Impact of Viral Hepatitis on Apo- and Lipoprotein Status in Blood

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Key Words

Hepatitis • Apoprotein • High-density lipoprotein • Lipoprotein (a) • Low-density lipoprotein

Abstract

Objectives: To investigate the effect of viral hepatitis on serum levels of apo- and lipoproteins in different forms of liver diseases. **Materials and Methods:** One hundred and thirty adult patients and 100 healthy age- and gender-matched control individuals participated in this study. Patients were grouped according to four types of liver disease: acute viral hepatitis, chronic active hepatitis (CAH), cirrhosis of the liver and fulminant hepatic failure. **Results:** Hepatitis B virus, C virus and E virus (HEV) infections were recorded in different combinations in these patients, but viral infections of hepatitis A and D were not seen in any of the patient groups. The results of lipo- and apoprotein analysis showed different patterns. The low-density lipoprotein value was high in the CAH group. In the other three groups, low-density lipoprotein level was comparable to the control value. The high-density lipoprotein level ($p = 0.02$) was significantly low in all groups except in the cirrhosis group. Apo-A was significantly reduced in the acute viral hepatitis and fulminant hepatic failure groups, whereas Apo-B level was low in the CAH and cirrhosis groups. The lipoprotein (a) level in these groups was low, compared to control. **Conclusion:** No apparent relationship was observed between etiological viruses and ensuing changes in lipid/lipoprotein profile.

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Introduction

As early as in 1862, Austin Flint [1] suggested that blood cholesterol level was affected by the liver diseases. He found raised blood cholesterol level in parenchymal liver disease and ascribed this hypercholesterolemia to the failure of the liver to remove cholesterol from blood. Similarly, Man et al. [2] demonstrated that changes in liver phospholipid levels followed the cholesterol pattern. The liver serves as the centre of metabolism of apoprotein, lipoprotein and lipid. An important function of liver in relation to lipid metabolism includes synthesis of apoprotein and lipoprotein, catabolism of various lipids as well as excretion of cholesterol and phospholipids through bile. Alteration in hepatic function associated with hepatocellular injury or cholestasis results in changes in the concentration and composition of apoproteins, lipoprotein and lipid levels, both in the liver and blood.

Viral hepatitis is a common cause of hepatic damage. Some earlier reports have demonstrated clear changes in lipid and apoprotein composition of serum in patients with viral hepatitis [3–5]. However, none of these reports demonstrated any relation between changes in blood apoprotein levels and etiology of the disease. The present study evaluated plasma levels of apoproteins and lipoprotein in patients with various types of liver diseases to establish a relationship between plasma levels of apo- and lipoprotein and viral etiology as well as type of liver disease.

Material and Methods

Patients and Blood Samples

Of the 130 male and female patients included in this study, 50 (mean age 36, range 22–44 years) were diagnosed with acute viral hepatitis (AVH), 30 (mean age 42, range 25–54 years) had chronic active hepatitis (CAH), 30 (mean age 39, range 28–53) had cirrhosis and 20 (range 19–47, mean age 32) had fulminant hepatic failure (FHF). All the patients attended either the outpatient department or were admitted to the Liver Unit, All India Institute of Medical Sciences, New Delhi, India, from July 2001 to November 2003. They were evaluated clinically and sera were tested for viral hepatitis viral markers. The diagnoses of different types of liver diseases were based on the accepted clinical, biochemical and histological criteria, as outlined previously [6]. AVH was diagnosed when patients exhibited overt jaundice and/or increased alanine aminotransferase levels (three times above the normal value) documented at least twice, at a 1-week interval without any history of pre-existing liver disease. The patients with CAH and cirrhosis of liver were diagnosed by histopathological criteria set by the international study group on chronic hepatitis [7]. All the CAH patients had persistent elevated transaminase levels for more than 6 months, histological evidence of chronic hepatitis and exclusion of other possible causes of chronic liver diseases. Cirrhosis patients did not have any history of chronic alcohol intake. The diagnosis of FHF was made when patients developed signs and symptoms of acute liver failure and encephalopathy within 8 weeks of onset of disease with no pre-existing liver problem [6]. One hundred age- and gender-matched healthy subjects were used as control subjects.

