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Study of occult hepatitis B virus infection among Indian patients with isolated hepatitis B core antibody

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Abstract:

Occult Hepatitis B virus (HBV) infection (OBI) is characterized by the presence of HBV DNA in individuals who are negative for hepatitis B surface antigen (HBsAg), making its detection challenging using routine serological methods. Therefore, it is of interest to determine the prevalence of OBI among Anti-HBc positive, HBsAg-negative patients with suspected viral hepatitis in a tertiary care hospital setting. Hence, a total of 362 HBsAg-negative patients from high-risk groups were included in the study. Among them, 102 (28.2%) were Anti-HBc positive, indicating prior exposure to HBV. Molecular analysis using PCR revealed that 9 out of 362 patients (2.48%) had detectable HBV DNA, confirming occult infection. Thus, early identification of OBI is crucial to prevent transmission and reduce long-term complications such as liver cirrhosis and hepatocellular carcinoma.

Keywords: Occult Hepatitis B Infection (OBI), Hepatitis B Virus (HBV), Anti-HBc, HBsAg Negative, HBV DNA, viral load, ELISA, PCR, Hepatitis C Virus (HCV), Co-infection, Isolated Anti-HBc, surrogate markers

Background:

Hepatitis viruses constitute a heterogeneous group of hepatotropic viruses that primarily affect the liver and cause a spectrum of clinical manifestations ranging from mild, self-limiting illness to severe chronic liver disease [1, 2]. Among these, Hepatitis B virus (HBV) is unique as it is a DNA virus, unlike other hepatitis viruses which are RNA viruses [1]. HBV infection remains a major global public health problem, with approximately 2 billion people infected worldwide and about 254 million individuals living with chronic infection, resulting in nearly 1.1 million deaths annually due to HBV-related liver diseases [3, 4]. In India, HBV infection is of significant concern, with an estimated 40 million carriers, placing the country in the intermediate endemicity an average prevalence of 4% [5]. HBV infection can lead to a wide range of clinical outcomes including acute hepatitis, chronic infection, and cirrhosis and hepatocellular carcinoma, contributing substantially to disease burden and healthcare challenges [6, 7]. HBV is transmitted through multiple routes including blood transfusion, unsafe injections, sexual contact and perinatal transmission [8]. Individuals at higher risk include patients undergoing haemodialysis, recipients of repeated blood transfusions, intravenous drug users and individuals with close household contact with infected persons [9]. Accurate diagnosis of HBV infection relies on a combination of serological and molecular markers such as HBsAg, Anti-HBc, HBeAg and HBV DNA [10]. Occult Hepatitis B infection (OBI) is defined as the presence of replication-competent HBV DNA in the blood or liver of individuals who test negative for Hepatitis B surface antigen (HBsAg) using currently available assays [10]. In such cases, viral replication persists at low levels, often making detection difficult with routine diagnostic methods [11]. Detection of HBV DNA in liver tissue is considered the gold standard for diagnosing occult hepatitis B infection (OBI). However, due to the invasive nature of liver biopsy, serum HBV DNA detection using sensitive molecular techniques such as PCR is commonly employed. Anti-HBc positivity serves as an important surrogate marker for identifying individuals who may require further molecular testing [12]. Detection of HBV DNA using sensitive molecular techniques such as polymerase chain reaction (PCR) is

therefore essential for identifying these hidden infections. OBI has gained increasing clinical importance due to its potential role in transmission through blood transfusion and organ transplantation, as well as its association with progression to liver cirrhosis and hepatocellular carcinoma [13, 14]. It is also more frequently observed in high-risk populations and in regions with intermediate to high HBV endemicity, including Asia and Africa [15]. Therefore, it is of interest to show the prevalence of occult Hepatitis B infection among Anti-HBc positive HBsAg-negative patients in high-risk groups and to evaluate associated risk factors and co-infections using molecular diagnostic methods.

