Clinical Significance of Occult Hepatitis B Infection in Progression of Liver Disease and Carcinogenesis

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Abstract

Occult hepatitis B infection (OBI) is defined as long-lasting persistence of hepatitis B virus (HBV) DNA in the liver of patients with hepatitis B surface antigen (HBsAg)-negative status, with or without serological markers of previous exposure (antibodies to HBsAg and/or to hepatitis B core antigen). Over the past two decades, significant progress has been made in understanding OBI and its clinical implications. OBI as a cause of chronic liver disease in patients with HBsAg-negative status is becoming an important disease entity. In conditions of immunocompetence, OBI is inoffensive in itself and detection of HBV DNA in the liver does not always indicate active hepatitis. However, when other factors that cause liver damage, such as hepatitis C virus infection, obesity and alcohol abuse are present, the minimal lesions produced by the immunological response to OBI might worsen the clinical course of the underlying liver disease. Several lines of evidence suggest that OBI is associated with progression of liver fibrosis and the development of hepatocellular carcinoma in patients with chronic liver disease. The major interest in OBI is primarily associated with the growing, widely discussed evidence of its clinical impact. The aim of this review is to highlight recent data for OBI, with a major focus on disease progression or carcinogenesis in patients with chronic liver disease.

Key words: hepatitis B virus, occult hepatitis B infection, chronic liver disease, carcinogenesis, clinical outcome

Introduction

Hepatitis B virus (HBV) belongs to a family of closely related DNA viruses called the hepadnaviruses and it contains a 3.2-kb partially double-stranded DNA genome with four open reading frames encoding seven proteins.[1] HBV is a major causative agent of hepatocellular carcinoma (HCC) and it remains a major public health problem worldwide.[2] Up to 400 million people worldwide have hepatitis B surface antigen (HBsAg)-positive chronic viral infection, which is primarily acquired by vertical transmission in high-endemicity countries. However, in individuals with low HBV viral load, HBV may not be detectable by commonly used serological assays based on detection of HBsAg and this unapparent infection makes the true incidence of HBV infection difficult to estimate. Thus, the pathogenic importance of HBV infection may be greater than that presently assumed.[3,4] During the natural history of chronic hepatitis B, seroconversion from HBsAg to anti-HB surface antibody (anti-HBs) is related to the remission of active hepatitis and improvement of liver function and pathological features. Individuals who have recovered from acute hepatitis B or those who have lost serum HBsAg during their clinical course might carry HBV genomes for several years without presenting any clinical evidence of chronic liver disease.[5-7]

In a small proportion of individuals, detectable HBV DNA in the serum and/or the liver is observed

in the absence of circulating HBsAg.[8–11] Occult hepatitis B infection (OBI) is defined by the presence of HBV DNA in the liver tissue of individuals who test negative for HBsAg, regardless of the detection of HBV DNA in serum.[8] OBI was first reported in the 1980s when hybridization techniques for the detection of HBV DNA became available.[12] Prevalence of OBI has been demonstrated to be associated with the overall prevalence of HBV infection in a given country (i.e., individuals from countries highly endemic for HBV are more likely to develop OBI).[13] Prevalence is higher in seropositive patients, particularly those who are positive for antibody to HB core antigen (anti-HBc), than in seronegative patients who are negative for anti-HBc as well as anti-HBs.[9,14] Anti-HBc positivity is thus reported to be a surrogate marker for such latent HBV carriers.[15] OBI is observed in up to 30% of serum samples and 50% of liver biopsies of patients with chronic hepatitis C virus (HCV) infection.[7,14–17]

