

Viral hepatitis in India: Current status

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Abstract

Background: Viral hepatitis is a major cause of liver diseases and poses a serious public health problem throughout the world including India.

Objectives: Present study was planned to investigate the current status of viral hepatitis in patients with different liver and renal diseases.

Methods: A total number of 1043 patients, selected from an adult population with both the sexes, were included in this study plan. After clinical examination, their sera were analyzed for the presence of different hepatitis viral markers. The diagnosis of acute infection was based on the presence of IgM type antibody for hepatitis A, B, D & E infections. HCV-RNA or total anti-HCV was used for the diagnosis of HCV infection.

Results: We found the presence of hepatitis A virus (HAV) infection to be in 1-2% cases. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections were recorded to be the major cause of acute and chronic liver diseases. At the same time, hepatitis E virus (HEV) was responsible for causing acute liver diseases without showing its presence in chronic liver diseases. Hepatitis D virus (HDV) infection was not detected in this group of patients. Occurrence of co-infection was recorded merely with HBV-HCV without any other type of co-infection. A clinical follow-up of patients having co-infection or super-infection demonstrated a relatively more derangement of liver functions in the patients.

Conclusion: All types of hepatitis viral infections are prevalent in Indian patients' populations. The relative incidences of these infections vary slightly from place to place.

Introduction

Viral hepatitis is caused by a number of viruses some of which have already been characterized and named as hepatitis viruses A, B, C, D, E and G, respectively [1-3]. In addition, there are patients who are not accounted by these viral infections but show all clinical symptoms suggestive of viral hepatitis. Such cases are assumed to be caused by a group of viruses classified as non-A-G hepatitis viruses. Nearly 15% patients of viral hepatitis belong to this group. These viruses need to be characterized for their molecular structure and types of disease caused. Viral hepatitis is a serious problem in India too with a high proportion of liver ailments caused by hepatitis viruses [4-6]. Based on various studies, all types of known and unknown viruses causing hepatitis have been reported in Indian populations [7].

Some of these viral infections have sufficiently long incubation period and remain asymptomatic in initial phase of disease. At the same time, it is not possible to detect any serological marker during window period. Today, there are assays available to diagnose hepatitis viral infections with high accuracy during total course of disease [8-10]. These assays are able to detect viral markers both in an early phase of the disease, as well as during the window period. These diagnostic assays focus mainly on detecting viral nucleic acid in addition to serological markers. Also, the newly developed assay systems can differentiate between the past and present infection [11-13]. A combination of both serology and nucleic acid based assays help in early detection and accurate diagnosis of infection.

The previous reports have demonstrated the relative prevalence of different viral infections in various categories of liver diseases including acute/chronic liver diseases, cirrhosis of liver and hepatocellular carcinoma (HCC) from different parts of our country [7,14,15]. All these reports clearly indicate the prevalence of all types of hepatitis viral infections that present from mild and asymptomatic form to severe

liver ailments requiring emergency treatment. In addition, occurrence of water borne epidemics of hepatitis E viral infections is also not uncommon in this country [16,17]. Although there has been a decline in occurrence of such epidemics in last few years, however, it cannot be completely ruled out even today.

In view of viral hepatitis being a serious public health problem in India, it was assumed worth to conduct studies to assess the current status of viral hepatitis in this country, particularly with introduction of new and advanced diagnostic assays in this area. Therefore, we planned to find out the relative prevalence of hepatitis viral infections in different types of liver and renal diseases using sensitive and specific assay systems including higher generation immunoassays and PCR techniques. Sera from a large population of patients under each category of disease were analyzed to know the status of each individual type of infection as well as co-infections in these groups of patients.

Material and methods

Patients and blood samples

Total number of 1043 patients were included in the present study after obtaining their informed consent. They belonged to both the sexes and adult age group. Application of diagnostic criteria indicated 191

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patients (age range: 23-49 years) with acute viral hepatitis (AVH), 321 patients (age range: 21-45 years) with chronic viral hepatitis (CVH), 223 patients (age range: 34-58 years) with cirrhosis of the liver (CIR), 88 patients (age range: 32-51) with fulminant hepatic failure (FHF) and 76 patients (age range: 43-54) with hepatocellular carcinoma (HCC). In addition, 144 patients with chronic kidney diseases (CKD) with or without liver diseases were also included in the panel of patients for analysis. Those suspected with alcoholic or auto immune liver diseases were excluded from the study plan. These patients were treated at liver unit of All India Institute of Medical Sciences, New Delhi.

