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Genotypes and Markers of Hepatitis B and Antibodies to Hepatitis C virus in Patients with Chronic Liver Disease in a Tertiary Care Hospital in Puducherry

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ABSTRACT

Background: Chronic liver disease leading to cirrhosis, hepatocellular carcinoma or hepatic failure have been associated with Hepatitis B and C virus infections. Detection of various viral markers of Hepatitis B have been reported to be useful tools to assess the progression of disease. In view of chronic liver disease being high in this population, an attempt was made to detect Hepatitis B viral markers and antibodies to Hepatitis C in patients reporting to this hospital.

Methods: Serum samples from a cross section of hundred clinically confirmed cases of chronic liver disease were screened for HBsAg, total anti-HBc, total anti-HBs and HCV Ab. The patients who were positive for HBsAg were further screened for HBeAg, total anti HBe and IgM anti-HBc. Genotyping of Hepatitis B virus was done by nested PCR.

Results: HBsAg positive patients accounted for 10% of the patients admitted to this hospital. HCV antibodies were also seen in 10% of the patients. A single patient tested positive for both HBsAg and HCV antibodies. Anti HBs was present in 11%, total anti HBc was detected in 42% and 87.5% had anti HBe. Two patients had IgM to HBc. Genotype C was detected in all nine HBV DNA positive samples.

Conclusion: Hepatitis B and C markers may be used to detect recent infection, active replication and carriers among cases of chronic liver disease. Genotype C is associated with faster progression to hepatocellular carcinoma, hence there is a need to monitor these patients.

Key Words: Hepatitis B, Hepatitis B markers, Chronic liver disease, HBV genotypes

INTRODUCTION

Hepatitis B and C are associated with chronic hepatitis and are also associated with the development of cirrhosis and chronic liver disease. Various markers have been shown to predict progression to chronicity and other complications.[1]

HBsAg positivity is compatible with acute or chronic HBV infection. If this marker remains positive for more than 6 months, it denotes a chronic HBV infection.[2] Among viral causes of hepatitis, Hepatitis B virus (HBV) is the major cause of chronic hepatitis, cirrhosis and primary liver cell cancer in India. About 50% of chronic liver disease (CLD) is due to HBV and 20% is due to Hepatitis C virus (HCV) infection. About 90% of HBV infected patients who acquire infection during birth are prone to develop chronic liver disease in later stages of life.[1]

Genotypes of HBV has a varied distribution in the world with specific geographic distribution. Certain genotypes are

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associated with disease severity, outcome as well as response to antiviral therapy. Treatment outcomes can be predicted and response to therapy will vary based on the genotypes detected among these patients.[3] A knowledge of the existing genotype in our locality would greatly influence the course of management in these patients. There is a paucity of data on association of Hepatitis B and C in patients with chronic liver disease. Data is also lacking on predominant genotypes of Hepatitis B. Hence this study was undertaken in this group of patients to document the genotypes.

METHODS

This cross sectional study was carried out during the period from October 2013 to April 2015 in a tertiary care centre in Pondicherry. This study was approved by the institute`s research and ethics committees. (Ref no. IEC: RC/13/62)

Clinical details and laboratory investigations including liver profile, coagulation profile, ultrasound abdomen findings and other investigations were obtained and documented. Following the screening process, samples found to be positive for HBsAg were stored at -70°C for HBV DNA amplification and genotyping.

Enzyme Linked Fluorescent Assay (ELFA) was performed to detect all Hepatitis B markers using commercially available kits (VIDAS Anti-HBs Total Quick, Belgium).

Detection of Genotypes (A-F) of Hepatitis B was done by Nested PCR. Using type specific primers of Hepatitis B genotyping kit provided by Helini Biomolecules, Chennai, viral nucleic acid was extracted according to the manufacturer`s instructions by using 200µl serum sample by column centrifugation method. The extracted nucleic acid was used as template for PCR assay.

Amplification of the target HBV DNA and genotyping was carried out by PCR in Eppendorf Mastercycler Gradient, Germany. The results were recorded on a UVP gel documentation system (UK).

RESULTS

Among hundred patients with chronic liver disease who were screened, 92 (92%) were males and 8 (8%) were females. A majority of 61% patients were middle aged individuals (41 – 60 years). The demographic details and risk factors observed among these patients are listed in Table.1

Fifty percent of patients were positive for one or more markers of hepatitis such as HBsAg, anti HBs, IgG anti HBc, HBeAg, anti HBe, IgM anti HBc or HCV Ab.