The clinical implications of OBI involve different clinical contexts as follows: (1) harboring potential risk of HBV transmission through hemodialysis, blood transfusion, and organ transplantation; (2) causing cryptogenic liver disease; (3) contributing to the progression of liver disease or even HCC; and (4) affecting response to antiviral therapy for patients with chronic HCV infection.[18–21] Thus, OBI as a cause of chronic liver disease in patients with HBsAg-negative status is becoming an important disease entity.[10,14,21,22] Despite its potential clinical significance, knowledge of the effect of OBI in patients with chronic liver disease is limited because HBV-DNA detection may require liver tissue, and liver biopsies are not routinely performed in the majority of such patients in clinical settings. In addition, most previous studies addressing this issue have included small sample sizes or heterogeneous populations, or are cross-sectional, and the use of different methods with variable sensitivity for HBV-DNA determination in the liver or serum may explain the discrepancies in the influence of OBI in patients with chronic liver disease.[23–26] The aim of the current review is to highlight recent evidence of OBI with a focus on its progression or carcinogenesis in patients with chronic liver disease.

Definition of OBI

Chronic HBV infection is defined by the persistence of serum HBsAg for >6 months. The gold standard for diagnosis of OBI is analysis of HBV-DNA extracts from the liver and blood samples.[11,27] However, extracted liver samples are only available in a minority of cases, therefore, diagnosis of OBI is most often based on analysis of serum samples.[9] In general, OBI is defined by the presence of HBV DNA in the liver tissue of individuals who test negative for HBsAg, regardless of the detection of HBV DNA in the serum. Highly sensitive commercial assays should be preferred for HBsAg and HBV-DNA testing. When HBV DNA is detectable, its level in the serum is usually low (<200 IU/mL). Depending on the HBV antibodies detected, OBI may be seronegative (anti-HBc and anti-HBs negative) or seropositive (anti-HBc and/or anti-HBs positive).[9] More than 20% of individuals with OBI have no seropositive markers.[8,14] In addition, viral replication appears to be regulated by different mechanisms: for patients with anti-HBc positivity, by a T-cell response of protective memory; whereas anti-HBc-negative patients have no HBV-specific T-cell expansion, indicating that persistent low HBV-DNA infection is insufficient for protective memory to mature (Figure 1).[28]
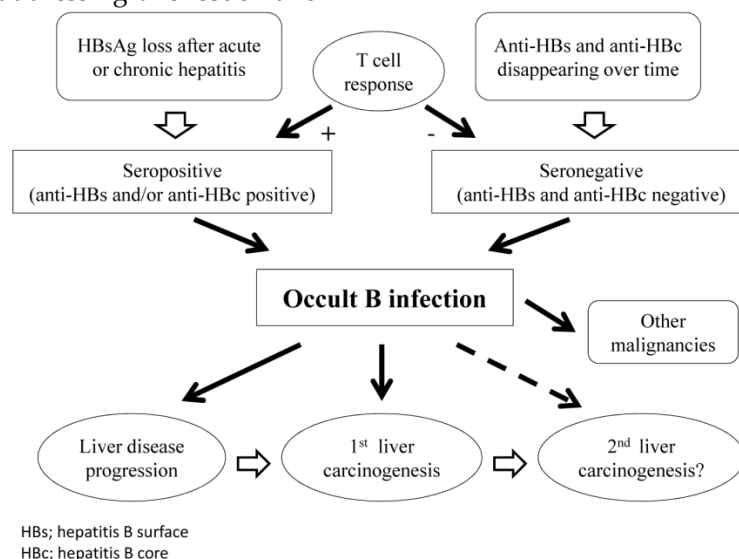

Figure 1. Schematic representation of clinical course in patients with OBI.

