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Occult Hepatitis B Virus Infection Among HBsAg-Negative Maintenance Haemodialysis Patients in Malaysia: Association with Anti-HBs Serostatus

Xin Yi Jolynne Tan
Cardiff University
Jun Min Em
University of Malaya
Man Le Ng
University of Malaya
Sze Er Lim
University of Malaya
Azwani Abdullah
University of Malaya
Lim Soo Kun
limsookun@um.edu.my

University of Malaya

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Abstract

Background

Occult Hepatitis B infection (OBI)—defined as the presence of replication-competent HBV DNA in HBsAg-negative individuals—poses a severe transmission and reactivation risk in haemodialysis units. This study aims to investigate the prevalence and clinical characteristics of detectable HBV DNA among HBsAg-negative maintenance haemodialysis patients, particularly those with anti-HBc positivity.

Methods

A retrospective observational study was conducted involving 2,146 patients who attended the dialysis unit at Universiti Malaya Medical Centre (UMMC) from 1 January 2019 to 30 June 2024. Patients established on maintenance haemodialysis were included, while those with HBsAg positivity or missing data were excluded. Patient demographics, clinical variables, and biochemical information were extracted from the Electronic Medical Record (EMR). Data collection was conducted using REDCap version 14.3.13, and statistical analysis was performed using SPSS version 29.0 and Python version 3.10.16. A Firth penalised multivariable binary logistic regression model was constructed utilising OBI status as the dependent variable to identify independent clinical predictors, addressing potential small-sample bias inherent in standard logistic regression.

Results

Among 2,146 screened patients, 1,635 patients were included. Anti-HBc IgG positivity was identified in 23.5% (N = 384) of the cohort. HBV DNA testing was not performed in 146 patients under the testing practices prevailing during the study period. Within the tested core-positive subgroup (N = 238), overall OBI prevalence was 9.2% (N = 22). In patients with positive HBV DNA, 54.6% were concurrently Anti-HBs IgG positive (≥ 10 IU/L), and 18.2% possessed an Anti-HBs IgG level ≥ 100 IU/L. The multivariable Firth penalised logistic regression model revealed that while an Anti-HBs titre ≥ 10 IU/L was a significant negative predictor of OBI (OR 0.18, 95% CI 0.07–0.50, p = 0.001), it failed to exclude active viraemia entirely.

Conclusion

Our study identified an overall OBI prevalence of 9.2% in a tested Malaysian ESKD cohort. Firth penalised logistic regression model utilising OBI status as the dependent variable confirmed that relying solely on Anti-HBs IgG to infer viral clearance is inadequate in this immunocompromised population, especially given that over half (54.6%) of OBI patients maintained Anti-HBs IgG positivity.

SUMMARY AT A GLANCE

Our study identified that the overall prevalence of Occult Hepatitis B Infection (OBI) was 9.2% (22/238) among tested ESKD patients on maintenance haemodialysis. They were anti-HBc-positive with detectable HBV DNA. Among these patients, over half of OBI patients were Anti-HBs IgG positive, suggesting that anti-HBs positivity should not be interpreted or used to exclude occult viraemia.

INTRODUCTION

Occult hepatitis B infection (OBI) is clinically defined according to the 2019 Taormina consensus as the presence of replication-competent HBV DNA in the liver and/or blood at low levels in individuals who test negative for the hepatitis B surface antigen (HBsAg) [1]. In clinical settings, OBI is categorised primarily into seropositive OBI, characterised by the presence of hepatitis B core antibodies (Anti-HBc) with or without surface antibodies (Anti-HBs), and seronegative OBI, where no standard serological markers are detectable [2]. Seropositive OBI, which accounts for about 80% of all OBI cases, is associated with an increased risk of Hepatitis B (HBV) reactivation, while seronegative OBI carriers are at an especially high risk in blood or organ donors due to the higher risk of HBV transmission [1].

For the nephrology community, OBI represents a formidable clinical and epidemiological challenge. Patients with end stage kidney disease (ESKD) undergoing regular maintenance haemodialysis (HD) exhibit an impaired cellular immunological response, significantly increasing their susceptibility to nosocomial infections. Reactivation of HBV in immunocompromised individuals, particularly those subjected to immunosuppressive therapies for comorbid conditions or subsequent kidney transplantation, can lead to rapid liver damage, cirrhosis, and a significantly higher risk of hepatocellular carcinoma [3, 4]. Furthermore, the shared environment of a haemodialysis unit, combined with repetitive vascular access, heightens the risk of patient-to-patient transmission of blood-borne pathogens.

