

Insight on the hepatitis B virus and host immune mechanisms in the context of occult hepatitis

Manal El Said^{1,2}, Asim M. Alshanberi³, Faten Abouelmagd^{2,4}, Mohammed Shaikhomer⁵, and Fatma E. Hassan^{6,7}

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¹Department of Microbiology and Infection Prevention & Control Unit, Theodor Bilharz Research Institute, Giza 12411, Egypt.

²General Medicine Practice Program, Department of Microbiology, Batterjee Medical College, Jeddah 21442, Saudi Arabia.

³Department of Community Medicine & Pilgrims Health Care, Umm Alqura University, Makkah, Saudi Arabia.

⁴Department of Medical Parasitology, Faculty of Medicine, Sohag University, Sohag, Egypt.

⁵Department of Internal Medicine, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.

⁶General Medicine Practice Program, Department of Physiology, Batterjee Medical College, Jeddah 21442, Saudi Arabia.

⁷Department of Medical Physiology, Kasr Alainy, Faculty of Medicine, Cairo University, Giza11562, Egypt.

Corresponding author: Manal El Said, Department of Microbiology and Infection Prevention & Control Unit, Theodor Bilharz Research Institute, Giza 12411, Egypt, and General Medicine Practice Program, Department of Microbiology, Batterjee Medical College, Jeddah 21442, Saudi Arabia.
Email: microbiology1.jed@bmc.edu.sa

Abstract

Hepatitis B virus infection is the 10th leading cause of death around the world. Occult HBV infection (OBI) represents those with a viral load of < 10⁴ IU/ml. Among permanent blood recipients, OBI appears to be the most common cause of posttransfusion hepatitis. Moreover, OBI may reduce hepatitis C virus (HCV) treatment responsiveness in people with chronic HCV infection, and OBI may become acutely reactivated when on immunosuppression or potentially contribute to chronic liver disease. Since most previous studies used either small sample sizes, diverse populations, or were cross-sectional in design, it is possible that using multiple techniques with varying sensitivity for HBV DNA measurement in the liver or serum will shed light on discrepancies in the impact of OBI in cases with chronic liver disease. The purpose of this research is to review many elements of OBI, such as its prevalence, the hepatitis B virus and host immune mechanisms, diagnostic procedures, clinical implications, therapy, and prevention strategies.

Keywords: Hepatitis B virus, occult, classification, immune mechanisms, clinical impact, diagnosis.

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Introduction

Hepatitis B virus (HBV) infection is a critical international epidemic. HBV infection affects 260 million people globally¹ and is the tenth

leading cause of mortality, with 890,000 people dying each year from its complications.^{2,3}

HBV infection can cause a variety of symptoms, including inactive carrier status, fulminate hepatitis, cirrhosis, and hepatocellular

cancer⁴. Serological indications of previous or current HBV infection are present in around one-third of the world's population.⁵ The recognition of hepatitis B surface antigen (HBsAg) in serum is frequently used to diagnosis chronic HBV infection.

In contrast, highly sensitive molecular techniques have indicated that HBV genome persistence is feasible even in HBsAg-negative patients who show no serological signs of past infection, such as antibodies to HBsAg (anti-HBs) and/or HB-core antigen (anti-HBc).⁶

This sort of persistent infection is referred to as "occult HBV infection" (OBI). The viral load in OBI is often less than 10^4 IU/ml. OBI has been recognized since the early 1980s, when detection of HBVDNA became possible, but it has only recently been properly researched as HBV DNA and HBsAg assays become more sensitive and specific.^{7,8}

HBV transmission by HBsAg-negative blood components has been confirmed, and HBV transmission remains the most frequent virus spread by transfusions.⁹ OBI appears to be the most common reason of posttransfusion hepatitis among permanent blood recipients, such as those with hemophilia, thalassemia, and those on hemodialysis. Because OBI is undetectable by standard HBV tests, it has the potential to cause substantial complications for blood transfusion systems.^{10,11}

OBI has clinical significance in a variety of therapeutic settings. In individuals with concurrent causes of liver damage, the existence of OBI may increase the risk of developing hepatocellular carcinoma (HCC) and speed the progression of liver fibrosis.¹²

Furthermore, OBI may reduce the responsiveness to hepatitis C virus (HCV) management in people with HCV infection.¹³ In addition, OBI may become acutely reactivated when an immunosuppressive state arises. Finally, OBI can be spread by blood transfusions, hemodialysis, and organ donation, resulting in classic hepatitis B.^{14,6}

As a result, OBI as a reason of chronic liver disease in HBsAg-negative individuals is gaining importance. Despite the possibility that OBI has a substantial role in the development or progression of chronic liver disease.¹⁵ More

research on the efficacy of OBI in chronic liver disease patients is needed because HBV DNA detection requires a liver biopsy, which is not regularly performed in clinical settings on most of these patients.¹⁶

In addition, since most prior studies either had small sample sizes, heterogeneous populations, or were cross-sectional in nature, using multiple methodologies with varying sensitivity for HBV DNA determination in the liver or serum may clarify inconsistencies in the impact of OBI in patients with chronic liver disease.^{17,18,19.}

The goal of the present review is to emphasize many elements of OBI, such as its prevalence, the HBV and host immune mechanisms, diagnostic procedures, clinical implications, therapy, and prevention strategies.

Methods

Electronic databases including PubMed/Midline, Web of Science, and Google Scholar, were searched for case reports and series, case-control, cohort, and cross-sectional studies, as well as reviews from the inception of the database to April 2023. The inclusion criteria included studies written in English and relevant to our objectives, without any restrictions regarding time, population category, and/or detection assay.