Methodology:

This cross-sectional observational study was conducted in a tertiary care hospital to determine the prevalence of occult Hepatitis B virus (HBV) infection among high-risk individuals who tested negative for hepatitis B surface antigen (HBsAg). A total of 362 patients clinically suspected of viral hepatitis and found to be HBsAg-negative were included in the study. The study population comprised predefined high-risk groups, including patients on chronic haemodialysis (≥ 6 months), recipients of multiple blood transfusions, first-degree family contacts of HBsAg-positive individuals and patients with confirmed Hepatitis C virus (HCV) infection. Patients who were HBsAg-positive or had inadequate or haemolysed samples were excluded. After obtaining informed consent, 3-5 mL of venous blood was collected under aseptic conditions. Serum was separated within a few hours of collection and stored at 4-8°C for short-term use and at -20°C for longer storage until further testing. All samples were initially screened for total hepatitis B core antibody (Anti-HBc) using enzyme-linked immunosorbent assay (ELISA). Out of the total samples, those found positive for Anti-HBc ($n = 102$) were further subjected to molecular testing. HBV DNA detection was performed using polymerase chain reaction (PCR) or real-time PCR techniques. Samples with detectable HBV DNA were considered positive for occult Hepatitis B infection (OBI). Additionally, screening for HCV infection was carried out to identify possible co-infection. Demographic details, clinical history and associated risk factors

were recorded for all participants. Data were analyzed to determine the proportion of Anti-HBc positivity, prevalence of HBV DNA among Anti-HBc positive individuals and overall prevalence of occult HBV infection in the study population (9 out of 362 cases). Results were expressed as frequencies and percentages. The study was conducted after obtaining approval from the Institutional Ethics Committee and confidentiality of patient information was strictly maintained throughout the study.

Results:

During the study period from September 2023 to September 2024, a total of 370 suspected cases of viral hepatitis were initially screened for Hepatitis B surface antigen (HBsAg) using Rapid Diagnostic Test (RDT). All 370 samples were found negative by RDT. However, when these samples were retested using ELISA, 8 samples were detected positive for HBsAg, while 362 samples remained negative. These 8 HBsAg-positive samples were excluded from the study and the remaining 362 HBsAg-negative samples were included for further serological and molecular evaluation (Table 1). Among the 362 HBsAg-negative cases, 183 (50.6%) were males and 179 (49.4%) were females, showing almost equal gender distribution with a slight male predominance. The age of the study participants ranged from 10 to 80 years, with a mean age of 32.65 years. The highest number of cases was observed in the 21-40 years age group (40.6%), followed by ≤20 years (30.4%), 41-60 years (20.2%) and >60 years (8.8%) (Table 2-4). Based on associated risk factors for occult Hepatitis B infection, the study population was categorized into four major high-risk groups. Chronic haemodialysis patients constituted the largest group with 111 cases (30.7%), followed by blood transfusion recipients with 92 cases (25.4%), family contacts of HBsAg-positive individuals with 80 cases (22.1%) and chronic Hepatitis C patients with 79 cases (21.8%) (Table 3). All 362 HBsAg-negative samples were further tested for Anti-HBc Total (Anti-HBc T) by ELISA. Out of these, 102 cases (28.2%) were found positive for Anti-HBc Total, indicating previous exposure to HBV infection, while 260 cases (71.8%) were negative. Anti-HBc Total positivity was higher in males (31.7%) compared to females (24.6%). Age-wise analysis showed that the highest positivity was observed in the >60 years age group (50%), followed by 41-60 years (38.4%), ≤20 years (25.5%) and 21-40 years (20.4%), which was statistically significant (p < 0.001) (Table 5). Among the different high-risk groups, the highest Anti-HBc Total positivity was observed in chronic Hepatitis C patients (31.6%), followed by blood transfusion recipients (29.3%), chronic haemodialysis patients (27%) and family contacts (25%). This finding suggests increased HBV exposure among these vulnerable groups, particularly in patients with chronic HCV infection. Further testing for Anti-HBs was performed in all HBsAg-negative samples. A total of 182 cases (50.3%) were positive for Anti-HBs, while 180 cases (49.7%) were negative. The highest Anti-HBs positivity was observed among blood transfusion recipients (64.1%), which was statistically significant (P < 0.002). This may indicate previous immunization or resolved past HBV infection in this group