While strict infection control measures and mandatory HBsAg screening have drastically reduced overt HBV outbreaks and despite the World Health Organization's (WHO) mandate to eliminate viral hepatitis by 2030 [5], standardising OBI screening in dialysis cohorts remains controversial due to the absence of a universally applied gold-standard screening methodology [6]. According to the National Haemodialysis Quality Standards 2026 (3rd Edition) by the Ministry of Health (MOH) Malaysia [7], routine screening for Hepatitis B in dialysis facilities remains primarily based on HBsAg and anti-HBs surveillance. Universal Anti-HBc testing is not currently compulsory; however, it is highly recommended upon admission, before kidney transplantation and the use of immunosuppression, and specifically in patients who fail to mount an appropriate response (anti-HBs < 10 mIU/mL) after two courses of the Hepatitis B vaccine [7]. HBV DNA is subsequently checked only if Anti-HBc is positive. Consequently, reliance on isolated HBsAg and Anti-HBs metrics to dictate primary isolation protocols may leave significant diagnostic blind spots.

The Malaysian context presents specific unanswered questions and three critical clinical gaps. First, there are limited contemporary data on the prevalence of OBI specifically among Malaysian ESKD patients on maintenance HD. Second, we would like to explore the clinical relevance of seroprotection.

The relationship between anti-HBs titre and detectable HBV DNA in anti-HBc-positive dialysis patients remains clinically relevant yet insufficiently characterised in this cohort. Third, the blind spots in current screening and diagnosis algorithms. The current screening strategies may not identify every HBsAg-negative patient with detectable HBV DNA, as routine anti-HBc IgG testing is not universally mandated, leaving a subset of at-risk patients unidentified. This study therefore aims to determine the prevalence and clinical characteristics of detectable HBV DNA among HBsAg-negative maintenance haemodialysis patients with anti-HBc positivity, and to examine the association between anti-HBs status and HBV DNA detection.

METHODS

Study Design and Patient Selection

A retrospective, single-center, observational study was conducted utilising data from the dialysis registry at Universiti Malaya Medical Centre (UMMC) between January 2019, and July 2024. A total of 2,146 patients were initially identified. To ensure a homogenous infection-risk profile, inclusion was strictly limited to adult ESKD patients established on regular maintenance haemodialysis. Patients managed with peritoneal dialysis, kidney transplant recipients, and those receiving pre-dialysis conservative or palliative care were explicitly excluded. Patients with a known diagnosis of chronic hepatitis B (HBsAg positive) or missing critical baseline data were also excluded. Ultimately, 511 patients (23.8%) were excluded, leaving a final cohort of 1,635 strictly defined maintenance haemodialysis patients. (Fig. 1)

Clinical Definitions

The following clinical definitions were used for standardisation in this study. ESKD was defined as an estimated Glomerular Filtration Rate (eGFR) of less than 15 mL/min/1.73m², with management exclusively through regular maintenance haemodialysis [6]. Chronic hepatitis B was defined by the presence of hepatitis B surface antigen (HBsAg) in the blood for more than six months following infection. OBI was defined as the detection of any quantifiable HBV DNA in a confirmed HBsAg-negative patient [1].

Laboratory Definitions and Assays

Patient demographics, including age, ethnicity, primary cause of ESKD, and associated risk factors, were extracted from hospital Electronic Medical Record (EMR). Laboratory data collected included hepatitis B virology markers (HBsAg, Anti-HBs IgG, Anti-HBc IgG, HBV DNA), liver function tests (LFTs) such as ALT, AST, ALP, GGT, total bilirubin, and albumin, as well as complete blood count parameters (haemoglobin, white cell count, and platelets). For Hepatitis B serology, HBsAg, Anti-HBs IgG, and Anti-HBc IgG were measured using standardised commercial chemiluminescent microparticle immunoassays (CMIA). In alignment with national guidelines, Anti-HBs IgG was defined as positive at levels ≥ 10 IU/L. For

molecular testing, HBV DNA quantification was performed using highly sensitive real-time polymerase chain reaction (PCR) assays (e.g., COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0, Roche Molecular Systems), calibrated to detect trace viraemia with a lower limit of detection (LOD) of 10 copies/mL or 59 copies/ml in plasma or serum.