The search was performed by using terms related to "Occult hepatitis B" OR "OHB" OR "Occult hepatitis B infection" OR "OBI" OR "Hepatitis B" AND "Immune mechanisms" OR "Immunity". The OBI high-risk groups for instance HIV-positive patients, patients with chronic liver disease, patients on hemodialysis, patients with hematological disorders, patients with malignancies, and organ recipients were considered.

HBV structure and replication

HBV is a small DNA virus that belongs to the Hepadnaviridae viral family. HBV is classified into 8 genotypes, which range from A to H. Every genotype has its own geographical location.^{20,21} A 42-nm-diameter infectious HBV virion is comprised of a lipid envelope

containing HBsAg and an inner nucleocapsid containing hepatitis B core antigen (HBcAg) complexed with virally encoded polymerase and the viral DNA genome. Four partially overlapping open reading frames (ORFs) make up the HBV genome, which is 3,200 nucleotides long and predominantly double-stranded relaxed circular DNA.²²

These ORFs are designated as pre-S/S, pre-C/C, P, and X. The HbsAg-corresponding viral surface proteins preS1, preS2, and S are all encoded by the Pre-S/S ORF. Both the core antigen (HBcAg) and the soluble "e" antigen (HBeAg) are encoded by the PreC/C ORF (HBeAg).²² A viral polymerase, consisting of DNA polymerase, reverse transcriptase, and ribonuclease H, is encoded by the P ORF. For the virus to replicate, the X ORF must encode the regulatory X protein. This protein can transactivate the expression of several genes in both cells and viruses.²²

Many of the noteworthy features of the HBV replication cycle (Figure 1) are briefly discussed here. Following viral attachment to cell surface receptors, the following sequence of events

occurs: the HBV genome is released into the nucleus, where it is transformed into covalently closed circular DNA, the viral core nucleocapsid is transferred to the nuclear membrane, and the virus then exits the nucleus as covalently closed circular DNA (cccDNA).²³ In the cytoplasm, host RNA polymerase II uses HBV transcripts as templates to produce the viral envelope, core, "e," polymerase, and X proteins (IV). Step VI involves the viral reverse transcriptase synthesizing new viral DNA from pregenomic RNA (pgRNA) contained inside nucleocapsids; step VII involves a small amount of nucleocapsids being recycled into the nucleus to maintain a stable cccDNA reservoir; and step VIII involves viral replication. While in the endoplasmic reticulum, most nucleocapsids gain viral surface proteins that they will need during viral maturation and release.²⁴

HBV DNA can integrate into the host hepatic cells' genome; however, this process is unrelated to the HBV replication cycle and only includes parts of the viral genome. Even if an infected person is HBsAg-negative, integrated HBV may stay in their liver cells indefinitely.²⁵

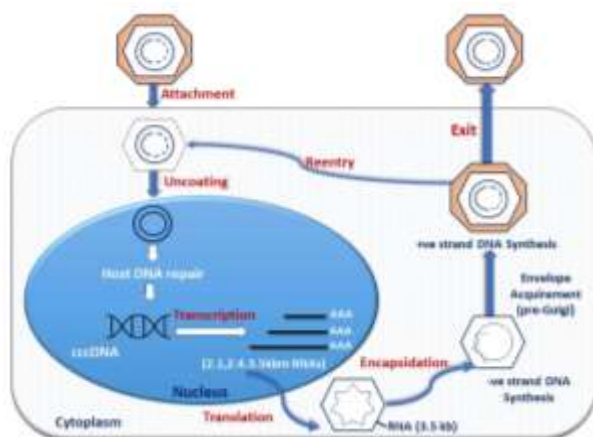


Figure 1. The replication cycle of Hepatitis B virus.

Definitions and classification of OBI

Many different authors have proposed different definitions of OBI. In a study by Bremer et al., 2009,²⁶ the phrase "occult hepatitis B virus infection" was initially applied to characterize a state in which replication-competent HBV DNA is present in the liver but no detectable HBsAg is identified in the blood. This is common since HBsAg levels decline over time after infection,

but some people will always be "low-level carriers".²³

Allain, 2024²⁷ first recognized that HBV DNA might be present outside of the acute phase window even in the absence of HBsAg and/or HBV antibodies. The presence of low amounts of HBV DNA in the serum (200 IU/mL), lymphatic (immune) cells, and/or hepatic tissue, along with the presence of anti-HBc and/or anti-HBs antibodies, defined patients as having "OBI"

at the 2008 Taormina Consensus Conference.^{28,29,30}

OBI is divided into two categories seropositive and seronegative. Seropositive people have anti-HBc with or without anti-HBs, whereas seronegative ones do not.³¹ Even though anti-HBc is positive in most cases, 30% to 50% of OBI patients are negative for all HBV blood indicators except HBV DNA.³²

Therefore, cases with serum HBV DNA levels comparable to those usually detected in the different phases of serologically evident (overt) HBV infection have to be considered as "false OBI" and are usually due to infection by HBV variants.³³ Hollinger & Sood, 2010³⁴ laid the groundwork for this idea, which necessitates the presence of infectious viral clones. The authors argued that the term "OHB" should be used instead of "occult hepatitis B infection" (OBI) since the presence of HBV DNA does not necessarily reflect infectivity or the number of HBV progeny viruses released from hepatocytes.^{35,36}

It is not appropriate to consider an occult infection when integrated viral DNA is present in HBsAg-negative people because this condition is primarily related to the intrahepatic long-term persistence of entire viral genomes as free episomal forms and the persistence of viral cccDNA in infected cells' nuclei.²⁵

Prevalence of OBI

OBI is anticipated to be widely used around the world. The presence of a particular risk for HBV infection, sampling process, test sensitivity, and geographical location are all elements that influence the observed prevalence rate of OBI.³⁷

Independent of geography, high OBI prevalence rates are noticed between hepatitis C patients (1% -20%),^{38,39} and human immunodeficiency virus (HIV) (10- 40%) because the transmission form is comparable, and HCC (>40%). The high frequency of OBI among these patients could be owed to performing more frequent and periodic investigations.

