Occult Hepatitis B virus (HBV) infection: A hidden threat

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Abstract

Occult hepatitis B infection (OBI) is described as the existence of detectable hepatitis B virus (HBV) DNA by polymerase chain reaction (PCR) in serum or liver, in patients who test negative for HBsAg. According to the presence of serum markers of HBV exposure, OBI can be categorised as seropositive or seronegative, based on serological profiles. Furthermore, OBI may occur because of the infrequent infection by strains that have escape mutations in the S gene, modifications that may cause structural abnormalities in HBsAg, making the infection difficult to detect by the commonly used serological detection tests. OBI's treatment is exceptionally difficult. Its purpose is to enhance patients' quality of life by delaying and preventing the development of liver failure, cirrhosis, and hepatocellular carcinoma. HBV DNA carriers with OBI may transmit HBV infection via blood transfusion. The more sensitive diagnostic tools used for blood screening can significantly reduce the risk of HBV transmission. To eradicate viral hepatitis by 2030, the World Health Organization adopted the Global Health Sector in 2016, but the current lack of effective treatment and detecting methods, challenge this goal. The need to discover new antiviral medications and treatment methods is the biggest problem facing HBV research today.

Key words: *Occult hepatitis B infection; OBI; HBV DNA; HBV surface antigen*

INTRODUCTION

HBV infection

Hepatitis B virus (HBV) is a hepatotropic virus that can provoke a persistent and chronic infection in individuals through immune anergy [1]. Approximately 33% of the current world population has previously been exposed to HBV. From those an estimated 240 million individuals are chronically infected, and approximately 800,000 people worldwide die each year from this infection [2]. Currently, 3.5% of the global population is chronically infected with HBV, although the incidence of HBV infections is decreasing owing to vaccination and, to a lesser extent, the use of antiviral therapy to reduce the viral load of chronically infected individuals [1]. Well-defined serum and liver biopsy diagnostic markers allow the evaluation of disease severity, viral replication, risk stratification for a patient, and the appropriate treatment decisions. Current treatment includes antiviral agents that directly affect viral replication and immunomodulators [1].

There are ten unique genotypes of HBV (A-J), and the geographical distribution of each HBV genotype is distinct. Different genotypes of HBV infection are linked to different chronicity after infection, disease progression, and IFNα treatment responses; nonetheless, the approved HBV vaccines are effective against all genotypes [1].

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A silent evolution characterises HBV infection's natural history, because typically, the disease is diagnosed decades after infection. An immunoenzymatic assay or a rapid test can detect HBV infection [3]. The presence of HBsAg in the serum is the key serological maker of both acute and chronic hepatitis B [1]. Laboratory confirmation is essential because it is impossible to distinguish hepatitis B from other viral hepatitis types clinically. Notably, HBV infection is asymptomatic in 80% of infected individuals. If the result is positive, the diagnosis is confirmed by carrying out complementary tests, such as liver biopsy, to search for other markers or using molecular tests to directly detect HBV DNA [3].

Acute HBV infection is characterised by the presence of HBsAg and an immunoglobulin M antibody for the core antigen. The HBeAg is found in the initial phase of infection. An immune-tolerant phase is characterised by a typically high viral load in the blood that is responsible for high infectivity [3].

Chronic infection is marked by the persistence of HBsAg for at least six months (with or without HBeAg), while the load of HBV DNA determines the rest serologic profile and, consequently, the need for treatment. The persistence of HBsAg is the main risk factor for the development of liver cirrhosis and hepatocellular carcinoma (HCC) [3].

Taking into consideration the presence of HBeAg, HBV DNA levels, alanine aminotransferase (ALT) values and the existence or absence of liver inflammation, chronic HBV infection can be classified into five stages (Table 1).

i) **HBeAg-positive chronic HBV infection** is characterised by the presence of serum HBeAg, very high levels of HBV DNA (>10^7 IU/m) and ALT within the normal range. In the liver, there is no or minimal liver necroinflammation or fibrosis but a high level of HBV DNA integration and clonal hepatocyte expansion. Due to high levels of HBV DNA, patients are highly contagious [4].
ii) **HBeAg-positive chronic hepatitis B** is characterised by the presence of serum HBeAg, high levels of HBV DNA (10^4 -10^7 IU/ml) and elevated ALT. In the liver, there is moderate or severe liver necroinflammation and rapid development of fibrosis [4].
iii) **HBeAg-negative chronic HBV infection** is characterised by the presence of anti-HBe, HBV DNA levels < 2,000 IU/ml and normal ALT. In the liver, minimal necroinflammatory activity and low fibrosis [4].
iv) **HBeAg-negative chronic hepatitis B** is characterised by the lack of serum HBeAg, normally with detectable anti-HBe, and moderate to high levels of serum HBV DNA (>2.000 IU/ml), alongside elevated ALT values. As regards the liver, there is necroinflammation and fibrosis [4].
v) **HBsAg-negative phase** is characterised by serum negative HBsAg and positive anti-HBc, with or without detectable anti-HBs. This phase is widely known as "occult HBV infection". In this phase, we have normal ALT values and ordinarily undetectable serum HBV DNA. HBV DNA (cccDNA) can be detected often in the liver [4].

