Available online at www.elixirpublishers.com (Elixir International Journal)

Virus Research

Elixir Vir. Res. 35 (2011) 2802-2808

ISSN: 2229-712X

Hepatitis C virus (HCV)-core expression: Role of serum-core protein in diagnosis of HCV infection and its relation with oxidative stress & liver steatosis

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ARTICLE INFO

Article history:

Received: 5 April 2011;

Received in revised form:

19 May 2011;

Accepted: 25 May 2011;

Keywords

HCV,

Core protein,

SOD,

Lp(a),

Pathogenesis,

Genotypes.

ABSTRACT

Objective: To investigate the role of hepatitis C virus (HCV) core protein expression, as indicated by the presence of HCV core protein in serum, in the diagnosis and pathogenesis of HCV infection.

Methods: A total number of 432 patients with various liver diseases including acute and chronic/end stage liver diseases were analysed for HCV-related markers comprising anti-HCV, HCV-RNA and HCV-core.

Results: HCV-core protein was found to be a better serum marker compared to anti-HCV and HCV-RNA for the diagnosis of HCV-infection in chronic liver diseases. HCV-core protein in serum shows a low prevalence in patients with acute infection. To explore the possibility of core-expression in liver pathology via its impact on oxidative stress and steatotic changes, we studied the sera level of lipids, lipoproteins, apoproteins and anti-oxidant levels in relation to HCV-core protein in serum. The analysis of data could not demonstrate any significant change in the level of these metabolites with presence or absence of HCV-core protein. This was explained as HCV-core expression having insignificant role in inducement of oxidative stress and development of steatosis to cause liver damage. This study also describes the varied prevalence of different HCV-genotypes in relation to HCV-core protein in these patients, thus, explaining that HCV-genotypes have little role in expression of HCV-core.

Conclusion: Serum HCV-core protein has important role in the diagnosis of HCV infection. However, we could not observe any significant relation between HCV-core expression and liver steatosis, oxidative stress or HCV-genotypes in liver diseases.

Introduction

Hepatitis C virus (HCV) causes liver diseases ranging from an asymptomatic carrier state to hepatocellular carcinoma (HCC).[1] HCV infection has been detected globally[2] and poses a serious public health problem throughout the world. It is a major cause of chronic hepatitis and leads to cirrhosis and to HCC over a period of time. While intravenous drug use has become the main route of transmission in most industrialized countries, blood transfusion still represents a major risk of HCV infection in developing countries.

Molecular biology of HCV has revealed it as a composite virus, having different structural components with diverse functions. Molecularly cloned in 1989, HCV was classified as a new member of the Flaviviridae family.[3] HCV has a positive sense, single stranded RNA genome that has a single long open reading frame (ORF) flanked by 5' and 3' untranslated regions (UTRs). It codes for a polypeptide (about 3000 amino acid long), consisting of 3-4 structural proteins at the amino-terminus (core, E1 and E2/P7) and six non-structural protein (NS2 [protease], NS3 [protease-helicase], NS4A [co-factor for NS3] NS4B, NS5A (serine phosphoprotein) and NS5B (RNA polymerase) at the carboxyl terminus. The structural component of HCV, the HCV core protein, has attracted a special attention after its characterization and various reports on its important role in HCV pathogenesis. Preliminary data on HCV core protein indicated its possible role in diagnosis and pathogenesis of HCV infection. Also HCV core was reported to contribute to the causation of hepatocellular carcinoma (HCC) after HCV infection.

Liver injury during HCV infection is supposed to be a multifactorial process involving immune-mediated cell lysis and organ damage caused by reactive oxygen species.[4] There are reports available to demonstrate that oxidative stress causes lipid peroxidation in HCV infected patients.[5] Recent in-vitro studies have suggested that Hepatitis C core protein and NS5A can induce oxidative stress in transfected cells.[6] Also, hepatic steatosis is a common histopathologic feature noted during HCV infection. There are several reports indicating that help "Reactive Oxygon Species (ROS)" in development of hepatic steatosis.[7] At the same time, there are some reports demonstrating an association of steatosis with HCV-core and HCV-genotypes.[8] Steatosis induced by HCV-core protein predisposes to lipid peroxidation with potential risk of genomic mutation[9] and thus, a possibility of oncogenesis. In view of all this, it becomes interesting to know how HCV-core shows