According to HBV serological profiling, the estimated incidence of OBI amongst anti-HBc-positive individuals is 4% - 25%.³⁸ OBI was shown to be more prevalent in African and

Asian countries, with reported frequency ranging from 1% to >50%.^{40,41}

With HBV endemicity, anti-HBc and OBI prevalence vary. In areas with low endemicity, 10-20% of those with HBV markers also have anti-HBc.⁴² High endemic sub-Saharan and Asian regions have anti-HBc prevalence rates of up to 50% of the general population.⁴³

HBV DNA and HBV seromarkers correlate positively in patients with OBI. The lowest HBV DNA levels were found in subjects with seronegative or negative for all seromarkers. Intermediate HBV DNA levels were found in subjects with anti-HBc negative and anti-HBs positive. While the highest HBV DNA levels were found in those with anti-HBc positive but anti-HBs negative or anti-HBc only, who are more likely to be infectious.⁴³ Such findings suggest that anti-HBc does not result in complete HBV exclusion.⁴⁴

HBV is highly prevalent in most African countries, such as Ethiopia which is a high endemic setting for HBV infection according to the World Health Organization (WHO) criteria ($\geq 8.0\%$).⁴⁵ Thus, this observed seroprevalence of HBsAg is comparable with previous reports investigating the national pooled prevalence (7.4%) and subgroup meta-analysis prevalence (8.0%) in community-based studies in Ethiopia (2016).⁴⁶ Even though the data on OBI prevalence are limited, there are studies conducted in Ethiopia disclosed that the prevalence in the Easter region was 6.4% (2020),⁴⁷ in the Northern region 19.1% amid HIV-positive individuals (2020),⁴⁸ and 20.3% among antenatal care women (2020).⁴⁹

Other East African countries revealed different HBV prevalences. The HBV prevalence was 2.1% in Kenya (2016),⁵⁰ 6.1% in the whole African region, and 3.5% worldwide among the general population (2021),⁵¹ 10% in Ivory Coast (2010),⁵² 16.48% in Sudan, among blood donors (2014),⁵³ 18.1% in Egypt among HCV patients (2017),⁵⁴ 18.7% in Kenya (2020),⁵⁵ 8% in Nigeria (2014),⁵⁶ and 6.9% in Cameroon (2016).⁵⁷

Little information on the prevalence of OBI in North America and Oceania has lately been published, emphasizing the need for more epidemiological research in these regions. Because of the scarcity of studies in healthy

populations and the lack of a standard diagnostic test, assessing the true prevalence of OBI is difficult.⁶

Hepatitis B Virus and Host Immune Mechanisms of OBI

Low amounts of HBV DNA can survive in the absence of detectable HBsAg for unknown

reasons, however both host and viral mechanisms are thought to have a role in restricting viral replication and keeping the infection under control.³⁴ (Table 1)

Table 1. Hepatitis B Virus and Host Immune Mechanisms of Occult HBV Infection (OBI).

Mechanisms	
1. Sequence variation in HBV genomes	Mutations in HBsAg "a" determinant Treatment-associated mutations RNA splicing - Pre-S mutants
2. Coinfection	Coinfection with HCV or HIV
3. APOBECs	Deamination-dependent - Deamination-independent
4. Host immune responses	T cell, VDR gene, Immunosuppression - Apoptosis
5. Epigenetic changes	Methylation - Acetylation
6. Genetic integration	Disruption and Rearrangement - Reduced HBV proteins and replication
7. Immune Complexes	- Lack of detectable HBs antigen

APOBECs: Apolipoprotein B mRNA- Editing Enzyme Catalytic Polypeptide

Sequence variations in HBV genomes

Mutations in the pre-S region, splicing, and a determinant of HBsAg have all been related to latent HBV infection, as have treatment-associated mutations.

- Mutations in the "a" determinant of HBsAg

One of the initially identified mechanisms leading to OBI was a mutation in the surface antigen's "a" determinant. HBsAg's "a" determinant is a two-loop structure. It has a lot of cysteine residues, which help create disulfide bonds and keep this region's shape.⁵⁸ Since HBsAg mutations cause conformational changes, some commercially available HBsAg assays are unable to identify protein.⁵⁹ Both unvaccinated and vaccinated people can be infected by these mutations.⁶⁰

- Treatment-associated mutations

A novel mutation was found in the "C" domain of the HBV polymerase, which was connected to a premature stop codon in the surface gene and the formation of HBsAg substitution in lamivudine-treated people.⁶¹ Mutations in the lamivudine-associated polymerase gene have been linked to decreased anti-HBs antibody binding, implying that these mutants might circumvent detection by numerous commercially available HBsAg assays as well as immunological neutralization by vaccine-induced anti-HBs antibodies. These mutations can infect vaccinated people who are resistant to lamivudine treatment.⁶²

- RNA splicing

Studies on HBV have indicated that splicing plays a critical function in modulating gene expression. The G to A mutation at location 458

in the surface gene affected splicing of the S gene mRNA. The poor replication phenotype and the lack of HBsAg expression are associated with this mutation.⁶³

- Pre-S mutants

Deletions in the pre-S region have been connected to a lack of detectable HBsAg in the blood. Deleting the pre-S region of HBV lowers surface protein expression and boosts viral persistence by eliminating antigen recognition molecules (e.g., human leukocyte antigens) that limit the virus' access to B cells and T cells.⁶⁴

