Original Article

A Study on Hepatitis B Viral Seromarkers and Associated Risk Factors among the Patients Suffering from Acute and Chronic Hepatitis B Infection

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Abstract

Background: Hepatitis B viral infection is the most common cause of hepatitis, and it leads to serious liver diseases such as cirrhosis and hepatocellular carcinoma. **Aim:** The aim of the study is to differentiate acute hepatitis B and chronic hepatitis B (CHB) among patients seropositive for hepatitis B surface antigen (HBsAg). **Materials and Methods:** This study was carried out in the Department of Microbiology, Chettinad Hospital and Research Institute, Kelambakkam, Tamil Nadu, India, for a period of 6 months (January 2018–June 2018). Blood samples were collected from 87 patients for the detection of hepatitis B virus (HBV) serological markers. HBsAg, hepatitis B e antigen (HBeAg), anti-HBc total, anti-HBc IgM, and antibody to hepatitis B surface antigen were screened using the ELISA method. Detailed demographic profile including history of previous hepatitis infection, previous blood transfusion, and other related details were collected and documented using a structured questionnaire. **Results:** A total of 87 patients were HBsAg seropositive; among them, 55 (63.2%) were male and 32 (36.9%) were female. Based on the serological markers tested, 24 and 63 were suffering from acute and chronic HBV infections, respectively. Among the acute hepatitis B patients, all samples were seropositive for HBsAg, anti-HBc total, and anti-HBc IgM. HBeAg seromarker was found in 15 patients (62.5%). Among the CHB patients, all samples were seropositive for HBsAg and anti-HBc total. HBeAg seromarker was found in 28 patients with 44.4%. Alcohol consumption was the major risk factor for the transmission of HBV infection. **Conclusion:** An increased sample size and detailed study of high-risk behavior will provide an alarming awareness of their association.

Keywords: *Acute hepatitis B virus infection, chronic hepatitis B infection, hepatitis B virus, serological markers*

Introduction

Hepatitis B virus (HBV) belongs to the *Hepadnaviridae* family, and it is a partially double-stranded DNA virus with 40–42 nm.[1,2] Hepatitis B viral infection is the most common causes of hepatitis, and it leads to serious liver diseases such as cirrhosis and hepatocellular carcinoma.[3-5] Serological markers tests done for the diagnosis of HBV infection include hepatitis B surface antigen (HBsAg), antibody to hepatitis B surface antigen (anti-HBs), anti-HBc IgM, anti-HBc IgG, hepatitis B e antigen HBeAg, and anti-HBe.[6] Most of the persons infected with acute hepatitis B (AHB) infection can recover by the clearance of HBsAg and the development of anti-HBs. In chronic carriers, HBeAg remains positive for several years.[7] The high risk of HBV transmission includes those requiring frequent blood transfusion, dialysis, intravenous drug users, health-care workers, as well as sexual contacts with an acute or chronically infected person.[8] This study aims to differentiate acute and chronic hepatitis B (CHB) among patients seropositive for HBsAg by testing other HBV serological markers.

Materials and Methods

This study was carried out in the Department of Microbiology, Chettinad Hospital and Research Institute, Kelambakkam, Tamil Nadu, India, for a period of 6 months (January 2018–June 2018). This study was conducted in patients who registered both in outpatient departments and inpatient departments. During the period of study, blood samples from patients were screened for HBsAg in microbiology laboratory by rapid immunochromatographic method.

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Blood samples seropositive for HBsAg were also screened for other HBV serological markers (HBsAg, anti-HBc Total, anti-HBc IgM, HBeAg, and antiHBs) by the ELISA method. These markers were used for the differentiation of acute and chronic HBV infection among the patients.

Blood samples (3 ml) were collected, and the detailed history of the patients was obtained in a questionnaire form. The questionnaire includes a history of previous hepatitis infection, previous blood transfusion, previous surgical interventions, dialysis, multiple sexual partners, tattooing, and family history of hepatitis infections. The forms were filled by the patient or the guardian along with the informed written consent from each patient. Ethical clearance was obtained from the Chettinad Academy of Research and Education Institutional Ethics Committee (Proposal No: 13/IHEC/3–16) to conduct the study. Clinical trial registration has been done for the study, and the registration number is CTRI/2018/01/011460.

