Lipoprotein Abnormalities in Cholestasis I. Electrophoretic and Ultracentrifugal Analyses

Makoto Watanabe*
Lipoprotein Abnormalities in Cholestasis I. 
Electro-phoretic and Ultracentrifugual 
Analyses* 
Makoto Watanabe

Abstract

The alterations of lipid composition in sera of patients with liver diseases, particularly intra-hepatic cholestasis and biliary obstruction, were studied by ultracentrifugation and polyacrylamide-gel disc-electrophoresis of lipoproteins and apoproteins. The elevation of serum cholesterol in intrahepatic cholestasis was greater than in biliary obstruction. The appearance of lipoprotein X in obstructive disease accounted for most of the increased cholesterol. The level of non-lipoprotein X cholesterol in intrahepatic cholestasis was significantly elevated, this being in part ascribed to the appearance of a new class of cholestatic lipoprotein, Slow-migrating HDL. The electrophoretic pattern of lipoprotein in cholestasis was generally characterized by a decrease in alpha band intensity and, in some types of cholestasis, by the appearance of Slow-migrating HDL. In addition, other abnormal lipoproteins exhibiting the characteristics of triglyceride-rich LDL (LP-Y), LP-X-like HDL and LDL-like HDL were found in some cases of intrahepatic cholestasis and biliary obstruction.

KEYWORDS: intrahepatic cholestasis, biliary obstruction, cholestatic lipoprotein, polyacrylamide-gel disc-electrophoresis, ultracentrifugation

*PMID: 227230 [PubMed - indexed for MEDLINE] 
Copyright (C) OKAYAMA UNIVERSITY MEDICAL SCHOOL
LIPOPROTEIN ABNORMALITIES IN CHOLESTASIS
I. ELECTROPHORETIC AND ULTRACENTRIFUGAL
ANALYSES

Makoto WATANABE

First Department of Internal Medicine, Okayama University
Medical School, Okayama 700, Japan
(Director: Prof. H. Nagashima)
Received March 20, 1979

Abstract. The alterations of lipid composition in sera of patients
with liver diseases, particularly intrahepatic cholestasis and biliary ob-
struction, were studied by ultracentrifugation and polyacrylamide-gel
disc-electrophoresis of lipoproteins and apoproteins. The elevation of
serum cholesterol in intrahepatic cholestasis was greater than in biliary
obstruction. The appearance of lipoprotein X in obstructive disease
accounted for most of the increased cholesterol. The level of non-lipo-
protein X cholesterol in intrahepatic cholestasis was significantly elevated,
this being in part ascribed to the appearance of a new class of
cholestatic lipoprotein, Slow-migrating HDL. The electrophoretic pat-
tern of lipoprotein in cholestasis was generally characterized by a
decrease in $\alpha$ band intensity and, in some types of cholestasis, by the
appearance of Slow-migrating HDL. In addition, other abnormal lip-
proteins exhibiting the characteristics of triglyceride-rich LDL (LP-Y),
LP-X-like HDL and LDL-like HDL were found in some cases of intra-
hepatic cholestasis and biliary obstruction.

Key words: intrahepatic cholestasis, biliary obstruction, cholestatic
lipoprotein, polyacrylamide-gel disc-electrophoresis, ultracentrifugation

Because of the importance of the liver as a major site of lipoprotein meta-
bolism, several derangements in serum lipoprotein pattern occur in hepatic
disorders. Altered concentrations of serum lipids, which are carried by the
lipoproteins, have been shown to characterize different groups of liver diseases,
e.g. increased total cholesterol level with reduced esterified fraction in obstruc-
tive jaundice, increased triglyceride concentration in acute hepatitis, and reduc-
tion of both cholesterol and triglyceride levels in cirrhosis of the liver (1–4).
These lipid abnormalities are even more clearly recognized as decrease or dis-
appearance of $\alpha$-lipoprotein and pre $\beta$-lipoprotein and increase of $\beta$-lipoprotein
(5–10).

