

## PREVALENCE OF OCCULT HEPATITIS B INFECTION AMONG KIDNEY DONORS WITH ANTIBODIES TO HEPATITIS B CORE ANTIGEN

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### ABSTRACT

**Background and Objectives:** Occult hepatitis B virus infection is defined as the presence hepatitis B virus DNA (HBV DNA) in the serum or tissues of persons who have negative test for hepatitis B surface antigen (HBsAg). It is believed that transmission of hepatitis B infection from blood and organ donations negative for HBsAg is due to HBV occult infection. The aim of this study was to determine the prevalence of occult hepatitis B infection and the level of viremia among these individuals with occult infection.

**Methods:** Data on hepatitis B profile, HBV-DNA titer and alanine aminotransferase (ALT) levels of 398 subjects enlisted for donor nephrectomy from June 2006 to June 2008 at the National Kidney and Transplant Institute (NKTi) were reviewed.

**Results:** One hundred twenty five of the 398 subjects (31.4%) included in the study were positive for anti-HBc; 12/125 (9.6%) has detectable serum HBV DNA levels. Antibody to hepatitis surface antigen (anti-HBs) was detected in 8/12 (66.7%) of the HBV-DNA positives. ALT levels in seven of the subjects (58.3%) with occult hepatitis B were normal.

**Conclusion:** There is high prevalence rate (9.6%) of occult hepatitis B infection among kidney donors at the NKTi. Patients with occult hepatitis B infection have low viremia (<6 IU/mL). There is no reverse correlation between the levels of HBV DNA and anti-HBs titer. The level of ALT also does not correlate with the amount of HBV DNA in the serum.

**Keywords:** anti-HBc; HBsAg; Hepatitis B virus; HBV DNA; prevalence

### INTRODUCTION

About 5% of the world's population is infected with the hepatitis B virus (HBV).<sup>1</sup> One of the greatest challenges in transfusion medicine is the prevention of transmission of HBV through blood transfusions. Since the dawn of HBV surface antigen (HBsAg) screening for all blood products for transfusion in the 1970s, the risk of transfusion-transmitted HBV infection has been greatly reduced. But recently, there have been reports of post-transfusion hepatitis B infection from blood products which were tested negative for HBsAg.<sup>2</sup> There have been cases of seroconversion of cancer patients who were previously HBsAg negative becoming HBsAg positive after or during chemotherapy. In areas with low endemicity for HBV, such as the United States and Canada, studies have shown that some individuals who test negative for HBsAg but positive for antibody to hepatitis core antigen (anti-HBc) were found to have low viremia for HBV. This was made possible when sera were tested for HBV DNA using the polymerase chain reaction technology (PCR).<sup>3</sup> The term occult hepatitis B infection was then introduced.

Occult hepatitis B infection is generally defined as the detection of HBV-DNA in the sera or tissues of subjects who have negative tests for HBsAg, with or without anti-HBc or antibody to HBV surface antigen (anti-HBs), outside the pre-seroconversion window period.<sup>4</sup> It is related to low level of HBV infection with non-detectable levels of HBsAg in the serum, and may also be due to infection with HBV variants or mutants.

Occult hepatitis B infection has been reported in populations asymptomatic for any signs of liver disease, that is, in healthy blood donors in low endemic countries like Canada, Germany, Brazil and Italy.<sup>5,2,6,7</sup> In both high and low endemic areas with HBV infections, positive HBV DNA results in subjects with negative HBsAg have been shown.<sup>8</sup>

In the Philippines, a country highly endemic for HBV infection, there is limited data available on occult hepatitis B infection. Therefore, risk of transmission of HBV because of occult infection, whether through blood transfusion or organ donation,

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is high. Reactivation of hepatitis infection in patients with occult infection during immunosuppression is possible.

The aim of this study was to determine the prevalence of occult hepatitis B among those who tested positive to anti-HBc but negative for HBsAg. It also focused on determining the level or titer of HBV DNA among individuals with occult hepatitis B infection.

## MATERIALS AND METHODS

All donors enlisted for donor nephrectomy at the out-patient department of the National Kidney and Transplant Institute (NKTi) and Human Organ Preservation Effort (HOPE) from June 2006 to June 2008 were included in this study. All subjects included were negative for HBsAg and antibody for hepatitis C (anti-HCV). A systematic computer search for all the subjects' information regarding their hepatitis B profile (which includes HBsAg, anti-HBc, anti-HBs), HBV-DNA titer and alanine aminotransferase (ALT) levels was done using the NKTi's laboratory database. Standard or normal values used were as prescribed by NKTi's laboratory.

All subjects were screened for anti-HBc, HbsAg and anti-HBs with an assay using Generation Microparticle Enzyme Immunoassay (MEIA). HBV DNA testing was done using PCR (Chain Reaction) technique in Real Time, which detected HBV particle as low as 6 IU/mL.

Records were analyzed and results were tallied. The prevalence rates were then computed.

## RESULTS

A total of 398 subjects were included in the study, 300 males with mean age of 39.1 years and 98 females with mean age of 41.1 years. One hundred twenty five (125) of all the subjects (31.4%) were positive for anti-HBc. Twelve of the anti-HBc positives, 12/125 or 9.6%, has detectable serum HBV DNA levels. Of the twelve who tested positive for HBV DNA, ten has levels <6 IU/mL, while the other two has levels >50 IU/mL. Anti-HBs were detected in 8/12 (66.7%) of those who were HBV-DNA positives, with titers of anti-HBs >10 IU/mL. In all subjects who tested positive for HBV DNA, only 4/12 (33.3%) has elevated ALT (mean value of 102.25 IU/mL).