Statistical Analysis

Data collection was conducted using REDCap version 14.3.13, and statistical analyses were performed using SPSS version 29.0.2.0 and Python programming language (version 3.10.16) utilising the Pandas (version 2.0.0) and Matplotlib (version 3.6.0) libraries. Parametric data were summarised as means and standard deviations (SD), while non-parametric continuous data were expressed as medians and interquartile ranges (IQR). Bivariate comparisons utilised the independent samples t-test, Mann-Whitney U test, and Chi-square or Fisher's exact tests as appropriate.

To identify independent clinical predictors of OBI, a multivariable regression model was constructed utilising OBI status as the dependent variable. Due to the rare nature of the primary outcome (22 OBI events) relative to the number of evaluated clinical predictors (n = 5), standard logistic regression based on Maximum Likelihood Estimation (MLE) was deemed inappropriate. In small-sample or rare-event scenarios, standard MLE is highly susceptible to small-sample bias and data separation (complete or quasi-complete), which can produce artificially inflated odds ratios and unreliably wide confidence intervals. To mitigate this statistical limitation, a Firth penalised multivariable binary logistic regression model was employed. Firth's method introduces a mathematical penalty term to the log-likelihood function (equivalent to Jeffrey's invariant prior), which effectively shrinks the parameter estimates, providing robust, unbiased odds ratios even in the presence of rare outcomes. A p-value of < 0.05 was considered statistically significant.

Ethical Considerations

The study protocol was conducted in accordance with the Declaration of Helsinki and approved by the University of Malaya Medical Centre Medical Research Ethics Committee (Approval No. 2024327-13594). The requirement for informed consent was waived due to the retrospective nature of the study and patient de-identification.

RESULTS

Cohort Demographics and Prevalence of OBI

In this study, a total of 1,635 (76.2%) HBsAg-negative patients were included (Refer to Fig. 1). Among the 1,635 included patients, they are predominantly male (56.7%) and of Malay ethnicity (46.2%), followed by Chinese (30.6%), Indian (21.2%), and others (2.0%). The predominant etiology of ESKD was diabetes mellitus (DM) (64.9%), followed by hypertension (18.3%), glomerulonephritis (7.3%), and others (5.7%).

Baseline serology revealed that 384 (23.5%) individuals tested positive for Anti-HBc IgG (Refer to Fig. 1), indicating a previous or resolved hepatitis B exposure. Subsequent HBV DNA testing was performed in a subset of the Anti-HBc IgG positive patients based on unit protocol. Within this core-positive subgroup, the 22 HBV DNA positive patients accounted for an OBI prevalence of 5.7% out of all core-positive patients, and a prevalence of 9.2% among those actively tested for HBV DNA (22/238). HBV DNA testing was not performed in 146 patients (38.0%) who were Anti-HBc IgG positive, as universal reflex DNA testing was not mandated by Malaysia's standard guidelines during the entirety of the study period.

The median HBV DNA load among OBI patients was 10.0 IU/ml (IQR: 54.0), corresponding to approximately 1.83 log copies/mL (IQR: 1.80). Notably, 21 of the 22 OBI cases presented with low-level viraemia (86.2 IU/ml or < 500 copies/mL). Only a single patient presented with an unusually high HBV DNA level exceeding 1,000 copies/mL, reaching a maximum of 35,560 copies/mL (equivalent to 6,110 IU/mL or 4.55 log copies/ml). Such an extreme viral load in an HBsAg-negative individual is atypical for classic OBI and strongly raises the suspicion of a false-negative HBsAg result—potentially due to an immune escape mutant—or a recent acute infection. (Refer Supplementary Table 1 for more information)

Table 1
Baseline Characteristics of the Anti-HBc Positive Tested Cohort (N = 238)