Occult HBV infection

Definition of Occult HBV infection

HBV DNA can be found only in serum or the liver in cases of occult hepatitis B infection (OBI) where hepatitis B surface antigen testing is negative. This is described as the existence of detectable HBV DNA by polymerase chain reaction (PCR) in patients who test negative for HBsAg. According to the international Taormina statement, occult HBV infection is defined as the presence of replication-competent HBV DNA (i.e. episomal HBV

Table 1: *Stages of chronic HBV infection, classified by the presence of HBeAg, HBV DNA levels, ALT values and the existence or absence of liver inflammation.*

	HBeAg positive		HBeAg negative	
	Chronic infection	Chronic hepatitis	Chronic infection	Chronic hepatitis
HBsAg	High	High/intermediate	Low	Intermediate
HBeAg	+	+	-	-
HBV DNA	>10^7 IU/ml	10^4 -10^7 IU/ml	<2.000 IU/ml	>2.000 IU/ml
ALT	Normal	Elevated	Normal	Elevated
Liver disease	None or minimal	Moderate/severe	None	Moderate/severe

Abbreviations: HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase

covalently closed circular DNA [cccDNA]) in the liver - in individuals who test negative for HBsAg (whether or not HBV DNA is present in the blood or liver) [5].

OBI may occur as a result of the infrequent infection by strains that have escape mutations in the S gene; these modifications cause structural abnormalities in HBsAg, making the infection difficult to detect by the commonly used serological detection tests. OBI may also result from mutations (substitutions, deletions, and insertions of nucleotides) in the pre-S1 and pre-S2 regions, which reduce or prevent HBsAg expression [3].

In a small minority of OBI subjects, the absence of serum HBsAg can be associated with an infection with HBV genetic variants containing mutations at the S gene level that result in the production of modified HBsAg which cannot be detected even by commercially sensitive testing. In these cases, serum HBV DNA levels may be as high as those normally detected in subjects with "overt" HBsAg-positive infection. Also, HBV DNA can be integrated into the host's genome in individuals with OBI. However, integrated viral sequences are not involved in HBV replication, and their existence does not affect the diagnosis of OBI, as OBI usually occurs in cases with persistence of replication-competent HBV DNA [5].

According to the presence of serum markers of HBV exposure, occult hepatitis B can be categorized as seropositive or seronegative OBI, based on serological profiles. About 80% of all instances of OBI are referred to be seropositive, which is defined as those who have antibodies to the HBV core antigen (anti-HBc) and/or antibodies to HBsAg (anti-HBs) that are detectable in the serum. A smaller proportion (between 1% and 20% of instances) of OBIs are referred to as seronegative OBIs [3,5].

Albeit the clinical features that distinguish OBI-seropositive from OBI-seronegative cases remain completely cryptic, OBI can be exhibited in one of three clinical forms:

(a) during an acute HBV infection window period, (b) detectable HBV DNA and undetectable HBsAg in patient serum without an overt HBV infection previous history, (c) in individuals who have a history of chronic HBV infection [6].

The main reason behind the growing interest in OBI is related to accumulating evidence of its clinical impact. Indeed, (a) it can be transmitted to the recipient, primarily through blood transfusion or liver transplant; (b) in case of immunosuppression, it may result in viral reactivation; (c) it might have a detrimental effect on the progression of chronic liver disease of various causes into more advanced clinical stages; (d) It significantly contributes to the development of HCC [7].

Prevalence of Occult HBV infection

Although occult hepatitis B infection was first described more than 30 years ago [8], there are several important aspects of this disease, including its natural history, factors involved and effective diagnostic tools, that have not been fully elucidated yet. Occult hepatitis B infection is widespread worldwide, with high prevalence in high-risk populations for HBV or living in areas where HBV infection is prevalent [3].

Occult hepatitis B infection is commonly associated with classical hepatitis B in infected individuals consistent with the expected clinical picture (i.e. with typical serological and clinical patterns of infection). It can occur in multiple clinical situations, such as cases where HBV infection is reactivated after the development of an immunodeficiency, which can result in acute hepatitis or fulminant hepatitis [9]. The sensitivity of HBsAg and HBV DNA assays, the existence of risk factors for HBV exposure, the prevalence of HBV among the general population in different geographic areas, the existence and adherence of anti-HBV vaccination programs in various countries, and the presence and severity of liver disease in the examined populations are just a few of the many factors that influence the worldwide epidemiology of OBI [6].