In the present study, the alteration of serum lipid concentration in chole-
stasis was studied by polyacrylamide-gel disc-electrophoresis (PAGE) of lipo-
proteins in an attempt to correlate changes in serum lipid composition with lipoprotein abnormalities. In some cases, lipoprotein classes were further separated by preparative ultracentrifugation and their lipid composition and apoprotein pattern analyzed. In addition to the known abnormal lipoproteins, LP-X (11, 12) and LP-Y (13, 14), several unusual lipoproteins with marked derangement in apoprotein composition in cholestasis are reported in the present paper.

MATERIALS AND METHODS

Blood samples were taken after 12-14 h fasting from 25 cases of intrahepatic cholestasis (viral 5, drug-induced 1, drug-induced, suspected 11, primary biliary cirrhosis (PBC) 4, PBC, suspected 1, anicteric cholestasis with biliary enzyme elevation (PBC-related) 1 and cause unknown 2), 17 cases of malignant, complete biliary obstruction, 44 cases of parenchymal liver diseases (acute viral hepatitis 9, chronic viral hepatitis 4, post-hepatic liver cirrhosis 12, primary hepatoma 10, alcoholic fatty liver 2 and alcoholic liver cirrhosis 7) and 1 case of Caroli's syndrome. Diagnoses were made by clinical and laboratory findings and further confirmed in most cases by peritonendoscopy and histological examination and/or percutaneous transhepatic choledangiography (PTC) or endoscopic retrograde cholangiography. EDTA (disodium salt, Sigma Chemical Co., 1 mg/ml blood) was used as an anti-coagulant in blood collection when preparative ultracentrifugation was subsequently performed, otherwise serum was separated and used for assay within one week after storage at 4°C.

Lipoprotein fractions, very low density lipoprotein (VLDL, 0.95<d<1.006), low density lipoprotein (LDL, 1.006<d<1.063) and high density lipoprotein (HDL, 1.063<d<1.21), were separated by preparative ultracentrifugation at 4-6°C by the method of Yasugi and Homma (15) using a Hitachi ultracentrifuge, Model 65P.

PAGE of lipoproteins was performed by the method of Naito et al. (16), Cellogel (Chemetron, Milano) electrophoresis by a routine laboratory technique, and PAGE of apoprotein by the methods of Kane (17) and of Weber and Osborn (18).

Concentrations of LP-X were determined by the method of Ritland et al. (19), i.e., phosphorus content in LP-X-containing agar gel separated after electrophoresis was measured and converted to LP-X concentrations.

Concentrations of total cholesterol (TC) and cholesteryl esters (EC) were determined by the method of Zak (20), triglycerides (TG) by Van Handel and Zilversmit (21), phospholipids (PL) by Bartlett (22), free fatty acids (FFA) by Itaya and Ui (23), and protein by Lowry et al. (24).

The concentrations of TC and PL deriving from lipoproteins other than LP-X (non-LP-X TC and PL) were calculated by subtracting the concentration of TC or PL contained in LP-X from that of TC or PL in serum.
Lipoprotein Abnormalities in Cholestasis

RESULTS

TC, PL, TG and FFA concentrations in the patients with intrahepatic cholestasis and biliary obstruction are shown in Fig. 1. The mean concentra-

![Graph showing distribution of lipid levels in sera of patients with intrahepatic cholestasis and biliary obstruction. IHC, intrahepatic cholestasis; BO, biliary obstruction; NS, not significant; hatched area, normal range; and horizontal bar, mean value.]

Fig. 1. Distribution of lipid levels in sera of patients with intrahepatic cholestasis and biliary obstruction. IHC, intrahepatic cholestasis; BO, biliary obstruction; NS, not significant; hatched area, normal range; and horizontal bar, mean value.

tions of TC and PL in intrahepatic cholestasis were significantly higher than in biliary obstruction, while those of TG and FFA were not significantly different in these two groups, although most of the cases in intrahepatic cholestasis had FFA levels above normal. The distribution of cases with lower, normal and higher values for TC, PL and TG in intrahepatic cholestasis and biliary obstruction is shown in Table 1A. The frequently increased lipid was cholesterol.