Serological markers for HBV were done by commercially available ELISA kits. The assay for each serological marker (HBsAg, anti-HBc total, anti-HBc IgM, HBeAg, and antiHBs) was done according to the manufacturer instruction. ELISA HBsAg (Transasia Bio-Medicals LTD, Daman, India) was done based on the sandwich principle for the determination of HBsAg in human plasma and sera. ELISA anti-HBc total (General Biologicals Corporation, Taiwan) was confirmed by the ELISA method for *in vitro* qualitative detection of total antibody to HBV core antigen (anti-HBc total) in human serum or plasma (heparin, EDTA, or citrate), and this assay is based on a competitive principle. ELISA anti-HBc IgM (General Biologicals Corporation, Taiwan) is a solid-phase enzyme immunoassay and is based on the noncompetitive principle. ELISA HBeAg (DIA. PRO, Diagnostic BioprobesSrl, Italy) was done based on the sandwich principle for the determination of HBV "e" antigen in human plasma and sera. ELISA HBsAb (Dia. Pro, Diagnostic BioprobesSrl, Italy) was done based on the indirect immunoenzymatic principle for qualitative determination of antibodies to the surface antigen of HBV in human plasma and sera by indirect method.

Statistical analysis

Data were analyzed using computer software IBM Statistical Package for Social Sciences ver. 10 SPSS (SPSS Inc, Chicago, IL, USA). The Chi-square test (2 × 2 contingency table) was used to compare the risk factors associated with AHB- and CHB-infected patients. Odds ratio and 95% confidence interval (CI) were used to measure the strength of the association. Statistical significance was set at $P < 0.05$.

Results

Patients' samples were screened for HBV seromarkers. A total of 87 patients were HBsAg seropositive; among them, 55 (63.2%) were male and 32 (36.9%) were female. Based on the serological markers, acute and chronic infection status of the patients was identified. A total of 24 patients were seropositive for HBsAg. These patients were seropositive for anti-HBc IgM, anti- HBc total, HBeAg and negative for anti-HBs seromarkers. This indicates acute infection. A total of 63 patients who were seropositive for HBsAg for more than 6 months and were positive for anti-HBc total, HBeAg and negative for anti-HBc IgM and anti-HBs serological marker indicate CHB infection. Among them, nine patients had a history of early hepatitis infection [Table 1 and Figure 1].

Patients suffering from acute infection were 100% (n = 24) seropositive for HBsAg, antiHBc total and anti-HBc IgM. Nearly 62.5% (n = 15) of them were seropositive for HBeAg seromarker. Presence of HBeAg in acute patients indicates active viral replication and they are highly infectious. All AHB patients were negative for anti-HBs seromarker [Table 2]. The CHB patients (n = 63) were 100% seropositive for HBsAg and anti-HBc, and n = 28 (44.4%) of them were seropositive for HBeAg seromarker. Chronically infected hepatitis B patients were seronegative for anti-HBs and anti-HBc IgM seromarker. The comparison between the HBV serological marker in acute and CHB infection is shown in Table 2.

Table 1: Screening of seromarkers among the study groups

Seromarkers	Result	Interpretation
HBsAg	Positive	Acutely infected
Anti-HBc total	Positive	
Anti-HBc IgM	Positive	
HBeAg	Positive/negative	
Anti-HBs	Negative	
HBsAg	Positive	Chronically infected
Anti-HBc total	Positive	
Anti-HBc IgM	Negative	
HBeAg	Positive/negative	
Anti-HBs	Negative	

HBsAg: Hepatitis B surface antigen; Anti-HBs: Antibody to hepatitis B surface antigen; Anti-HBc: Antibody to hepatitis B core antigen; HBeAg: Hepatitis B e antigen

Table 2: Hepatitis B virus serological marker in acute hepatitis B virus and chronic hepatitis B infection

Serological markers	AHB patients (n=24), n (%)	CHB patients (n=63), n (%)
HBsAg positive	24 (100)	63 (100)
Anti-HBc IgM positive	24 (100)	0
Anti-HBc total positive	24 (100)	63 (100)
HBeAg positive	15 (62.5)	28 (44.4)
Anti-HBs	0	0

AHB: Acute hepatitis B virus; CHB: Chronic hepatitis B; HBsAg: Hepatitis B surface antigen; Anti-HBs: Antibody to hepatitis B surface antigen; Anti-HBc: Antibody to hepatitis B core antigen; HBeAg: Hepatitis B e antigen

The behavioral risk factors among the HBV patients were habit of alcohol consumption (n = 34; 39%), sharing nail clippers (n = 18; 21%), tattooing (n = 10; 12%), and intravenous drug use and multiple sex partners (n = 4) 5% each. The past medicosurgical parameters and their association among the HBV patients were history of surgery (n = 29; 33%), history of previous hepatitis infection (n = 9; 10%), and dialysis (n = 4; 5%).

