

Occult Hepatitis B Virus Infection: A Comprehensive Review

Osama Ebrahim Ali Elghamry¹, Sami Eissa¹, & Mohamed Refaey¹

¹Department of Tropical Medicine, Faculty of Medicine, Zagazig University, Alsharquia, Egypt.

Correspondence: osamaelgamry234@gmail.com

ABSTRACT

Occult hepatitis B (OBI) was defined as the detection of hepatitis B virus (HBV) DNA in the liver (with or without HBV DNA in serum) without HBsAg[1].

The prevalence of OBI varies from region to region worldwide. This variability relies upon the sensitivity of HBV DNA detection assays, the sample size, and the detection of HBV DNA in liver tissue and serum by nested PCR or real-time PCR. The prevalence of OBI varies from 1% to 87% in different regions of the world[2], there is no standard assay for diagnosis of OBI in liver tissue or in serum, and the only reliable method is the detection of HBV DNA by nested PCR or real-time PCR[3].

The mutations in the HBsAg gene have been observed among patients coinfecting with hepatitis C virus (HCV)[4].

It has been described that about one-third of patients with chronic HCV infection had detectable serum HBV DNA but undetectable HBsAg[5]. When the coexistence of both HBV and HCV genomes occurs in the same hepatocyte, the replication of HBV is inhibited due to the interference of HCV molecules, which therefore results in the creation of OBI with low replication of HBV DNA[6].

The presence of OBI in chronic HCV infected patients increases the risk of HCC[7]. Blood transfusion is a main risk factor for transmission of OBI and the prevalence of OBI among blood donors varies from country to country provided that the screening of blood donors is done with less security[8].

1. Occult Hepatitis B Virus Infection

1.1. Definition: OBI was defined as the presence of HBV DNA in the liver (with or without HBV DNA in serum) without HBs Ag as determined by using the currently available assays. Serum HBV DNA can be either detectable or undetectable, and when detectable, the level of HBV DNA is usually very low (< 200 IU/mL) [9].

OBI has also been defined as a serological condition characterized by the presence of isolated hepatitis B core antigen (anti-HBc) in the absence of HBsAg and anti-HBs antibody[10].

Detection of anti-HBc antibody, a surrogate marker of OBI, is useful when an HBV DNA test is not available or when intermittent viremia is suspected[9].

Serum HBV DNA can be either detectable or undetectable, and when detectable, the level of HBV DNA is usually very low (< 200 IU/MI[11].

1.2. Classification

OBI can be classified into 2 groups,

- I. **seropositive OBI** [anti-HBc and/or anti-hepatitis B surface (antiHBs) positive], Seropositive-OBI develops when serum test results for HBsAg become negative after acute hepatitis or when HBsAg is cleared during the course of chronic hepatitis B.
- II. **seronegative OBI** (anti-HBc and antiHBs negative), on the basis of the HBV antibody profile[12].

In fact, annual HBsAg seroclearance rates are reported to be 0.50%-2.26% per year in chronic hepatitis B patients, and persistent HBV DNA in the liver was detected in some of these patients [13].

Seronegative-OBI is caused by primary occult of anti-HBs or anti-HBc from the beginning of the infection because of the mutation or due to progressive loss of anti-HBs [14].

Most OBIs are seropositive OBIs, but > 20% of patients with OBI are seronegative for OBI, representing a population negative for all serum markers of HBV infection [15].

1.3. Epidemiology

The prevalence of OBI is reported to range from 1% to 95% worldwide. These prevalence rates are influenced by several factors as follows: (1) geographic differences (endemicity), (2) different patient characteristics, including the presence of comorbid diseases such as chronic hepatitis C; and (3) and the different diagnostic techniques used, which have different sensitivity[16].

The prevalence of OBI differs according to the endemicity of HBV infection. OBI was reported at a higher rate in an HBV endemic area such as East Asia where 41%-90% of the population had prior exposure to HBV, and less frequently in the low endemic areas such as North America, where only 5%-20% of the population had previous exposure.

OBI is more commonly noted in patients at high risk for parenterally transmitted infections such as hepatitis C virus (HCV) infection or immunosuppression condition such as human immunodeficiency virus (HIV) infection[17].

In particular, OBI prevalence is high in patients with HCV infection. OBI prevalence was also high in patients with other chronic liver diseases at 20%-30% [18].

in hemophilia patients and in intravenous drug users, Among HIV-infected patients [19],[20].

The prevalence of OBI also differs according to the sensitivity of HBV DNA or HBsAg testing. There are various amplification methods for detecting HBV DNA,

and the HBV genome target sites are also different. Some commercial assays are more sensitive than others at detecting HBsAg mutants. The type of sample used (liver or serum) or number of samplings can also have some effect on the diagnosis of OBI. Indeed, as serum HBV DNA levels seem to fluctuate in OBI, serial sample is more useful to identify OBI [16].