Table 1. (A) Distribution of cases in different serum lipid levels in intrahepatic cholestasis and biliary obstruction

<table>
<thead>
<tr>
<th></th>
<th>Lower</th>
<th>Normal range</th>
<th>Higher</th>
<th>Total No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intrahepatic cholestasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>8(11.1)</td>
<td>7(25.9)</td>
<td>17(63.0)</td>
<td>27</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>6(22.2)</td>
<td>8(29.6)</td>
<td>13(48.1)</td>
<td>27</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1(5.6 )</td>
<td>10(55.6)</td>
<td>7(38.9)</td>
<td>18</td>
</tr>
<tr>
<td><strong>Biliary obstruction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>3(17.6)</td>
<td>7(41.1)</td>
<td>7(41.1)</td>
<td>17</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>3(16.7)</td>
<td>10(35.6)</td>
<td>5(27.8)</td>
<td>18</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0(0.0 )</td>
<td>2(22.2)</td>
<td>7(77.8)</td>
<td>9</td>
</tr>
</tbody>
</table>

The number in parentheses indicate the percentage.
(63.0%) in intrahepatic cholestasis and triglycerides (77.8%) in biliary obstruction. The high cholesterol level was closely associated with the high PL level in intrahepatic cholestasis (Table 1B), as is also demonstrated by the high correlation between them (Figs. 2A, B and C). On the other hand, the high TG level in biliary obstruction had no correlation with the elevated TG.

<table>
<thead>
<tr>
<th>Intrahepatic cholestasis</th>
<th>Cholesterol</th>
<th>Phospholipids</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Biliary obstruction</td>
<td>Cholesterol</td>
<td>Phospholipids</td>
<td>Triglycerides</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Fig. 2. Correlations among TC, PL and TG. A, TC vs PL; B, TG vs PL; and C, TG vs TG. ○, IHC; and ●, BO.

LP-X values in patients with intrahepatic cholestasis, biliary obstruction and other liver diseases are presented in Fig. 3. The lower detection limit for LP-X with our technique was 10 mg/dl (blank values ± 2 S. D. with 20 different assays). Thus, 87 cases of parenchymal liver diseases had no detectable level of LP-X. In biliary obstruction, 63.0% of the cases had LP-X levels above 100 mg/dl, while in intrahepatic cholestasis only 17.1% of the cases had LP-X values above this level. The cases of intrahepatic cholestasis with the high LP-X levels were: 4 cases of PBC, 1 case of PBC-related condition and 1 case of intrahepatic cholestasis of acute onset (probably caused by drug allergy).

In order to see whether the increased cholesterol is derived from LP-X or other lipoproteins, these lipid or lipoprotein levels in serum were followed in a case with complete common bile duct obstruction receiving PTC drainage (Fig. 4). It was apparent that the increase in cholesterol level was mostly due
Watanabe: Lipoprotein Abnormalities in Cholestasis I. Electro-phoretic

Lipoprotein Abnormalities in Cholestasis

Fig. 3. LP-X values in intrahepatic cholestasis, biliary obstruction and parenchymal liver diseases. The numbers, 87 and 28, surrounded by squares indicate the number of cases whose LP-X values were 0 to 10 (the lower limit of detection in our laboratory) and 11 to 20 mg/dl, respectively.