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hepatotoxic effect via its impact on ROS generation and hepatic steatosis in relation to HCV-genotypes. Present study was planned to understand the diagnostic relevance of HCV core protein and its merit over anti-HCV antibodies and HCV-RNA in diagnosing HCV infection. Also, our findings are supposed to extricate the mechanism of liver necrosis by HCV demonstrating the role of HCV core in HCV pathogenesis through ensuing changes in lipid & lipoproteins, steatosis and its impact on the level of oxidative stress during HCV infection. In a more simplistic way, this study will show how core protein affects the level of lipids and anti-oxidants demonstrating the oxidative damage of liver through steatosis.

Material & Methods

Patients and blood samples:

Four hundred and thirty-two patients of both gender and in age group of more than 18 years were included in the present study. 110 (age range 23-49 years) were diagnosed as having acute viral hepatitis (AVH); 104 patients (age range 21-45 years) with chronic viral hepatitis (CVH), 110 patients (age range 34-58 years) were found to have liver cirrhosis, 52 patients (age range 32-51) with fulminant hepatic failure (FHF) and 56 patients (age range 43-54) had hepatocellular carcinoma (HCC). These were the patients who attended outpatient department and subsequently admitted to the liver unit of All India Institute of Medical Sciences, New Delhi. After clinical evaluation, their blood samples were tested for hepatitis viral markers. The diagnosis of AVH was based on overt jaundice and / or increased alanine aminotransferase levels without previous history of liver disease. The history of alcohol intake or using drug was excluded in this. Similarly, the evidence of autoimmune diseases or biliary infection was also excluded. The patients with CVH and liver cirrhosis were diagnosed by histopathological criteria. CVH patients had persistent elevation of transaminases level for more than six months and histologic evidence of chronic hepatitis on liver biopsy at the beginning of follow-up and exclusion of alcohol abuse and other possible causes of chronic liver diseases. Cirrhotic patients were not found to have history of chronic alcohol intake. Fulminant hepatic failure was diagnosed if the patients developed hepatic encephalopathy within 4 weeks of the onset of acute hepatitis. Patients with HCC were diagnosed on the basis of liver histology. One hundred age and both gender matched healthy subjects were used as controls. This study plans in human subjects has been approved by our Institute's Ethics Committee.

The venous blood (6-10 mL) was drawn and transfered in plain tubes without anticoagulant. Serum was separated after centrifugation and then stored at -70°C. These sera samples were used to analyze liver function tests (LFT), hepatitis markers and HCV-core protein.

Hepatitis viral markers

Sera were investigated for hepatitis B surface antigen (HBsAg) and IgM antibodies to hepatitis A virus (IgM anti-HAV), hepatitis B core antigen (IgM anti-HBc), 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 obtained from internationally known firms. Kits for HBsAg, IgM anti-HBc and IgM anti-HAV were purchased from Abbot Laboratories, Illinois, USA. Anti-HCV was tested using highly sensitive third generation ELISA kit from Ortho-Clinical Diagnostics, Inc.(Raritan, New Jersey, USA). This anti-HCV kit used peptides versus core, NS3, NS4 and NS5 regions of HCV genome, as antigen to coat the ELISA plate. IgM antibody to hepatitis D virus (HDV) was tested using an enzyme immunoassay kit from Wellcome (Dartford, U.K). Similarly, IgM anti-HEV was tested using third generation ELISA kit from Genelabs Diagnostics Pte Ltd (Science Pk Dr, Singapore).

Diagnosis of viral hepatitis

The diagnosis of different types of viral hepatitis was established as follows: The diagnosis of hepatitis A virus (HAV) infection was confirmed by the presence of IgM anti-HAV in serum. Hepatitis B virus (HBV) infection was established by finding IgM anti-HBc in sera of AVH and FHF patients and by the persistent HBsAg antigenemia in sera of CVH and cirrhosis cases. Similarly, total anti-HCV and IgM anti-HDV in sera samples were used for the diagnosis of HCV and recent HDV infections, respectively. Active or recent hepatitis E virus (HEV) infection was diagnosed by the presence of IgM anti- HEV in serum. Sera positive for HBsAg but negative for all other viral markers were labeled as HBV-carriers. Absence of all the markers including HBsAg labeled the patients with hepatitis non-ABCDE infection on exclusion criteria.

