Research Article

Occult hepatitis B infection among chronic liver disease patients in Tamil Nadu Is screening mandatory?

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Abstract

Background: Occult hepatitis B infection (OBI) is characterised by the presence of hepatitis B virus deoxyribonucleic acid (HBV DNA) in the liver, but negative for Hepatitis B surface antigen (HBsAg), irrespective of the presence of HBV DNA in blood. Chronic liver disease (CLD) is a significant contributor to mortality. Globally, OBI prevalence varies from 1% to 87%. This study aims to determine OBI prevalence among chronic liver disease patients in Tamil Nadu by screening for anti-HB total core antibodies (anti-HBc) and HBV DNA among HBsAg-negative patients.

Methods: One hundred and sixty serum samples were obtained from CLD patients and tested using an enzyme-linked immunosorbent assay (ELISA) for HBsAg. The negative samples were tested for anti-HBc antibodies. The anti-HBc positive samples were tested for HBV DNA using real-time polymerase chain reaction (PCR).

Results: Among the 160 samples tested by HBsAg ELISA, 98 were negative for surface antigen. These samples underwent anti-HBc testing, of which eight samples were positive. HBV DNA tested positive in five of the eight anti-HBc positive samples. The prevalence of OBI in the present study was 3.13%.

Conclusion: In this study, 5% of CLD patients were diagnosed as OBI by anti-HBc assay and 3.13% by HBV DNA PCR, suggesting evidence of past exposure to HBV. To conclude, it is mandatory to diagnose OBI to prevent early progression to cirrhosis or hepatocellular carcinoma.

Keywords: *Hepatitis B virus. Hepatitis B surface antigens. Hepatocellular carcinoma. Liver Cirrhosis. Real-time polymerase chain reaction.*

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Introduction

Hepatitis B virus infection is prevalent worldwide and is categorised into high, intermediate, and low endemic areas.[1] India has a disease burden of roughly 50 million people and is in the intermediate endemicity zone with a prevalence of 4 - 5%.[2]

In 2021, the World Health Organisation (WHO) estimated 58.4 million new cases of chronic liver disease with 1.4 million deaths globally.[3] Chronic liver disease (CLD) was the third and tenth most common cause of death in men and women, respectively, accounting for 1.3 million deaths in 2017. The leading causes of CLD worldwide include alcohol, non-alcoholic steatohepatitis (NASH), hepatitis B virus (HBV), and hepatitis C virus (HCV).[4] In India, HBV is still the common cause of CLD (40.8%) and hepatocellular carcinoma (46.8%) among the general population.[5]

Occult hepatitis B infection (OBI) prevalence varies from 1% - 87% across the globe. The clinical features of OBI remain unknown, and further research is essential to recognise OBI characteristics among the general population and high-risk groups, including CLD patients. Immunosuppression or chemotherapy may reactivate an undetected HBV infection in people with a prior history of the virus.[6]

De novo infection with naturally occurring HBV mutations may be the cause of OBI since chronically infected persons are the main reservoirs of HBV infection.[7] Hepatitis B surface antigen (HBsAg) detection in serum is the primary method used in India to diagnose HBV infection. Commercial assays may not detect HBsAg in OBI as a consequence of mutations in the viral S-gene.[6] On the contrary, HBV core antibodies (anti-HBc) can be detected in both current and past HBV infection. Thus, in lower-income and middle-income nations, anti-HBc is utilised as an ideal surrogate marker to diagnose OBI.[8]

The present study was aimed to detect OBI prevalence in Tamil Nadu by screening for anti-HB core antibody (anti-HBc) among HBsAg-negative CLD patients and to detect the presence of HBV deoxyribonucleic acid (DNA) among the anti-HBc seropositive individuals by polymerase chain reaction (PCR). Additionally, anti-HB surface antibodies (anti-HBs) among the HBV DNA positives were detected using chemiluminescent immunoassay (CLIA).

Methods:

This descriptive cross-sectional study was conducted from September 2023 to February 2024.

Operational definitions:

- Chronic liver disease (CLD) is a progressive deterioration of liver functions for more than six months, which leads to inflammation, destruction and regeneration of liver parenchyma.
- Occult hepatitis B infection (OBI) is defined as the presence of replication-competent covalently closed circular DNA (cccDNA) in the liver regardless of hepatitis B virus DNA presence in the blood, but negative for hepatitis B surface antigen (HBsAg) using commercially available serological assays.
- Seropositive OBI is characterised by the detection of anti-HBc antibodies with or without anti-HBs antibodies.

- Seronegative OBI is described as undetectable anti-HBc and anti-HBs antibodies.

The study included patients above 18 years of age with a CLD diagnosis. Patients with history of taking antiviral drugs for HBV were excluded from the study. The demographic details and biochemical parameters including liver enzymes were recorded after obtaining written informed consent from the patients.