Fig. 4. Time course of LP-X, TC and PL concentrations in a case with complete common bile duct obstruction. The concentrations of TC and PL deriving from lipoproteins other than LP-X were calculated by subtracting the TC or PL contained in LP-X and shown by the shaded area.

to the increase in TC in LP-X, although the level of TC in lipoproteins other than LP-X increased to some extent while LP-X was detected in serum. Similar results were also obtained with PL. In another case of complete obstruction of common bile duct analyzed for lipid compositions in ultracentrifugally separated lipoprotein fractions at different stages of serum LP-X levels, 1,112 mg/dl and 0 mg/dl, the total increases of TC and PL concentrations were slightly less than the increases of TC and PL accounted for by LP-X (Fig. 5). For ex-
Fig. 5. TC and PL concentrations in each lipoprotein fraction and LP-X. Analyses were made when LP-X levels were 1,112 mg/dl and 0 mg/dl after PTC drainage. Darkened area of the column represents LP-X TC or PL. 1. LP-X level 1,112 mg/dl; and 2. LP-X level 0 mg/dl.

Fig. 6. Non-LP-X TC and PL concentrations in IHC and BO. IHC and BO, see abbreviations in the legend to Fig. 1.

Example, the TC concentration in HDL was somewhat diminished. In further cases of intrahepatic cholestasis and biliary obstruction, the alterations of TC and PL in lipoproteins other than LP-X was studied by subtracting the TC and PL contained in LP-X based on the assumption that the TC in LP-X constitutes 25% [11] of total LP-X (Fig. 6). Non-LP-X TC and PL concentrations were higher in intrahepatic cholestasis than in biliary obstruction; the cases with increased cholesterol in non-LP-X lipoproteins being 61.1% in intrahepatic cholestasis and 9.1% in biliary obstruction. The values for PL were 50.0% and 6.3%, respectively. The results suggest that the abnormalities in lipoproteins with increased TC and PL other than LP-X, occur more frequently in intrahepatic cholestasis than in biliary obstruction.

Because of the importance of analyzing the change in whole lipoprotein pattern to explain TC or PL-rich lipoproteins in cholestasis, electrophoretic patterns of lipoproteins were studied with two different supporting media in
cholestasis and other liver diseases.

The serum obtained from a case of PBC with increased non-LP-X TC or PL concentration gave unusual lipoprotein patterns upon electrophoresis on Cellogel (Fig. 7); the absence of normal α, β and pre-β-bands and the presence of slow-α (Fig. 7A) and broad fast β-bands were noted (Fig. 7C). Since the serum from a case of complete obstruction of common bile duct without increased non-LP-X TC level yielded a single band of intermediate mobility between β and pre-β (Fig. 7B), other unusual bands appeared to contribute to the increased concentration of non-LP-X TC.

Fig. 7. Cellogel electrophoretic patterns of lipoproteins. A, a case of PBC in a stage of improved liver function tests; B, a case of complete common bile duct obstruction; C, the same case as A in exacerbation; and D, a healthy control. O, origin.

Similar studies were made by PAGE including cases with parenchymal liver diseases (Fig. 8). The electrophoretoogram of the same sample as in Fig. 7A is given in Fig. 8A, where pre-β, β and α-lipoprotein bands with usual mobilities and an additional slow-migrating α (HDL-S) band were noticed. The single pre-β-migrating band on Cellogel (Fig. 7B) resolved into two bands of pre-β and β-mobilities on PAGE (Fig. 8B), whereas the broad band on Cellogel (Fig. 7C) gave practically a single β-band with virtual absence of an α-band. The PAGE pattern of elevated pre-β and β-bands and an intact α-band was also present in cases with cholestasis, as is shown in Fig. 8B'. Decreased pre-β and α-bands with elevated β-band is shown in Fig. 8C and intact pre-β, elevated β and de-
creased $\alpha$-bands in Fig. 8D. The incidence of these representative lipoprotein patterns in liver diseases is shown in Table 2. Decreased $\alpha$ and increased $\beta$-band intensities were commonly observed in liver diseases irrespective of their pathogenesis (severe parenchymal liver diseases or biliary obstruction). The intensity

$$\text{pre} \quad \beta \quad \beta \quad \text{HDL-S} \quad \alpha$$

![Image showing lipoprotein patterns](image)

**Figure 8.** Representative PAGE patterns of lipoproteins in cases of liver diseases. A, serum for Fig. 7A; B, serum for Fig. 7B; B', a case of intrahepatic cholestasis; C, a case of liver cirrhosis; and D, a case of biliary obstruction.