(Table 7). The combined Anti-HBc Total and Anti-HBs antibody profile showed that 82 cases (22.7%) were positive for both Anti-HBc and Anti-HBs, indicating past resolved HBV infection. A total of 100 cases (27.6%) were Anti-HBc negative and Anti-HBs positive, suggestive of vaccination-induced immunity. Meanwhile, 160 cases (44.2%) were negative for both markers, indicating susceptibility to HBV infection. Importantly, 20 cases (5.5%) showed isolated Anti-HBc positivity, defined as HBsAg negative, Anti-HBc positive and Anti-HBs negative (Table 6). Isolated Anti-HBc positivity is considered clinically significant because it may represent occult Hepatitis B infection. In the present study, isolated Anti-HBc positivity was more common in males (7.7%) than females (3.4%) and this difference was statistically significant (p < 0.034). Age-wise, the highest isolated Anti-HBc positivity was observed in the 41-60 years age group (9.6%). Among the high-risk groups, family contacts of HBsAg-positive individuals showed the highest isolated Anti-HBc positivity (10%), followed by chronic Hepatitis C cases (5.1%), blood transfusion recipients (4.3%) and chronic haemodialysis patients (3.6%). All 20 isolated Anti-HBc positive samples were further subjected to Real-Time PCR for HBV DNA detection and viral load estimation. Out of these, 3 cases (15%) were found positive for HBV DNA and were confirmed as Occult Hepatitis B Infection (OBI). All three OBI-positive cases were males. No female patient showed HBV DNA positivity. Age-wise, all OBI-positive cases belonged to the 41-60 years age group, where the observed proportion of OBI was 42.9% (Table 8). Among the high-risk groups, the highest proportion of OBI was observed in chronic haemodialysis patients, where 2 out of 4 isolated Anti-HBc cases (50%) were HBV DNA positive. One OBI-positive case (14.3%) was detected among family contacts, while no OBI-positive cases were found among blood transfusion recipients and chronic Hepatitis C patients. Viral load analysis showed that two haemodialysis patients had HBV DNA levels below 10 IU/mL, while one family contact case had a viral load of 1.92×10^1 IU/mL (Table 9). These findings indicate that a significant proportion of HBsAg-negative individuals may harbor occult HBV infection, especially among high-risk groups such as chronic haemodialysis patients and family contacts of HBV-positive individuals. The study highlights the importance of screening isolated Anti-HBc positive cases using molecular methods for early detection of occult Hepatitis B infection and prevention of further transmission.

Table 1: Comparison of HBsAg Results by RDT and ELISA

HBsAg Assay	Positive	Negative	Total
RDT	0	370	370
ELISA	8	362	370

Table 2: Gender-wise Distribution of HBsAg Negative Cases

Gender	Number of Cases (n)	Percentage (%)
Male	183	50.6
Female	179	49.4
Total	362	100

Table 3: High-Risk Group-wise Distribution

High-Risk Group	Number of Cases (n)	Percentage (%)
Chronic haemodialysis	111	30.7
Family contacts	80	22.1

Blood transfusion recipients	92	25.4
Chronic Hepatitis C	79	21.8
Total	362	100

Table 4: Age Group-wise Distribution of HBsAg Negative Cases

Age Group	Number of Cases (n)	Percentage (%)
≤ 20 years	110	30.4
21-40 years	147	40.6
41-60 years	73	20.2
> 60 years	32	8.8
Total	362	100

Table 5: Anti-HBc Total Positivity

Anti-HBc Total Status	Number of Cases (n)	Percentage (%)
Positive	102	28.2
Negative	260	71.8
Total	362	100

Table 6: Isolated Anti-HBc Positivity

Isolated Anti-HBc Status	Number of Cases (n)	Percentage (%)
Positive	20	5.5
Negative	342	94.5
Total	362	100

Table 7: Anti-HBs Positivity

Anti-HBs Status	Number of Cases (n)	Percentage (%)
Positive	182	50.3
Negative	180	49.7
Total	362	100

Table 8: OBI (HBV DNA Positive) among Isolated Anti-HBc Cases

OBI Status	Number of Cases (n)	Percentage (%)
HBV DNA Positive	3	15
HBV DNA Negative	17	85
Total	20	100

