Serum total lipids, lipoprotein cholesterol, and apolipoprotein A in acute viral hepatitis and chronic liver disease

C. VERGANI, G. TROVATO, A. DELÙ, M. PIETROGRANDE, AND N. DIOGUARDI

From the Third Department of Clinical Medicine, University of Milan, Italy

SUMMARY Serum total lipids, lipoprotein cholesterol, apolipoprotein A (Apo A), and liver function tests have been investigated in patients with acute viral hepatitis and chronic liver disease. Hypertriglyceridaemia, absence of α and pre β bands on the lipoprotein electrophoresis pattern, low level of Apo A, and presence of abnormal lipoproteins (β -VLDL and β_2 -LP) were observed in the early phase of acute hepatitis. A positive correlation was found between Apo A and high-density lipoprotein cholesterol, and a negative one between Apo A and triglyceride, bile acids, total bilirubin, and serum alanine aminotransferase. Lipoprotein abnormalities found in the early phase of acute hepatitis are probably due to low lecithin-cholesterol acyltransferase activity. The reappearance of α lipoprotein and the increase of Apo A are sensitive indices of improvement of liver function. In chronic liver disease low levels of cholesterol and Apo A indicate the severity of liver cell injury.

The liver is the major site of synthesis of plasma lipoproteins (Jackson *et al.*, 1976) and of lecithin-cholesterol acyltransferase (LCAT) (E.C.2.3.1. 43) (Simon and Boyer, 1971), a key enzyme involved in lipoprotein metabolism. The association of serum lipid and lipoprotein abnormality and liver disease has been known since 1862 (Flint, 1862).

We determined the fasting serum concentration of total cholesterol, total triglycerides, lipoprotein cholesterol, and apolipoprotein A (Apo A) during the course of acute viral hepatitis and in different types of chronic liver disease.

Material and methods

Fasting blood samples were drawn from 40 controls, medical students and hospital personnel aged between 20 and 39 years (20 men and 20 women), from 23 patients with acute viral hepatitis, and from 128 patients with chronic liver disease. In the patients with acute viral hepatitis blood was obtained weekly for about one month using the approximate date of onset of jaundice as the beginning of the disease. Patients with chronic liver disease included six sub-

jects with chronic persistent hepatitis (CPH), 24 with chronic active hepatitis (CAH), 47 with compensated cirrhosis (CC), 14 with decompensated cirrhosis (DC) (ie, cirrhosis with serum albumin $\leq$ 3 g/dl, ascites, and/or oedema), six in hepatic coma (HC), and eight with primary biliary cirrhosis (PBC). In these cases the diagnosis was proved by percutaneous liver biopsy (De Groote *et al.*, 1968), by laparotomy, and by clinical and laboratory evidence. Serum was separated and kept at + 4°C for one or two days before analysis. Total cholesterol was determined according to Röschlau *et al.* (1974), and total triglyceride according to Eggstein and Kreutz (1966). Lipoprotein electrophoresis was performed in agarose according to Noble (1968). LP-X₆ was investigated as previously described (Vergani *et al.*, 1973). High-density lipoprotein-cholesterol (HDL-cho) was measured by the heparin-manganese chloride precipitation method (Burstein and Samaille, 1960). Low-density lipoprotein-cholesterol (LDL-cho) was calculated according to Friedewald *et al.* (1972). Apo A was measured by immunoelectrophoresis according to Laurell (1972) (Fig. 1) using an antiserum which reacts with both Apo A-I (R-gln-I) and Apo A-II (R-gln-II).

An antiserum concentration of 2% in 1% agarose in barbital buffer, pH 8.6, ionic strength 0.02, was

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used. 2 μ l serum sample diluted 1:40 with buffer was applied in 2 mm diameter wells, and the electrophoresis was run for six hours at 14 V/cm in a cooled chamber, after which the gel was thoroughly washed in phosphate-buffered saline, dried, and stained with Coomassie brilliant blue R (Sigma Chemical Co, St. Louis, Ohio, USA).