Venous blood (6–10 ml) from each of the patients and controls was drawn and aliquoted in tubes without and with EDTA as anticoagulant. Anticoagulated blood was used to separate plasma. Serum was separated from blood after centrifugation and then stored at -70°C for analysis of various hepatitis markers. Repeated freezing and thawing of serum was avoided as far as possible.

Hepatitis Viral Markers

Sera were analyzed for hepatitis B surface antigen and IgM antibodies to hepatitis A virus (IgM anti-HAV), hepatitis D virus (IgM anti-HDV) and hepatitis E virus (IgM anti-HEV). Similarly, all these sera were also tested for total antibodies against hepatitis C virus (anti-HCV). The serological analysis was done using enzyme immunoassay kits of high sensitivity and specificity. Kits for hepatitis B surface antigen, IgM anti-HBc and IgM anti-HAV were purchased from Abbot Laboratories, USA. Anti-HCV was tested using highly sensitive third-generation enzyme-linked immunosorbent assay (ELISA) kits from Ortho Diagnostics, USA. This anti-HCV kit used peptides versus core, NS3, NS4 and NS5 regions of the HCV genome, as antigen to coat the ELISA plate. IgM anti-HDV was tested using an enzyme immunoassay kit from Wellcome, UK. Similarly, IgM anti-HEV was tested using third-generation ELISA kit from Genelabs and Diagnostics, Biotechnology, Singapore.

Estimation of Apo and Lipoprotein

Estimation of lipoprotein (a) [Lp(a)], apoprotein A (ApoA), apoprotein B (ApoB), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) was performed in fasting state using immunoturbidimetry (Hitachi 912, chemistry autoanalyzer and kits from Randox Laboratories, UK).

Statistical Analysis

The data entered into Microsoft Excel format were analyzed by Epi Info v6.04d package to carry out appropriate unpaired Student's *t* test/Mann-Whitney test and *Z* test. Log transformation was also applied to obtain normal distribution (significant at $p < 0.05$).

Results

Complete viral analysis of the four categories of liver diseases (AVH, CAH, cirrhosis and FHF) indicated that HAV infection was absent in all the patients. Hepatitis B infection was detected in 38 (76%) AVH patients, hepatitis C in 7 (14%), hepatitis D in none and hepatitis E in 8 (16%). None of the patients had mixed BCE infection, though 3 cases with BC, 2 with BE and 2 with CE were recorded. Four patients with AVH had no marker of hepatitis viruses and were labeled as hepatitis non-ABCDE. In the CAH group, hepatitis B, C and E was detected in 23 (76%), 14 (47%) and 5 (17%) cases, respectively. Three of the CAH patients had mixed BCE infection and 3 had non-ABCDE infection. Similarly in the cirrhosis group, 13 (43%) patients had hepatitis B, 9 (30%) hepatitis C and 6 (20%) hepatitis E infection. None had BCE infection. Seven had non-ABCDE infection. Patients in the FHF group presented with B infection in 11 (55%), C infection in 2 (10%), E infection in 5 (25%), mixed BCE in none and non-ABCDE in 4 (20%) cases.