AVH was diagnosed when patients exhibited overt jaundice and/or increased alanine aminotransferase levels (at least 3 times above the normal value) documented at least twice at a 1-week interval without any history of pre-existing liver disease. None of the patients diagnosed with AVH had a past history of alcohol intake or using any drug. We also could not find any clinical or serological evidence of autoimmune diseases or biliary infection in these patients. The patients with CVH and cirrhosis of liver were diagnosed by histopathological criteria laid down by International study group on chronic hepatitis [4]. All CVH patients had persistent elevation of transaminases level (at least 3 times the upper limit of normal range) for more than six months, histologic evidence of chronic hepatitis on liver biopsy at the beginning of follow-up. Cirrhosis patients were not found to have history of chronic alcohol intake. Fulminant hepatic failure was diagnosed when the patients showed signs of hepatic encephalopathy within 4 weeks of the onset of acute hepatitis. Patients with cirrhosis and HCC were diagnosed on the basis of clinical, histopathological and radiological criteria. CKD was diagnosed by serum creatinine level of more than >1.8 mg/dL after 3 months in the absence of other factors [7]. Venous blood was taken from each patient and analyzed for various hepatitis markers and liver function tests, as described earlier [4]. The study was approved by the Institutional Ethics Committee of the concerned place of work.

Diagnosis of viral hepatitis

The diagnosis of viral hepatitis was made on the basis of presence of viral markers in the sera of these patients analyzed as described earlier [7].

HCV-RNA detection

After extraction of total nucleic acid from 200 µl serum or plasma using High Pure Viral Nucleic Acid kit from Roche, Germany, complementary DNA (cDNA) was synthesized. Reverse transcription (RT) was performed using Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Germany) at 42°C for 50 min using 60 µmol/L of random hexamer primer. Patients sera were screened for HCV RNA using conventional PCR to amplify 240 bp fragment of the highly conserved 5' non-coding region (5' NCR). The nested PCR was performed with sense primer NF5 – 5' GTG AGG AAC TAC TGT CTT CAC GCA G 3' and anti-sense primer NR5 – 5' TGC TCA TGG TGC ACG GTC TAC GAG A 3', respectively. PCR reaction contained 3 µL of cDNA, 200 µM of each dNTP, 1 µM of each primer and 0.75 U Taq Polymerase (Qiagen, Germany). Amplification conditions were as follows: initial denaturation at 95°C for 7 min followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 54°C for 30 sec, extension at 72°C for 45 sec and final extension at 72°C for 5 min. The second round of PCR was performed using sense primer KF2–5' TTC ACG CAG AAA GCG TCT AG 3' and antisense primer NR4 – 5' CTA TCA GGC AGT ACC ACA AGG 3', respectively. Amplification conditions were same as in first round, except for annealing step which was performed at 50°C for 30 sec and the extension step which was performed at 72°C for 30 sec. Three µl of the PCR products were loaded on 2% agarose gel containing

ethidium bromide. A band of ~240 bp on agarose gel after visualization under UV light indicated the amplification of 5' NCR.

HCV core antigen assay

HCV core antigen was detected in sera samples using assay system described earlier [7].

Detection of TTV-DNA by PCR

TTV-DNA was detected in serum as described in our previous report [18].

Biochemical tests

Liver function tests including transaminases and renal function tests were performed on autoanalyzer Hitachi - 917 model by the established techniques.

Results

Total number of 1043 patients belonging to different categories of liver and renal diseases were included in this study. Based on the accepted criteria of diagnosis, 191 of them were diagnosed with AVH, 88 patients with FHF, 32 patients with CVH, 223 patients with CIR, 76 patients with HCC and 144 patients were diagnosed with CKD. All of them belonged to adult age group in the range of 21-60 years and were from both the sexes. These patients were referred from different parts of India for treatment at All India Institute of Medical Sciences, New Delhi. Their blood samples were tested in laboratories for routine liver functions, CBC, coagulation profile and hepatitis viral markers.

Results of hepatitis viral markers are shown in Table 1. Based on analysis of the results, the presence of HAV infection, as indicated by IgM anti-HAV in serum, was noticed in 6 of 191 (3.1%) patients with AVH. Hepatitis B virus infection was diagnosed by the presence of HBsAg and IgM anti-HBc in serum. Presence of HBsAg in serum was recorded in 100 of 191 patients (52.4%) with AVH, 34 of 88 patients (38.6%) with FHF, 151 of 258 patients (58.5%) with CVH, 201 of 223 patients (90.1%) with cirrhosis and 34 of 69 patients (49.3%) with HCC. IgM anti-HBc, a marker indicating exclusively acute HBV infection, was detected in 25.5% cases with AVH, 29.8% cases with FHF, 19.9% cases with CVH, 7.9% cases with cirrhosis and none with HCC.