Five ml of blood was collected from the patients under strict aseptic conditions and serum separated by centrifugation and stored at -80 °C. All 160 samples were subjected to HBsAg detection using ELISA (ErbaLISA SEN HBsAg, Transasia Bio-Medicals, Daman, India). The efficacy of the kit, as mentioned by the manufacturer, is 100%.

The anti-HBc antibodies ELISA (Dia. Pro HBc Ab ELISA kit) which has >98% sensitivity and specificity (Okhla Industrial Area Phase-II, New Delhi, India) was used to test all HBsAg negative samples. HBV DNA PCR was performed on the anti-HBc-positive plasma samples using the PathoDetect HBV Quantitative PCR Kit (MyLab Discovery Solutions, Pune, India), with lowest limit of detection of 5 IU/mL. Anti-HBs antibodies were tested using Chemiluminescent Immunoassay (Architect i1000SR, Abbott, Chicago, USA), which has 97% and 99% of sensitivity and specificity, respectively.

Statistical analysis: The data obtained were entered into Microsoft Excel, and the results were analysed using SPSS software version 27.0, IBM Corporation, Armonk, NY, USA. Categorical variables were presented as numbers and percentages, and the continuous variables were expressed as mean ± standard deviation. Chi-square test was used to analyse the differences between the qualitative data, and the p-value of <0.05 was used to state the statistical significance.

Results

This study included 160 samples collected from CLD patients, of whom 97 (60.6%) were males, and 63 (39.4%) females. Most of the patients were between 51 and 60 (33.1%), followed by 41 to 50 (32.5%) age groups (Table 1). The mean age among the participants was 49 ± 10.16 years.

HBsAg ELISA was performed on all 160 samples and 62 tested positive for HBsAg. The remaining 98 HBsAg-negative samples were tested for anti-HBc antibodies and eight (5%) were positive. The viral load was tested on eight anti-HBc positive samples using real-time quantitative PCR (rt-qPCR) of which HBV nucleic acid was detected in five samples. (Table 2). HBV DNA-positive samples tested negative for anti-HBs antibodies. The lower and the higher viral load values were 12 IU/ml and 416 IU/ml, respectively, in this study. The mean age of the OBI patients was 46.2 ± 9.8 years, of whom 3 were males and 2 were females.

The serum levels of liver enzymes, including the alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were elevated among the patients with both anti-HBc and HBV DNA positivity compared to those with only anti-HBc positivity. (Table 1)

Table 1: Demographic distribution of chronic liver disease patients

Variable	Category	Frequency (%) n=160	HBsAg positive n=62	Anti-HBc positive n=8	HBV DNA positive n=5
Gender	Male	97 (60.6)	35	5	3
	Female	63 (39.4)	27	3	2
Age group (years)	21-30	12 (7.5)	9	-	-
	31-40	27 (16.9)	17	2	1
	41-50	52 (32.5)	24	4	3
	51-60	53 (33.1)	10	2	1
	≥ 61	16 (10)	2	-	-
	Mean age	49 ± 10.16	48 ± 9.1	47 ± 10.3	46.2 ± 9.8
Correlation of biochemical parameters					
Variables		Anti-HBc positive		HBV DNA positive	
		n=8	Mean value	n=5	Mean value
AST	>30 IU/L	7 (87.5)	54 ± 16.6	4 (80)	64.6 ± 9.9
	p-value		0.0782		0.1868
ALT	>40 IU/L	6 (75)	42.5 ± 16.3	3 (60)	50.2 ± 16.2
	p-value		0.4536		0.4652
ALP	>120 IU/L	5 (62.5)	145.8 ± 40.3	2 (40)	167.2 ± 35.9
	p-value		0.8822		0.8504

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase,
Anti-HBc: Hepatitis B core antibodies, HBsAg: Hepatitis B surface antigen,
HBV DNA: Hepatitis B virus deoxyribonucleic acid, IU/L: International unit per liter.

Table 2: Frequency of occult hepatitis B infection among the study participants

Assay performed	Total samples tested	Number of positives (%)
HBsAg ELISA	160	62 (38.75)
Anti-HBc ELISA	98	8 (5.00)
HBV DNA real-time PCR	8	5 (3.13)

Discussion:

The present study was a cross-sectional estimation of OBI frequency among CLD patients attending a tertiary care hospital. Minimal research has been reported on OBI among chronic liver disease patients across India.