**Table 2. Lipoprotein patterns in PAGE**

<table>
<thead>
<tr>
<th></th>
<th>IHC</th>
<th>BO</th>
<th>FH</th>
<th>AH</th>
<th>CH</th>
<th>LC</th>
<th>HC</th>
<th>FL</th>
<th>AL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>13(7)</td>
<td>10(3)</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>42</td>
</tr>
<tr>
<td>B'</td>
<td>2(1)</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>D</td>
<td>3(3)</td>
<td>5(1)</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Others</td>
<td>5(2)</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>25(14)</td>
<td>17(4)</td>
<td>10</td>
<td>4</td>
<td>11</td>
<td>4</td>
<td>2</td>
<td></td>
<td>7</td>
<td>81</td>
</tr>
</tbody>
</table>

FH, fulminant hepatitis; AH, acute hepatitis; CH, chronic hepatitis; LC, liver cirrhosis; HC, primary hepatoma; FL, fatty liver and AL, alcoholic liver injury. First arrow, second arrow and third arrow indicate pre $\beta$, $\beta$ and $\alpha$ lipoprotein, respectively. Number of HDL-S positive case is indicated in parentheses.
of pre β-migrating band increased in most cases of cholestasis, acute hepatitis and alcoholic liver injury. The apparent difference between intrahepatic cholestasis and biliary obstruction was observed only in HDL-S, of which the appearance was predominant in intrahepatic cholestasis; 14/25 vs 4/17. It should be noted here that the appearance of HDL-S is not confined to Type A electrophoretic pattern, because the classification of the pattern is mainly based on the other lipoprotein bands. Accordingly, the rise in non-LP-X TC in intrahepatic cholestasis could be explained by the increased HDL-S level. In fact, among 11 cases with high levels of non-LP-X TC (cf. Fig. 6), 7 cases had positive HDL-S (1/1, 4/6, 1/1 and 1/3 in Types A, B, C, and D, respectively). This implies that HDL-S could appear in cases without increased non-LP-X TC and that TC-rich lipoproteins other than HDL-S also contributes to the increase in non-LP-X TC.

Because of the heterogeneity assumed to be present in each lipoprotein band on PAGE, as suggested from the above results, PAGE analysis was made after ultracentrifugal separation of lipoproteins in some cases, where unusual lipid distribution among lipoproteins, such as a high level of non-LP-X TC, was suspected. The lipid composition, protein content and LP-X concentration in the analyzed cases with characteristic lipoprotein patterns are summarized in Table 3 and their PAGE patterns of lipoproteins and apoproteins in Figs. 9–13. The results obtained with a healthy control are presented in Fig. 9. The lipoproteins in separated fractions gave each a single band, except the faint β-migrating band in HDL fraction (Fig. 9A). Apoprotein profiles and lipid composition (Table 3) agreed with those generally accepted (Fig. 9B and C) (25, 26).

Fig. 9. Lipoproteins and apoproteins on PAGE in a healthy control. A, lipoproteins. The columns stand from left to right for whole plasma, VLDL, LDL and HDL fractions. B, apoproteins separated on PAGE with urea. The columns stand from left to right for VLDL, LDL and HDL fractions. C, apoproteins separated with SDS. The columns stand from left to right for VLDL, LDL and HDL fractions.
High concentrations of non-LP-X TC, PL and protein in LDL fraction and of free cholesterol (FC), PL and protein in HDL fraction were noticed in the plasma from a case of PBC (K. K.) (Table 3). In PAGE of lipoproteins, normal pre β, β and α-bands and the appearance of HDL-S were present (Fig. 10A).