1.4. Mechanisms Leading to Occult Hbv Infection

There have been significant advances in understanding the molecular mechanisms underlying occult HBV infection in the last decade; an overview of these mechanisms is shown in Fig. 5

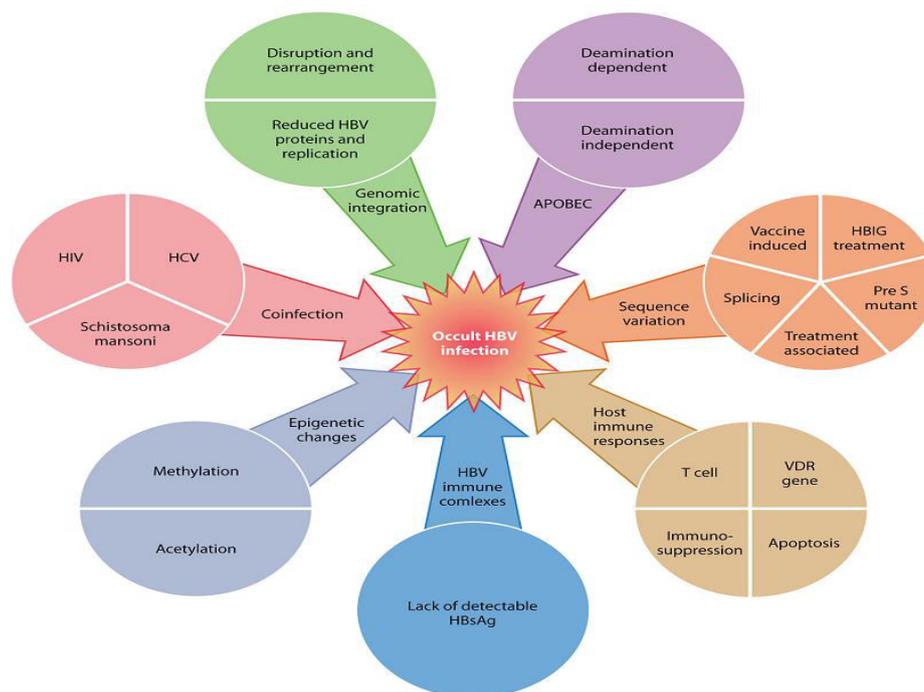


Figure (5): Mutations and Deletions in the HBV Genome

1.4.1. Mutations and Deletions in the HBV Genome

Sequence variation in HBV genomes, including

- (i) mutations in the “a” determinant of HBsAg,
- (ii) treatment-associated mutations,
- (iii) splicing,
- (iv) mutations in the pre-S region have been linked to occult HBV infection.

(Raimondo 2008)

1.4.2. Mutations in the “a” determinant of HBsAg.

A mutation in the “a” determinant of the surface antigen was one of the earliest recognized mechanisms leading to occult HBV infection. Mutations in HBsAg lead to

conformational changes rendering the protein undetectable by some of the commercially available HBsAg assays [9].

Occult hepatitis B virus infections are usually associated with surface gene mutants that are not detectable by some commercial HBsAg assays[21].

Individuals with isolated anti-HBc-positive status with virus loads of greater than 10^4 copies/ml frequently harbor HBsAg mutants [22].

Mutations in the “a” determinant of the surface protein are associated with HBV reinfection following liver transplantation despite HBIG prophylaxis. Withdrawal of HBIG after liver transplantation led to reversion of the mutant to wild type in majority of the patients, indicating the role of HBIG-induced immune pressure in leading to “a” determinant mutant[23].

A recent study has demonstrated that in addition to blocking HBsAg release, anti-HBs can also partially block virion release from infected hepatocytes, contributing to HBV clearance from circulation [24].

The emergence of “a” determinant mutants is a serious health concern not only because they are not detectable by some commercial HBsAg assays but also because they can infect both unvaccinated and vaccinated individuals[25].

1.4.3. Treatment-associated mutations.

Mutations in the HBV polymerase associated with the emergence of a mutation during lamivudine treatment result in amino acid changes in both the HBV polymerase and the surface gene [26].

1.4.4.RNA splicing.

Splicing has been shown to have a significant effect on gene expression in HBV [27].

1.4.5. Pre-S mutants

Mutations in the pre-S region, especially deletions, have also been associated with a lack of detectable HBsAg in the serum. Deletions in the pre-S region are associated with reduced expression of HBV surface proteins and also help in viral persistence by eliminating HLA-restricted B-cell and T-cell epitopes. Pre-S1/pre-S2 mutations are frequently detected in occult HBV infection [28].