OBI is characterized by the existence of pre-S1/pre-S2 mutations. Patients with occult HBV-related chronic liver disease had mutations in their pre-S2/S promoters while having undetectable blood HBsAg levels.⁶⁵ Deletions in the pre-S1/pre-S2 domains, as well as pre-S deletion mutants, were related with reduced HBsAg secretion after interferon therapy.^{66,67} Furthermore, the deletions in the pre-S region are seen in a substantially larger proportion of patients with low HBV DNA levels than in people with higher viral burdens.⁶⁸

Coinfection with other microbes

- Coinfection with HCV

HBV and HCV coinfection reduces HBV replication and HBsAg expression in the liver.¹⁵ HCV coinfection was connected to much greater spontaneous HBsAg clearance rates than HBV infection alone, showing that the two viruses/viral proteins interact.⁶⁹ Another research, on the other hand, showed no substantial interactions between the two viruses in coinfecting hepatocytes, showing that host responses play a crucial role in viral dominance in coinfecting cells.⁷⁰

HBV promoters and enhancers are activated by the hepatitis B virus X protein (HBx).⁷¹ The core protein of HCV is a viral capsid component that inhibits HBV encapsidation by preventing core and polymerase binding to the package signal in pgRNA within the hepatocyte.⁷² Repression of HBV enhancer I activity by HCV core protein is more significant than repression of HBV enhancer II activity.⁷³ HCV NS2, a non-structural protein, is capable of autoproteolysis.⁷⁴ The HCV NS2 protein inhibits

cellular and viral promoters as well as HBsAg and HBeAg secretion, replication, and HBV infection.⁷⁵

- Coinfection with HIV

OBI are more linked to HIV positive people, and HBV DNA is discovered sometimes in HIV positive people, necessitating many HBV DNA samples in this patient population.⁷⁶ HIV is expected to interfere with the natural progression of HBV infection by boosting HBV replication and HBV DNA levels while lowering HBeAg seroconversion.⁷⁷

- Coinfection with *Schistosoma mansoni*

HBV and schistosome co-infection are common in places in which both pathogens are endemic. HBV replication has been demonstrated to be significantly suppressed by *S. mansoni* infection.⁷⁸

Apolipoprotein B mRNA- Editing Enzyme Catalytic Polypeptide (APOBECs)

Cytidine deamination is a physiological function of APOBEC.⁷⁹ APOBEC3G expression in HBV-replicating cells decreases HBV DNA levels by 50 times. The APOBEC protein's deamination-dependent function in the HBV genome converts cytosine to uracil, resulting in OBI-related mutations. OBI is induced by APOBEC deaminases' deamination-independent action, which reduces DNA-RNA hybrid formation, increases sensitivity to nuclease digestion, and diminishes protein processing.⁸⁰

Host immune responses

The virus-host interactions essentially affect the outcome of HBV infection. Infection with HBV activates host immunological responses that help in viral clearance, viral survival, and immunopathogenesis. The host immune system's regulation of HBV replication and biogenesis has been associated with apoptosis, cytolytic and noncytolytic T-cell responses, and vitamin D receptor (VDR) polymorphisms (Figure 2).^{72,75}

The Fas (a death receptor) expression system regulates infected hepatocyte apoptosis and is essential for the clearance of old hepatocytes and the preservation of liver homeostasis.⁸¹ OBI has lower levels of soluble Fas (sFas) than

chronic HBV infection.⁸² Lower sFas levels in OBI indicate reduced apoptotic inhibition, which may be one of the reasons for HBsAg clearance and HBV replication suppression in OBI. Reduced expression of CXCL12, a chemokine that regulates apoptosis, has been reported to play a role in OBI.⁷⁹

T-cell responses and protected memory⁷⁹ are present in patients with OBI who test positive for anti-HBc but absent in those who test negative for anti-HBc. OBI has been postulated to arise via a non-cytolytic HBsAg-specific T-cell response, even in the face of very low and undetectable HBsAg levels. And the serum HBV DNA level correlates with the potency of the cytotoxic-T lymphocyte response. After

recovering from acute HBV infection, the cytotoxic-T lymphocyte response, which is essential for maintaining active viral replication control, may be sustained for years by the presence of trace amounts of the virus. It indicates that both the humoral and cellular immune responses have a role in OBI progression.⁷²

Involvement of vitamin D3 and its vitamin D receptor in regulating several cytokines makes them crucial determinants of the anti-HBV response. The outcome of persistent HBV infection has been linked to polymorphisms in the vitamin D receptor gene, which have been identified in OBI.⁷⁵

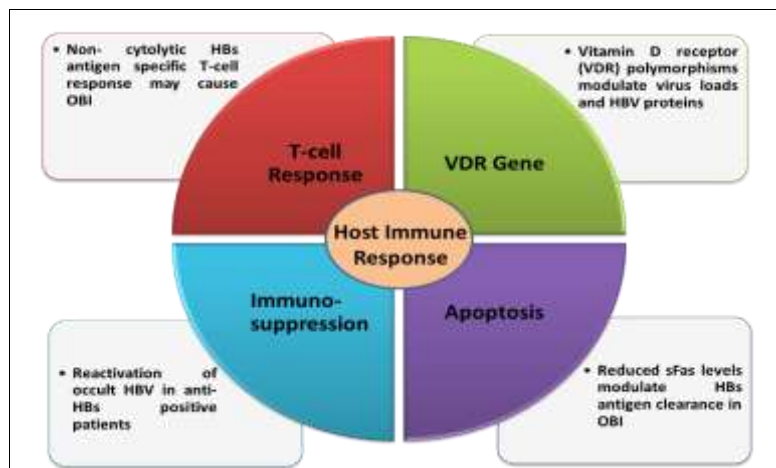


Figure 2. Host immune responses in Occult HBV Infection (OBI).