Variables	HBV DNA positive (N = 22)	HBV DNA negative (N = 216)	p-value
Age, years (mean ± SD)	68.7 ± 8.7	66.0 ± 11.8	0.298
Gender			
Male	15 (68.2%)	137 (63.4%)	0.817
Female	7 (31.8%)	79 (36.6%)	
Race			
Malay	8 (36.4%)	83 (38.4%)	0.511
Chinese	13 (59.1%)	101 (46.8%)	
Indian	1 (4.5%)	23 (10.6%)	
Others	0 (0.0%)	9 (4.2%)	
Primary Aetiology of ESKD			
Diabetes Mellitus (DM)	18 (81.8%)	145 (67.1%)	0.709
Hypertension (HTN)	3 (13.6%)	40 (18.5%)	
Glomerulonephritis (GN)	1 (4.5%)	11 (5.1%)	
Obstructive Uropathy	0 (0.0%)	4 (1.9%)	
ADPKD	0 (0.0%)	3 (1.4%)	
Others	0 (0.0%)	13 (6.0%)	
Risk factors			
Diabetes Mellitus (DM)	18 (81.8%)	165 (76.4%)	0.791
Hypertension (HTN)	21 (95.5%)	190 (88.0%)	0.483
Heart problem	13 (59.1%)	135 (62.5%)	0.819
Stroke	3 (13.6%)	33 (15.3%)	1.000
Chronic Liver Disease	1 (4.5%)	9 (4.2%)	1.000

Note: Baseline characteristics of the anti-HBc IgG-positive cohort (n = 238), stratified by HBV DNA status. Continuous variables were expressed as mean ± SD or median (IQR) while categorical variables were expressed as n (%). *Abbreviations*: ALP, alkaline phosphatase; ALT, alanine aminotransferase; anti-HBc/HBs IgG, hepatitis B core/ surface antibody IgG; AST, aspartate aminotransferase; DM, diabetes mellitus; ESKD, end-stage kidney disease; GGT, gamma-glutamyl transferase; GN, glomerulonephritis; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HTN, hypertension; IQR, interquartile range; SD, standard deviation.

Variables	HBV DNA positive (N = 22)	HBV DNA negative (N = 216)	p-value
Virology Results			
HBsAg Negative	22 (100.0%)	216 (100.0%)	-
Anti-HBs IgG Positive (< 10 mIU/m)	10 (45.4%)	30 (13.9%)	< 0.001
Anti-HBs IgG positive (10–99 mIU/m)	8 (36.4%)	61 (28.2%)	
Anti-HBs IgG positive (≥ 100 mIU/m)	4 (18.2%)	125 (57.9%)	
Anti-HBs IgG Negative	10 (45.4%)	30 (13.9%)	
Liver Function Test (LFT) median (IQR)			
ALT (U/L)	21.0 (21.5)	16.5 (13.0)	0.356
AST (U/L)	18.0 (13.0)	19.0 (14.0)	0.899
ALP (U/L)	84.0 (29.5)	88.0 (49.0)	0.069
GGT (U/L)	29.0 (38.0)	31.0 (34.0)	0.606
Total bilirubin (μmol/L)	6.0 (6.5)	5.5 (4.0)	0.448
Albumin (g/L)	29.0 (4.5)	26.0 (8.0)	0.187
Full Blood Count (FBC) median (IQR)			
Haemoglobin (g/L)	92.0 (26.5)	85.0 (23.8)	0.613
White Cell Count (10^9/L)	8.8 (3.7)	10.1 (6.0)	0.101
Platelet (10^9/L)	217.5 (106.0)	236.0 (127.5)	0.459

Note: Baseline characteristics of the anti-HBc IgG-positive cohort (n = 238), stratified by HBV DNA status. Continuous variables were expressed as mean ± SD or median (IQR) while categorical variables were expressed as n (%). *Abbreviations*: ALP, alkaline phosphatase; ALT, alanine aminotransferase; anti-HBc/HBs IgG, hepatitis B core/ surface antibody IgG; AST, aspartate aminotransferase; DM, diabetes mellitus; ESKD, end-stage kidney disease; GGT, gamma-glutamyl transferase; GN, glomerulonephritis; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HTN, hypertension; IQR, interquartile range; SD, standard deviation.