HCV Core Antigen Assay

HCV core protein was assayed in sera samples using EIA kit from Ortho-Clinical Diagnostics, Inc. (Raritan, New Jersey, USA). One hundred μL of samples and controls were mixed with 100 μL of a pretreatment buffer followed by their incubation for 95 minutes at 37°C with continuous shaking. The micro titer plates were washed and incubated for 30 minutes at 37°C with 200 μL of conjugate, washed again and incubated for 30 minutes at 37°C with 200 μL of substrate. The optical densities (ODs) were read at 490 nm using a 620 nm reference. The samples and controls were tested in duplicate and the mean OD of each duplicate testing was used. The cutoff value was established for each run and corresponded to the mean OD of the 2 negative controls plus 0.040. A sample was considered positive when the mean OD was higher than the cutoff OD of the corresponding run.

Detection of HCV-RNA by Reverse Transcriptase (RT) – Polymerase Chain Reaction (PCR)

HCV-RNA was isolated from 100-μL serum or plasma using High Pure Isolation kit from Roche, Mannheim, Germany, according to the method given in instructions manual. It was immediately used in RT-PCR experiments or stored at -70°C. Five μL of the isolated RNA was applied to reverse transcription and nested PCR with primers.

For first round:
Forward: 5'-GTG AGGAACTACTGTCTTCACGCAG-3',
Reverse: 5'-TGCTCATGGTGCACGGTCTACGAGA-3';
and for second round:
Forward: 5'-TTCACGCAGAAAGCGTCTAG-3',
Reverse: 5'-CTATCAGGCAGTACCACAAGG-3'

Located in the highly conserved 5` noncoding region (5` NCR) using BIOHCV kit (B&M Labs., Madrid, Spain). The reverse transcription mixture was incubated for 1 min at 85°C, followed by 30 min at 60°C. First PCR was performed in whole content after adding 40 μL of HCV amplification mixture. Thermal cycler was programmed as follows: 85°C for 30 s, 94°C for 2 min followed by 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s and then incubation of samples for 5 min at 72°C. Five μL of first PCR product was subjected to nested PCR using nested PCR mixture containing second round primer

and enzymes etc. The protocol on thermocycler was the same as mentioned in first PCR. The PCR product was identified by 2% agarose gel electrophoresis containing ethidium bromide followed by visualization of bands under UV. All positive and negative controls were tested in parallel with test samples.

HCV-genotyping

PCR products obtained by above method were subjected to HCV-genotyping using Restriction Fragment Length Polymorphism (RFLP) method. These amplicons were digested by different sets of restriction enzymes: *Rsa*I and *Hae*III; *Mva*I and *Hin*fI, incubated at 37°C for over night. These amplicons were further treated with restriction enzymes *Bst*UI (at 60°C, 4hr.) and *Scr*FI (37°C, 4hr.) for sub-typing 1a, 1b and 2a, 2b, 3a and 3b respectively. The fragments obtained after digestion were run on 2% high quality agarose gel and the pattern of bands were used to type the HCV-genotypes.

Estimation of lipoprotein [Lp (a)], apoprotein A-1 (Apo A-1) and apoprotein B (Apo-B)

Estimation of lipoprotein (a) [Lp (a)], apoprotein A-1 (Apo A-1), Apoprotein B (ApoB) in serum was performed by immunoturbidimetry methods using kits from Randox Laboratories, Crumlin, UK on Hitachi 912, chemistry autoanalyser.

Estimation of low density lipoprotein (LDL) and high density lipoprotein (HDL)

The estimation of LDL and HDL were performed by the routine established techniques. The test reagents for these parameters were purchased from Randox Laboratory (Crumlin, UK).