The lipoproteins and apoprotein levels in different disease groups are shown in table 1. Analysis of results showed a significantly high LDL value (100.9 ± 10.62 mg/dl) in the CAH group. LDL levels in the other three groups were comparable to the control value. However, HDL level was significantly reduced in all the groups except in that with cirrhosis (30.9 ± 24.3 mg/dl). Levels of ApoA were found to be low in both the AVH and FHF groups (60.5 ± 51.1 and 49.3 ± 42.8 mg/dl, respectively). No effect was seen in the CAH (87.7 ± 60.1 mg/dl) and cirrhosis (91.9 ± 63.7 mg/dl) groups, when compared to the control value (87.6 ± 21.2 mg/dl). On correlating HDL and ApoA level in these groups, almost similar patterns were noted. Similarly, the value of ApoB was low in CAH (58.1 ± 29.6 mg/dl) and cirrhosis (57.8 ± 39.3 mg/dl) patients, with no significant change in AVH (87.4 ± 41.4 mg/dl) and FHF (90.0 ± 55.6 mg/dl) patients.

The values of Lp(a) analysis in all these cases showed reduced Lp(a) level in AVH (7.4 ± 15.3 mg/dl), cirrhosis (6.7 ± 12.9 mg/dl), CAH (10.3 ± 18.2 mg/dl) and FHF (10.2 ± 14.9 mg/dl) compared to the control value (15.4 ± 13.1 mg/dl).

Table 1. Lipid parameters in different liver diseases

Parameter	AVH	CAH	Cirrhosis	FHF	Control
LDL, mg/dl	75.2 ± 39.2	100.9 ± 10.62*	66.8 ± 59.5	81.6 ± 57.8	80.2 ± 19.5
HDL, mg/dl	27.5 ± 12.7**	28.0 ± 12.4*	30.9 ± 24.3	26.4 ± 14.1*	35.6 ± 16.0
ApoA, mg/dl	60.5 ± 51.1**	87.7 ± 60.1	91.9 ± 63.7	49.3 ± 42.8**	86.7 ± 10.5
ApoB, mg/dl	87.4 ± 41.4	58.1 ± 29.6**	57.8 ± 39.3**	90.0 ± 55.6	87.6 ± 21.2
Lp(a), mg/dl	7.4 ± 15.3**	10.3 ± 18.2	6.7 ± 12.9**	10.2 ± 14.9	15.4 ± 13.1

* p < 0.05 (significant); ** p < 0.01 (highly significant).

Table 2. Levels of lipoprotein and apoprotein in relation to viral etiology in different forms of liver diseases