OBI and Chronic HCV Infection: Effect on Progression of Liver Fibrosis

HBV and HCV share common routes of transmission and this explains the high prevalence of OBI reported in patients with HCV.[8,14,29,30] Selim et al. reported that in 60 HBsAg-negative patients with chronic HCV infection, HBV DNA was detected in four (13.3%) of 30 patients with normal or slightly high alanine aminotransferase (ALT) levels.[31] However, in those with ALT flare (n = 30), HBV DNA was detected in 19 (63.3%) patients (P < 0.001).[31] This indicates that in patients with HCV, OBI may contribute to increased plasma HCV RNA loads and liver transaminase levels. Cacciola et al. reported that 66 (33%) of 200 patients with chronic HCV infection had HBV genomes, as did seven (14%) of 50 patients with liver disease unrelated to HCV (P = 0.01).[29] Among these 66 patients with HCV who had HBV genomes, 46 were anti-HBc positive and 20 were negative for all HBV markers (P < 0.001).[29] Twenty-two (33%) of these 66 patients had liver cirrhosis, as compared with 26 (19%) of the 134 patients with HCV infection but no HBV sequences (P = 0.04).[29] This suggests that patients with chronic HCV infection and OBI more frequently have liver cirrhosis than those with chronic HCV infection alone.[29] Likewise, Matsuoka et al. demonstrated that in patients with chronic HCV infection, the mean score of fibrosis stage and degree of inflammatory cell infiltration in patients with OBI were significantly greater than in those without OBI.[32] Furthermore, Ikeda, et al. found that 251 (43.6%) of 576 patients with chronic HCV infection had anti-HBc positivity, which is a surrogate marker for OBI, while 141 (52.2%) of 270 patients with HCV-related liver cirrhosis had anti-HBc positivity.[16] They concluded that the proportion of patients with chronic HCV infection with OBI increases in association with progression of liver fibrosis.[16]

However, these observations have not been confirmed by other investigations. Kao et al. reported that in patients with chronic HCV infection, the prevalence of OBI did not parallel the severity of liver disease.[33] OBI was seen in 16 (14.5%) of 110 patients with chronic hepatitis, four (8%) of 50 patients with liver cirrhosis, and 11 (22%) of 50 patients with HCC.[33] They concluded that OBI does not have clinical significance in patients with chronic HCV infection.[33] Hui et al. examined a retrospective cohort of 74 HCV-infected patients and reported that 11 (35.5%) of 31 with OBI compared with 12 (27.9%) of 43 without OBI had fibrosis progression (P = 0.608).[34] Also, six (19.4%) of 31 patients with OBI compared with eight (18.6%) of 43 without OBI developed severe fibrosis (P = 0.946).[34] They concluded that patients with chronic HCV infection with OBI do not seem to progress more than patients without OBI.[34] Similarly, Sagnelli et al. studied 89 patients [37 (41.6%) with OBI] with biopsy-proven chronic HCV infection and reported that there was no significant association between OBI and degree of liver necroinflammation and fibrosis.[23]

The predominance of certain HBV genotypes in patients with chronic HCV infection and OBI, and their potential role in determining clinical outcome, has been reported recently.[35] The authors suggest that genotype B or D may influence the outcome of OBI, which may lead to progression of liver disease.[35] In terms of HCV genotypes, a higher proportion of OBI was detected in patients with HCV genotype 1b than in those with genotype 2a.[36]

In view of previous reports, results of the combined effect of chronic HCV infection and OBI on progression of liver disease have yielded controversial results and no firm conclusion can be reached.

Mechanism of Carcinogenesis in Patients with OBI

The direct mechanism of carcinogenesis in HBV-related HCC includes mutagenesis and changes in proliferation and differentiation caused by integration of HBV DNA into the host genome (HCV is an RNA virus, therefore, it is not integrated into host hepatocyte DNA, which causes the mutation).[37,38] OBI may maintain these direct mechanisms of HBV-related carcinogenesis, such as the ability to be integrated into the host genome and production of transforming proteins, mainly including X and preS-S proteins.[11,32,39–41] In contrast, OBI may also exert pro-oncogenic properties through indirect mechanisms. These are associated with its propensity to induce persistent necroinflammation in the liver and to promote progression of chronic hepatitis to liver cirrhosis, which indicates the step preceding HCC occurrence in the majority of cases.[11,20–22,25,26,41] Furthermore, indirectly, continuous HBV replication caused by OBI can mediate alterations in transforming growth factor-β1 and α2-macrogloblin production, leading to liver carcinogenesis.[42]

Effect of OBI on Risk of HCC Development in Patients with Chronic HCV Infection

The risk of HCC development has been reported to increase up to 100-fold in patients with chronic HBV infection.[11,43] Chronic infection with HBV or HCV is the major cause of HCC worldwide.[2] Central questions include the influence of OBI on liver carcinogenesis in patients with HCV-related liver disease.