Epigenetic changes

Inhibiting HBV protein replication, and virion formation,⁸³ methylation of HBV DNA is a novel epigenetic mechanism that causes occult HBV infection. Histone acetylation controls HBV replication and transcription. In addition, elevated HBV replication is linked to hyperacetylation of histones that bind to cccDNA.⁸⁴

Genome integration

HBV DNA sequence integration into the host genome is typical in persons with chronic HBV infection, and it has been linked to the development of HCC.⁸⁵ Gene disruption and rearrangement during gene integration can result in a) a loss of HBsAg in the blood, b)

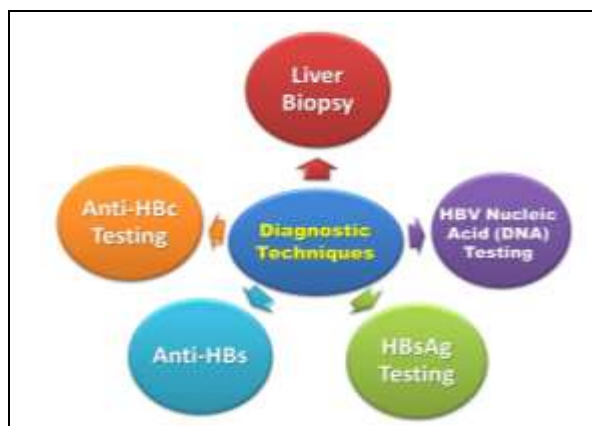
decreased virion production, and c) a lack of detectable HBV DNA in serum.⁶

During HBV DNA integration, the HBV core gene is frequently lost, resulting in HBV core protein decrease or loss. Poor viral assembly and hepatocyte accumulation of unencapsulated HBV DNA have both been linked to the lack of the HBV core protein. This may provide a possible explanation for the paradoxical finding that patients with HBV-related HCC have HBV DNA in their livers but none in their blood.⁷²

Immune Complexes

Entrapment of HBsAg in immunological complexes with anti-HBs antibodies can impede traditional serological assays. The presence or absence of HBsAg-containing immune

complexes is related to the formation of detectable anti-HBs. Asymptomatic HBsAg carriers, acute HBV infection, and chronic HBV infection have all been demonstrated to have circulating HBsAg-containing immune complexes. In the presence of anti-HBs antibodies, immune complexes containing HBsAg have also been found.^{72,85}



OBI diagnostic techniques

HBV DNA extracts from liver and blood samples are typically used to diagnose OBI. Because extracted liver samples are only available in a small percentage of cases, OBI is primarily diagnosed using serum samples (Figure 3).⁸⁶

Figure 3. Occult HBV Infection (OBI) diagnostic techniques.

Liver biopsy

The best technique for detecting OBI is to test for HBV DNA in a liver biopsy.¹⁶ Moreover, liver biopsy tissue is not always obtainable, and no USA Food and Drug Administration (FDA)-approved standardized and reliable procedures for detecting HBV DNA in liver tissue are available.⁸⁷

HBsAg testing

The "a" determinant (amino acids 99-160 of the HBsAg), which is encoded by the envelope (S) gene, is the primary target for diagnostic antibodies. The presence of mutations in this region may result in a failed diagnosis. To screen for HBsAg, enzyme immunoassays such as enzyme-linked immunosorbent tests and chemiluminescence immunoassays are utilized. These tests have a sensitivity range from 0.1 to 0.62 ng of HBsAg per ml. The introduction of OBI mutations in reagent manufacture would improve commercial analysis performance.^{29,88}

Anti-HBc testing

The first detected circulating antibody against HBV, anti-HBc Ab, is widely established and has the highest titer among antibodies. As a result,

antibody detection is a useful confirmatory method that can be employed in conjunction with HBsAg 89. Approximately, 90% of anti-HBc blood donors also carried anti-HBs signals, suggesting that they have recovered from HBV infection, according to Candotti & Allain, 2009.⁹⁰ Due to low test specificity and a lack of confirmatory assays, the remaining 10% are either anti-HBc alone or false-positive anti-HBc. Only samples with detectable anti-HBc from either cured infection with anti-HBs or advanced chronic infections with HBsAg can be utilized for testing. Relatively recent research has shown that OBI can be detected in anti-HBc-only samples. OBI develops in anti-HBc-positive patients with persistent HBV infection when HBsAg levels fall to undetectable levels and is sometimes accompanied by the emergence of anti-HBs.²⁹

HBV nucleic acid (DNA) testing

HBV DNA amplification is one of the most accurate approaches for diagnosing OBI.⁹¹ The real-time, nested polymerase chain reaction (PCR) is the current gold standard method for diagnosing HBV DNA extracts from plasma.^{92, 93} It depends on the use of PCR primers that span at least three genomic sections of the HBV

genome, such as the S, X, and core genes, and validate from at least two genomic regions to reduce the risk of obtaining erroneous findings.⁹³

The test should be repeated several times to increase the chances of detecting a small number of template sequences. Blood screening for HBV nucleic acid testing (NAT) is now possible because NAT now detects HIV RNA, HCV RNA, and HBV DNA simultaneously ("multiplex" NAT assays).⁹⁴ With a potency of 106 IU/mL (each vial contained 5×10^5 IU per vial/0.5 ml), the WHO International Standard for HBV DNA (NAT)-based testing was developed.²⁹ In OBIs, HBV DNA levels in plasma are frequently low (<200 IU/mL).⁹⁵ OBI detection requires tests with the highest sensitivity and

specificity, with HBV DNA detection limits of <12 IU/mL and HBsAg detection limits of < 0.1 ng/mL.^{96,97}

Role of Anti- HBs

OBI carriers with no detectable HBsAg antibodies are assumed to be infectious.²⁹ Anti-HBs from spontaneous infection, immunization, or passive immuno-prophylaxis protects transplanted patients with anti-HBc livers from de novo HBV infection.⁹⁸

Clinical impact of OBI

OBI may have an impact in several different clinical situations. Extensive studies have evaluated the risk of acquiring OBI in several clinical entities (Table 2) including the following:

Table 2. Clinical impact of Occult HBV Infection (OBI).