A total of 15 (17.2%) of the HBV patients had a family history of hepatitis infection. In our study, habit of cigarette smoking was seen in 52% (n = 45) of the HBV-infected patients. None of the female patients gave a history of cigarette smoking and alcohol consumption. The risk factors of hepatitis B infection among HBV patients are shown in Table 3. The behavioral risk factors were found high in patients with CHB compared with AHB-infected patients. Among the risk factors, the most common such as cigarette smoking (44%), alcohol consumption (31%), sharing nail clippers (12%), intravenous drug user, and multiple sex partners (5%) each were high in patients with CHB. The past medicosurgical parameters such as previous surgery (23%), previous hepatitis infection (10.3%), blood transfusion (8%), and dialysis (5%) were also found high in patients with CHB compared with AHB-infected patients.

Family history of hepatitis infection (37.5% vs. 9.52%; P = 0.000; odds ratio [OR] = 5.7; 95% CI: 1.75, 18.53) and tattooing (33.3% vs. 3.17%; P = 0.000; OR = 15.2; 95% CI: 2.94, 78.9) were the risk factors seen higher in AHB-infected patients than the CHB-infected patients with statistical significance. The other risk factors such as sharing nail clippers, alcohol consumption, and history of previous surgery did not show statistical significance [Figure 2 and Table 4]. All HBV patients had total serum albumin levels more than 1 mg/dl. All patients with AHB infection showed elevated alanine aminotransferase levels >49 U/L.

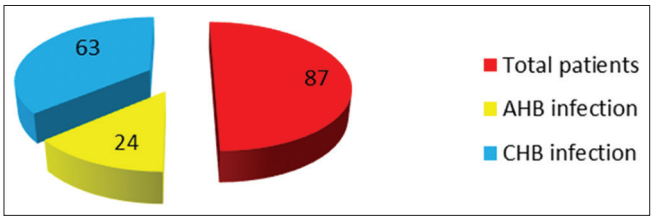

Figure 1: Seroprevalence of hepatitis B virus infection

Discussion

Globally, HBV infection is responsible for most of the acute and chronic liver diseases.[9] In our study, 72.4% of the patients were infected with CHB which was higher than the patients infected with CHB (63.4%) reported by Jiang *et al*.[10] HBV endemicity is divided into three categories based on HBsAg prevalence rate. The prevalence rate found in highly endemic areas showed 8%, intermediate areas showed 2%–7%, and low-endemic areas showed 0.5%–2%.[11]

In our study, 87 patients were positive for HBsAg; among them, 27.6% were acutely infected and 72.4% were chronically infected. The seroprevalence of HBsAg was higher in males (63.2%) than females (36.9%). This finding of higher prevalence of HBsAg among male gender is supported by other studies.[12-14]

In our study, both acute and chronic groups showed that the anti-HBc total was 100% seropositive and similar

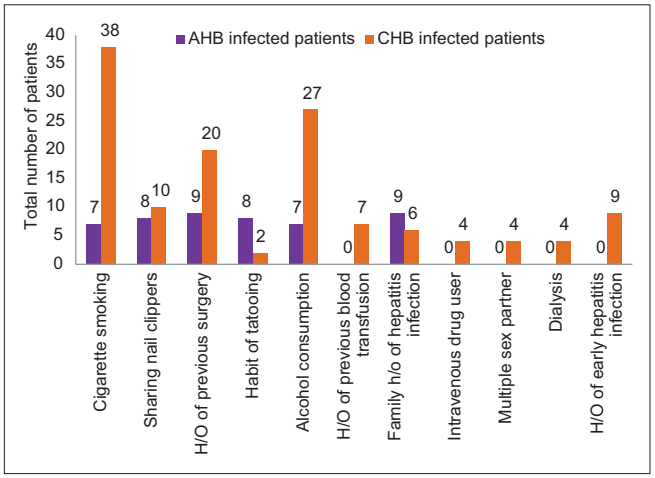

Figure 2: Risk factors among acute hepatitis B and chronic hepatitis B patients

Table 3: Genderwise distribution of risk factors

Risk factors	Male (n=55)	Female (n=32)	Total patients (n=87), n (%)
Cigarette smoking	45	0	45 (52)
Sharing nail clippers	10	8	18 (21)
History of previous surgery	23	6	29 (33.3)
Habit of tattooing	9	1	10 (12)
Alcohol consumption	34	0	34 (39.1)
History of previous blood transfusion	5	2	7 (8.0)
Family history of hepatitis infection	10	5	15 (17.2)
Intravenous drug use	4	0	4 (5)
Multiple sex partners	4	0	4 (5)
History of dialysis	2	2	4 (5)
History of early hepatitis infection	5	4	9 (10.3)

