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Molecular and Serological Evaluation of Occult Hepatitis B Virus Infection among Patients Undergoing Maintenance Hemodialysis at a Tertiary Teaching Hospital of Bangladesh

Nishad Sultana[1], Pompy Dey[2], Ayesha Ahmed Khan[3], Tabassuma Rahman[4], Masuma Jannat[5], Nusrat Fatema[6]

[1]Lecturer, Department of Dental Public Health, Chittagong Medical College, Dental Unit, Chattogram, Bangladesh; [2]Lecturer, Department of Microbiology, Rangamati Medical College, Rangamati, Bangladesh; [3]Assistant Professor, Department of Microbiology, Institute of Applied Health Sciences, Chattogram, Bangladesh; [4]Medical Officer, Seba Hospital, Chattogram, Bangladesh; [5]Lecturer, Department of Forensic Medicine, Noakhali Medical College, Noakhali, Bangladesh; [6]Associate Professor, Department of Virology, Chittagong Medical College, Chattogram, Bangladesh

Abstract

Background: Occult hepatitis B virus infection (OBI) represents a silent yet significant phase of chronic HBV infection (CHB), characterized by the presence of HBV DNA in the liver and/or serum without detectable hepatitis B surface antigen (HBsAg). It may occur in both seropositive and seronegative individuals and poses a risk of transmission via blood transfusion, organ transplantation, and hemodialysis. Immunocompromised patients, particularly those with chronic kidney disease (CKD) undergoing maintenance hemodialysis (MHD), are particularly vulnerable due to repeated transfusions, invasive procedures, and altered immune responses. Objective: The purpose of the present study was to determine the prevalence and serological patterns of OBI among CKD patients receiving MHD at Chittagong Medical College Hospital (CMCH), Bangladesh. Methodology: This cross-sectional study was conducted from January to December 2024 in the Department of Microbiology at Chittagong Medical College, Chattogram, Bangladesh including 176 CKD patients on MHD who were HBsAg-negative, as confirmed by ELISA. HBsAg was rechecked using immunochromatographic tests (ICT). Anti-HBc and anti-HBs antibodies were detected by enzyme-linked immunosorbent assay (ELISA). HBV DNA was quantified using real-time polymerase chain reaction (qPCR). Results: All patients were negative for HBsAg. Anti-HBc was positive in 53 (30.11%) and anti-HBs in 96 (54.55%) patients. HBV DNA was detected in 6 patients (3.41%), confirming occult hepatitis B virus infection. Among them, 4 (2.27%) were seropositive and 2 (1.14%) were seronegative OBI. No significant associations were observed between occult hepatitis B virus infection status and sociodemographic variables, dialysis duration, blood transfusion history, or serological profile. Conclusion: Integration of HBV DNA testing into routine screening protocols is essential for early detection, improved clinical management, and reduction of HBV transmission risks in dialysis units.

Keywords: Occult Hepatitis B Virus Infection (OBI); Maintenance Hemodialysis (MHD); HBV DNA; Chronic Kidney Disease (CKD)

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Introduction

Hepatitis B virus (HBV), a 42-nm DNA virus in the Hepadnaviridae family, is among the most prevalent and infectious blood-borne pathogens globally[1]. While HBV can lead to both acute and chronic liver disease, a particularly elusive and clinically significant form is occult hepatitis B infection (OBI), defined by the presence of HBV DNA in liver and/or serum in the absence of detectable hepatitis B surface antigen (HBsAg)[2]. Chronic HBV infection can progress to cirrhosis, hepatocellular carcinoma (HCC), and death, ranking it among the top ten global causes of mortality[3-4]. Despite the success of HBV vaccination programs, the virus remains a major public health

Correspondence: Dr. Nishad Sultana, Lecturer, Department of Dental Public Health, Chittagong Medical College, Dental Unit, Chattogram, Bangladesh; Email: nishadliramaher@gmail.com; Cell no: +8801672405351
ORCID: https://orcid.org/0009-0004-6071-9911;

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challenge, particularly in resource-limited countries[5,6].