Clinical and Serological Characteristics

Anti-HBs IgG data of the study population were rigorously evaluated against viraemia status (Refer Table 1). Among those who were Anti-HBc IgG positive and actively HBV DNA positive (OBI), 12 individuals (54.6%) were concurrently positive for Anti-HBs IgG, while 10 individuals (45.4%) were negative. In this viraemic group, Anti-HBs IgG levels of more than 100 IU/L were detected in 4 individuals

(18.2%), followed by 8 individuals (36.4%) with levels between 10–100 IU/L, and 10 individuals (45.4%) with levels of less than 10 IU/L.

In the Anti-HBc IgG positive and HBV DNA negative group, 186 individuals (86.1%) were Anti-HBs IgG positive, and 30 individuals (13.9%) were negative. In this group, Anti-HBs IgG levels of more than 100 IU/L were detected in 125 individuals (57.9%), followed by 61 individuals (28.2%) with levels between 10–100 IU/L, and 11 individuals with isolated levels < 10 IU/L. The high prevalence of elevated Anti-HBs IgG levels in the absence of detectable HBV DNA underscores effective immune clearance or control of HBV replication in these individuals.

Predictors of Occult Hepatitis B

A multivariable Firth penalised binary logistic regression model was constructed utilising OBI status as the dependent variable to identify independent clinical predictors within the tested core-positive cohort (Refer Table 2). After adjusting for age, gender, diabetes mellitus, and logarithmic ALT levels, possessing an Anti-HBs titre ≥ 10 IU/L remained a highly significant independent negative predictor of OBI (OR 0.20, 95% CI 0.07–0.52, P = 0.001). Notably, hepatocellular injury (log-ALT) was not a statistically significant independent predictor of viraemia in this cohort (OR 1.56, P = 0.402). While the presence of Anti-HBs significantly reduced the odds of active replication, it definitively failed to exclude the presence of viraemia entirely.

Table 2
Multivariable Firth Penalised Binary Logistic Regression Model for Predictors of OBI (N = 193)

Variable	Adjusted Odds Ratio (OR)	95% Confidence Interval (CI)	p-value
Age	1.02	0.98–1.07	0.352
Gender (Male)	1.24	0.45–3.39	0.682
Diabetes Mellitus	0.93	0.29–2.94	0.899
Anti-HBs IgG (≥ 10 IU/L)	0.20	0.07–0.52	**0.001**
Log-ALT	1.56	0.55–4.38	0.402

Note: Multivariable Firth penalised logistic regression model used to predict Occult Hepatitis B Infection (OBI) while mitigating small-sample bias. The model was adjusted for age, gender (male), presence of diabetes mellitus, Anti-HBs IgG (> 10 IU/L), and log-transformed alanine aminotransferase (Log-ALT). An Anti-HBs IgG > 10 IU/L remained a statistically significant independent predictor of reduced OBI odds (aOR 0.20, 95% CI 0.07–0.52, p = 0.001).

DISCUSSION

Principal Findings

This study identifies OBI in 22 of 238 anti-HBc IgG-positive patients who underwent HBV DNA testing, yielding a tested subgroup prevalence of 9.2% and a minimum detected proportion of 5.7% across all 384 Anti-HBc IgG positive patients, as 38.0% were untested and the true prevalence is likely higher. 54.6% of confirmed OBI cases concurrently had an Anti-HBs IgG ≥ 10 IU/L, which is conventionally regarded as indicative of immunity. Although multivariable Firth penalised binary logistic regression confirmed that Anti-HBs IgG ≥ 10 IU/L independently reduced the odds of OBI (aOR 0.20, 95% CI 0.07–0.52, p = 0.001), nearly half of all OBI cases occurred in patients who would be considered seroprotected under the current national guidelines [7], collectively underscoring the inadequacy of HBsAg and Anti-HBs surveillance alone.

Accurately determining the global prevalence of OBI remains challenging due to heterogeneity in PCR assay sensitivity, regional HBV endemicity and the population selected for molecular testing. A recent systematic review and meta-analysis by Im et al. estimated OBI prevalence at approximately 4.25% among haemodialysis populations [8]. Country-level estimates vary widely, from 7.3–10.4% in high-endemicity settings such as Ghana [9], Nepal [10] and southern Iran [11] to 0.3–3.2% in South Korea [12], Turkey [13] and Japan [14]. Currently in Malaysia, the HBsAg seroprevalence within the general population is 1.5–9.8% [15]. Hence, our overall cohort prevalence of 1.35% and 9.2% among the tested Anti-HBc IgG positive subgroups, is consistent with this profile and likely further shaped by Malaysia's vaccination programme and the restriction of molecular testing to core-positive patients rather than universal screening.