Fig. 1 Calibration curve for rocket immunoelectrophoresis of Apo A. The precipitates are shown.

A serial dilution (1:20, 1:40, 1:160) of pooled normal sera, frozen batchwise at -20°C and thawed only once, was used for the calibration curve. Each serum sample was tested twice. Both the within- and between-assay coefficients of variation were 5–9%. Bidimensional immunoelectrophoresis was carried out according to Ganrot (1972) using 1% agarose in barbital buffer, pH 8.6, ionic strength 0.05. The first dimension electrophoresis was run for one hour at 20 V/cm, and the second dimension electrophoresis was run for 16–18 hours at 4 V/cm on the same agarose containing 2% antiserum. Preparative ultracentrifugation was performed in an MSE Superspeed 65 Centrifuge, Rotor 59592 titanium, by layering serum at its native density of 1.006 under a sodium chloride solution of the same density. The serum was spun at 15°C for 18 hours at 105 000 g.

Total bilirubin, direct bilirubin, γ -glutamyl-transferase (γ -GT), alanine aminotransferase (SGPT), alkaline phosphatase, albumin, and IgG, IgA, and IgM were determined in the serum according to standard laboratory techniques. Bile acids were measured according to Schwarz *et al.* (1974). The coefficient of regression, mean, standard deviation, and Student's test of significance were calculated by standard techniques (Snedecor and Cochran, 1967).

Results

In controls, the normal range, based upon the 10th and 90th percentiles, was 78–128% for Apo A, 146–230 mg/100 ml for total cholesterol, and 58–145 mg/100 ml for total triglycerides. The early phase of acute viral hepatitis was characterised by disappearance of α and pre β lipoproteins on the lipoprotein electrophoresis pattern of 18 out of 23 patients, by low levels of Apo A, by hypertriglyceridaemia, and by the presence in the top fraction of the ultracentrifuged serum of a lipoprotein with a $d < 1.006$, which displayed a β electrophoretic mobility (β -VLDL) (Fig. 2-A). In four patients with pronounced cholestasis and hypertriglyceridaemia a lipoprotein with $d > 1.006$ with β electrophoretic mobility, which was different from LP-X, was found (Fig. 3). During the course of the hepatitis, except in the patients who died of acute liver failure (Fig. 4), a decrease of serum triglycerides, a reappearance of α and pre β lipoproteins (Fig. 2-B), and an increase of Apo A were observed (Fig. 5).

Fig. 2 Agarose gel lipoprotein electrophoresis: 1 = whole serum, 2 = top fraction after ultracentrifugation at d 1.006, 3 = bottom fraction after ultracentrifugation at d 1.006. A = early phase of acute viral hepatitis; B = recovery from acute viral hepatitis. In A absence of α and pre β lipoproteins and presence of β -VLDL can be seen.

Fig. 3 Agarose gel lipoprotein electrophoresis: 1 = whole serum, 2 = top fraction after ultracentrifugation at d 1.006, 3 = bottom fraction after ultracentrifugation at d 1.006. Absence of α and pre β lipoproteins and presence of β_2 -LP can be seen.

Fig. 4 Serum Apo A, triglyceride, SGPT, and direct bilirubin levels in a patient who died of acute liver failure.

Fig. 5 Concentration of serum Apo A during the course of acute viral hepatitis. N = normal subjects as controls.

Table 1 Correlation coefficients between Apo A, total triglyceride, and some biochemical parameters in acute viral hepatitis

	log SGPT	log TB	AP	γ GT	Bile acids	HDL-cho	TG
Apo A	-0.28**	-0.54***	0.10	0.28	-0.57**	0.38***	-0.48***
TG	0.26**	0.50***	-0.12	-0.19	0.52**	-0.28**	—

, * Significant correlations at the 1.0 and 0.1% levels, respectively.