Globally, HBV affects approximately 3.2% of the population[7], with regional prevalence ranging from ≥ 8% in hyperendemic zones to ≤1.9% in low-prevalence regions[8]. In Asia, rates vary widely 5.1% in Thailand, 4.0% in Malaysia, and 2% in Singapore; 6.9% in China versus 1.0% in Japan; and in South Asia, from 6.5% in Myanmar to 1.0% in Nepal[9-12]. These differences reflect regional disparities in vaccination, healthcare access, and infection control.

In Bangladesh, childhood HBV prevalence has dropped to below 1% due to the successful implementation of the Expanded Program on Immunization[13]. However, adult infection remains a concern, with a national prevalence estimated at 4%-above the global average[14,15]. This highlights the ongoing risk in adult and high-risk populations, including those undergoing hemodialysis.

The European Association for the Study of the Liver (EASL) defines five phases of chronic HBV infection, including the HBsAg-negative or occult phase[16,17]. Occult hepatitis B virus infection is characterized by low levels of replication-competent HBV DNA, particularly covalently closed circular DNA (cccDNA), in liver tissues, often below 200 IU/mL in serum, with undetectable HBsAg using current assays[18]. Mutations in the S gene may mask HBsAg expression, complicating diagnosis[19-21].

Occult hepatitis B virus infection is subclassified as seropositive (anti-HBc and/or anti-HBs positive) or seronegative (absence of both), with most cases (approximately 80%) falling into the former category[18]. Among patients undergoing maintenance hemodialysis (MHD), HBV prevalence is estimated at 7.32% globally, with OBI rates ranging from 0% to 58%, reflecting geographic and diagnostic variability[22,23]. MHD patients are particularly susceptible to OBI due to immunosuppression, repeated blood transfusions, poor vaccine responsiveness, and prolonged exposure to dialysis procedures[24-26].

While HBsAg is the standard screening tool, the CDC recommends a full panel-HBsAg, anti-HBc, and anti-HBs-to better capture past or ongoing infection[27]. Serological testing alone may miss OBI, especially in seronegative individuals. Therefore, nucleic acid testing (NAT) for HBV DNA is considered the gold standard, particularly in clinical contexts where transmission risks are high[18].

Despite low viremia, occult hepatitis B virus infection remains transmissible and capable of reactivation, leading to severe liver complications. Hemodialysis settings, characterized by vascular access, equipment sharing, and infection control gaps, are particularly vulnerable[28]. Integrating HBV DNA testing into routine screening could mitigate these risks.

This study was aimed to assess the prevalence of occult hepatitis B virus infection among maintenance hemodialysis patients using both serological and molecular assays. The results may guide infection control practices and screening protocols in dialysis units, especially in high-burden regions like Bangladesh.

Methodology

Study Design and Setting: This was a cross-sectional study conducted over a 12-month period (January to December 2024) in the Department of Microbiology, Chittagong Medical College, Chattogram, Bangladesh. A total of 176 patients undergoing maintenance hemodialysis (MHD) at Chittagong Medical College Hospital (CMCH), Chattogram, Bangladesh was enrolled based on predefined eligibility criteria. Patients with chronic kidney disease (CKD) undergoing maintenance hemodialysis for ≥3 months with the age of ≥15 years, patients with negative for HBsAg by ELISA within the preceding 3 months with provided informed written consent were included in this study. Known HBV-positive CKD patients on maintenance hemodialysis, patients on immunosuppressive therapy or chemotherapy were excluded from this study.

Study Procedure: Sociodemographic and clinical data, including age, sex, duration of dialysis, transfusion history, and comorbidities, were collected using a structured questionnaire and verified with medical records. Prior to dialysis sessions, 5 mL of peripheral blood was collected aseptically in plain vacutainer tubes. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -80°C until further analysis.

Laboratory Investigations: Occult HBV Infection (OBI) was defined as the presence of HBV DNA (<200 IU/mL) in serum in the absence of detectable HBsAg. Based on serological status, OBI cases were categorized as Seropositive OBI which was presence of anti-HBc and/or anti-HBs[18] and seronegative OBI was absence of both anti-HBc and anti-HBs[18].

HBsAg Detection: Performed using a commercial immunochromatographic test (ICT). The commercially available kit (EXCEL® China) was used for the

detection of HBsAg with the detection limit of 1PEI ng/ml (1IU/ml) of HBsAg in serum.