Mutations in the pre-S2/S promoters were detected in patients with occult HBV-related chronic liver disease; serum HBsAg was not detectable in these patients [29].

The association of mutations and deletions in the pre-S gene with a lack of secreted HBsAg and low levels of HBeAg and HBV DNA was demonstrated using functional analysis by transfection into hepatocyte cell lines [30].

1.5. Diagnosis

The diagnosis of OBI is based on the sensitivity of assays used in the detection of HBV DNA. Several methods using liver tissue, DNA extracts from liver or blood, or other serologic markers such as antiHBc IgG have been used to diagnose OBI. The

gold standard for OBI diagnosis is the detection of HBV DNA in the DNA extraction from the liver, as cccDNA persists in the hepatocytes and HBV DNA is sometimes detected in the liver in the absence of HBV DNA in the serum [31].

However, obtaining liver tissue is an invasive procedure; therefore, obtaining hepatic HBV DNA is difficult in clinical practice. In addition, real-time PCR based assays for serum (or plasma) HBV DNA detection have been used with sufficient sensitivity to detect OBI in many cases; hence, serum HBV DNA assays are widely used to diagnose OBI (table.3) [9].

Periodic testing for HBV DNA will improve diagnosis of OBI especially in high-risk patients, as intermittent viremia can occur in occult HBV infection[32], [14],[16].

Anti-HBc could be used as a possible surrogate marker for identifying potential seropositive OBI in cases of blood and organ donation or those receiving immunosuppressive therapy. In this case, seronegative OBI or false-negative anti-HBc in an immunocompromised host should also be considered (table .3)[17].

Since the diagnosis of OBI is dependent on detection of HBV DNA by PCR, which is an expensive tool and the Egyptian budget for health care and scientific research is very low , many Egyptian studies depend on anti-HBc positivity for diagnosis of OBI leading to missing of HBV DNA positive/anti-HBc negative cases and consequently underestimating the problem[33].

Table (3): Interpretation for diagnosis of OBI

Anti-HBc	HBV DNA	Interpretation
+	+	Occult hepatitis B infection (seropositive)
-	+	Occult hepatitis B infection (seronegative)
+/-	+++ (>2-3 logs)	1. Chronic overt hepatitis B infection with acquired pre-S/S mutation 2. Window period of acute HBV infection
-	-	1. Non-infected 2. Occult hepatitis B (seronegative) with intermittent HBV DNA detection/low viraemia
+	-	1. Non-infected 2. Occult hepatitis B (seropositive) with intermittent HBV DNA detection/low viraemia 3. False positive anti-HBc

[34]

1.6. RISK OF OBI TRANSMISSION

Transfusion: Although the risk of HBV transmission through blood transfusion has decreased owing to the development of sensitive and specific diagnostic assays, transfusional transmission of HBV still occurs. Transmission of HBV by transfusion occurs in 3 situations: (1) blood from a donor with OBI; (2) blood from patients in the

infectious window period of HBV infection; or (3) blood from a donor infected with S-escape mutant HBV infection not detected by the routinely used diagnostic HBsAg assay. The prevalence of OBI in blood donors is variable depending on the geographic area and is higher in HBV endemic areas [35].

The risk of HBV transmission may depend on the presence of anti-HBsAb. Among occult HBV-infected donors, those with high anti-HBs levels (recovered) are unlikely to transmit the infection, whereas those without anti-HBs (anti-HBc only) may transmit the infection [36],[37].

Organ transplantation:OBI in a transplantation donor is important because there is a risk of HBV transmission from an OBI-seropositive donor, and severe HBV reactivation can occur in some of these cases during immunosuppression. As the hepatocytes are the reservoir of HBV cccDNA, the rate of transmission is higher in orthotopic liver transplantation compared to other organ transplantations such as kidney, bone marrow, and heart [38].

Hemodialysis:Hemodialysis patients are at increased risk of parenterally transmitted infections because they are in an immunosuppressed state and exposed to invasive procedures, share the same dialysis machine, and receive more transfusions than the general population. The relatively low acceptance and response rates to the HBV vaccine among dialysis patients also likely contributes to OBI transmission in hemodialysis patients [39], [40].

Several studies suggest that OBI could be a source of viral spread both to other patients and staff within the hemodialysis units [39].

Therefore, patients and staff need HBV vaccine boosts to maintain levels of protective antibody to HBsAg (anti-HBs). Strict dialysis-specific infection-control programs, including avoidance of dialyzer reuse and use of dedicated dialysis rooms and machines, should be implemented. Staff for infected patients should be educated on preventive methods to limit HBV transmission within dialysis units. Furthermore, regular screening for HBV DNA with sensitive PCR-based assays in all dialysis patients should be considered, and more attention should be given to patients who receive immunosuppressant drugs after renal transplantation [41].