Three different diagnostic markers including anti-HCV antibodies, HCV-RNA and HCV-core antigen, were tested in sera for the diagnosis of HCV infection. Anti-HCV antibody, tested with third generation EIA, was detected in high proportion of patients with chronic liver diseases as compared to acute liver diseases. As such, anti-HCV was found positive in sera from 19 of 191 patients (9.9%) with AVH, 2 of 64 patients (3.1%) with FHF, 95 of 321 patients (29.6%) with CVH, 85 of 120 patients (70.8%) with cirrhosis and 34 of 76 patients (44.7%) with HCC. The presence of HCV-RNA in serum with replicating virus was noted in 14.3% cases with AVH, 1.6% cases with FHF, 80% cases with CVH, 43.3% cases with cirrhosis and 66.6% cases with HCC, respectively. HCV core antigen, reported to be another important marker of active HCV infection, was noted in 3.3%, 27.8%, 43.3%, 32.5%, and 27.3% with above liver disease groups, respectively. HDV infection, demonstrated by the presence of IgM-HDV antibodies in serum, was not detected in present panel of sera. On the contrary, HEV infection, showing its presence by IgM anti-HEV positivity in serum, was recorded in 28.4% patients with AVH and 41.9% with FHF. Similarly, TTV infection, detected by the presence of TTV-DNA in serum, shown a high prevalence in all categories of diseases.

Analysis of 144 patients with CKD admitted for treatment, demonstrated the presence of mainly HBV & HCV infections of the known hepatitis-causing viruses. Whereas HBV was detected in approximately 4% cases, HCV infection on the contrary, was found in about 28% cases. Other hepatitis viruses like HAV, HDV or HEV could not show their presence in this disease group. The data shown in Table 2 demonstrate the presence of co-infection, i.e. simultaneous presence of two or more viral markers in serum. Co-infection with HBV and HCV was predominant in chronic liver diseases as compared to acute liver diseases. On the other hand, HBV and HEV co-infection was noted in higher percent cases of AVH as compared to other category of diseases. Presence of HBV with HEV was noted in 6.7% cases with CVH also, possibly due to exposure of these patients to contaminated water or food products. Other types of co-infections were very rare and insignificant in both liver and renal diseases. Co-infections usually result in severe form of infection causing serious disease. The results from this study also point out towards a relation between co-infection and the severity of diseases. We observed more deranged organ function tests in patients carrying co-infection as compared to those with any single infection.

Discussion

After characterization of different hepatitis viruses in last few decades, studies were conducted to investigate the relative role of these viruses in causing acute and chronic liver diseases. At the same time, attempts were made to find out their role in outbreak of hepatitis in different countries and also their oncogenic potency in causing liver cancer. Today, hepatitis caused by these viruses is a serious public health

problem throughout the world and WHO has developed a framework for the prevention and control of viral hepatitis [19]. Whereas HAV and HEV cause asymptomatic, benign and self-restricted diseases in majority of cases [20,21], HBV and HCV infection may lead to serious liver diseases including cirrhosis of liver and hepatocellular carcinoma in significant proportion of cases [22,23]. In India, studies were conducted periodically and reported the presence of all these viruses in our populations [4,5,14]. They are responsible both for epidemic as well as sporadic cases of hepatitis in this country. Moreover, their pattern of disease causation as well as the final outcome of diseases was noticed more or less same as seen in other parts of the world, except their differential prevalence in patient's populations. An evolution tree of diagnosis in the area of hepatitis has ever prompted the researcher to have a new look at their status with induction of novel diagnostic assays. Since studies in last few years have inducted new techniques with high accuracy detecting presence of markers in window period as well as differentiating past from present infection, our team made fresh attempts to understand the recent status of hepatitis viral infection in different categories of liver and renal diseases in India. Present report includes a large population of patients with serious liver and renal diseases for treatment and describes the status of prevalence of hepatitis viral infection in these disease groups.

Based on the results of our study, it has been observed that HAV shows its presence in minor percentage (1-2%) of adult population. The previous reports, both from our centre as well as from other studies, demonstrate HAV to be a problem of children and development of protecting anti-HAV antibodies in large population by the age of 12 years [24]. This may be an important reason of protection against

Table 1. Status of hepatitis viral infections in different liver and renal diseases

Type of infection	Viral Marker	AVH (n= 191)			FHF (n= 88)			CVH (n= 321)			CIR (n= 223)			HCC (n= 76)			CKD (n= 144)		
		No. tested	+ve	%age	No. tested	+ve	%age	No. tested	+ve	%age	No. tested	+ve	%age	No. tested	+ve	%age	No. tested	+ve	%age
HAV	IgM Anti-HAV	191	6	3.1	60	0	0	200	0	0	200	0	0	70	0	0	80	0	0
HBV	HBsAg	191	100	52.4	88	34	38.6	258	151	58.5	223	201	90.1	69	34	49.3	131	5	3.8
	IgM Anti-HBc	149	38	25.5	47	14	29.8	156	31	19.9	114	9	7.9	14	0	0	87	3	3.4
HCV	Anti-HCV	191	19	9.9	64	2	3.1	321	95	29.6	120	85	70.8	76	34	44.7	144	16	11.1
	HCV-RNA	56	8	14.3	64	1	1.6	85	68	80	30	13	43.3	9	6	66.6	108	30	27.7
	HCV-Core Ag	30	1	3.3	18	5	27.8	127	55	43.3	40	13	32.5	22	6	27.3	48	23	47.9
HDV	IgM Anti-HDV	100	0	0	20	0	0	50	0	0	46	0	0	21	0	0	2	0	0
HEV	IgM Anti-HEV	183	52	28.4	62	26	41.9	60	9	15	60	12	20	ND	---	---	29	0	0
TTV	TTV-DNA	90	45	50	24	9	37.5	114	45	39.5	66	31	46.9	13	6	46.2	68	50	73.5