In fact, the majority of studies on OBI prevalence have been carried out on liver disease patients and blood donors, so, they do not truly represent the population as a whole. There are a few studies that indicate a low OBI prevalence in Asian and African regions with high endemicity of HBV infection, despite the fact that OBI prevalence is higher in areas of the world where hepatitis B is endemic. High OBI prevalence has been found in groups of patients with risk factors for HBV infection, for instance, intravenous drug users (45%), subjects with HCV co-infection (15-33%) or HIV co-infection (10-45%), patients on dialysis (27%), and patients with co-existing liver disease, for example, those with HCC (63%), cryptogenic cirrhosis (32%), or liver-transplant patients (64%) [7]. The relatively high prevalence reported in HCV or HIV-infected populations and HCC patients may be biased by the fact that clinical investigations are being conducted more frequently in these populations. Hepatitis B virus serological profiles suggest that the estimated OBI rate in anti-HBc- positive patients varies between 4% and 25% [3,5].

Risk of Occult HBV infection-Transmission - Blood Transfusion

Numerous studies have demonstrated that HBV DNA carriers with OBI may transmit HBV infection via blood transfusion, resulting in typical hepatitis B in the recipient. The more sensitive diagnostic tools used for blood screening have significantly reduced the risk of HBV transmission over the past 30 years through blood transfusion [7]. However, despite the widespread availability of anti-HBc tests and nucleic acid testing (NAT), the transmission of HBV from OBI blood donors remains a significant public health concern in low- and middle-income nations [7]. Because the minimum dose of infectious HBV DNA is below the lower limit of detection of the NAT assays that are currently in use, there is still a minimal risk of OBI transmission through transfusion in developed countries [7].

If the donor is an OBI carrier, the transmission relies upon many variables, for example, how much plasma is transfused, the immune status of the recipient, and the HBV serology status of both donor and recipient [10]. Additionally, an OBI carrier may be intermittently infectious due to the fact that OBI is characterized by periods of transient viremia alternated with phases of absence of serum viral replication [10]. HBV DNA positive OBI donors with an isolated anti-HBc serological marker have been shown to be more infectious than anti-HBs positive OBI carriers, and the recipient's anti-HBs positivity significantly lowers the risk of infection [10].

Diagnosis of Occult HBV infection

The detection of HBV DNA in the blood or liver of individuals who test negative for HBsAg is necessary for the diagnosis of OBI. The detection of HBV genomes in DNA extracts from the liver is regarded as the gold standard [7]. HBV DNA testing in the blood, on the other hand, is a much more common diagnostic method and generally easier to perform. Anti-HBc testing may be used as a substitute marker for the diagnosis of OBI primarily for the purpose of identifying potential seropositive OBI subjects in cases of blood, tissue, or organ donation and when immune suppressive therapy has to be initiated [7].

Currently available HBsAg assays have a limit of detection of 0.05 IU/mL, and some recent studies showed that between 1% and 48% of samples testing negative in these assays resulted positive using more sensitive HBsAg assays with a lower limit of detection of 0.005 IU/mL [11,12]. Another issue is the different ability of commercial HBsAg assays to detect S-escape variants [9]. The lower limit of detection of most currently available commercial HBV DNA assays is 10 to 20 IU/mL. In addition, HBV DNA is usually present at low levels in people with OBI and can only be detected intermittently, so blood samples collected at multiple time points and testing DNA extracts from no less than 1 mL of serum or plasma, is recommended for the diagnosis of occult HBV infection [5]. Furthermore, NAT assays that are highly specific (99.9%) and sensitive (LLOD 2–4 IU/mL) are used for blood donations. However, sensitivity significantly decreases when NAT screening is performed on minipools of multiple donations [7].

Reliable methods for detecting OBI include nested PCR techniques, real-time PCR methods whereas digital techniques include droplet PCR assays. NATs are increasingly being applied using primers containing three or more, conserved regions covering the S, X and core genes [3,13].

Escape mutations in the S region is a main challenge in OBI detection. HBsAg assays that are insufficiently sensitive or unable to detect variants in this region, usually give false negative HBsAg results and therefore misdiagnosis. Molecular tests for HBV may also be inadequately sensitive. There is currently no standardized and validated test in order to detect OBI. Several studies have attempted to standardize a technique based on internal tests, but differences in sensitivity and specificity have limited their successful application [3,5].