Ikeda et al. demonstrated in a multicenter prospective study that in patients with HCV-related liver cirrhosis (*n* = 270), HCC developed in 85 (60.3%) of 141 patients with anti-HBc and 58 (45.0%) of 129 patients without anti-HBc.[16] Their multivariate analysis of factors contributing to HCC occurrence identified that anti-HBc positivity was an independent risk factor with a hazard ratio (HR) of 1.58.[16] Similarly, Adachi et al. reported in a prospective study of 123 patients with HCV-related liver cirrhosis that cumulative 5-year HCC development rates were 59.2% in the anti-HBc-positive group (*n* = 96) and 37.8% in the anti-HBc-negative group (*n* = 27) (*P* = 0.0039).[24] Their multivariate analysis identified that anti-HBc positivity was a significant adverse predictor linked to development of HCC with an HR of 3.111 (*P* = 0.001).[24] They concluded that serum anti-HBc positivity, namely potential OBI, has clinical significance in hepatocarcinogenesis in patients with HCV-related liver cirrhosis.[24] In another large prospective study, Matsuoka et al. prospectively studied the effect of OBI on the histopathological features and clinical outcomes of 468 patients with HBsAg-negative chronic HCV infection.[32] They demonstrated that the cumulative probability of development of HCC was significantly higher for HBV-DNA-positive patients than for HBV-DNA-negative patients, and that HBV-DNA positivity was an independent risk factor for the occurrence of HCC.[32] Miura et al. investigated the influence of OBI, focusing on HCC development in patients in whom HCV eradication by interferon therapy had failed.[44] Their multivariate analysis revealed that low platelet count, OBI, high ALT levels after interferon therapy, and liver fibrosis stage were important independent factors associated with HCC development.[44] They concluded that OBI is a risk factor for HCC development in patients with chronic HCV infection in whom HCV eradication has failed. [44]

Obika et al. suggested in a prospective study that a high amount of HBV DNA in liver tissue of HBsAg-negative patients with HCV-related liver disease was associated with HCC development.[45]

In contrast, Anna et al. reported that there was no difference in prevalence of anti-HBc in serum or HBV DNA in liver between patients who did or did not develop HCC.[46] Anti-HBc was present in the serum of 41.8% of HCC cases and 45.6% of controls (*P* = 0.54), and HBV DNA was detected in the liver of 10.7% of HCC cases and 23.6% of controls (*P* = 0.18).[46] They reached the conclusion that OBI is not an important factor in HCC development among patients with advanced chronic HCV infection.[46] In addition, Tsubouchi et al. found in a community-based cohort study that there was no significant difference in the incidence of HCC and cumulative mortality associated with liver-related death between HCV carriers who were positive and those who were negative for anti-HBc.[47]

Recently, Shi et al. conducted a meta-analysis of prospective and retrospective studies to investigate whether OBI increases the risk of HCC.[48] They demonstrated in 12 eligible studies that OBI increases the risk for HCC in HCV-infected populations [risk ratio (RR) = 2.83, 95% confidence interval (CI) = 1.56–4.10].[48]

In summary, these results suggest that OBI may increase the likelihood of developing HCC in patients with chronic HCV infection. However, to reach the conclusion that OBI plays a pivotal role in the development of HCC in patients with HCV-related liver disease, further studies on molecular pathogenesis and prospective molecular epidemiological studies will be required. Previous prospective studies evaluating the effect of OBI or potential OBI on liver carcinogenesis in patients with HCV-related liver disease are listed in Table 1.

Table 1. Prospective studies evaluating the effect of occult B infection (OBI) or potential OBI on carcinogenesis in patients with chronic hepatitis C.