Estimation of Superoxide Dismutase (SOD) and total anti-oxidants

SOD level in hemolysate prepared from red blood cells (RBCs) was estimated using Ransod kit supplied by Randox Laboratories (Ardmore, Northern Ireland, UK). This assay employs xanthine and xanthine oxidase to generate superoxide anion which reacts with 2-(4-indophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride (INT) to form a red formazan dye. SOD activity is then measured by the degree of inhibition of this reaction. The values of SOD were expressed in units/g hemoglobin (Hb). Like SOD, the total antioxidant (TAO) level in plasma was also estimated using kit from Randox Laboratories. In this assay, 2,2`-azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS), is incubated with metmyoglobin and H_2O_2 to produce the radical cation colour, which is measured at 600 nm. Anti-oxidants in the added sample cause suppression of this colour production to a degree which is proportional to their concentration. Values of TAO were expressed as mmol/L plasma.

Biochemical tests

Sera samples from all the patients were analyzed for liver function tests including transaminases levels by the established laboratory techniques.

Statistical analysis

The analysis of data to determine statistical difference among different values was done using student's *t* test by SPSS software 16.0 (SPSS Inc, Chicago, IL, USA). *P* value of less than 0.05 was taken as a significant difference.

Results

The analysis of results described in Table-1 demonstrate that in AVH group, HCV-core protein and HCV-RNA were present in 21 of 110 (19.1%) patients each. However, anti-HCV was recorded in only 12 of 110 (10.9%) cases. Presence of anti-HCV in CVH patients was detected in relatively higher number, i.e. 36 of 104 (34.6%) as compared to HCV-core (21.2%) and HCV-RNA (19.2%). Similarly, anti-HCV was detected in 31.8% patients with liver cirrhosis as compared to the presence of HCV-core (25.5%) and HCV-RNA (29.1%) respectively. Patients with FHF had HCV-core in 21.2% cases as compared to HCV-RNA (9.6%) or anti-HCV (9.6%) antibody. A similar pattern was noted in HCC cases also. Simultaneously, presence of anti-HCV with HCV-core or HCV-RNA shown variable results with no definite trend maintained in liver disease groups. The sera from 100 healthy controls were found to be negative for all hepatitis viral markers.

To establish a relation of HCV-core expression with status of lipids and lipoproteins, the sera in each disease group were tested for lipids, lipoproteins and apoproteins A1 and B. The results were analysed to investigate the level of lipids and lipo/apoprotein in relation to presence of HCV-core (Table-2 and 3).

The serum level of total cholesterol, triglyceride, high density lipoprotein (HDLc) and low density lipoprotein (LDLc) in AVH patients were found to be comparable both in HCV-core positive and HCV-core negative patients. However, Lp(a) level was found to be significantly reduced (P = 0.043) in HCV-core positive patients as compared to core negative patients in AVH group. Exactly, a similar pattern was noted in CVH patients also. In other disease groups, on the contrary, the levels of lipids and lipoproteins were comparable and not affected by the presence of HCV-core protein.

Studies were conducted to investigate another important aspect i.e., the effect of HCV-core expression on anti-oxidant levels in these liver diseases. The number of sera analysed and results achieved are given in Table-4. Blood samples from patients and healthy controls were tested for SOD, total anti-oxidant (TAO) and uric acid levels. Table 4 presents the comparative results for anti-oxidant levels in presence and absence of HCV-core in blood samples from various liver diseases. The results indicate that there is no difference in the values of SOD and TAO in all the disease groups in relation to presence or absence of HCV-core. However, Uric Acid levels were significantly reduced (P = 0.043) in HCV-core positive patients from AVH, CVH, liver cirrhosis and FHF as compared to HCV-core negative patients. HCC had no effect of core positivity on Uric Acid level.

HCV-core expression was also studied in relation to HCV-genotypes detected in these patients. It was aimed to find out whether core-expression is influenced by the genotyping. All the sera positive for HCV-RNA were assayed for HCV-genotypes/isotypes and presence of HCV-core protein. The results were analysed to relate genotypes with presence / absence of HCV-core. Our findings indicate that genotypes are variable and include genotype-1, 1a, 1b, 3a, 3b and 4a distributed in a random fashion without showing any concrete relation with HCV-core. However, the sample size is too low to reach the logistic conclusion.