TB = total bilirubin; AP = alkaline phosphatase; γ -GT = γ glutamyltransferase; TG = total triglyceride.

Fig. 6 Relationship between HDL-cholesterol and Apo A in acute viral hepatitis.

Fig. 7 Concentration of serum triglycerides in the different types of chronic liver disease. For explanation see text. The horizontal lines represent the mean value. P values for difference between patients and normal subjects: * < 0.05, ** < 0.01, *** < 0.001.

A positive correlation was found between Apo A and HDL-cholesterol (Fig. 6). Apo A was negatively correlated with triglycerides, log SGPT, log total bilirubin, and bile acids. Triglycerides were positively correlated with log SGPT and log direct bilirubin and negatively correlated with HDL-cholesterol. Table 1 summarises these results.

In chronic liver disease triglyceride levels were not modified (Fig. 7). We also observed a clear trend towards low levels of total cholesterol (Fig. 8) and Apo A (Fig. 9), reflecting the severity of liver injury. The absence of α and pre β lipoproteins, and very low concentrations of total cholesterol and Apo A were found in hepatic coma. Primary biliary cirrhosis behaved quite differently, in that total cholesterol was high, LP-X was present, and Apo A was nearly normal in five out of eight patients. Table 2 shows the correlation between Apo A and some biochemical parameters in chronic liver disease.

Discussion

Marked alteration of lipid and lipoprotein com-

Fig. 8 Concentration of serum cholesterol in the different types of chronic liver disease. For explanation see text.

Table 2 Correlation coefficients between Apo A and some biochemical parameters in chronic liver disease

Apo A	SGPT	γ GT	AP	Bile acids	Albumin	IgG	IgA	IgM	TG	Chol	HDL- chol	LDL- chol	VLDL chol
CPH (n = 6)	0.06	-0.06	0.04	-0.82	-0.50	0.28	0.34	0.19	0.26	0.72	0.12	0.72	0.37
CAH (n = 24)	-0.12	0.06	0.03	0.01	-0.16	-0.20	-0.15	0.18	-0.02	0.32	0.23	0.25	-0.06
CC (n = 47)	*** 0.57	0.31	-0.16	-0.26	*** 0.56	-0.08	-0.40	-0.28	-0.10	0.29	*** 0.44	0.04	-0.06
DC (n = 14)	0.03	0.33	-0.16	0.38	0.47	-0.35	-0.53	0.82	0.28	0.17	0.30	-0.01	0.27
HC (n = 6)	-0.56	-0.31	0.06	-0.10	0.50	-0.38	-0.61	-0.34	-0.38	0.06	0.74	0.09	-0.38
PBC (n = 8)	0.15	0.21	0.01	-0.54	0.48	-0.09	-0.84	-0.16	-0.15	0.33	0.79*	0.29	-0.15

*, **, *** Significant correlations at the 5, 1.0, and 0.1% levels, respectively. See footnote to Table 1.

Fig 9 Concentration of serum Apo A in the different types of chronic liver disease: ● = male; ▲ = female. For explanation see text.

position in acute viral hepatitis has been reported (Mendenhall *et al.*, 1962; Klör *et al.*, 1972; Schmitz and Kahlke, 1973; Müller *et al.*, 1974; Thalassinou *et al.*, 1975; Vogt and Enghardt, 1975). The absence of α lipoprotein has been attributed to the impaired lipid-binding capacity of Apo A (Seidel *et al.*, 1972). According to our results, Apo A is decreased during the early phase of the disease and its cholesterol content is normal. In bidimensional immunoelectrophoresis we were also able to see a decrease of Apo A. In normal subjects Apo A shows one peak with an anodal shoulder (Fig. 10a). In the early phase of acute hepatitis the peak is greatly reduced while α_1 antitrypsin, an acute phase reactant protein synthesised by the liver, which was used as a marker, increases (Fig. 10b). During recovery the Apo A peak returns towards a near normal height (Fig. 10c).