Diagnostic criteria :

- HBV infection : IgM anti-HBc in AVH & FHF ; HBsAg / IgM anti-HBc in CVH, Cirrhosis & HCC
- HCV infection : Anti-HCV in all groups.
- HDV infection : IgM anti-HDV in presence of HBsAg in all groups.
- HEV infection : IgM anti-HEV in all groups.
- TTV infection : TTV-DNA in all groups.
- Non A-E infection : Absence of all markers in all groups.

n. = Total number ; +ve No. = Number of sera detected positive; %age = Percentage of cases detected positive; ND= Not done.

Table 2. Status of hepatitis viral co-infection in liver and renal diseases.

Co-infection	AVH	FHF	CVH	CIR	HCC	CKD
HBV + HCV	5.8%	2.8%	9.7%	13.8%	28.6%	1.4%
HBV + HDV	Nil	Nil	Nil	Nil	Nil	Nil
HBV + HEV	14.1%	5.7%	6.7%	Nil	Nil	Nil
HCV + HEV	3.7%	Nil	8.3%	1.6%	Nil	Nil
HBV + HCV + HEV	1.8%	Nil	3.3%	Nil	Nil	Nil

- (i) Co-infection of HBV & HCV is indicated by presence of IgM anti-HBc& anti-HCV in AVH & FHF and HBsAg / IgM-anti-HBc and anti HCV in CVH, cirrhosis and HCC cases.
- (ii) Co-infection of HBV, HCV & HEV is indicated by IgM-anti-HBc with anti-HCV and IgM anti-HEV in acute cases and HBsAg/ IgM-anti-HBc with these markers in CVH, cirrhosis and HCC cases.
- (iii) % value was computed on the basis of total number of cases tested in each disease group.

HAV infection in adults. However, it is a common observation that co-infection of HAV with other hepatitis virus(es), usually takes a formidable form causing very serious diseases [25,26].

We found presence of HBV infection both in acute as well as chronic liver diseases. Its presence was recorded in about one-fourth population of acute viral hepatitis cases. Similarly, HBV infection was quite common in patients with all types of liver diseases. However, its presence was noticed to be low in patients with CKD. HCV infection, which is responsible to cause chronic liver diseases, was recorded in high proportion of patients with CVH, cirrhosis and HCC. Acute liver diseases could not show HCV infection in significant number of cases. The prevalence of HCV infection was based on the analysis by combined assays including EIA and PCR detecting anti-HCV and HCV RNA. This finding is in total consonance with results reported from several other countries as well [2,11]. Similarly, HCV infection was recorded in about one-third of patients with CKD. Use of HCV core antigen could not show a direct relationship with other markers like anti-HCV and HCV-RNA and therefore, was not considered important for diagnostic purpose. Presence of HDV, HEV and TTV infection in all these disease groups is the same as reported earlier on several occasions [27,28]. Hepatitis viruses do not appear to change a scenario of endemicity in this country and so infect the population and cause diseases pattern determined by their molecular characteristics and host response to their invasion. In India, HEV is more popular for causing waterborne epidemics in certain pockets and during particular seasons [14]. Their sporadic invasion often remains benign until HEV becomes a part of co-infection or targets pregnant women where this infection manifests in serious diseases [29]. On a similar pattern, TTV infection, known hitherto as a possible component of unknown hepatitis viruses, is extensive in this country infecting large population but causing no symptoms [30]. Therefore, its routine diagnosis or the treatment of TTV infection is never taken seriously in India.

Conclusion

Present study, thus, concludes that viral hepatitis in India follows a pattern seen in many other regions of the world. Whereas hepatitis A is rarely noticed in adults, hepatitis B and C infections are responsible for causing both acute and chronic liver diseases in sizeable proportions of patients. Hepatitis E causes acute manifestation whereas HDV infection remains to be a rare entity in this country. Viral hepatitis is really a serious concern in India and needs a strategic control on global pattern.

Author contributions

Irshad M planned the study, wrote the manuscript and edited it. Rest other authors conducted the study and analyzed the data.

Conflict of interest statement

There is no conflict of interest among the authors.

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