Among 100 chronic liver disease patients, 11/100 (11%) patients were positive for anti HBs and 42/100 (42%) patients were positive for IgG anti HBc. Eight chronic liver disease patients (8%) were positive for both anti HBs and IgG anti HBc. Among the 11 patients who were positive for HBsAg, 9/11 (9%) were also positive for IgG anti HBc and none were positive for total anti HBs. Among 100 patients with chronic liver disease, 10/100 (10%) were positive for HBsAg and 10/100 (10%) were positive for antibodies to HCV. Apart from this, one patient tested positive for both HBsAg as well as HCV antibody. The remaining 79/100 (79%) patients were non reactive for both HBsAg and HCV antibodies. (Table.2)

Among eleven HBsAg positive individuals, 2 (18.2%) were positive for HBeAg, 9 (18.8%) were positive for anti HBe and 2 (18.2%) were positive for IgM anti HBc. One patient with IgM anti HBc also tested positive for anti HBe, while another who was IgM anti HBc positive tested positive for HBeAg (Fig.1)

Liver profile of HBV and HCV positive patients were compared using t test and Mann Whitney tests, which did not show any statistically significant difference. (Table.3)

Among the 11 patients who tested positive for HBsAg, DNA was detectable in nine patients (81.8%). Two patients (18.2%) did not have detectable levels of virus in the serum. Genotyping of these nine HBV DNA showed that all the nine patients belonged to Genotype C.

Clinical prognosis and survival rates of patients were estimated using two scoring systems, Child – Pugh score and MELD score.

DISCUSSION

Various factors contribute to the severity of disease progression in Hepatitis B infected individuals. HBsAg prevalence in the general population is estimated to be ranging from 2 to 8 % in India.[4] However, HBV carrier numbers are exponential and is above 50 million, thus constituting the large global pool of chronic HBV infections in East Asia.[4] As India is an intermediate HBV endemic zone, large screening studies of communities are necessary to determine the true burden of HBV infection. Investigations on HBV genotypes will help in understanding the molecular epidemiology of the virus in any given area.

The results of the present study revealed that the majority of chronic liver disease patients (92%) were males due to higher rate of alcohol intake in males. Among these, 9% of males and 1% of females were positive for HBsAg and HCV antibodies were present in 9% males and 1% females. Alcoholics are known to be more susceptible to liver damage due to Hepatitis B and C because ethanol has adverse effects on humoral and cellular immune responses thereby impairing the ability of the host to generate viral specific CD 4 and CD 8 immune responses.[5]

In the present study, prevalence of both HBV and HCV was found to be 11%. A recent study from India by V Singh et al reported higher prevalence of HCV (48%) than HBV (30%) among patients with chronic liver disease. Majority of patients included in this study had cirrhosis of liver and 3% individuals had co infection of HBV and HCV.[6] The co infection rate in the present study is also very negligible (1%).

In the absence of HBsAg and anti HBc, presence of anti HBs indicates protective immunity against HBV acquired by vaccination. Anti HBs was seen in 11% patients indicating immunity to Hepatitis B infection, whereas only 3 patients gave a history of Hepatitis B vaccine. The 89% of patients negative for anti HBs are therefore more susceptible to acquire Hepatitis B infection. Hence it is prudent to advise immunization with Hepatitis B vaccine for chronic liver disease patients.[7,8]

IgG antibodies against HBc was detected in 42/100 (42%) thus denoting past exposure to infection. Among these 42 patients, 9 were positive for HBsAg denoting that they were chronic carriers. However, 24/100 (24%) were negative for HBsAg. Isolated anti-HBc is seen only in two clinical conditions which are occult hepatitis and resolved hepatitis B infection. In patients with occult HBV, anti-HBc is presumed to be the only serological marker to suggest HBV infection. In these patients, there is failure in expression of detectable HBsAg. However the diagnosis of occult hepatitis can be confirmed in these cases only when HBV DNA is detected either in serum or liver tissue.[7,8] In this study, isolated anti-HBc in 24% individuals suggests that these patients might have occult HBV infection.

All patients with antibodies to HBe should also be considered infectious as studies suggest that HBV DNA is present in 26-64% of these patients, suggesting active viral replication.[9] The reason for this condition is the development of pre core mutants, where there is failure to express HBeAg in serum, due to mutation in the basal core promoter region.[6] This was observed among 9 patients where HBeAg was negative but anti HBe was present in the present study. It is highly likely that these 9 patients harboured pre core mutants.

There was no statistically significant difference in prognosis of chronic liver disease in HBV DNA positive and negative patients (p value : 0.709 and 1.000 respectively) by the Child score and MELD scoring systems.

HBV DNA was found in the serum in 81.8% out of eleven HBsAg positive samples. Presence of HBV DNA directly indicates that the virus is replicating; however, the amount of DNA does not correlate directly with the degree of HBV–induced liver disease. Highest levels of HBV DNA are seen in patients who have a positive HBeAg.[10] One reason for undetectable DNA in two of our patients could be that the number of viral copies were less than detection threshold.

The commonest genotypes of HBV reported from India are D,A,C and B in descending order. However there are variations among the various geographical regions, wherein certain places like Vellore report D and C as the commonest. Genotype A has been reported as second commonest genotype after genotype D in many places across India.[11,12] Interestingly genotype B is only reported recently from Hyderabad though they are predominantly reported from far east and South East Asian countries. A recent study from Chennai reported all the four common genotypes in equal number.[13]

All the HBV infected patients in our study were found to have Genotype C. Genotyping of HBV is important in predicting the disease severity and outcome as well as in predicting response to antiviral therapy in patients with chronic HBV infection. HBV genotype C is independently associated with a higher risk of hepatocellular carcinoma and is associated with more rapid progression to cirrhosis than other HBV genotypes.[14,15] One HBV infected patient in our study had an established hepatocellular carcinoma at presentation.