Discussion

In continuation to a brief report published earlier[10,] present study discusses in detail the diagnostic and pathogenic significance of HCV-core expression during HCV infection. HCV infection is a global problem and remains a major cause of all categories of liver diseases including acute, chronic and malignant hepatic diseases. It is diagnosed by the presence of anti-HCV antibody in sera samples. The detection of HCV-RNA

in serum, though supposed to be a better marker, is quite expensive and not feasible in many routine investigative laboratories. The presence of HCV-core protein in serum was studied and found as an alternate HCV-marker. It indicated its presence in nearly 80-92% patients positive with anti-HCV antibody.[11,12] At the same time, the concordance between HCV core and HCV-RNA was noted upto 93-95%.[11] It implies that anti-HCV antibody detection overestimates HCV infection in at least 8-10% cases. Despite several merits of HCV core assay over HCV-RNA detection, core assay has been reported to be a specific but less sensitive in different studies. Whereas the sensitivity of HCV-RNA assay was found to be 99%, that of HCV core assay was noted as 98%[13]**.** Similarly, Zanetti et al[14] found only 82% sensitivity of core as compared to that of HCV-RNA assay.

When we analysed our data to find out diagnostic significance of HCV-core in relation to anti-HCV antibodies and HCV-RNA in different liver diseases, we noted varied results. In majority of patients, the presence of HCV-core was more common as compared to that of HCV-RNA. In AVH patients, HCV-core was noted more frequently as compared to anti-HCV. In patients with CVH and cirrhosis, on the contrary, anti-HCV remains positive in high percent population as compared to HCV-core. It may imply that the patients with AVH express core protein much earlier than the development of anti-HCV, which is quite likely due to delayed immune response in these patients. In chronic liver diseases, persistent infection keeps the immune response and production of antibody at a steady level with presence of antibody in circulation at all time. This shows that detection of HCV-core in serum is more promising for diagnosis of acute HCV infection than chronic infection.

In order to explore all possible factors responsible for liver damage during HCV-infection, the studies conducted globally have reported a change in lipid fabric of liver and status of oxidant species.[15] It is known that HCV as such is not cytopathic[16] and therefore, its damaging potential lies in the level of HCV-induced immunocytolysis,[17] deranged lipid metabolism, particularly the development of steatosis[18] and inducement of pro-oxidant reactions.[19] Our study attempted to show how HCV-core antigen expression during HCV infection plays a role in cellular damage by affecting the metabolic changes related to status of lipid and lipoproteins and the state of oxidative stress in these patients. [20-21] It was aimed to investigate the impact of HCV core expression on liver cell damage through steatosis and oxidative stress.

Steatosis is an established fact to bring about a slow and steady liver damage.[22] Therefore, any factor responsible to increase liver steatosis, helps in liver damage. Although, steatosis is detected histologically, present study uses lipid and lipoprotein levels in serum to demonstrate steatosis indirectly.[10] Changes in serum level of these lipids and lipoproteins may indicate the stage of liver steatosis. Our findings on the changes in lipid and lipoproteins in relation to HCV-core protein indicate the contradictory results. We could not find any change in lipid status, both with and without core, during HCV infection. This means, that during HCV-induced damage, the lipid level is not significantly changed. In other words, HCV-infection does not cause liver damage by increasing steatosis of liver. Absence of changes in relation to HCV-core further supports that HCV-core expression does not affect liver damage by inducing liver steatosis. Minor lipid level variation, like decreased level of triglyceride or Lp(a) in some cases, may be an impact of severity of liver disease rather than the cause of disease.

There are several reports to relate HCV-core expression with oxidative stress which ultimately contribute to the development of steatosis.[23] Okuda et al[24] in their study demonstrated increased reactive oxygen species (ROS) in Huh-7 or HeLa cyclin-inducible expression system. Another study[25] reported that acute core expression leads to increased lipid peroxidation and induction of antioxidant enzymes. However, it is worth to note here that all these reports indicate an in vivo change with very little information on viral replication and protein production during HCV infection.[26] Of course, available evidence indicates that core protein promotes oxidative stress in vivo and cause hepatic injury.[27] This meant that pro-oxidant actions of core may have a mechanistic role in the pathology of the virus.