Author	Country	Year	Study design	No. of patients	No. of HCC occurrence / OBI or potential OBI positive patients (%)	No. of HCC occurrence / OBI or potential OBI negative patients (%)
Hasegawa, et al[18]	Japan	2005	Prospective	140	2 / 11 (18.2%)	16 / 129 (12.4%)**
Squadrito, et al[39]	Italy	2006	Prospective	124	8 / 50 (16.0%)	1 / 74 (1.4%) *
Ikeda, et al[16]	Japan	2007	Prospective	846	130 / 392 (33.2%)	107 / 454 (23.6%) *
Matsuoka, et al[32]	Japan	2008	Prospective	468	29 / 204 (14.2%)	9 / 264 (3.4%) *
Obika, et al[45]	Japan	2008	Prospective	167	2 / 25 (8.0%)	10 / 142 (7.0%) **
Anna, et al[46]	USA	2011	Prospective	273	38 / 121 (31.4%)	53 / 152 (34.9%) **

HCC; hepatocelluar carcinoma, *; statistically significant, **; statistically not significant

Influence of OBI on Carcinogenesis in Patients with Cryptogenic Liver Disease

OBI can be detected in patients with cryptogenic chronic liver disease and may be related to the progression of liver fibrosis.[49-51] However, specific guidelines for the management of OBI-related liver disease are yet to evolve. OBI has also been demonstrated in patients with autoimmune hepatitis and nonalcoholic steatohepatitis.[52, 53] The frequency of OBI in patients with cryptogenic liver cirrhosis has ranged from 4.8% to 40% in previous studies, depending on the prevalence of HBV and the type of specimen examined (liver or serum).[54-56] Interestingly, a previous study has suggested that HBV DNA is integrated into the human genome in non-B and non-C (NBNC) HCC tissues, indicating that OBI might play a role in hepatocarcinogenesis in patients with NBNC liver disease.[57] OBI has thus a negligible role in the pathogenesis of cryptogenic chronic liver disease.

Ikeda et al. reported that in 82 patients with NBNC liver cirrhosis, the presence of HBc and HBx DNA, which is a transactivator of viral regulatory elements and host gene promoters with a potential role in carcinogenesis, was an independent factor associated with liver carcinogenesis (RR = 8.25, P = 0.003).[17,58,59] Yotsuyanagi et al. studied HBV DNA in serum and liver tissues in 42 patients with NBNC HCC and reported that HBV DNA was detected in the sera of 20 (47.6%). This was significantly higher than in age-matched controls without liver disease (P < 0.001), suggesting that HBV is associated with a substantial proportion of NBNC HCC cases.[60] In contrast, Kusakabe et al. found a weak association between OBI and HCC development in patients with NBNC liver disease.[61] Furthermore, Shi et al. demonstrated in a meta-analysis of four eligible studies that OBI increased the risk of HCC development with an HR of 10.65 (95% CI = 5.94–10.98) in patients with chronic liver disease unrelated to hepatitis virus.[48]

Overall, OBI may play a significant role in promoting tumor growth unrelated to hepatitis virus. In patients with hepatitis-unrelated chronic liver disease, the risk of HCC might be entirely attributed to OBI; however, further studies will be needed to confirm these hypotheses.

Effect of OBI on Survival in Patients with HCC Who Underwent Curative Therapy

HCC is diagnosed in more than half a million people worldwide each year, and therefore it is a major global health problem. HCC is the fifth most common cancer in the world and the third most common cause of cancer-related death.[62-64] HCC often

recurs. The prognosis of HCC is generally poor because of a high post-treatment recurrence rate. Recurrence only occurs at intrahepatic sites in 68–96% of patients. The identification of prognostic factors for HCC recurrence is thus essential for improving survival, even after curative treatment.[2,65-67] The pathogenesis of HCC recurrence has been extensively examined and various risk factors associated with HCC recurrence have been identified.[68-70] However, whether potential OBI can be a risk factor linked to HCC recurrence after curative therapy for HCC remains elusive.