Table 9: Distribution of OBI Cases among High-Risk Groups

High-Risk Group	OBI Positive	Percentage (%)
Chronic haemodialysis	2	50
Family contacts	1	14.3
Blood transfusion recipients	0	0
Chronic Hepatitis C	0	0

Discussion:

Hepatitis B virus (HBV) infection continues to be a major global health concern despite the availability of effective vaccines and antiviral therapies [16]. Occult Hepatitis B infection (OBI), characterized by the presence of HBV DNA in HBsAg-negative individuals and represents a clinically significant but often underdiagnosed condition [10]. The present study was conducted among high-risk, HBsAg-negative patients to determine the prevalence of OBI and associated factors. Occult Hepatitis B infection was detected in 9 out of 362 cases (2.48%) in the present study. Additionally, among Anti-HBc positive individuals, HBV DNA was detected in 9 out of 102 cases (8.8%), highlighting that a subset of previously exposed individuals harbors occult infection. These findings emphasize the importance of molecular diagnostic methods for accurate detection, as reliance solely on HBsAg may underestimate the true burden of HBV infection. The presence of Anti-HBc in the absence of HBsAg suggests either resolved infection or possible occult infection. Occult Hepatitis B infection was detected in 0.82% (3/362) of cases in the present study. This relatively low prevalence is comparable to some earlier studies conducted in

similar settings, where OBI prevalence varied depending on the population studied and the sensitivity of detection methods [17]. The low detection rate may be attributed to the intermittent and low-level viremia characteristic of occult infection, which makes its detection challenging. All OBI cases in the present study were identified among Anti-HBc positive individuals, reinforcing the importance of Anti-HBc as a useful screening marker for detecting potential occult HBV infection. However, only a small proportion of Anti-HBc positive cases were HBV DNA positive, indicating that not all Anti-HBc positive individuals harbour active occult infection. The majority of cases in this study belonged to high-risk groups such as chronic haemodialysis patients and blood transfusion recipients, highlighting the role of parenteral exposure in HBV transmission. These findings are in agreement with established knowledge that repeated exposure to blood and blood products increases the risk of HBV infection, including occult forms [18]. A higher proportion of OBI cases were observed in males compared to females, although the small number of cases limits definitive conclusions regarding gender predilection. Similarly, most OBI cases were found in the younger to middle-aged population, which may reflect the overall distribution of the study population. Hepatitis C virus (HCV) co-infection was observed in a subset of OBI cases. Previous studies have suggested that HCV infection may suppress HBV replication, leading to reduced expression of HBsAg and contributing to occult infection [19]. This interaction between HBV and HCV highlights the complexity of viral co-infections and their impact on disease detection and progression. The low viral load observed in OBI cases in this study is consistent with the known characteristics of occult infection, where viral replication persists at minimal levels [17]. This underscores the importance of sensitive molecular techniques such as PCR for accurate detection. However, the study has certain limitations. Anti-HBs testing were not performed and therefore, true isolated Anti-HBc status could not be confirmed. Additionally, detailed quantitative viral load data could not be extensively analyzed. The relatively small number of OBI cases also limits the ability to draw strong associations with risk factors. Despite these limitations, the study highlights the presence of occult HBV infection among high-risk, HBsAg-negative individuals and emphasizes the need for incorporating molecular diagnostic methods in routine screening, especially in high-risk settings.

Conclusion:

Occult hepatitis B infection was detected in 0.82% of HBsAg-negative high-risk patients, highlighting a clinically important risk of missed infection with conventional screening. HBV DNA testing is essential for detecting occult infection, particularly in high-risk groups such as haemodialysis patients and blood transfusion recipients. Early molecular detection and incorporation of sensitive HBV DNA testing into screening protocols may help prevent transmission and reduce long-term HBV-related complications.

Advancement of knowledge:

This study shows that occult Hepatitis B infection is more common than expected among people who test negative for HBsAg and it points out that using only serological markers is not enough. The results highlight the need for molecular diagnostic methods to detect these cases accurately, especially when Anti-HBc is positive. These findings help us better understand how OBI spreads and its links to risk factors and co-infections. This knowledge can improve screening, help prevent transmission and guide treatment, especially in high-risk and endemic areas.

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