Table 4: Analysis of risk factors of hepatitis B virus transmission

Variables	AHB (*n*=24), *n* (%)	CHB (*n*=63), *n* (%)	OR (95% CI)	*P*
Sharing nail clippers				
Yes	8 (33.3)	10 (15.8)	2.65 (0.89-7.84)	0.133
No	16 (66.6)	53 (84.1)		
Alcohol consumption				
Yes	7 (29.1)	27 (42.8)	0.54 (0.20-1.51)	0.355
No	17 (70.8)	36 (57.1)		
Tattooing				
Yes	8 (33.3)	2 (3.17)	15.2 (2.94-78.9)	0.000
No	16 (66.6)	61 (96.8)		
Multiple sex partners				
Yes	0	4 (6.34)	NA	NA
No		59 (93.6)		
Intravenous drug use				
Yes	0	4 (6.34)	NA	NA
No		59 (93.6)		
Family history of hepatitis infection				
Yes	9 (37.5)	6 (9.52)	5.7 (1.75-18.5)	0.000
No	15 (62.5)	57 (90.4)		
History of previous hepatitis infection				
Yes	0	9 (14.2)	NA	NA
No		54 (85.7)		
History of dialysis				
Yes	0	4 (6.34)	NA	NA
No		59 (93.6)		
History of blood transfusion				
Yes	0	7 (11.1)	NA	NA
No		56 (88.8)		
History of previous surgery				
Yes	9 (37.5)	20 (31.7)	1.29 (0.48-3.44)	0.799
No	15 (62.5)	43 (68.2)		

AHB: Acute hepatitis B virus; CHB: Chronic hepatitis B; OR: Odds ratio; CI: Confidence interval; NA: Not available

documentation was done by Fayyadh and Ma.[15] Anti-HBc total develops in all HBV infection which appears shortly after HBsAg in the acute infection.[16] The HBeAg seropositive in acute and CHB patients were 62.5% and 50.9%, respectively; this result indicates that those patients were highly infectious. It is a marker of replication and infectivity. Marcus *et al*. have recorded 100% seroprevalence in AHB groups for anti-HBc IgM, which is similar to our study.[17] Anti-HBc IgM is the best serological marker of AHB viral infection, and it is detected at the onset of clinical illness. Its presence in serum indicates viral replication. It appears in the serum 1–2 weeks after the presence of HBsAg.[16]

In our study, anti-HBs were found to be seronegative among the acute and chronic groups. Seronegative to anti-HBs indicates that no recovery will be possible in the groups. Our study was in concordance with the study of Weber. Since it reflects long-term immunity, it is also known as a neutralizing antibody.[18] In our study, history of alcohol consumption was more among the patients suffering from chronic infection and there is no statistical significance found between them. Similar documentation was done by Krishnasamy *et al*. (2015) regarding alcohol consumption.[19] Several studies reported that alcohol consumption is independent risk factors for hepatitis B viral infection.[20]

In our study, a family history of hepatitis infection and tattooing were the risk factors highly associated with AHB-infected patients with significant correlation. A study by Köse *et al*. found that HBV transmission was highly associated with patients having HBV-positive family members.[21] The risk of developing hepatitis B infection among family members is high. It can be through blood-tinged fluid, saliva, skin lesions, fluid from open sores, contact with chronic carriers, and other household instruments.

A study by Eke *et al*. reported that tattooing was the significant risk factor for HBV infection. Nonprofessional practice and reused needles may be the reason for transmission.[22] For the prevention of HBV transmission among the tattoo recipients, it is necessary to implement the education programs among the tattoo artists and tattoo parlor owners regarding the importance of universal precaution. The measures comprise single use of sterile tattoo needles and the use of suitable disinfectants.[23]

Sharing nail clippers among friends and household members were 33% in AHB patients and 16% in CHB patients. The similar finding was documented in the previous study. The unawareness of the people regarding the practice of sharing the nail clippers can be reduced by educating the population about the routes of transmission of HBV.[23] According to Stroffolini *et al*., Kupek, and Al-Nassiri and Raja'a, history of blood transfusion was identified as a risk factor of HBV infection.[24-26] In our study, history of blood transfusion was documented only in CHB patients (11%). This study was similar to the study documented by Naqshbandi *et al*.[27]

In our study, history of multiple sexual partners was documented in 6.34% of the CHB patients. None of the AHB-infected patients gave the history of multiple sex partners. The reliability of the information obtained is questionable because people are generally unwilling to confer their sexual relationships.[23] Similar finding was documented by Drazilova *et al*.[28]

Conclusion

HBV screening is important to prevent chronic hepatitis and its complications. Diagnosis of HBV infection is achieved using serological markers to determine acute and chronic infection to establish preventive measures and to initiate antiviral treatment.

Proper governmental education and media campaign should be conducted to the general population to know about the risk factors of HBV infection and its routes of transmission. Further, a detailed study of high-risk behavior with increased sample size would provide an alarming awareness of their association.

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Conflicts of interest

There are no conflicts of interest.

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