Group	Cases	HDL	LDL	ApoA	ApoB	Lp(a)
AVH						
HBV only	33	27.6 ± 10.7	77.5 ± 36.3	60.3 ± 47.3**	92.7 ± 43.9	8.4 ± 16.9**
HCV only	2	15.14 ± 7.6	47.2 ± 29.6	54.3 ± 53.1*	66.4 ± 30.4	11.2 ± 19.0
HEV only	4	15.1 ± 7.6	47.2 ± 29.6	30.4 ± 33.8**	54.5 ± 28.7**	2.2 ± 5.8*
BC	3	24.3 ± 16.0	66.3 ± 28.4	80.2 ± 53.0	71.6 ± 14.4	18.6 ± 23.1
BE	2	22.5 ± 16.6	82.2 ± 27.8	75.7 ± 32.8	81.8 ± 30.9	7.7 ± 10.9
CE	2	9.0 ± 1.4	17.3 ± 7.0	14.0 ± 3.6**	22.1 ± 13.9**	5.0 ± 7.07
BCE	0	—	—	—	—	—
Non-BCE	4	36.1 ± 11.3	80.1 ± 37.8	94.2 ± 5.4	94.3 ± 33.7	5.8 ± 9.2
CAH						
HBV only	12	32.3 ± 25.9	103.2 ± 117.9*	89.9 ± 65.2	59.6 ± 32.1**	9.8 ± 20.0
HCV only	3	26.07 ± 9.7	74.50 ± 51.8	72.8 ± 32.8	58.4 ± 23.4**	9.7 ± 18.5
HEV only	0	—	—	—	—	—
BC	7	28.0 ± 9.2	97.1 ± 44.3*	68.0 ± 37.7	58.5 ± 11.4**	0.25 ± 0.5
BE	1	32.6	97.1*	82.7	60.7**	8.3
CE	1	30.6	92.1	80.7	61.7	8.4
BCE	3	30.0 ± 10.1	57.6 ± 12.7	82.1 ± 30.8	56.6 ± 12.6	0.33 ± 0.57
Non-BCE	3	21.0	18.2	83.5	33.2**	10.0
Cirrhosis						
HBV only	9	30.6 ± 20.05	75.6 ± 60.9	93.4 ± 6.5	64.3 ± 48.0**	6.6 ± 12.5*
HCV only	6	55.4 ± 42.1	111.6 ± 65.1	78.2 ± 29.7**	46.0 ± 14.4**	4.4 ± 5.5
HEV only	3	22.3 ± 9.9	34.9 ± 20.1	136.6 ± 100.9	60.5 ± 52.3**	1.8 ± 2.8*
BC	2	40.0 ± 9.6	61.3 ± 19.1	117.0	46.0	2.3
BE	2	16.0 ± 9.8	40.3 ± 42.0	93.5 ± 16.2	39.9 ± 28.3**	7.7 ± 10.8
CE	1	55.0	153.0	118.1	55.7**	0.1*
BCE	0	—	—	—	—	—
Non-BCE	7	25.5 ± 17.2	63.4 ± 68.8	68.2 ± 40.1	51.1 ± 26.4**	13.1 ± 20.2
FHF						
HBV only	9	25.1 ± 12.0	80.5 ± 49.1	43.8 ± 42.0**	98.3 ± 55.1	4.8 ± 8.9*
HCV only	1	27.5 ± 9.1	106.8 ± 9.4	43.4 ± 23.3**	135.0 ± 45.5	23.7 ± 33.5
HEV only	4	34.4 ± 20.6	77.5 ± 59.1	50.5 ± 59.4**	78.7 ± 42.5	7.5 ± 12.0
BC	1	34.0	100.2	26.9	167.2	0
BE	1	39.0	80.0	10.0	112.0	0
CE	0	—	—	—	—	—
BCE	0	—	—	—	—	—
Non-BCE	4	26.4 ± 14.1	81.6 ± 57.8	50.5 ± 36.8**	83.4 ± 78.8	16.4 ± 18.6*
Control	100	35.6 ± 16.0	80.2 ± 19.5	86.7 ± 10.5	87.6 ± 21.2	15.4 ± 13.1

* p < 0.05 (significant); ** p < 0.01 (highly significant). Plasma levels are in mg/dl.

Detailed lipo- and apoprotein levels in the etiological subgroups of the four categories of hepatitis are shown in table 2. Essentially, there were no statistically significant differences in the values of HDL and LDL in relation to etiological variation in each disease group. Analysis of ApoA, -B and Lp(a) in relation to etiology in each disease group showed distinct changes in ApoA and -B levels, but again no relation with etiology was observed. Also, the same finding was noted in Lp(a) cases.

Discussion

The present study focuses on the effect of viral hepatitis on the blood level of apo- and lipoprotein in various forms of liver diseases. Liver diseases have been reported to show a varying effect on lipid parameters in blood [8]. Moreover, although the mechanism of action is not fully understood, changes in lipid parameters are supposed to vary with the nature of the causative agent.

In the present series of patients, the results of hepatitis viral analysis indicated the absence of HAV infection in all the groups, as supported by earlier reports [9, 10]. Hepatitis B virus (HBV) infection is common, not only in acute but also in chronic liver diseases, including cirrhosis of the liver. This finding supports our earlier findings showing HBV infection in nearly 70% of cases with CAH [11]. Like HBV, hepatitis C virus (HCV) infection was also recorded in all categories of patients. This is in agreement with findings by Tandon et al. [12]. HCV plays a major role in the causation of chronic liver diseases, also in India.