We envisaged some possibilities of reactive oxidant species to be increased or being more active during HCV infection and core-expression affecting oxidant-antioxidant levels in blood. However, we could not find any major change in the level of SOD and total anti-oxidants in presence or absence of HCV-core protein. The slight rise in Uric Acid level may be interpreted as an adaptive rise to counteract minor alterations in oxidative stress during liver diseases or an effect of disease on purine metabolism. At least oxidative changes had no role in liver damage in these patients. Further, there was hardly any relation between anti-oxidant levels and presence of HCV-core, thus, stressing that core expression does not affect the level of reactive oxygen species (ROS). This shows that oxidant species are not responsible of cellular damage. Secondly, HCV-core expression does not change this dynamism and keeps the responsible damaging action unaffected.

HCV is not a single entity but a group of various molecular species varying differently along its genome. These species have been named as genotypes, isolates and / or quasispecies depending on the type and extent of genomic variation in HCV-genome. As such HCV has six genotypes and more than 50 isotypes. It is already proved that all HCV-species do not respond equally to the anti-viral treatment using peg-interferon and ribavirin. Whereas some selected genotypes may be completely eradicated by antiviral agents, others do not respond to treatment and thus, show long term persistence leading to end stage liver diseases like cirrhosis of liver and hepatocellular carcinoma.[28-30] Based on these observations, it is assumed that HCV-genotypes in a parallel way have some potential role in cellular damaging activities and response to treatment. The data achieved under this study plan in a few number of cases show a variable prevalence of HCV-genotypes and their subtypes having no concrete relation with the presence or absence of HCV-core in different liver diseases. It is mere a preliminary information and needs more extensive and elaborated studies to establish the role of HCV-genotypes in pathogenicity of HCV-infection.

Acknowledgement

The authors thank and appreciate the financial aid provided by ICMR, New Delhi, India to conduct this study. Authors are also thankful to Mrs. Suman Rawat for preparing this manuscript.

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Table 1. Hepatitis C virus (HCV) core protein in relation to anti-HCV antibodies in different liver disease

Disease Group	No. tested	HCV Core	HCV-RNA positivity	Anti-HCV positivity	HCV Core & Anti-HCV positivity	HCV-RNA & Anti-HCV positivity
Acute viral hepatitis	110	21 (19.1%)	21 (19.1%)	12 (10.9%)	3 (2.7%)	7 (6.4%)
Chronic viral hepatitis	104	22 (21.2%)	20 (19.2%)	36 (34.6%)	10 (9.6%)	14 (13.5%)
Liver cirrhosis	110	28 (25.5%)	32 (29.1%)	35 (31.8%)	7 (6.4%)	32 (29.1%)
Fulminant hepatic failure	52	11 (21.2%)	5 (9.6%)	5 (9.6%)	2 (3.8%)	3 (5.8%)
Hepatocellular Carcinoma	56	6 (10.7%)	2 (3.6%)	5 (8.9%)	0 (NIL)	0 (NIL)
Healthy Control	100	NIL	NIL	NIL	NIL	NIL

*Values in parenthesis indicate percent positivity in relation to total number tested in each disease group.
**The column with HCV Core & Anti-HCV positivity shows the cases simultaneously positive for both the markers.

Table 2. Levels of lipids and lipoproteins in relation to hepatitis C virus (HCV) core protein

Liver disease (No. tested)	HCV Core positive cases						HCV Core negative cases					
	No. tested	T. Chol. (mg/dL)	TG (mg/dL)	HDLc (mg/dL)	LDLc (mg/dL)	Lp(a) (mg/dL)	No. tested	T. Chol. (mg/dL)	TG (mg/dL)	HDLc (mg/dL)	LDLc (mg/dL)	Lp(a) (mg/dL)
AVH (102)	21	139±48	164±39	33±9	105±31	1.4±2.1*	81	138±51	152±81	34±9	89±32	8.9±7.0
CVH (104)	22	119±53	112±25	26±7	93±54	1.9±1.0*	82	162±42	124±41	28±11	74±26	7.9±8.0
Liver cirrhosis (105)	26	120±27	131±38	29±15	93±44	7.5±6.2	79	121±14	105±21	32±11	118±15	8.0±4.0
FHF (52)	11	146±70	318±95*	34±28	104±24	6.8±9.0*	41	118±60	108±44	39±14	78±60	6.0±2.4
HCC (54)	6	115±21	132±24	29±8	96±29	8.0±0.2	48	128±17	105±18	30±9	82±12	8.5±1.2
Healthy Controls (100)	0	---	---	---	---	---	100	137±24	112±46	38±22	86±43	8.8±6.0