CONCLUSION

Hepatitis B and C are well known causes of chronic liver disease. We found a dual etiology in 21% patients underlining the importance of screening for multiple etiologies (hepatotropic viruses) in all patients with chronic liver disease. Serological markers of Hepatitis B and C may be used to detect recent infection, active viral replication and carriers among cases of chronic liver disease. Diagnosing the stage of hepatitis in these patients will aid in choice of antiviral therapy. Treating HCV infected patients with conventional and newer antivirals may be considered in order to prevent progressive fibrosis of liver tissue. Genotype C of HBV is associated with faster progression to hepatocellular carcinoma, hence these patients need to be monitored throughout the course of illness. Further genotyping studies should be conducted on a larger population in this geographic area. All chronic liver disease patients need to be vaccinated to prevent HBV infection.

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Table 1: Demographic characteristics of patients with chronic liver disease (n=100)

	Chronic liver disease patients (number of patients)
Alcoholics	75
Past history of jaundice	35
Co morbidities :	
Diabetes mellitus	25
Hypertension	15
Ascites	74
Hepatic encephalopathy	16
History of Hepatitis B vaccine :	
Immunized	3
Unimmunized/status unknown	97

Risk factors commonly found in chronic liver disease patients were alcohol, past history of jaundice. Most patients were unimmunized.

Table 2: Distribution of HBV and HCV markers among patients with chronic liver disease (n=100)

Hepatitis markers	Number (Percentage)
HBsAg	11 (11%)
IgG anti HBc	42 (42%)
Total anti HBs	11 (11%)
HCV Ab	11 (11%)
HBsAg + IgG anti HBc	9 (9%)
HBsAg + total anti HBs	Nil

Presence of HBsAg and HCV Ab were equal with a co infection of 1%. Markers suggesting past infection, spontaneous clearance were also found.

Table 3: Liver profile of HBV (n=11) and HCV (n=11) infected individuals

Liver enzymes	HBV (mean ± SD)	HCV(mean ± SD)	p value	Statistical test applied
Serum albumin	2.84 ± 1.05	2.55 ± 1.02	0.55	t test
SGOT	119 ± 75.56	126.2 ± 72.1	0.84	
Serum bilirubin	5.41 ± 7.51	8.32 ± 8.93	0.37	Mann Whitney test
SGPT	95.2 ± 127.96	36.14 ± 13.9	0.07	
ALP	116.3 ± 43.97	182 ± 147.97	0.63	

Liver enzymes among infected and uninfected patients was not statistically significant

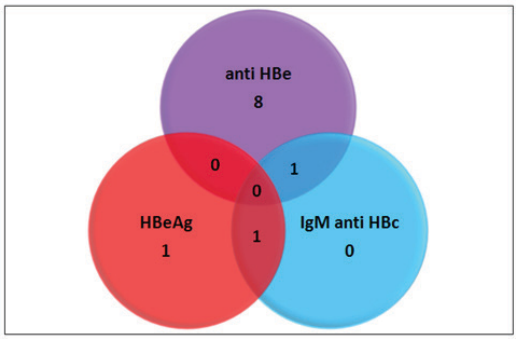
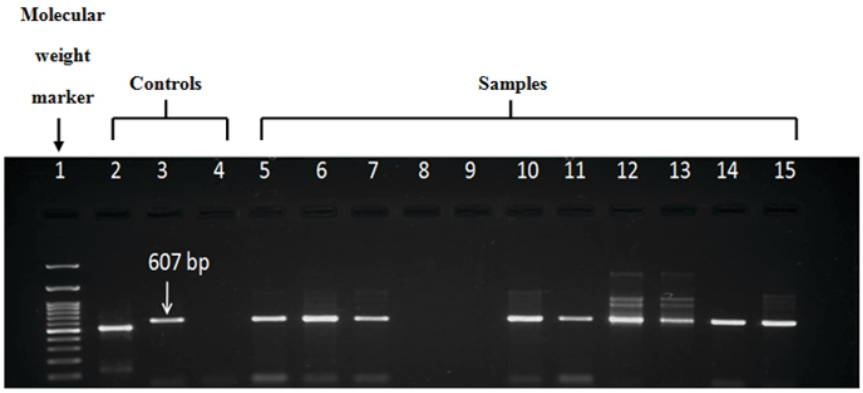


Figure 1: Distribution of HBeAg, anti HBe and IgM anti HBc among HBsAg positive patients with chronic liver disease (n=11).

Most patients (9%) had anti HBe in serum. HBV DNA was also present in the serum probably suggesting presence of pre core mutants.



Lane 1 → Molecular weight marker (100 bp ladder), Lane 2 → Control for Genotype A

Lane 3 → Control Genotype C, Lane 4 → Control for Genotype D

Lane 5 – 15 → Samples 1 to 11

The bands correspond to 607 bp of Genotype C.

Figure 2: Gel electrophoresis of HBV genotyping by nested PCR.

All the samples in which HBV DNA was detected tested positive for Genotype C.