Hepatitis D virus (HDV) infection was absent in all the patients. This is in agreement with our earlier studies demonstrating HDV infection to be rare in Indian patients [10, 13]. However, hepatitis E virus (HEV) infection was demonstrated in all four patient groups [14].

Analysis of sera samples of patients from the four study groups showing effect of disease and etiological viruses on lipo- and apolipoprotein levels is of interest.

LDL level was raised only in the CAH group. HDL on the other hand, showed a decline in all the groups except in cirrhosis. Similarly, ApoA levels were found to be low in the AVH and FHF groups, when compared to the control. The levels of ApoB were normal in the AVH and FHF groups but they were reduced in CAH and cirrhosis patients. The observed changes in the levels of these lipo- and apoproteins show both agreement as well as disagreement with the results reported in earlier studies. Ciconnani et al. [15] showed that LDL was significantly re-

duced in patients with cirrhosis compared to the CAH and the control group, thus supporting findings of this study that LDL is not reduced in CAH. The same group also showed that HDL level is reduced in liver failure groups without cholestasis. This again supports the finding of this study, showing a decline in HDL level in these disease groups. Sposito et al. [16] further reported that ApoA and ApoB were reduced in chronic liver failure. A differential reduction in ApoA and ApoB levels in different disease groups was noted in this study. Whereas ApoA was significantly reduced in AVH and FHF, ApoB exhibited a significant decline in only the CAH and cirrhosis groups. It is not possible at this stage to explain precisely the importance of these results. For this, further work is required.

Analysis of data at several levels of HDL, LDL, ApoA and ApoB in relation to viral etiology in all four disease groups did not show any relationship between etiology and changes in blood levels of these parameters. It can be inferred that the stage of disease influences lipid metabolism and lipid profile of the blood and not the virus. Viral hepatitis had almost similar impact on blood lipid levels in all cases.

Lp(a) was first demonstrated by Berg [17] as a circulating particle, with apolipoprotein B-I00 linked to apolipoprotein(a) with a disulphide bridge. Liver is the major source of circulating Lp(a) [18–21]. Although several studies report a close association of serum Lp(a) level with cardiovascular and thrombotic disorders, only few studies have described a possible relation between Lp(a) and liver diseases [22]. These studies demonstrated AVH with diminished Lp(a) serum concentration [22]. Similarly, Lp(a) was reported to be low in chronic liver diseases of different etiology [23]. This study made further attempts to understand the impact of severity and etiology of liver diseases on Lp(a) level in blood.

The data here show that both acute and chronic liver diseases, including cirrhosis of liver, have lowered plasma lipid concentrations. This was the case in large proportions of patients with more severe forms of liver diseases like FHF and cirrhosis of liver, compared to acute and CAH. Lp(a) could not be detected in 20 of the 50 cases with AVH, 14 of 30 with CAH, 21 of 30 with cirrhosis and 16 of 20 with FHF. The absence of detectable Lp(a) level in these liver diseases is in agreement with the findings of several other studies [14, 24–27]. The data here indicate that there is an apparent decline in Lp(a) levels in all types of viral hepatitis. Though, as such, reduction in Lp(a) level does not appear to follow any trend in relation to the

causative hepatitis virus, it seems that reduction in Lp(a) level is not dependent on the nature of the causative virus but on the degree of liver damage. The same is further confirmed by our findings, where in several cases the initial low level of Lp(a) was raised as the severity of the disease increased.

As observed in this study, that patients with all liver diseases studied here have a very low level of circulating Lp(a), it may be concluded that patients suffering from liver diseases are at a low risk of cardiovascular diseases. A similar view was also put forward in another study [28], where patients with cirrhosis were considered as being at lower risk of coronary heart diseases owing to their low Lp(a) levels.

Conclusion

Viral hepatitis variably raises the levels of serum apo- and lipoproteins. This effect is not explainable. No apparent relationship can be observed between etiological viruses and ensuing changes in lipid/lipoprotein profile.

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