



## Review Article

# Occult Hepatitis B : A Hidden Threat

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### Abstract

Occult hepatitis B (OHB) is defined as the presence of HBV-DNA in liver with or without detectable HBV DNA in serum in patients who are HBsAg negative in currently available assays. Diagnosis of occult HBV infections requires sensitive HBV-DNA PCR assay. Though reports of occult hepatitis B infections are coming from almost every corner of the world, the exact prevalence of this hidden menace is still not know. Several aspects of occult hepatitis B infection are still not resolved including its clinical significance relating to the molecular basis, risk of transmission, reactivation and progression to chronic liver disease. In this review article, we will try to explore the current concepts and clinical implications of occult hepatitis B virus infection.

TAJ 2010; 23(1): 109-113

### Introduction

Hepatitis B virus (HBV) infection is one of the major global human health problems. It is estimated that more than 350 million people world-wide are affected by HBV infection.<sup>1</sup> The spectrum of HBV-related disease ranges from acute hepatitis B, asymptomatic HBV carrier, chronic hepatitis, to rarely fulminant hepatitis. Chronic HBV infection is also associated with cirrhosis and hepatocellular carcinoma. Early studies revealed that clearance of HBsAg in patients with HBV infection is associated with disappearance of viraemia and remission of the disease. However, accumulated data indicated that a low level of HBV-DNA remains detectable in serum and liver tissue in some patients who cleared HBsAg from either acute self-limited or chronic HBV infection, or even after a successful

anti-HBV treatment. Demonstration of this clinical entity has resulted in introduction of the concept of occult, silent, or latent' HBV infection, which defines presence of HBV infection with undetectable HBsAg. Occult hepatitis B as a concept is not a new one. In 1978, Hoofnagle et al reported that transfusion with blood containing anti-HBc, but not HBsAg and anti-HBs, resulted in HBV infection in the recipients.<sup>2</sup> This provided direct evidence that patients with positive anti-HBc alone may harbor HBV and transmit infection. Subsequently, it was found that HBV-DNA in the liver and blood of HBs Ag-negative individuals that were positive for anti HBc has been published. Blum et al described presence of HBV-DNA in a patient with HBsAg-negative chronic hepatitis who was positive for anti-HBc.<sup>5</sup> Michalak et al demonstrated the long-term

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persistence of HBV DNA in serum and peripheral blood mononuclear cells of patients up to 70 months after complete clinical, biochemical, and serological recovery from acute viral hepatitis.<sup>6</sup> These findings suggest that recovery from acute hepatitis B virus may not result to complete virus elimination, but rather the immune system keeps the virus at very low level and routine serological profiles are not always reliable in determining status of HBV infection. These findings further confirm the functional competence and infectivity of HBV present HBsAg-negative patients. It is now clear that HBsAg-negative i.e. occult HBV infection is a genuine clinical entity.

### **Serological patterns of occult hepatitis B**

About 20% of OHB sera are negative for all serological markers of HBV infection except HBV DNA, 50% are positive for hepatitis B core antibody ( $\pm$ anti-HBc), and 35% are positive for hepatitis B surface antibody ( $\pm$ anti-HBs). On the basis of these HBV antibody profiles, occult hepatitis B may be further stratified into seropositive or seronegative categories with the seronegative subjects being negative for both anti-HBc and anti-HBs. The HBV DNA Levels are lowest in these subjects. Seropositive individuals can be further divided into two groups: Anti-HBc positive, with or without anti-HBs negative, and these individuals are more likely to be infectious. When present, HBV DNA levels are intermediate in anti-HBc- and anti-HBs-positive persons.<sup>7</sup>

### **Molecular basis of occult hepatitis B infection**

The molecular basis of OHB is linked to the peculiar life cycle of HBV. A key step in replication of the virus is conversion of the 3.2-kb circular DNA into a covalently closed circular DNA (cccDNA) in the nucleus of infected hepatocytes. The cccDNA is the template for transcription leading to production of new virions. This highly stable cccDNA is resistant to enzymatic digestion or to current antiviral agents and is the basis for persistence of HBV infection. The median cccDNA copies/hepatocyte is estimated to be approximately 1.5, but ranges from  $<0.01$  to  $>50$  copies/cell.<sup>9</sup> These levels strongly correlate with intracellular and serum HBV DNA

and are lowest in patients with occult hepatitis B.<sup>10</sup> The reasons for persistence of low levels of HBV DNA in the absence of detectable HBsAg remain largely undefined, but it is conjectured that both host and viral factors are important in suppressing viral replication and keeping the infection under control.<sup>11</sup> In HBV sequences obtained from serum samples of HBsAg seronegative carriers, a plethora of mutations has been observed. Point mutations, deletions and splicing alternatives have been associated with occult HBV, infection. Many of these occult infection associated mutations reside in the S gene and/or regions governing the regulations of S gene expression, but they have also been documented for the core (C) and polymerase (P) genes.<sup>12</sup>

### **Diagnosis of occult hepatitis B infection**

Based on current definition of occult hepatitis B infection endorsed by the European Association for the Study of the Liver extracts for HBV DNA appears to be best approach for diagnosis. But liver tissue is not always available, and standardized and valid assays for detection of HBV DNA in liver tissue are not FDA approved. At present, the optimal standard for diagnosis is the analysis of HBV DNA extracts from plasma performed by real-time, nested polymerase chain reaction (PCR) techniques. To avoid false-negative and false-positive results, these assays should employ PCR primers that span at least three genomic regions of the HBV genome such as the S, X and core genes, and validation should require detection from at least two regions of the genome.<sup>13</sup> The preferred lower limit of detection of HBV DNA is 30 copies/mL. This is the smallest concentration of an analyte that can be distinguished from a blank or negative specimen in a single test with a 95% level of confidence.