Serological Markers: Anti-HBc total and anti-HBs were assessed using third-generation ELISA kits. For the detection of anti-HBc total and anti-HBs total, commercially available kit (Biolab, UK) was used.

HBV DNA Detection: Quantified by real-time polymerase chain reaction (qPCR) using a standard viral DNA extraction kit (Bosphore viral DNA Extraction Spin Kit). The qPCR was done by a commercially available Bosphore HBV quantification kit with an analytic sensitivity of 10 IU/ml.

Statistical Analysis: Data were analyzed using IBM SPSS version 26.0. Descriptive statistics (frequencies and percentages) were used to summarize demographic and clinical variables. Associations between OBI and risk factors were evaluated using chi-square tests. A p-value <0.05 was considered statistically significant.

Ethical Consideration:

Ethical approval was obtained from the Ethical Review Committee of Chittagong Medical College (Memo no: 59.27.0000.013.19. PG.2024/1071, dated 14.03.2024). Written informed consent was obtained from all participants or guardians of minors, following the Declaration of Helsinki, Good Clinical Practice guidelines, and national regulations. Study aims and procedures were explained in Bengali or the local language. Confidentiality and anonymity of all participants were strictly maintained. The collected data were used solely for the purpose of this study and will not be used for any other research or purpose without appropriate permission.

Results

Among the 176 study participants, none tested positive for HBsAg (0%). Anti-HBs was detected in 96 (54.55%) samples, while anti-HBc total was positive in 53 (30.11%) samples (Table 1).

Table 1: HBsAg, anti-HBs, and anti-HBc Total Biomarker Status among study participants (N=176)

Biomarker	Test method	Non-reactive	Positive
HBsAg	ICT	176(100%)	0(0%)
Anti-HBs	ELISA	80(45.45%)	96(54.55%)
Anti-HBc total	ELISA	123(69.89%)	53(30.11%)

Note: ICT=Immunochromatographic test, ELISA = Enzyme-linked immunosorbent assay, non-reactive = Negative

A subset of 37 (21.02%) samples showed positivity for both anti-HBs and anti-HBc total. Conversely, 64 (36.36%) samples were negative for both markers (Figure I).

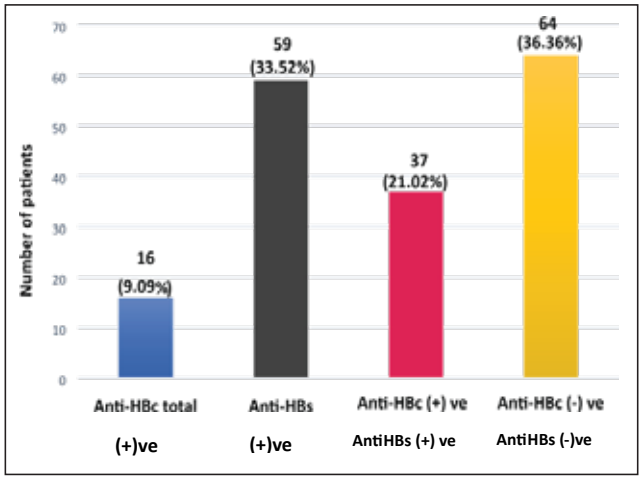

Figure I: Distribution of anti-HBc total and anti-HBs serostatus among study participants (N=176)

Quantitative real-time PCR detected HBV DNA in 9 (5.11%) patients; 6 (3.41%) had viral loads <200 IU/ml (true occult HBV infection), and 3 (1.70%) had viral loads ≥200 IU/ml (false OBI). HBV DNA was undetectable (<10 IU/ml) in 167 (94.89%) patients (Table 2, Figure II).

Among OBI-positive patients, 3 (1.7%) were anti-HBs positive, 1 (0.57%) was anti-HBc total positive, and none were positive for both antibodies. Two (1.14%)

Table 2: Quantitative HBV DNA levels detected by qPCR in study participants (N=176)

Viral Load Category	Number of Cases n (%)	Mean Viral Load (IU/ml) ± SD
<200 IU/ml (True OBI)	6 (3.41)	43.59 ± 32.86
≥200 IU/ml (False OBI)	3 (1.70)	374.80 ± 29.33
Undetected (<10 IU/ml)	167 (94.89)	Not detected

Note: True OBI is defined as HBV DNA <200 IU/ml; False OBI as ≥ 200 IU/ml; detection limit = 10 IU/ml.