![Fig. 10. Lipoproteins and apoproteins on PAGE in a case of PBC (K. K.). A, lipoproteins. The columns are identical with those in Fig. 9. B, apoproteins with urea and C, with SDS. The columns are identical with those in Fig. 9.](image)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Lipoprotein</th>
<th>FC</th>
<th>EC</th>
<th>TG</th>
<th>PL</th>
<th>LP-X</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. T.</td>
<td>Plasma</td>
<td>56.3</td>
<td>148.9</td>
<td>87.0</td>
<td>226.1</td>
<td>0</td>
<td>7,250</td>
</tr>
<tr>
<td></td>
<td>VLDL</td>
<td>1.6</td>
<td>4.6</td>
<td>31.6</td>
<td>15.3</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>23.2</td>
<td>45.0</td>
<td>23.0</td>
<td>45.0</td>
<td>31.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>32.5</td>
<td>98.3</td>
<td>26.5</td>
<td>163.3</td>
<td>163.4</td>
<td></td>
</tr>
<tr>
<td>K. K.</td>
<td>Plasma</td>
<td>98.2</td>
<td>219.7</td>
<td>121.0</td>
<td>363.4</td>
<td>0</td>
<td>7,640</td>
</tr>
<tr>
<td></td>
<td>VLDL</td>
<td>4.6</td>
<td>10.2</td>
<td>34.5</td>
<td>16.1</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>31.9</td>
<td>88.1</td>
<td>30.4</td>
<td>97.8</td>
<td>95.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>59.1</td>
<td>108.8</td>
<td>32.1</td>
<td>242.0</td>
<td>226.7</td>
<td></td>
</tr>
<tr>
<td>S. S.</td>
<td>Plasma</td>
<td>205.8</td>
<td>50.2</td>
<td>263.0</td>
<td>319.0</td>
<td>148.0</td>
<td>10,900</td>
</tr>
<tr>
<td></td>
<td>VLDL</td>
<td>0.7</td>
<td>6.8</td>
<td>46.9</td>
<td>9.3</td>
<td>0</td>
<td>25.4</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>160.2</td>
<td>32.5</td>
<td>169.3</td>
<td>201.3</td>
<td>148.0</td>
<td>194.5</td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>44.9</td>
<td>10.8</td>
<td>46.9</td>
<td>27.1</td>
<td>0</td>
<td>34.6</td>
</tr>
<tr>
<td>H. Y.</td>
<td>Plasma</td>
<td>70.8</td>
<td>39.2</td>
<td>211.0</td>
<td>175.0</td>
<td>10.0</td>
<td>6,040</td>
</tr>
<tr>
<td></td>
<td>VLDL</td>
<td>0.6</td>
<td>5.7</td>
<td>40.1</td>
<td>29.8</td>
<td>0</td>
<td>23.5</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>20.8</td>
<td>7.3</td>
<td>167.0</td>
<td>114.0</td>
<td>10.0</td>
<td>131.0</td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>49.2</td>
<td>26.3</td>
<td>3.9</td>
<td>31.1</td>
<td>0</td>
<td>20.8</td>
</tr>
<tr>
<td>M. K.</td>
<td>Plasma</td>
<td>112.9</td>
<td>118.6</td>
<td>98.0</td>
<td>266.6</td>
<td>47.1</td>
<td>5,920</td>
</tr>
<tr>
<td></td>
<td>VLDL</td>
<td>0.3</td>
<td>0.6</td>
<td>10.0</td>
<td>4.4</td>
<td>0</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>74.3</td>
<td>87.7</td>
<td>51.9</td>
<td>147.6</td>
<td>47.1</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>39.4</td>
<td>29.3</td>
<td>28.9</td>
<td>108.3</td>
<td>0</td>
<td>128.2</td>
</tr>
</tbody>
</table>
Unusual apoprotein distributions were: the presence of Apo A, C and E in LDL fraction (Fig. 10B and C) and a relative increase in Apo E (Fig. 10C). PAGE of lipoproteins in Case S. S. (Fig. 11A), diagnosed as complete common bile duct obstruction, revealed HDL with fast β-mobility and lack of HDL of usual α-mobility. The HDL had a low ratio of protein to lipids, decreased EC and relatively high TG content, and its apoprotein was characterized by a relative increase in Apo E. The LDL fraction contained some pre β-migrating lipoprotein and the apoprotein pattern resembled that of HDL. Characteristic features in a case with complete common bile duct obstruction (Case H. Y.) were elevations of TG, PL and protein with decrease of EC levels in LDL fraction, of which the lipoprotein gave a broad β-band extending to the pre β-position (Fig. 12A) with an apoprotein pattern similar to that of HDL in addition to usual Apo B (Fig. 12B). Marked reduction of lipids and protein in HDL were found in this case. In M. K., a case in convalescent stage of acute exacerbation of chronic hepatitis with cholestasis, had a near normal β-band and a trace of pre β-band in LDL fraction in addition to the appearance of HDL-S in HDL fraction (Fig. 13A). The apoprotein pattern in LDL was, however, practically identical with that of HDL (Fig. 13B) and TC, TG, PL and protein contents were apparently increased (Table 3). Similarity of this LDL to that in Case S. S. was also noted. VLDL fractions in those cases demonstrated only quantitative alterations in lipids and apoproteins. The incidence of those abnormal lipoproteins among 12
Fig. 12. Lipoproteins and apoproteins on PAGE in a case of complete bile duct obstruction (H.Y.). A, lipoproteins and B, apoproteins separated with urea. Columns are identical with those in Fig. 9.