### **Prevalence of occult hepatitis B in different parts of the world**

Occult HBV infection has been reported in 0.1-2.4% of Hs Ag-negative, anti-HBc-positive ( $\pm$ HBs) blood donors in Western countries such as the United States.<sup>14</sup> Among the general population in Asia with normal ALT levels, the prevalence of occult HBV has ranged from 7.5% to 16%.<sup>15</sup>

### **Clinical implications of occult hepatitis B infection**

Transmission of HBV infection been documented from HBsAg-negative, anti-HBc- positive blood and organ donors. The risk is variable (0.4%-90%,) It is highest when livers from anti-HBc-positive donors are transplanted to seronegative recipients.<sup>2,16</sup> In the United States, blood and organ donors are routinely screened for anti-HBc. In general, blood and organs from HBsAg-negative, anti-HBc-positive donors are not used.<sup>17</sup>

However, the risk of transmission appears to be negligible when concurrent anti-HBs is present in the blood above a certain level (100-200mIU/mL). In this regard, we observed that blood containing level (100-200mIU/ML). In this regard, we observed that blood containing detectable anti-HBs carries no increased risk of transmitting hepatitis B when compared with blood that lacks this antibody.<sup>18</sup> The majorities of OHB cases are secondary to overt HBV infection and represent a residual low viraemia level suppressed by strong immune response together with histological derangements occurred during acute or chronic HBV infection. Moreover, immune response to hepatocytes sustaining a low HBV replication level may contribute to chronic liver damage in the setting of OHB. Berasain et al showed that approximately 50% of patients with persistent hypertransaminasaemia of unknown etiology have chronic hepatitis or cirrhosis due to occult HBV or hepatitis C virus (HCV) infection.<sup>19</sup> The proportion of OHB among cryptogenic liver disease patients varies based on HBV endemicity and the methods used for HBV DNA and HBsAg detection. In patients with HBsAg-negative chronic hepatitis, cirrhosis or HCC, HBV DNA has been detected in serum and/or liver in approximately 19-67% regardless of the presence of other seromarkers.<sup>8,20</sup> In the occult HBV study by Chemin and colleagues, HBV DNA was detected in the serum as well as in the liver in 15 of 50 patients (30%) with chronic hepatitis of unknown aetiology. Among these patients, the viral load was invariably less than  $10^4$  copies/ML with a median level of 400 copies/ML.<sup>20</sup> In a recent study from China, HBV DNA was detected in the sera of

28% of 159 subjects with cryptogenic liver disease, and all of these patients were positive for anti-HBc.<sup>15</sup> As these were not prospective studies in which disease could be chronicled from the beginning, conclusions regarding progression of liver disease in patients with occult HBV should be judged accordingly. The presence of OHB in chronic HCV infection is well established. Both viruses share common routes of infection, and both are transmitted parenterally. Hence, coinfection with HCV and HBV is frequent, particularly in areas of high endemicity and among individuals at high risk for parenteral infections. OHB has been reported in up to 65% of patients with HCV and is a risk factor for cirrhosis and HCC.<sup>21</sup> OHB is also known to decrease the response to interferon therapy when employed in patients with chronic hepatitis C.<sup>22</sup> In a recent study from China, HBV DNA was detected in 70% of 135 HBsAg-negative patients with HCC in the absence of chronic HCV.<sup>15</sup> Liver grafts from donors who are HBsAg negative but anti HBc positive can transmit HBV to susceptible recipients after transplantation at an incidence rate of 17-94%.<sup>23</sup> Liver transplant recipients with serological evidence of past infection to hepatitis B (anti-HBc positive) May have reactivation of OHB under immunosuppression in the post-transplant period. In two retrospective studies, reactivation of HBV was mild in the transplant recipients with previous serological immunity without any impact on patient or survival. None of these recipients received any prophylaxis after transplantation.<sup>24</sup> In patients with OHB, there is a change of viral reactivation following immunosuppression or transplantation. In a recent study from Japan, de novo HBV reactivation (reverse seroconversion) was observed in 4.2% of 48 HBsAg-negative, anti-HBc-positive patients who had received intensive chemotherapy including steroid-containing regimens plus rituximab for lymphoma.<sup>25</sup> Reactivation with or without liver disease is lowest in recipients of hematopoietic stem cell from donors who are immune and highest in anti-HBc-positive and anti-HBs-negative allogeneic hematopoietic stem cell transplantation patients receiving immunosuppressive therapy.<sup>26-27</sup>

## Treatment of occult hepatitis B

Treatment should be considered during reactivation and cirrhosis settings. The reactivation of OHB in hemato-oncological malignancies (<5%), although at a lower rate than that of HBsAg positive cases, carries a significant risk of mortality and morbidity, which is much higher in the setting of stem cell transplantation.<sup>28, 29</sup> Many fatalities especially due to rituximab containing regimens have also been reported.<sup>30</sup> Although a definitive conclusion cannot be reached at the moment, targeted therapy via HBV DNA monitoring or even routine pre-emptive nucleoside analogue prophylaxis was offered to all HBsAg negative/anti-HBc positive patients in recent consensus reports.<sup>31</sup> Moreover, recent guidelines offer therapy for cirrhotic patients with a detectable HBV DNA level.<sup>32</sup>

## Conclusion

Though known to the scientific community for a long time, clinical significance of occult hepatitis B at community level is still under evaluation. It is not also certain whether OHB infection. Further research is needed to explore the molecular basis, correlation with chronic liver disease and optimum treatment of OHB.

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