## DISCUSSION AND CONCLUSION

In the Philippines, testing for the presence of HBsAg is the initial diagnostic examination used to determine HBV infection. Anti-HBc was used to determine previous exposure to the hepatitis B virus but not as a determinant of infection.

In this study, it was found that 9.6% of those who are HBsAg negative but anti-HBc positive have detectable levels of HBV DNA in their serum. This prevalence rate of occult hepatitis B is higher compared to non-endemic countries like Germany (1.59%),<sup>2</sup> Italy (4.96%)<sup>7</sup> and Brazil (0.85%).<sup>6</sup>

In occult HBV infection, higher rates of detection and titers of HBV DNA are found in the liver or peripheral mononuclear cells, as compared with serum or plasma.<sup>9</sup> Of those who tested positive for HBV DNA in our study, they showed only very low viremia (<6 IU/mL). The sensitivity of the PCR technique corresponds to the load of intact virus particles which could form immune complexes with antibodies, particularly with anti-HBs. It seems likely that there would be more HBV DNA positive donors and possibly higher titers of HBV DNA detected if larger quantities of plasma will be tested.<sup>2</sup>

The rationale for the presence of traces of HBV in the blood even after many years from clinical recovery from acute hepatitis suggest that sterilizing immunity to HBV frequently fails to occur. Traces of virus can maintain the presence of serum antibodies and HBV-specific cytotoxic T lymphocytes (CTL) response for decades, apparently creating a negative feedback loop that keeps the virus under control for life.<sup>2</sup> Persistence of HBV DNA in HBsAg negative subjects may mean presence of HBsAg levels but undetectable by standard immuno-assays due to low level of viral replication.<sup>8</sup> In this pool of patients, they usually test positive for anti-HBs. Another mechanism that could explain presence of HBV DNA in the absence of any HBV serologic markers is the genetic variability of HBV. Mutations in the S gene of HBV has been described that could count for negative HBsAg assay.<sup>8</sup>

Anti-HBs was believed to be an indicator of immunity because of its neutralizing effect on HBV, especially if found in high titers. It was previously believed that with anti-HBs titer more than 10 IU/mL, HBV should be suppressed, if not entirely eradicated.<sup>9</sup> In our study, we could not find any reverse correlation between the levels of HBV DNA and anti-HBs. Eight of the 12 (66.7%) HBV DNA positives has anti-HBs levels >10 IU/mL.

In standard practice, the best diagnostic test to assess liver inflammation is liver biopsy. But ALT has been used as a surrogate marker for liver inflammation. In this study, the levels of ALT do not correlate with the presence of HBV DNA titer in the serum. Seven of the 12 subjects with low HBV viremia (58.3%) has normal ALT levels (<65 IU/mL). Because only low level of viremia in occult hepatitis B exists, this may also mean low level, if not absence, of liver inflammation.

The major implication of occult hepatitis B infection is the sustenance of chronic HBV infection because of its low replication rate, undetectable with standard immunoassays for HBsAg determination. Because of this, patients who will undergo immunosuppression, that is, those who will undergo organ transplantation or chemotherapy, who have undiagnosed occult hepatitis B infection, will have high risk of viral reactivation followed by mild hepatitis, even fulminant hepatitis and death. Viral reactivation in HBV carriers undergoing immunosuppression ranges from 20-70%.<sup>11</sup> In such cases, pre-emptive antivirals for hepatitis B play a significant role in preventing occurrence of reactivation which may result to a fatal fulminant course.<sup>12</sup>

Liver inflammation and fibrosis solely due to occult hepatitis B infection may be debated. However, it can occur if associated with concomitant hepatitis C infection and/or alcoholism which increase the risk for liver cirrhosis and hepatocellular carcinoma.

**Table I. The Rates of HBV Markers and HBV-DNA**

Total no. of subjects	HBsAg (-) rate	HBsAg (-) and anti-HBc (+) rate	HBV-DNA (+) rate	Anti-HBs (+) rate
398	398/398 (100%)	125/398 (31.4%)	12/125 (9.6%)	8/12 (66.7%)

**Table II. HBV titer, anti-HBs and ALT levels among subjects with occult hepatitis B**

Subjects	HBV DNA titer (IU/mL)	anti-HBs level (IU/mL)	ALT level (IU/mL)
1	<6	276.9	180
2	<6	663.9	71
3	<6	NR	38
4	<6	365.4	85
5	58	166.4	47
6	55	NR	38
7	<6	56.8	32
8	<6	NR	45
9	<6	>1000	40
10	<6	471.2	(-)
11	<6	NR	40
12	<6	223.2	73

## RECOMMENDATIONS

Obligatory anti-HBc screening is recommended in all transplantation settings (solid organ and bone marrow transplant) for both donors and recipients. All patients who will receive chemotherapy should also be screened for anti-HBc. If positive for anti-HBc, HBV DNA titer should be tested to determine level of viremia which will serve as baseline measurement prior to initiation of immuno-suppression. Pre-emptive use of antivirals to prevent hepatitis B reactivation is highly recommended.

The potential infectivity of blood units which are HBsAg negative but anti-HBc positive is debated. But their use cannot be considered safe at least in immunocompromised patients. Therefore, it is also recommended to include HBV Nucleic Acid Testing (HBV NAT), which is cheaper than PCR, as a screening test for blood donors to prevent possible transfusion of blood products with occult hepatitis B.

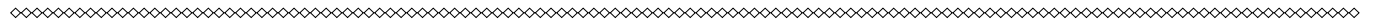
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