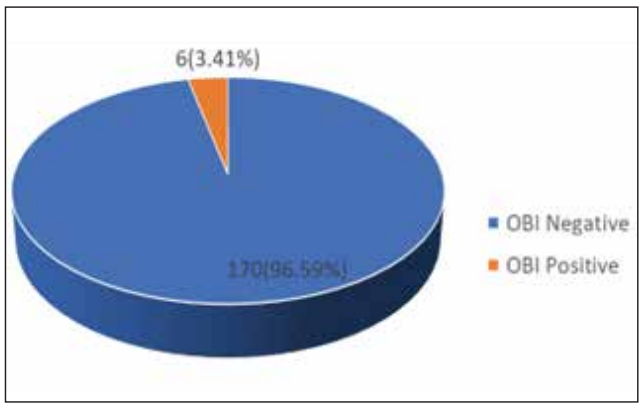

Figure II: Frequency of occult HBV Infection Among Study Participants (n=176)

OBI-positive patients were negative for both markers. Among OBI-negative patients, 56 (31.8%) were anti-HBs positive, 15 (8.52%) were anti-HBc total positive, 37 (21.02%) were positive for both antibodies, and 62 (35.28%) were negative for both (Table 3, Figure III). No statistically significant differences were observed (p = 0.529).

Table 3: Distribution of anti-HBc total and anti-HBs serostatus by OBI detection (N=176)

Serostatus	OBI Detected	OBI Undetected	Total	P value
Anti-HBs positive	3 (1.7%)	56 (31.8%)	59(33.5%)	0.529*
Anti-HBc total positive	1(0.57%)	15(8.5%)	16(9.1%)	
Both positive	0(0.0%)	37 (21.0%)	37(21.0%)	
Both negative	2(1.1%)	62(35.3%)	64(36.4)	

Note: *Chi-square test performed; p > 0.05 considered not significant.

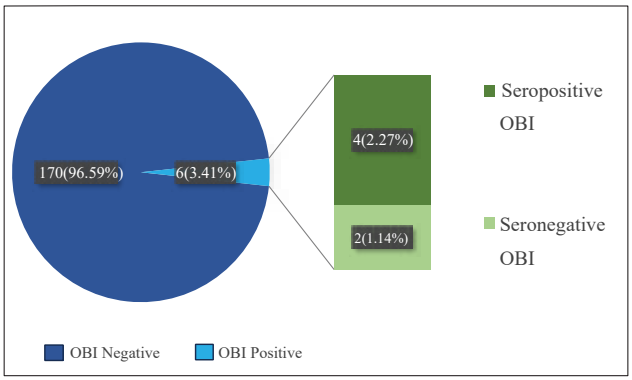

Figure III: Seropositive and seronegative OBI distribution among study participants.

Discussion

This study investigated the prevalence of occult hepatitis B virus infection (OBI) among chronic kidney disease (CKD) patients undergoing maintenance hemodialysis (MHD) at a tertiary hospital in Bangladesh. OBI, characterized by replication-competent HBV DNA in liver and/or serum (<200 IU/mL) despite negative HBsAg status[29], presents a diagnostic challenge and public health concern, especially in resource-limited settings where reliance on HBsAg testing is routine. Given the dependence of socioeconomically disadvantaged populations on government healthcare facilities such as Chittagong Medical College Hospital (CMCH), understanding the epidemiology of OBI in this context is critical.

Among the 176 HBsAg-negative participants, 30.11% were anti-HBc total positive, and 54.5% were anti-HBs positive. These figures are consistent with previous studies, reported anti-HBc total and anti-HBs positivity rates of 23.5% and 66.3%, respectively among Iranian MHD patients30, 43% anti-HBs, 3.0% anti-HBc total, and 35% double positivity in Brazil[31]. The presence of anti-HBc suggests previous or occult HBV exposure[32,33], while anti-HBs indicates either vaccine-induced or natural immunity, though neither marker reliably excludes viral persistence[34]. These results reinforce the importance of a comprehensive screening panel including HBsAg, anti-HBc, and anti-HBs, as recommended by global guidelines[27].