Clinical impact of OBI	References
1. Blood donors	7, 9, 14, 29, 83, 99, 100, 101
2. Chronic liver diseases	29, 33, 103-107
3. Fulminant hepatic failure	34, 60
4. Hepatocellular carcinoma	12, 108- 115
5. Hematological malignancy	4, 59, 116-122
6. Hemodialysis	30, 83, 123
7. Organ transplantation	14, 34, 119, 124-128
8. HIV-infected patients	129-133
9. Cryptogenic cirrhosis	134-137

OBI in blood donors

In HBV-naïve patients, OBI can be transmitted through blood transfusion.⁹ Although post-transfusion HBV infection still develops. It has been reported that the use of HBsAg screening in blood donors has considerably reduced it. A recent comparable study found that when only HBsAg was used for screening, a considerable number of HBV-infected donors were unobserved.⁷

OBI in blood donors can have a range of reasons depending on the infection's natural history. It could be due to the outcome of earlier infections that resulted in the production of anti-HBs antibodies. However, it could also be due to persistent, low-level viral replication

and/or escape mutations that are undetected by HBsAg testing, or it might be due to healthy chronic carriage. The latter state is the most typically generated by anti-HBc. Over time, antibody markers may become undetectable, leaving HBV DNA as the major indicator of infection.²⁹

However, there is some evidence that HBsAg carriage and post-transfusion hepatitis are not generated by OBI transfusion in HBV-hyperendemic locations.⁸³ Since in endemic areas most recipients already had HBV, the risk of contracting the virus through a blood transfusion was likely reduced there.¹⁴ Immunization against HBV may reduce the prevalence of overt HBV infection, but it may also increase OBI.⁹⁹

Patients with impaired immune systems should exercise caution when accepting blood donations from those who test positive for both anti-HBc and anti-HBs.¹⁰⁰ Donors with HBV infection will be screened out using anti-HBc tests, which will help reduce HBV transmission and its potential consequences, especially in the immunocompromised population.¹⁰¹

HBV NAT must be performed with extreme sensitivity or on individual donors to eliminate HBV DNA-containing units. HBV residual risk can be minimized by the development of more sensitive HBsAg tests, the application of anti-HBc screening when appropriate, and the implementation of HBV NAT using mini pools or more effectively in individual samples.¹⁰² However, there are two big limitations to this method. Firstly, it does not consider infections that are not detectable by a viral antigen or antibody marker during the seronegative window period. Furthermore, in regions of the world where the frequency of anti-HBc is higher than 10%, it would be impractical since too many otherwise healthy donors would be ruled out.²⁹

OBI and chronic liver diseases

Multiple studies have found that HCV-coinfected individuals had lower levels of HBV replicative intermediates.⁷³ According to a study by Cacciola et al., 1999 nearly a third of those with chronic HBV liver disease had detectable HBV DNA but no HbsAg.¹⁰³ They found that traumatic brain injury increased the risk of cirrhosis among HCV carriers. In patients with chronic HCV infection, OBI has been associated with an increased risk of HCC.¹⁰⁴

OBI was more prevalent among HCV genotype 1b patients than among those infected with HCV genotype 2a.¹⁰⁵ Elevated liver enzymes in HCV-infected patients have been related with higher HBV DNA levels with OBI, pointing to a possible mechanism for liver damage.¹⁰⁶

Long-term viral persistence in the liver has already been linked to a mild but persistent necro-inflammation that when combined with other causes of liver disease, can eventually lead to the progress of cirrhosis.²⁹ A rise in alanine transaminase (ALT) levels is a common

symptom of HCV infection, and it is believed that HBV replication is a common cause.³³ Also, it is known that OBI accelerates the advance of cirrhosis, hepatic decompensation, and HCC in individuals with chronic HCV and that it reduces their response to interferon treatment.²⁹

However, some researchers have found no OBI-related clinical consequences in individuals with HCV-related liver disorders. The occurrence of OBI was unrelated to the extent to which the liver was affected by HCV.^{73,107} Furthermore, in individuals with and without OBI, therapeutic response to combination therapy (interferon and ribavirin) against HCV infection was comparable.

OBI and fulminant hepatic failure (FHF)

Hepatitis B typically has a severe and occasionally fatal prognosis,⁶⁰ although this drop in viral replication and gene expression may be lost in an immunosuppressive situation. Between 30% and 35% of patients with apparent non-A, non-B (NANB) fulminant hepatic failure (FHF) in three North American trials, included a total of 36 individuals, had HBV DNA detected in the liver but not in the serum of NANB-FHF patients. Contrarily, in liver tissue from 45 cases with NANB-FHF in the United Kingdom, no HBV DNA was identified, and in French patients, the percentage was less than 5%. These differences are likely because of the sensitivity and specificity of the assays used to classify patients,³⁴ as well as the difference in study populations.