Because in acute hepatitis the synthetic capacity of the liver is not impaired, the low level of Apo A is probably due to an increased catabolism of the apolipoprotein after reduction of LCAT activity described by several authors (Simon and Scheig, 1970; Calandra *et al.*, 1971; Gjone *et al.*, 1971) in acute hepatitis.

The mechanism of LCAT deficiency in acute viral hepatitis is obscure; it could be related to bile salt interactions with apoprotein activator or to subtle derangement in lipoprotein metabolism (Sabesin *et al.*, 1977).

It seems probable that LCAT plays a necessary role in the normal conversion of the nascent HDL to

the 'mature' HDL (Hamilton, 1972). Electron microscopic studies of HDL in acute alcoholic liver disease revealed the presence of stacked discs, which resemble nascent HDL (Sabesin *et al.*, 1977). The same modification present in acute hepatitis, such as hypertriglyceridaemia, absence of α lipoprotein, and presence of β -VLDL, has been reported in familial LCAT deficiency (Norum and Gjone, 1967). Because Apo A-I (R-gln-I) is an activator of LCAT (Fielding *et al.*, 1972), a decrease of Apo A may in turn impair LCAT activity. Apo C-II (R-glu), which is exchanged between HDL and VLDL (Eisenberg and Levy, 1975), is an activator of the lipoprotein lipase (LaRosa *et al.*, 1970). A reduced recycling, due to a deficiency of Apo A, may cause an impairment of the enzyme activity, which results in hypertriglyceridaemia.

Apo A is a trace component of VLDL. According to Levy *et al.* (1966), the pre β mobility of normal VLDL is derived from its Apo A component.

There is evidence that the electrophoretic mobility of VLDL returns to normal when acted upon by LCAT (Seidel *et al.*, 1972; Glomset and Norum, 1973). The abnormal LDL that we found in four patients with marked cholestasis has been described by Klör *et al.* (1972) as LDL III and by Müller *et al.* (1974) as β_2 -LP. Müller *et al.* (1974) suggested that the β_2 -LP represents remnant particles derived from the metabolism of VLDL and of chylomicrons. The remnant particles are due to a marked decrease in hepatic lipase activity. In acute hepatitis the improvement of liver function was accompanied by a

Fig. 10 Bidimensional immunoelectrophoresis in agarose containing anti-Apo A and anti- α_1 antitrypsin (α_1 AT) immune sera: (a) normal subject; (b) patient in the early phase of acute viral hepatitis; (c) patient during recovery from acute viral hepatitis.

gradual return of triglyceride and Apo A towards normal levels. This was first noted as the reappearance of α and pre β bands on the lipoprotein electrophoretic pattern.

The prognostic value of these parameters during the course of acute viral hepatitis has been pointed out (Thalassinou *et al.*, 1975). In chronic liver disease, impairment of the synthetic ability of the liver, as indicated by routine biochemical tests, was associated with low levels of total cholesterol and

Apo A. Increased levels of cholesterol in primary biliary cirrhosis have been attributed to LP-X (Seidel *et al.*, 1969; Vergani *et al.*, 1973). Protein synthesis is enhanced in cholestatic liver tissue (Stakeberg *et al.*, 1974), which may explain the nearly normal levels of Apo A found in some patients with primary biliary cirrhosis. High levels of α lipoprotein in primary biliary cirrhosis have been reported by Blomhoff *et al.* (1974). Cross-incubation studies showed that cholestatic serum stimulates LCAT activity (Kepkay *et al.*, 1973). This effect has been related to the presence of LP-X, which may act as an unusually good substrate (Kepkay *et al.*, 1973). However, the normal levels found in primary biliary cirrhosis of Apo A, which is the preferable substrate for LCAT (Glomset and Norum, 1973), may explain the enhanced enzyme activation.

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Requests for reprints to: Professor C. Vergani, Third Department of Clinical Medicine, University of Milan, Via Pace 15, 20122 Milano, Italy.