Our findings directly challenge the conventional understanding that Anti-HBs IgG reliably indicates absolute viral clearance [1, 3, 4]. While Anti-HBs ≥ 10 IU/L significantly reduced the probability of OBI, it did not exclude it entirely, as 54.6% of confirmed OBI cases maintained a detectable Anti-HBs response. This is because HBV establishes a stable intrahepatic reservoir of covalently closed circular DNA (cccDNA) that persists within hepatocyte nuclei and is resistant to immune-mediated clearance [1, 3]. Surface antibody production reflects humoral immunity against circulating HBsAg but does not eliminate intrahepatic cccDNA, permitting ongoing low-level replication [1]. This is evidenced by the Anti-HBs titre gradient in our data, whereby among OBI patients, 45.4% had Anti-HBs < 10 IU/L, 36.4% had 10–100 IU/L, and only 18.2% had > 100 IU/L, compared with 57.9% of HBV DNA-negative patients with Anti-HBs > 100 IU/L, further suggesting that higher titres correlate with better viral suppression, but confer no absolute protection. Those with Anti-HBs < 10 IU/L likely represent patients with waning or insufficient humoral immunity secondary to uraemia [2, 4].

HBV DNA levels among our OBI cases ranged from 10 to 35,560 copies/mL. While most cases exhibited levels between 10 and 100 copies, a subset presented with notably elevated levels, highlighting the episodic nature of viral replication in this population [3, 4]. Uraemia-related immune dysfunction, impaired T-cell responses, reduced natural killer cell activity and diminished vaccine responsiveness may periodically fail to contain intrahepatic replication [3, 4]. Atypically high viral loads should prompt consideration of early OBI reactivation, incomplete resolution of prior acute HBV infection, or false-negative HBsAg due to immune escape mutants [16].

Clinical implications

These findings carry immediate clinical implications for Malaysian practice. Under the 2026 National Haemodialysis Quality Standards, routine HBV screening relies primarily on HBsAg and Anti-HBs, with Anti-HBc testing reserved for vaccine non-responders or patients commencing immunosuppression [7]. Our data demonstrates that this algorithm would have missed 54.6% of OBI cases, all of whom maintained Anti-HBs levels ≥ 10 IU/L. This risk is particularly significant before kidney transplantation or immunosuppression therapy, where unrecognised OBI poses a well-established reactivation risk capable of precipitating on-chronic liver failure [3, 4]. To align with international best practices in Singapore [17] and Hong Kong [18], and to support Malaysia's National Strategic Plan for Hepatitis B and C [19], we propose incorporating routine Anti-HBc IgG screening for all patients entering a dialysis programme, with reflex HBV DNA testing for those who test positive, irrespective of Anti-HBs status. Since the World Health Organisation's (WHO) 2022–2030 Global Health Sector Strategy mandates elimination of viral hepatitis by 2030 [5], expanded OBI screening within a high-risk population is a tangible step towards this goal.

Strengths and limitations

Several limitations of this study warrant consideration. First, although our criteria restricted the study cohort to patients on regular maintenance haemodialysis, granular data regarding hemodialysis sub-modalities (e.g., online hemodiafiltration) and dialysis vintage (duration on dialysis) were not captured in the extracted records. Dialysis vintage is an important clinical parameter, as prolonged time on dialysis is well-documented to exacerbate uremic immune dysfunction, increase cumulative nosocomial exposure within the dialysis unit, and accelerate the waning of antibody titers over time [3, 4]. Secondly, comprehensive Hepatitis B vaccination histories were not retrieved. Hence, we were unable to definitively distinguish between vaccine-induced humoral immunity, natural immunity following resolved infection, or an anamnestic response [2, 4], nor can we formally categorise vaccine non-responders [7]. Third, HBV DNA testing was not universally performed across all Anti-HBc positive individuals, considering that in all 384 anti-HBc positive patients, 5.7% had documented HBV DNA positivity. However, this should be regarded as a minimum detected proportion as 38.0% (N = 146) did not undergo HBV DNA testing due to historical clinical protocols. There would be risk of introducing selection bias and likely underestimating the true OBI prevalence [4]. Fourth, seronegative OBI was beyond the scope of this evaluation as molecular testing was only restricted to core-positive patients. Finally, this retrospective single-centre study spanning 2019 to 2024 limits generalisability to facilities with differing HBV endemicity, patient demographics or testing protocols, and findings may not be fully applicable beyond the Malaysian healthcare setting context or its predominant ethnic groups.