OBI and Hepatocellular Carcinoma

Since OBI keeps the pro-oncogenic features of overt infection, it is considered a major risk factor for the development of HCC. HBV facilitates the development of HCC through one or more of the immunopathogenic pathways. Firstly, in OBI patients, inflammation can lead to repeated cycles of cell death and regeneration, promoting fibrosis, cirrhosis, and the formation of a mutagenic environment.¹² Second, the HBV genome could integrate into host DNA and alter genes that code for proteins essential in cell signaling, proliferation, and death.¹⁰⁸

HBV DNA integration may also increase chromosomal instability in the host, resulting in

significant inverted duplications, deletions, and chromosomal translocations.¹⁰⁹ Additional evidence suggests that HBV DNA incorporation can either activate or suppress growth-regulating genes in cells.¹¹⁰ Moreover, the expression of some HBV proteins potentially aids in the progression of cancer. For instance, during the oncogenic process, hepatitis B x antigen (HBx) binding to p53 inactivates it. HBx suppresses DNA repair, caspase-3, and anti-Fas antibody-dependent apoptosis.¹¹¹

Muroyama et al., 2022,¹¹² resulted in a change in the transcription of endoplasmic reticulum stress response genes ATF3, ATF4, and ATF6 due to fusion HBx. To emphasize, fusion HBx had the same impact on the endoplasmic reticulum stress signaling pathway as C-terminal shortened HBx but not as wild HBx. Their results suggested that fusion HBx is an important player in hepatocarcinogenesis through altering the endoplasmic reticulum stress response and could be a useful therapeutic target for HBV-induced HCC. Although OBI mono-infection still has a carcinogenic potential,¹¹³ OBI may hasten the progression of cirrhosis in HCV patients.¹¹⁴ Cirrhosis is the most significant risk factor for HCC.¹¹⁵

OBI and Hematological Malignancy

The incidence of OBI in cases undergoing chemotherapy for hematological malignancies is the most therapeutically significant element of OBI. Since the mid-1970s, chemotherapy-induced HBV reactivation has been known.^{116,117} This occurrence is particularly noteworthy because reactivation generally requires a break from chemotherapy, and it has been related to serious liver failure and deadly fulminant hepatitis.¹¹⁸

Chemotherapy-induced immunosuppression is thought to cause fast viral replication. A T-cell-mediated immune response produces inflammation and liver necrosis as the immune system is being reconstituted.⁵⁹ Although there are no universal criteria for HBV reactivation, one widely recognized criterion includes an ALT better than 3 times the upper limit of normal, as well as a ten-fold increase in HBV DNA or an absolute value larger than 20,000 IU/mL.¹¹⁹

Transmitting and reactivating OBI is crucial in the setting of organ transplantation, particularly liver transplants.¹⁴ Occult HBV infection was not connected with increased episodes of acute rejection, coinfection with hepatotropic viruses, altered responses to HBV vaccination, or the development of de novo hepatitis B in HBsAg-negative liver transplant patients.¹²⁴ However, Hollinger and Sood, 2010³⁴ found that OBI reactivation can occur in immunocompromised post-transplant patients with serological evidence of a past hepatitis B infection (anti-HBc-positive). According to two studies, liver transplants from anti-HBsAg-positive donors are safe for use in HBsAg-positive or anti-HBc/anti-HBs-positive recipients.¹²⁵

OBI and Hemodialysis

HBV and HCV share common transmission channels, so individuals on hemodialysis are more likely to be exposed to infected blood and tools and contract both illnesses.¹²³ Through hemodialysis, OBI poses a risk of HBV transmission.⁸³ It has been hypothesized that status of chronic uremia in hemodialysis patients may suppress the inflammatory reactions in the liver and consequently, no hepatocyte destruction will occur. Therefore, the evaluation of quantitative HBV DNA was found to be the most efficient method to evaluate OBI in HD patients.³⁷

OBI and organ transplantation

Transmitting and reactivating OBI is crucial in organ transplantation, particularly liver transplantation.¹⁴ A study by Ghisetti et al., 2004, reported that in HBsAg-negative liver transplant patients, concealed HBV infection was not linked with additional episodes of acute rejection, coinfection with hepatotropic viruses, altered responses to HBV vaccination, or the development of de novo hepatitis B.¹²⁴ However, Hollinger and Sood, 2010,³⁴ postulated that OBI reactivation may occur in immunocompromised post-transplant patients with serological evidence of past hepatitis B infection (anti-HBc-positive). Liver transplants from anti-HBsAg-positive donors are safe for use in HBsAg-positive or anti-HBc/anti-HBs-

positive recipients, according to two investigations.¹²⁵

OBI transmission is rare after kidney, heart, or bone marrow transplants.²⁹ This was proved from transplanting hearts from anti-HBc-positive donors. Heart transplant recipients from HBcAb-positive donors have little serological data. Occult HBV infection in HBcAb-positive donors is rare but possible. Some recipients had HBsAb and HBcAb changes, indicating acute hepatitis B, although the reason is uncertain. After immunization or infection, HBsAb titers or pharmacological therapies can avoid infection.¹²⁶

Bone marrow transplant recipients seldom develop HBV infection, but in rare cases, the latent virus can become active and cause severe liver damage or even death.^{119,127} Infected hematological malignancies protracted immune reconstitution, and immunosuppressive therapy all contribute to the increased morbidity of HBV reactivation during allogeneic hematopoietic stem cell. Immunosuppression results in the reactivation of HBV replication from cccDNA residing in hepatocytes.

OBI in HIV-infected patients

HIV patients should be screened for HBV serological markers as well as HBV DNA tests to detect OBI.¹²⁹ The prevalence of OBI in HIV-positive patients varies significantly by geographical region, ranging from 0% to 89.5%.¹³⁰ It is also highly common among those who have HIV and HCV co-infection. OBI is most common in patients who only have an anti-HBc antibody as an HBV serological marker. However, in some people, no anti-HBc or anti-HBs antibodies were found.¹³¹ HIV-positive people who are immunocompromised may not respond to the recombinant HBV vaccine or have a low immune reaction to HBsAg.¹³² HBV DNA should be tested prior to initiating high-dose antiretroviral therapy in HIV patients who screen negative for HBsAg so that anti-HBV antiretrovirals can be added if necessary.¹³³