Normal ranges : T. cholesterol : 150-210 mg/dL ; Triglyceride : Upto 200 mg/ dL ; HDLc : 28-62 mg/ dL ; LDLc : 91-178 mg/ dL ; Lp(a) :<30 mg/ dL
* Values statistically significant in comparison to core negative values ($P = 0.043$)
AVH : acute viral hepatitis HDLc : high density lipoprotein associated cholesterol Lp(a) : lipoprotein a T. chol : Total cholesterol ;
CVH : chronic viral hepatitis LDLc : low density lipoprotein associated cholesterol TG : Triglyceride
The number of patients included in each disease groups in above table is the actual number of sera tested for that disease group. For rest of the cases, serum was not adequate for the test.

Table 3. Levels of apolipoproteins in relation to hepatitis C virus (HCV) core protein

Liver disease	HCV core positive cases			HCV core negative cases		
	No. tested	Apo A1 (mg/ dL)	Apo B (mg/ dL)	No. tested	Apo A (mg/ dL)	Apo B (mg/ dL)
AVH (102)	21	32±5.0*	88±0	81	88±41	91±39
CVH (104)	22	41±18*	36±3	82	105±38	72±24
liver cirrhosis (105)	26	115±12	68±32	79	78±14	54±22
FHF (52)	11	22±8*	128±41	41	58±38	74±31
HCC (54)	6	112±9	124±14	48	82±15	60±24
Healthy controls (100)	---	---	---	100	93±14	65±18

Normal ranges : Apo A 1 : 120–176 mg/ dL ; Apo B : 63–114 mg/ dL

* Indicates the values statistically significant in comparison to core negative values in the same disease group ($P = 0.043$)

AVH : acute viral hepatitis
CVH : chronic viral hepatitis
FHF : fulminant hepatic failure
HCC : hepatocellular carcinoma
Apo A & B: apolipoprotein A and B

The number of patients included in each disease groups in above table is the actual number of sera tested for that disease group. For rest of the cases, serum was not adequate for the test.

Table 4. Oxidations status in relation to hepatitis C virus (HCV) core protein in patients with liver diseases

Liver disease	HCV core positive patients				HCV core negative patients			
	No. tested	SOD (U/g Hb)	TAO mmol/L	U. Acid mg/ dL	No. tested	SOD Hb(U/g)	TAO mmol/L	U. Acid mg/ dL
AVH (102)	21	1189±151	1.44±0.31	9.2±1.2*	81	1068±202	1.39±0.35	6.2±1.65
CVH (104)	22	1015±85	1.39±0.41*	11.2±1.3*	82	1058±120	1.71±4.1	8.1±5.1
Liver cirrhosis (105)	26	1305±121	1.54±0.38	8.2±0.05*	79	1104±109	1.74±0.40	7.1±1.1
FHF (52)	11	1114±105	1.65±0.30	9.1±0.40*	41	1201±85	1.69±0.30	7.5±0.4
HCC (54)	6	1052±113	1.36±0.40	7.8±0.30	48	1145±46	1.36±0.40	6.8±0.34
Healthy Controls (100)	---	---	---	---	100	1035±78	1.64±0.22	6.5±0.40

Normal ranges : SOD : 1092-1817 U/g Hb ; TAO : 1.3-1.77 mmol/L ; U.Acid : 2.0-7.4 mg/ dL

* Indicates statistically significant values compared to core negative value in the same disease group ($P = 0.043$)

AVH: acute viral hepatitis SOD: Superoxide dismutase
CVH: chronic viral hepatitis TAO: Total antioxidant
FHF: fulminant hepatic failure U. Acid: Uric acid
HCC: hepatocellular carcinoma

The number of patients included in each disease groups in above table is the actual number of sera tested for that disease group. For rest of the cases, serum was not adequate for the test.