Six participants (3.4%) were identified as OBI cases, with HBV DNA levels ranging from 11 to 94 IU/mL (mean 43.6 IU/mL). This is in line with studies from Ghana[35] (7.3%) and Nepal[36] (8.0%). Three additional patients exhibited viral loads ≥200 IU/mL despite negative HBsAg and were classified as false OBI potentially attributable to surface gene escape mutants that compromise antigen detection[37,38]. A similar result was found in a study of Iran, where one true OBI and four false OBI cases were found among 118 participants[39].

The serological profiles of OBI patients varied: three were anti-HBs positive (two vaccinated without a booster, one unvaccinated), one was anti-HBc positive, and two were seronegative for both markers. These findings highlight the limitations of serological screening and the possibility of OBI in both vaccinated and naturally exposed individuals. Waning immunity or low seroconversion rates in CKD patients on dialysis may contribute to vulnerability[8,40]. Persistence of HBV in hepatocytes as covalently closed circular DNA (cccDNA) or in peripheral blood mononuclear cells further underlines the potential for reactivation, particularly in immunocompromised patients[41].

The OBI prevalence in this study (3.4%) is comparable to 4 to 8% reported in similar populations across Iraq, Iran, and Nepal[30,36,42]. Variability may stem from differences in population demographics, diagnostic methods, and the sensitivity of molecular assays[43]. Importantly, all OBI patients in this cohort had histories of multiple blood transfusions, reinforcing transfusion as a key risk factor, even when donors are HBsAg-screened[44].

This study has several limitations. First, HBsAg testing was conducted using an immunochromatographic test (ICT) rather than ELISA or chemiluminescence assays, which may have reduced sensitivity. Second, anti-HBc IgM testing was not performed, limiting the differentiation between past and recent infections. Third, HBV DNA

sequencing was not undertaken, restricting analysis of potential viral mutations or escape variants. The cross-sectional nature of the study may have underestimated OBI prevalence due to intermittent viremia. Additionally, liver biopsy the definitive diagnostic method for OBI-was not feasible in this immunocompromised population. Lastly, this single-center study may not reflect the broader hemodialysis population in Bangladesh, limiting generalizability.

Conclusion

This study reveals a notable prevalence of occult hepatitis B virus infection among hemodialysis patients in Bangladesh, despite negative HBsAg results by routine screening. The detection of HBV DNA in both seropositive and seronegative individuals underscores the limitations of relying solely on serological assays for HBV surveillance. Incorporation of HBV DNA PCR into routine screening of hemodialysis patients is essential for early detection of occult hepatitis B infection (OBI), complemented by booster vaccination programs for individuals with suboptimal anti-HBs titers. Future research should emphasize full-length HBV genome sequencing in OBI cases to identify immune escape variants, drug resistance, and genotype distribution, while diagnostic accuracy may be enhanced through ultrasensitive HBsAg assays and nested PCR targeting multiple genomic regions. Additionally, public health strategies, including awareness campaigns and policy-level interventions, are necessary to promote comprehensive HBV vaccination, particularly in rural and underserved populations.

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Conflict of Interest
The authors declare no competing interests.

Financial Disclosure
No external funding was received.

Authors' contributions
The study was conceptualized by Nishad Sultana and Nusrat Fatema. Data collection was conducted by Nishad Sultana and Tabassuma Rahman. Formal analysis and methodology were prepared by Ayesha Ahmed Khan and Masuma Jannat. Laboratory work was performed by Nishad Sultana, and Pompy Dey drafted the manuscript. Dr. Nishad Sultana reviewed and edited the final draft. All authors revised the content and approved the manuscript.

Data Availability
Data are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate
Ethical approval

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ORCID
Nishad Sultana: https://orcid.org/0009-0004-6071-9911
Pompy Dey: https://orcid.org/0009-0002-3145-9626
Ayesha Ahmed Khan: https://orcid.org/0000-0003-4101-2035
Tabassuma Rahman: https://orcid.org/0009-0008-8507-459X
Masuma Jannat: https://orcid.org/0009-0001-8867-1655
Nusrat Fatema: https://orcid.org/0009-0008-9754-0906

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