1.7. Pregnancy and OBI transmission:

OBI may occur in newborns from HBsAg positive mothers despite proper active/passive immunoprophylaxis at birth[42].

1.8. Occult HBV reactivation

The definition of HBV reactivation in patients with OBI generally includes i) HBsAg seroreversion and/or an increase of serum HBV DNA by at least 1 log above the lower limit of detection of the assay in a person who had previously undetectable HBsAg and HBV DNA in serum, and ii) a more than 1 log increase in serum HBV DNA in people who had detectable HBV DNA at baseline[43].

People with OBI can experience reactivation of HBV replication when they receive cancer chemotherapy or other immunosuppressive therapies. Although the incidence is lower than in those with chronic HBV infection, HBV reactivation can occur in up to 40% of people with OBI when potent immunosuppressive therapies are used[44].

Patients prone to OBI reactivation are shown in table (4).

The risk of HBV reactivation is very low in individuals with OBI receiving direct-acting antiviral therapy for hepatitis C[45].

The underlying mechanism of reactivation is thought that chemotherapy induced immunosuppressive state triggers rapid viral replication because of the loss of the immunological control. After immune system reconstitution, cytotoxic T-cell-mediated hepatocyte injury may occur, leading to the development of hepatic inflammation and concomitant hepatic necrosis[46].

Hematological malignancies, hematopoietic stem cell transplantation, liver transplantation from anti-HBc positive donors, and treatment with anti-CD20 (rituximab) seem to be the factors associated with the highest risk of OBI reactivation [17].

Other immunosuppressive conditions, including HIV infection, kidney or bone marrow transplantation, systemic chemotherapy, and rheumatologic diseases or inflammatory bowel disease treated with biological agents or high-dose steroids for prolonged treatment, also have been reported as possible causes of viral reactivation in OBI patients [9]. Most studies on HBV reactivation in people with OBI relied on detection of anti-HBc (table.4) [47].

All patients receiving chemo- and immunotherapy should be tested at least once for anti-HBc antibodies before starting therapy and monitored periodically for ALT elevations. In case of ALT elevation, further diagnostic workup is required pending initiation of antiviral therapy upon establishing the diagnosis of HBV reactivation [48].

Monitoring in such cases should be extended for months or even years after discontinuation of immunosuppression[9].

Table (4): Categories of occult HBV carriers known to be prone to viral reactivation

Patients with	Patients who have undergone
<ul style="list-style-type: none"> • Haematological malignances • HIV infection 	<ul style="list-style-type: none"> • Bone marrow transplantation • Liver transplantation • Renal transplantation • Chemotherapy

	<ul style="list-style-type: none"> • Treatment with anti-CD20 (Rituximab) • Treatment with anti-CD52 (Alemtuzumab) • Treatment with anti-TNF-α(Infliximab)
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[49]

2. HBV/HCV coinfection

2.1. Epidemiology

Infection with either (HBV) or (HCV) virus is one of the major causes of chronic liver disease globally [50].

Due to shared routes of transmission, coinfection with HBV and HCV is common among individuals in areas of high HBV prevalence and among individuals at high risk of parenterally transmitted infections, such as people who inject drug (PWID), those with an increased number of lifetime sexual partners, patients on hemodialysis, patients undergoing organ transplantation and HIV positive individuals [51].

Due to a lack of largescale population-based studies the exact number of people coinfecting with HBV/HCV is unknown. Dual infection ranges from 9% to 30%, depending on the geographic region [52].

These numbers may underestimate the true number of people with HBV/HCV coinfection, as there is a well-known entity of occult HBV infection (patients with negative hepatitis B surface antigen [HBsAg] but detectable serum HBV DNA) in patients with chronic HCV [53].

Screening for HBV/HCV coinfection

People with a first episode of acute hepatitis should be screened for all viral causes including HBV and HCV as Some patients may be inoculated with both viruses simultaneously and will present with acute hepatitis due to both viruses. In addition, HBV superinfection in patients with chronic HCV, and HCV superinfection in patients with chronic HBV have both been reported [54].

Therefore, episodes of acute hepatitis in patients with known chronic HBV or HCV infection, especially those with ongoing risk behavior for hepatitis infections such as injecting drug use or multiple sex partners, should undergo screening for superinfection. In addition, in patients with chronic HCV, ruling out occult HBV infection beyond HBsAg testing, by polymerase chain reaction (PCR), should be done when clinically indicated [46].

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