Treatment of occult HBV infection

Indications for treatment in chronic HBV infection are based on the levels of serum HBV DNA, ALT and severity of liver disease. Treatment indications are typically similar for HBeAg-positive and HBeAg-negative diseases [7]. Treatment is generally indicated for those with HBV DNA levels >2000 IU/mL, and elevated levels of liver enzymes, 1-2 times the upper limit of normal [14]. In the presence of cirrhosis, treatment is frequently advised in cases of detectable HBV DNA levels independent of the ALT levels. Furthermore, patient's age, a family history of liver cancer, comorbidities, risk of HBV transmission and extrahepatic manifestations of hepatitis B, are some factors we can take into consideration for the indication of treatment. Treatment for acute HBV infection is primarily supportive, and antiviral treatment is often not necessary unless patients have fulminant liver failure [7].

OBI is exceptionally difficult to be treated because current HBV therapeutic strategies fail to eliminate the

HBV minichromosomal reservoir (cccDNA / integrated HBV DNA) from all infected cells [3]. In order to find a cure for HBV, the current research and clinical trials aim to develop novel antiviral strategies. It is probably impossible to completely sterilize all tissues with the elimination of cccDNA and integrated HBV DNA. As a result, the current focus is on achieving functional cure, which is defined as HBsAg clearance in a high percentage of patients after a limited course of treatment [15].

In patients with chronic HBV infection, spontaneous or treatment-induced HBsAg clearance has been shown to reduce liver necroinflammation and, consequently, the risk of cirrhosis, HCC, and HBV-related mortality [5].

However, HBV DNA positive patients with elevated levels of liver enzymes, during or after direct acting oral antivirals (DAA) therapy should be monitored at regular intervals (once every 4 weeks) for possible reactivation of HBV [3].

To eradicate HBV from people with OBI, HBV infected hepatocytes would either need to be eliminated or cured. Theoretically, there are several options:

-Elimination of cccDNA within infected hepatocytes, using cccDNA targeting procedures like CRISPR/Cas9 technologies or gene editing techniques [5].

-Killing infected hepatocytes by using strategies directed at restoring HBV-specific T cell responses, therapeutic vaccination strategies, engineered T cell treatments like chimeric antigen receptor (CAR-T) cell technologies or HBV-T cell receptor (TCR) engineered T cells to kill the remaining infected liver cells [5].

This would require not only adapting these innovative technologies to this clinical application, but also gaining a deeper comprehension of the biology of cccDNA and immune control, along with the number of infected hepatocytes in the case of occult HBV infection [5].

With the primary goals of: (a) stopping treatment with no risk of virological relapse and no risk of liver disease progression; and (b) further decreasing the risk of HCC, numerous research programs are currently underway to develop new treatment concepts that concentrate on the clearance of HBsAg in a significant proportion of patients [16].

Direct antivirals and immunotherapeutic drugs can be used to classify the innovative treatment alternatives currently undergoing pre-clinical and early clinical research. HBV entry inhibitors, drugs aiming at cccDNA destruction or silencing, approaches targeting viral transcripts by siRNA or anti-sense oligonucleotides, nucleocapsid assembly modulators, approaches to decrease HBsAg release in serum are all examples of direct-acting antivirals. This list is not meant to be comprehensive as many viral targets are currently being screened for drug discovery. First phase clinical trials are ongoing for several of these agents [16].

Occult HBV infection and challenges to the HBV elimination strategy

To eradicate viral hepatitis by 2030, the WHO adopted the Global Health Sector Strategy (GHSS) in 2016. With this strategy WHO aims to decrease by 2030 the frequency of hepatitis to 0.9 million cases and will reduce annual deaths because of hepatitis from 1.4 million to 0.5 million [2].

The WHO is assisting a number of nations in the development of hepatitis control programs in this regard. In order to eliminate hepatitis by 2030, five strategic areas are listed in the GHSS document. Due to our current lack of an effective treatment, most of them target HBV. The following are the focus areas: (a) expansion of HBV vaccination coverage (b) prevention of transverse transmission of HBV (c) parental transmission care (d) reducing harm and co-infection, and (e) broadening the availability of HBV and HCV testing and treatment [2].

CONCLUSION

The WHO proposes increasing early HBV diagnosis and treatment in addition to expanding preventive vaccination coverage [2]. To aid in the treatment of OBI, coordinated clinical studies of therapies that eliminate cccDNA are required. The detection of "false OBI" would also benefit from an increase in diagnostic sensitivity for HBsAg detection. One of the main challenges associated with OBI and the elimination of HBV, is the significant unmet need to develop specific, sensitive and standardized tests for the detection of OBI. Screening for anti-HBc, HBV DNA NAT with a LOD of 0.8 copies/ml (0.15 IU/ml), or pathogen reduction of blood components, are some methods that can increase HBV blood safety. Treatment is not necessary for OBI unless it is complicated [3].

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