OBI and Cryptogenic Cirrhosis

Among individuals with cryptogenic cirrhosis who have HBV or HCV serologic indicators, HBV DNA was noticed in the serum or liver.¹³⁴ The

prevalence of OBI in cryptogenic cirrhosis varies widely between 4.8% and 40%,¹³⁵ depending on the occurrence of HBV in the research area and the kind of material studied. No connection was found between the presence of OBI and demographic, biochemical, or serologic factors in people with cryptogenic cirrhosis, suggesting that these criteria are not useful for differentiating OBI-positive from OBI-negative patients.¹³⁶ A study observed that OBI prevalence tends to rise in anti-HBc positive individuals isolates.¹³⁷

OBI treatment

A recently published approach for treatment of chronic hepatitis B mentioned OBI but did not discuss therapy. The researchers reported that treatment is indicated for HBeAg-negative chronic HBV patients with HBV DNA levels >2000 IU/mL and increasing ALT (1-2 times the upper limit of normal).¹³⁸

Since HBV DNA and ALT tests are limited, the question of treating occult HBV arises. Investigating the prevalence of histologic alterations in occult HBV would be recommended. Occult HBV can be treated with oral regimens for chronic HBV, which are well tolerated.¹¹⁹

OBI preventive measures

Due to the higher risk of HBV reactivation in patients with OBI, antiviral drugs should be started before chemotherapy, especially if anti-HBs are not available, and should be continued for 6 months after immunosuppressive treatment is completed.¹³⁹ Antiviral prophylaxis prevents OBI reactivation in most transplant cases involving HBsAg-negative and anti-HBc-positive donors.¹⁴⁰ A prior hepatitis B vaccine's capacity to modify or prevent infection is also unknown.⁸³

Anti-HBV prophylaxis, independent of HBV DNA and anti-HBs status, is recommended for patients with hematological disorders and/or rituximab-containing regimens who are HBsAg-negative but anti-HBc-positive because of the considerable risk of HBV reactivation. Patients with non-hematological illnesses or rituximab-free regimens may not require anti-HBV

prophylaxis if HBV DNA is undetectable and anti-HBs are positive.¹⁴¹ The Japanese guidelines propose serial HBV DNA screening (monthly during and after therapy for at least a year).³⁰

Patients who test positive for HBsAg and receive immunosuppressive drugs should take preventative antiviral therapy as a matter of course. Prophylactic anti-viral medication is often advised in HBsAg-negative patients receiving immunosuppressive therapy/hematopoietic stem cell transplant who have detectable HBV DNA, regardless of whether they are anti-HBc/anti-HBs positive or negative. Patients with undetectable HBV DNA at baseline should be monitored at regular intervals of 1-3 months until at least 12 months after termination of immunosuppressive cure. The timing of the checks has not been established. Patients taking rituximab, for instance, should be monitored more often than those not receiving the drug.¹⁴²

Reactivation begins with HBV DNA. HBsAg assessed with a sensitive one-step immune complex transfer technology with chemiluminescence enzyme immunoassay (ICT-CLEIA) (Sysmex Corporation, Kobe, Japan) and HBcrAg assessed by lumipulse, G1200 automated analyzer were investigated for their predictive abilities in HBV reactivation in OBI.^{143,144} Both were very prognostic. Until further evidence is available, antiviral therapy should begin when HBV DNA is detected, not when ALT levels rise.¹⁴⁵

Nucleotide analogues are quite effective at preventing HBV reactivation. It has also been proposed that patients be treated prophylactically in clinical settings when routine HBV DNA testing is not practicable.¹⁴⁶ Recent prospective trials employing four weekly HBV DNA screening in HBsAg-negative, anti-HBc positive patients having rituximab or HSCT and immediate entecavir therapy once HBV DNA is discovered resulted in improved control with no hepatitis flares. Nonetheless, the cost-effectiveness of various protocols has not been well explored.^{147,148}

Summary and Conclusion

OBI refers to the occurrence of HBV DNA in the liver/serum despite the lack of detectable

HBsAg. OBI has been recognized for almost three decades, but many questions about its prevalence, pathogenesis, and clinical relevance remain unsolved. Both host and viral mechanisms are thought to have a role in restricting viral replication and keeping the infection under control. Researchers were able to determine the global dissemination, potential therapeutic implications, and various virological characteristics of OBI because of the availability of very sensitive molecular biology techniques.

However, in countries with high HBV endemicity and the expensive pricing of NAT detection tests, OBI remains a serious concern, accounting for the great majority of the remaining cases of transfusion-transmitted HBV hepatitis. When a person receives a liver transplant from a donor who tests positive for OBI, they may be exposed to HBV. The debate centers on whether OBI contributes to the progress of cirrhosis in patients with persistent hepatitis. Because OBI infection is so prevalent in HIV/HCV co-infected patients, the virological picture of this subset of people is far more nuanced than was previously assumed. There is substantial evidence that OBI is a contributor to the emergence of HCC, and it appears to retain most of the pro-oncogenic features generally associated with overt HBV infection.

Even if anti-HBc and anti-HBs tests are negative, HBV DNA nucleic acid testing should be utilized to identify OBI, particularly in endemic regions and presumed high-risk patients as well as in hemodialysis and cryptogenic chronic hepatitis.

Before beginning chemo or immunotherapy, all patients must be examined for anti-HBc antibodies at least once, and ALT levels should be monitored on a regular basis. We will only be able to destroy this dreadful opponent of human health if we expand newborn immunization to all nations, particularly those where the virus is endemic. Investigating OBI is crucial to gaining a complete knowledge of HBV infection, and it is a promising new field of viral hepatitis research. More research in larger patient cohorts with long-term clinical and laboratory follow-up is required to better understand the biological basis and implications of OBI infection.

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ME, was responsible for the main concept of the review and was contributor in data collection and analysis and revised the manuscript. FEH and FA, were contributors in writing the manuscript and revised it. AMA and MS, revised the final manuscript. All authors read and approved the final manuscript.

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ORCID iD

Manal El Said  <https://orcid.org/0000-0002-1090-1713>.

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