Kubo et al. examined the effect of anti-HBc positivity on survival in 59 patients who underwent liver resection for HCV-related HCC (<2.0 cm in greatest dimension).[71] Tumor-free survival tended to be lower in patients with anti-HBc positivity than in those without anti-HBc positivity (P = 0.0758).[71] The cumulative survival rate was significantly lower in patients with anti-HBc positivity than in those without anti-HBc positivity (P = 0.0224), with an RR of anti-HBc for shorter survival of 3.817.[71] We also analyzed the influence of anti-HBc positivity on survival in 222 patients with HCV-related, HBsAg-negative HCC who underwent curative surgical resection [119 anti-HBc-positive patients (53.6%) and 103 anti-HBc-negative patients (46.4%)].[72] The 3-year cumulative overall survival rate was 70.2% in the anti-HBc-positive group and 77.1% in the anti-HBc-negative group (P = 0.300). The corresponding recurrence-free survival rates were 33.0% in the anti-HBc-positive group and 38.5% in the anti-HBc-negative group (P = 0.482).[72] We thus concluded that anti-HBc positivity cannot be a useful predictor for survival in patients with HCV-related HCC after curative surgery.[72] Whether anti-HBc positivity constitutes an additional risk in terms of survival after curative therapy for HCV-related HCC therefore appears to be controversial.

In patients with NBNC HCC who undergo curative therapy, it is also unclear whether anti-HBc positivity is a predictor for survival. In our single-center experience, in 260 patients with NBNC HCC who underwent curative therapy [124 anti-HBc-positive patients (47.7%) and 136 anti-HBc-negative patients (52.3%)], the 1-, 3-, and 5-year recurrence-free survival rates were 65.1, 29.8, and 14.0%, respectively, in the anti-HBc-positive group and 78.9, 43.0, and 36.7% in the anti-HBc-negative group (P = 0.001).[49] By multivariate analysis, anti-HBc positivity was an independent predictor linked to HCC recurrence with an HR of 1.567 (P = 0.006).[49] Anti-HBc positivity may be a useful predictor for recurrence in patients with NBNC HCC after curative therapy; however, because there have been few reports regarding the effect of OBI on

survival in patients with hepatitis-unrelated HCC, further studies will be needed.

OBI and Risk of Other Malignancies

OBI has been reported as a potential risk factor for the development of other malignances. Wang et al. conducted a meta-analysis involving 5883 patients with pancreatic cancer.[73] They demonstrated that the RR for development of pancreatic cancer was 1.76 (95% CI = 1.05–2.93) in anti-HBc-positive but anti-HBs-negative individuals, and concluded that OBI may increase the risk of pancreatic cancer.[73] In patients with hematological malignances, the presence of OBI cannot be ignored because HBV reactivation may occur in patients with OBI undergoing immunosuppressive therapy or chemotherapy, where it is often associated with the fulminant course of hepatitis, as well as in those with HBsAg positivity.[4,9,11] Liu et al. found in 405 patients with non-Hodgkin lymphoma that the positive rate of anti-HBc was 47.9%, which was significantly different from that in the general population (*P* < 0.01).[74] Furthermore, Rossi et al. examined the prevalence of OBI in 173 patients with chronic lymphocytic leukemia.[75] OBI was observed in 17/173 (10%) patients and 5/173 (3%) age- and sex-matched controls (RR = 3.6, 95% CI = 1.37–9.79, *P* = 0.014). The tumorigenic mechanisms caused by OBI need to be elucidated.[75] Clinicians should also be alert to the presence of OBI in patients with other malignances.

Conclusions

OBI is a complex disease entity comprising different situations. The definition of OBI should be based on high-sensitivity HBsAg and HBV-DNA testing. The cumulative evidence has revealed its possible implication in various clinical contexts. The oncogenic potential of OBI has become progressively evident. OBI may exert oncogenic activity through direct and indirect mechanisms, and it can affect liver disease progression and hepatocarcinogenesis in patients with other viral causes, particularly HCV, as well as in those with hepatitis-unrelated liver disease. Whether OBI is associated with secondary carcinogenesis in HCC patients after curative therapy remains unclear. Further studies examining this issue should be conducted in the future. OBI may be a potential risk factor for development of other cancers.

Acknowledgments

Competing Interests

References

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