Despite these limitations, several methodological strengths support the validity of our findings. The study enrolled a large cohort of 1,625 HBsAg-negative maintenance haemodialysis patients under a standardised institutional protocol, reducing selection heterogeneity. Besides that, molecular testing was performed using a highly sensitive real-time PCR platform, which ensures adequate analytical sensitivity

for the detection of low-level viraemia characteristic of OBI. Furthermore, the serial stratification of Anti-HBs IgG titres into three clinically defined thresholds (< 10, 10–99 and ≥ 100 IU/L) permitted a more granular characterisation of the humoral immune gradient in relation to HBV DNA detectability beyond conventional binary seropositive thresholds. Lastly, the application of Firth penalised multivariable logistic regression with adjustment for key clinical covariates strengthens the internal validity of the observed association by correcting for potential small-sample bias.

Recommendations

Prospective multicentre studies with universal HBV DNA testing in all HBsAg-negative maintenance haemodialysis patients, regardless of Anti-HBc serostatus, are needed to establish the true OBI prevalence and characterise longitudinal reactivation risk. Health-economic evaluation of Anti-HBc guided reflex testing strategies would inform implementation in resource settings. Ultimately, these findings support a re-examination of national haemodialysis screening guidelines to address the diagnostic blind spots identified.

CONCLUSION

Our study identified an overall OBI prevalence of 9.2% in a Malaysian ESKD cohort on maintenance haemodialysis. Firth penalised multivariable logistic regression utilising OBI status as the dependent variable confirmed that relying solely on Anti-HBs IgG to infer viral clearance is inadequate in this immunocompromised population, especially that over half (54.6%) were Anti-HBs IgG positive among OBI patients.

Declarations

Declarations Conflicts of Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability De-identified participant data and the analytic code (Python/SPSS) used for this study will be made available upon reasonable request to the corresponding author, subject to institutional and Medical Research Ethics Committee approval.

Author Contributions (Credit Statement)

J.M.E. and **X.Y.J.T** conceptualised the study, performed formal data analysis, and wrote the original manuscript draft. **M.L.N, S.E.L, A.A** conducted data curation and clinical investigations. **J.M.E** and **X.Y.J.T** provided methodological review and validated statistical models. **S.K.L.** provided clinical supervision, resources, and critical review of the final manuscript. All authors approved the final submitted version.

Ethics Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Medical Research Ethics Committee (MREC), University of Malaya Medical Centre and approved under the approval number 2024327-13594, in accordance with the Declaration of Helsinki 1964 and its later amendments or comparable ethical standards.

Consent to Participate Declaration

The requirement for informed consent was waived by the Medical Research Ethics Committee (MREC), University of Malaya Medical Centre due to the retrospective nature of the study and the use of de-identified patient data. All data were accessed and analysed in accordance with relevant privacy regulations and guidelines.

