (mmol/l)</td>
<td>2.9 / 0.13</td>
<td>20.9 / 0.27</td>
<td>1.46 / 0.07</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFP</td>
<td>3.4 x 10⁵ ng/ml</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Pathology | Micro / Macrovesicular steatosis, Fibrosis (mild - moderate) in portal area (P-P bridging) |
| Transplantation | 8 months | Not performed | Scheduled | Not performed |
| Status | Alive | Dead | Alive | Dead |

<table>
<thead>
<tr>
<th>Case 5</th>
<th>Case 6</th>
<th>Case 7</th>
<th>Case 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset age</td>
<td>5 months</td>
<td>2 months</td>
<td>4 months</td>
</tr>
<tr>
<td>Family history</td>
<td>Not particular</td>
<td>Not particular</td>
<td>Not particular</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Failure to thrive MR</td>
<td>Failure to thrive Hypoglycemia</td>
<td>Heart Failure (EFE)</td>
</tr>
<tr>
<td>Liver function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GOT / GPT (IU/l)</td>
<td>489 / 176</td>
<td>209 / 92</td>
<td>unknown</td>
</tr>
<tr>
<td>T-Bil / D-Bil (mg/dl)</td>
<td>5.7 / 4.3</td>
<td>3.7 / 2.2</td>
<td>35.4 / 25.6</td>
</tr>
<tr>
<td>ChE (U/l)</td>
<td>105</td>
<td>198</td>
<td>66</td>
</tr>
<tr>
<td>PT (%)</td>
<td>60</td>
<td>33.2</td>
<td>22.1</td>
</tr>
<tr>
<td>Lactate / pyruvate (mmol/l)</td>
<td>3.2 / 0.1</td>
<td>1.6 / 0.1</td>
<td>63.3 / -</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFP &gt; 5.0 x 10⁴ ng/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Pathology | Macrovesicular steatosis, Extensive fibrosis | Macrovesicular steatosis | Extensive fibrosis |
| Transplantation | Scheduled → Stopped | 9 months | Not performed | Not performed |
| Status | Dead | Alive | Dead | Dead |

**Determinations of enzyme activities**

Activities of respiratory chain complexes I, II, III and IV were assayed for the crude post-600 g supernatant of the liver samples as described previously (Rahman et al., 1996; Murayama et al., 2008). Each complex activity was presented as a percent ratio relative to the mean value obtained from 35 healthy controls. Together, for each patient, the percent ratios of complex I, II, III and IV...
activities to the citrate synthase (CS) as a mitochondrial enzyme marker or complex II activity were calculated.

**BN-PAGE western blotting**

Expressions of the mitochondrial respiratory chain complex I, II, III and IV proteins in the liver were examined by western blotting using blue native polyacrylamide gel electrophoresis (BN-PAGE) according to the methods described previously (Dabbeni-Sala et al., 2001; Schägger et al., 1988). Ten μg weight of the protein in mitochondria-enriched fraction was separated by BN-PAGE. Immunostaining was performed using a monoclonal antibody specific for the 39 kD subunit of complex I, 70 kD subunit of complex II, core 1 subunit of complex III and subunit 1 of complex IV (Molecular Probes, Eugene, OR, USA).

**Quantitative PCR**

The quantitative estimation of mtDNA was performed by the real-time amplification of fragments of nicotinamide adenine dinucleotide dehydrogenase 1 (ND1) in mtDNA genome, as previously described (Pagnamenta et al., 2006; He et al., 2002). To determine the overall abundance of mtDNA, we compared the real-time amplification of ND1 with single-copy nuclear reference gene (exon 24 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene; chosen on account of the lack of single-nucleotide polymorphisms). For both experiments, DNA from six adult liver samples (from needle biopsies, obtained with informed consent) was used as controls, and results are the means of four independent runs, with samples assayed in triplicate in each run.

**RESULTS AND DISCUSSION**

8 cases with liver failure showed extremely low activities and protein levels of complex I, III and IV (Figs. 1 and 2). We also performed qPCR and estimated the

<table>
<thead>
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<th>Table 2. The cases with metabolic liver disease which were investigated respiratory chain enzymes</th>
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<tr>
<td></td>
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<tr>
<td>6 cases of OTC Deficiency</td>
</tr>
<tr>
<td>Wilsonian fluminant</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
</tr>
<tr>
<td>4 cases of Wilson Disease</td>
</tr>
<tr>
<td>2 cases of Neonatal Hemochromatosis</td>
</tr>
<tr>
<td>3 cases of Methylmalonic Aciduria</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Table 3. Respiratory chain enzyme analysis</th>
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<tbody>
<tr>
<td>i)  In vitro respiratory chain enzyme assay</td>
</tr>
<tr>
<td>ii) BN-PAGE in gel enzyme staining</td>
</tr>
<tr>
<td>iii) BN-PAGE western blotting</td>
</tr>
<tr>
<td>iv) Quantitative PCR</td>
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</tbody>
</table>

![Fig. 1](#)  The results of enzyme assay of respiratory chain in eight cases. Complex I and III were extremely low activities.
The percentage of mtDNA/nDNA was calculated by the real-time PCR. The ND1 probe of mtDNA and Exon 24 of the CFTR probe of nuclear DNA were used for this calculation (He et al., 2002).

<table>
<thead>
<tr>
<th>Case</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%)</td>
<td>2.3</td>
<td>2.8</td>
<td>6.6</td>
<td>3.3</td>
<td>11.5</td>
<td>23.7</td>
<td>4.6</td>
<td>18.4</td>
</tr>
</tbody>
</table>

Normal range: 60-150%

Diagnosis: Mitochondrial DNA Depletion Syndrome

Fig. 3. The ratio of mtDNA/nDNA by the real-time PCR. All cases are significantly low ratio of mtDNA (normal range: 40-150%).
Mitochondrial function in liver disease

![Graph showing CS ratio (%)](image)

**Fig. 4.** The result of enzyme assay of respiratory chain in 12 cases with metabolic liver disease (OTC deficiency, Wilson disease, and Neonatal hemochromatosis).

**OTCD; OTC deficiency, NH; Neonatal hemochromatosis, WD; Wilson disease**

**Fig. 5.** BN-PAGE and Western blotting in the cases with metabolic liver disease (OTC deficiency, Wilson disease, and Neonatal hemochromatosis).

**Fig. 6.** Metabolic pathway about methylmalonic aciduria.
mitochondrial disease, but Kirby et al. (1999) reported that blood lactic acid level was normal in about 20 percent of patients with complex I deficiency. About a half of our patient usually showed normal blood lactate level.

Recently, BN-PAGE immunoblotting and enzyme staining have been shown to be a powerful diagnostic tool for mitochondrial disorders. We confirmed their utility in studying these patients. More investigation is required to acquire a better understanding of liver disease mitochondrial respiratory chain disorders.

The present study strongly suggested that considerable disturbance of mitochondrial respiratory chain occurs in children with metabolic diseases and possibly modifies the pathophysiology of the liver disease.

**ACKNOWLEDGMENT**

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**REFERENCES**


Mitochondrial function in liver disease