Fig. 13. Lipoproteins and apoproteins on PAGE in a case of chronic hepatitis with intrahepatic cholestasis (M.K.). A, lipoproteins and B, apoproteins separated with urea. Columns are identical with those in Fig. 9.

cases studied by lipoprotein and apoprotein analyses after ultracentrifugal separation of lipoprotein class is summarized in Table 4. Except for the TG-rich LDL in biliary obstruction and the HDL-S in intrahepatic cholestasis, the ap-
Lipoprotein Abnormalities in Cholestasis

The appearance of abnormal lipoproteins had disease specificity as far as the cases studied are concerned.

**Table 4. Prevalence of abnormal lipoproteins found by analyses after ultracentrifugal separation**

<table>
<thead>
<tr>
<th>HDL-S</th>
<th>LP-X like HDL</th>
<th>TG-rich LDL</th>
<th>HDL-like LDL</th>
<th>total case No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC</td>
<td>2&quot;</td>
<td>1</td>
<td>2&quot;</td>
<td>4</td>
</tr>
<tr>
<td>BO</td>
<td>3</td>
<td>2</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>LC</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>HG</td>
<td>1&quot;</td>
<td>1&quot;</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>AL</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

*a In each case, two abnormal lipoproteins were found. The terms applied to abnormal lipoproteins are to be conferred to those under DISCUSSION. See the legends to Table 2.*

**DISCUSSION**

Hypercholesterolemia in patients with obstructive jaundice has been known since 1862 (27). Besides the increase in cholesterol, which is due to unesterified cholesterol, phospholipids are also elevated in biliary obstruction (1, 2). The alterations in lipoproteins in obstructive jaundice are the increase in \( \beta \)-lipoprotein or LDL and the decrease in \( \alpha \)-lipoprotein or HDL (2, 28). Eder et al. (2) reported the increase of Cohn fraction IV–VI and the presence of an abnormal lipoprotein was shown by Russ and others (29) in Cohn fraction VI. The abnormal LDL was shown by Switzer (30) not to react with antibodies to LDL and was later characterized by Seidel and his groups as LP-X (11). Heterogeneity of LP-X (LP-X₁, LP-X₂ and LP-X₃) has been also demonstrated by Gotto's group (31). The usefulness of LP-X assay in differentiating biliary obstruction from intrahepatic cholestasis was proposed by Magnani and Alaupovic (32). The results of the present study obtained analyzing sera of 176 cases for LP-X confirmed their observation; namely, values higher than 100 mg dl were rarely found in intrahepatic cholestasis except PBC in contrast with relatively high levels in biliary obstruction. The increased serum cholesterol level in biliary obstruction was explained in most cases by the appearance of LP-X, which contains relatively large amount of unesterified cholesterol and phospholipids, whereas in intrahepatic cholestasis the elevation of serum cholesterol was only partially accounted for by the appearance of LP-X.