The prevalence of OBI in Southeast Asia and other Asian countries was 9% and 4%, respectively. Also, the OBI prevalence among the general population and chronic liver disease cases was 1% and 18%, respectively.[9] OBI is transmitted by blood transfusion, organ transplantation and from HBsAg positive mother to child through vertical transmission.[10] Mechanisms of OBI include mutation in the "a" determinant of HBsAg, mutations in the pre-S_1 and pre-S_2 regions of the 'S' gene and mutation in the surface region involved in the mRNA splicing of the 'S' protein.[6]

There are many challenges associated with the diagnosis of OBI, including the following:

i. A knowledge deficit about OBI among clinicians and the general population due to the paucity of research on the condition, which may result in the unwillingness of CLD patients to be screened for other HBV serological markers.
ii. For the confirmation of OBI, real-time quantitative PCR is required, which is unavailable in most resource-limited settings.
iii. As most OBI patients have low viremia, detection of intrahepatic cccDNA is needed, but is less frequently performed due to the risk associated with the procedure and its complications.
iv. A referral system that is inadequate in resource-poor countries, when an expert opinion from a gastroenterologist is mandated.[11]

The average age in this study was 49±10 years which is concordant with a study by Faria et al. (2022),[12] with a mean age of 47.8 years. Among OBI cases, the mean age was 46.2±9.8 years, which is similar to Im YR *et al* (2022),[8] study, which stated the median age as 51 years. Of the five OBI cases, four patients had < 200 IU/ml of HBV DNA. A study by Makvandi M (2016) suggested that OBI cases may have a less than 200 IU/ml of HBV DNA.[6]

In this study, OBI was observed in 3.13% of the CLD patients. Similar results were noted in other studies from India which reported 2.2% (Madhavan A et al. 2021)[13] and 3.4%, (Srivastava A et al. 2015)[14] respectively of OBI cases in their study population.

These disparities in prevalence are probably related to (i) variations in the efficiency of serological and PCR assays used, (ii) the geospatial difference in HBV prevalence in different regions of India, (iii) type of study population, and (iv) fluctuation in HBV DNA levels.[4,13]

In the current study, anti-HBc seropositivity was 5% among the study population which is concordant with a study conducted in India (Dhawan HK *et al:* 2008)[15] of 8.4%. Yet another study from India reported 34.8% of anti-HBc antibodies among children with CLD.[14] A meta-analysis conducted by Yang Y *et al* (2025), quoted that the distribution of anti-HBc seropositivity across the globe varies with the prevalence of HBV in the geographical region and the commercial assays used. The anti-HBc distribution in Asian countries were 2.3% and 75.8% in Saudi Arabia and Hong Kong, respectively. Other countries, such as Canada, Colombia, Italy, Brazil and Cameroon had 0.53%, 2.16%, 4.8%, 22.2% and 48.7% of anti-HBc seropositivity, respectively.[16]

The anti-HBs antibodies were negative for all the OBI-positive cases, correlating to the definition of 'seropositive-OBI'. A consensus conducted at Taormina, Italy stated that anti-HBc and anti-HBs antibodies may become negative due to progressive loss in hepatitis B specific antibodies or may be negative for hepatitis B antibodies from the beginning (primary occult infection), which is similar to the woodchuck model of hepadnavirus infection with the woodchuck hepatitis virus. In such cases, only real-time qPCR using sera or hepatocytes can rule out OBI.[17]

The mechanism behind the development of OBI is based on viral, genetic, host immune factors and any comorbid conditions. The OBI-induced liver damage may be caused by HBV cccDNA persistence and transcription within hepatocytes, as well as the generation of inflammatory cytokines like tumour necrosis factor - α [TNF-α] and interferon-gamma, which can cause subsequent hepatocyte destruction.[18]

Detecting HBV nucleic acid from liver tissues and plasma samples using real-time or nested PCR is the gold standard technique for diagnosing OBI. Anti-HBc antibody assays could be performed if the HBV DNA PCR is not feasible in the setting and can be considered an ideal surrogate for OBI diagnosis.[19]

The limitations of the study are a single-centric study with a small sample size, only plasma was used for viral load detection, and other causes for chronic liver disease were not identified.

Conclusion:

In the current study, occult hepatitis B infection was diagnosed in 5% by anti-HBc assay and 3.13% by HBV DNA PCR, suggesting evidence of exposure to HBV in the past. To conclude, it is mandatory to diagnose OBI by performing nucleic acid testing/ or anti-HBc, to minimise HBV transmission, initiate appropriate medical management and prevent early progression to cirrhosis or hepatocellular carcinoma.

Declarations

Conflict of interest: There are no conflicts of interest.
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Ethics statement: The study proposal was approved by the Institutional Research Ethics Committee (Tirunelveli Institute Research Ethics Committee ID No: 20222402 dt.28.09.2022).
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Author contributions:
Dr. I.A : Conceptualisation, Study design, Data acquisition, Data analysis, Writing and editing of manuscript
Dr. C.B : Study design, Data acquisition
Dr. P.S : Study design, Writing and editing of manuscript, Research visualization

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