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Figures

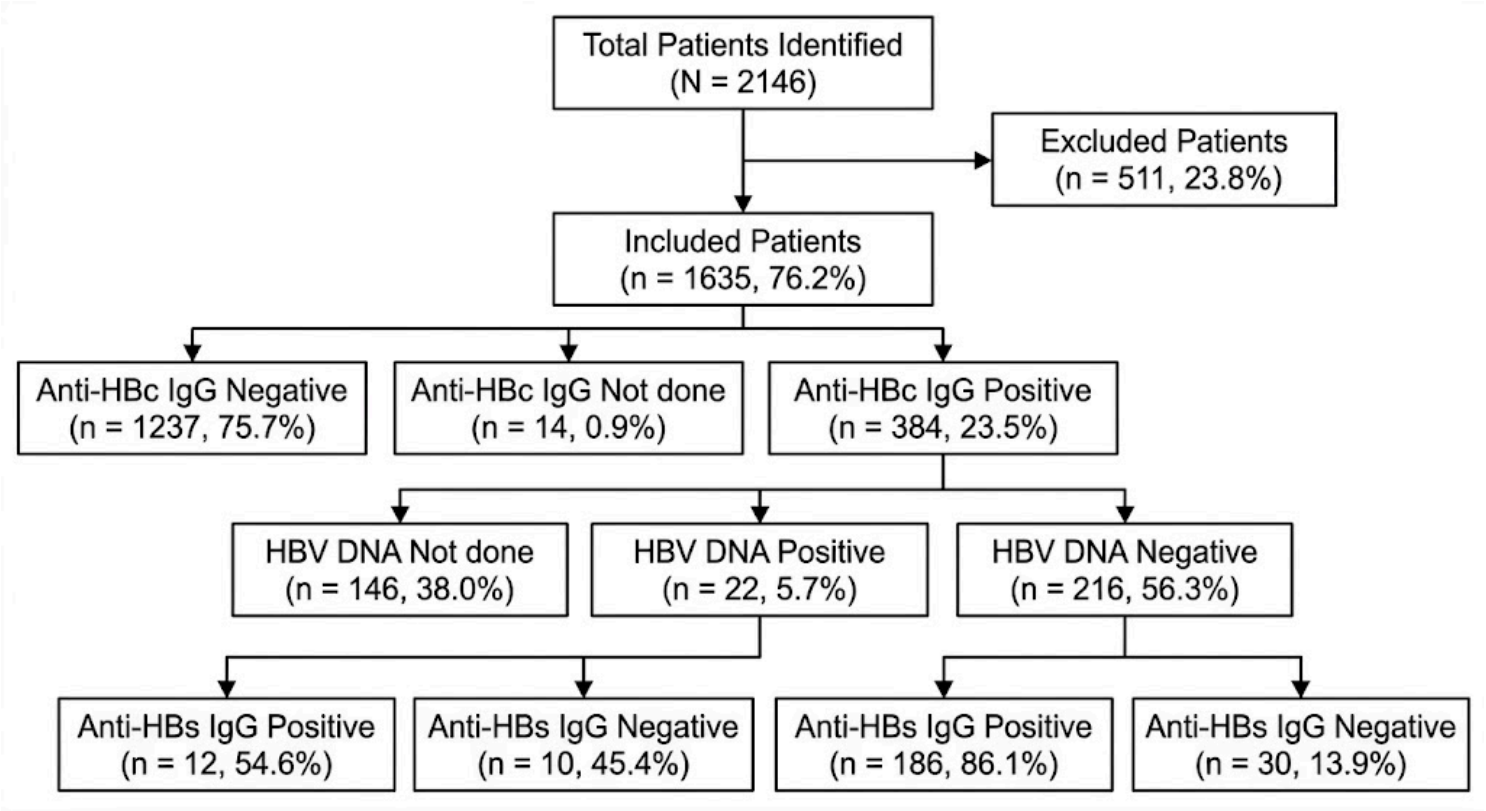


Figure 1

Flow Diagram of the Study Population

Note: Flow diagram of the study population. Of 2,146 patients identified, 511 (23.8%) were excluded, yielding 1,635 (76.2%) included patients. Patients were then stratified by anti-HBc IgG status: negative (n=1,237, 75,7%), not done (n=14, 0.9%), and positive (n=384, 23.5%). Among the anti-HBc IgG-positive patients, HBV DNA testing was categorised as not done (n=146, 38.0%), positive (n=22, 5.7%), and negative (n=216, 56.3%), and these subgroups were further stratified by anti-HBs IgG status. Percentages for HBV DNA and anti-HBs IgG subgroups are expressed as proportions (%) of their respective parent group. *Abbreviations:* Anti-HBc IgG, hepatitis B core antibody immunoglobulin G; anti-HBs IgG, hepatitis B surface antibody immunoglobulin G, HBV DNA, hepatitis B virus deoxyribonucleic acid.

Supplementary Files

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- SupplementaryTable1.docx