Several methods of electrophoretic separation of lipoproteins are available, such as those employing agar, agarose or cellulose-acetate membrane. None of them was satisfactory in separating and characterizing the abnormal lipoproteins.
in cholestasis except LP-X, which could be effectively separated and quantitated on agar gel (12, 19). PAGE of lipoproteins gave a more discrete resolution of lipoprotein bands and further provided some information on the molecular size of lipoprotein due to a sieving effect of PAG upon electrophoresis. By this technique, the author identified a slow-migrating HDL termed as HDL-S, which characterizes a specific group of intrahepatic cholestasis, fully described elsewhere (33). The appearance of HDL-S, which accounted for some of the increase in non-LP-X TC or PL in intrahepatic cholestasis, is a clinically important new observation in terms of differentiating cholestatic diseases. Furthermore, the PAGE technique was found useful in a sense that it revealed lipoprotein patterns characteristic of different liver diseases. The decreased α-band was observed in severe biliary obstruction and parenchymal liver injury and the intense pre β-migrating band was relatively specific to hepatitis particularly caused by alcohol, as has been reported for other techniques (34). However, the difficulty inherent to the electrophoretic separation of lipoprotein lies in most instances in poor resolution of β-migrating lipoproteins. This could result from either one of both of the following mechanisms: 1) the abnormal lipoproteins appearing in several liver diseases tend to have a property of β-migration or 2) the change in lipoproteins in hepatic injury is directed at convergence to a relatively uniform molecular species with β-mobility, the latter possibility being suggested in the present study as discussed below.

By combining ultracentrifugal separation of lipoproteins with PAGE of lipoproteins and apoproteins, each abnormal lipoprotein class appearing in liver diseases, particularly in intrahepatic cholestasis and biliary obstruction, could be identified. The results disclosed that the lipoproteins of β-mobility were distributed in fractions other than LDL and that the original apoprotein patterns of those fractions separated by ultracentrifugation were lost or markedly distorted, or rather a common apoprotein pattern similar to that of HDL was found in the LDL fraction. Among the lipoproteins thus identified as constituting a specific class of lipoprotein were: 1) TG-rich LDL with β-mobility as in Case H. Y., which is probably identical with TG-rich LDL or LP-Y appearing as a result of impaired hepatic TG lipase as studied by several workers (13, 14): 2) HDL with fast β-mobility as in Case S. S., this being considered as LP-X-like HDL, which had a low ratio of protein/lipids and decreased esterified cholesterol content with a relatively high amount of TG (35, 36); 3) LDL with apoproteins nearly identical with that of HDL as in Case M. K, which is regarded as HDL-like LDL (37) and 4) HDL-S (33, 38). The appearance of these abnormal lipoproteins was found to be less specific to liver diseases as summarized in Table 4 but rather related to the pathological state of liver diseases. Among those abnormal lipoproteins, HDL-S and LP-X-like HDL were relatively high in TC.
content even though they were not present in LDL, whereas LP-X is present. Thus, the presence of these TC-rich lipoproteins other than LP-X in intrahepatic cholestasis more frequently than in biliary obstruction would explain the elevated non-LP-X TC in intrahepatic cholestasis. TG-rich LDL might also contribute to the increased TC to some extent. The proportion of intact LDL remaining in the LDL fraction in cases with cholestasis could not be determined from the present study. In order to fully elucidate this problem, a further fractionation of LDL by gradient (or rate-zonal) ultracentrifugation, column chromatography or immuno-electrophoretic technique is required in future studies.

Acknowledgment. The author wishes to thank Prof. H. Nagashima and President K. Kosaka for their instruction and Dr. K. Taketa for his excellent guidance and advice.

REFERENCES


Lipoprotein Abnormalities in Cholestasis


