Iranian Journal of Pathology (2009)4 (2), 80 - 84

# Original Article

# Significance of Hepatitis B Core Antibody as the Only Marker of Hepatitis B Virus Infection in High Risk Patients

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## ABSTRACT

***Background and Objective:*** **Presence of hepatitis B core antibody (anti-HBc) in the absence of hepatitis B surface antigen (HBsAg) and hepatitis B surface antibody (anti-HBs) is defined, as isolated anti-HBc. little is known about the clinical significance of the isolated anti-HBc in hepatitis B virus (HBV) infections. The aim of this study was to assess the significance of anti-HBc as the only marker of HBV infection in high risk patients.**

***Patients and Methods:*** **In this cross-sectional study, 395 patients including 289 patients on chronic hemodialysis (HD) and 106 HIV infected subjects were enrolled. HBsAg, anti-HBs, anti-HBc, Hepatitis C antibody (anti-HCV) and Alanine aminotransferase (ALT) levels were tested in all subjects. The presence of HBV-DNA was determined quantitatively in plasma samples of patients with isolated anti-HBc by real-time PCR.**

***Results:*** **Of 395 patients, 40 (10.13%, 95% CI, 7.1%-13.1%) had isolated anti-HBc. HBV-DNA was detectable in 12 of 40 patients (30%, 95% CI, 15.8%-44.2%) who had isolated anti-HBc.**

***Conclusion:*** **Our study showed that detection of isolated anti-HBc could reflect unrecognized HBV infection; hence, screening of these patients is useful to preventing of HBV transmission.**

**Keywords: Hepatitis B Core Antigens, Hepatitis B Antibodies, Immunologic Marker, Iran**

## Introduction

Hepatitis B Virus (HBV) infection is a serious global health problem, with 2 billion people infected worldwide and 350 million suffering from chronic HBV infection. Of these, 75% are Asians (1).

Presence of hepatitis B core antibody (anti-HBc) in the absence of hepatitis B surface antigen (HBsAg) and hepatitis B surface antibody (anti-HBs) is defined as isolated anti-HBc (2). This serological pattern is observed in 10%-20% of individuals from areas of low endemicity for HBV (2). Now Iran is in the low endemic area of HBV infection (3). In one study on 4930 subjects in Iran, 5.13% were only positive for anti-HBc, without any detectable HBsAg (4).

The significance of this serological pattern is unclear. It may reflect past infection with HBV, after which anti-HBs either did not develop (5) or decreased to an

*Received: 12 September 2008*
*Accepted: 20 January 2009*
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undetectable level (2). Also, this serological pattern can be observed in the window phase of a resolving case of acute hepatitis B. Finally it may represent occult chronic HBV infection, with levels of the HBsAg below the limits of detection (6).

Occult HBV infection is characterized by presence of HBV infection with undetectable HBsAg. Serum HBV level is usually less than $10^4$ copies/ml in these patients. Diagnosis of occult HBV infection requires sensitive HBV-DNA PCR assay (7). A number of explanations for the persistence of HBV-DNA in HBsAg negative samples have been proposed, including integration of HBV-DNA into host's chromosomes (8), genetic variations in the S gene (9,10) and the presence of immune complexes in which HBsAg may be hidden (11;12). Occult hepatitis B may also be due to the window period following acute HBV infection, poor laboratory detection of HBsAg due to low level of HBs antigenemia, underlying hepatitis C virus (HCV) co-infection, immunosuppression, or other host factors (2,7).

The clinical implications of occult HBV infection involve different clinical aspects. First of all, occult HBV infection harbors potential risk of HBV transmission through blood transfusion, hemodialysis, and organ transplantation. Second, it may serve as the cause of cryptogenic liver disease; contribute to acute exacerbation of chronic hepatitis B, or even fulminant hepatitis. Third, it is associated with development of hepatocellular carcinoma. Fourth, it may affect disease progression and treatment response of chronic hepatitis C (7).

Occult HBV infection is most frequently seen in patients with anti-HBc as the only HBV serological marker (13). It is estimated that in individuals with HBV infection, 10-20% present with a positive anti-HBc as the only HBV maker (2).

Little is known about the clinical significance of the isolated anti-HBc in HBV infections. The aim of this study was to assess the significance of anti-HBc as the only marker of HBV infection in high risk patients.

## Material and Methods

In this cross-sectional study, 395 patients including 289 patients on chronic hemodialysis (HD) and 106 HIV infected subjects were enrolled. A questionnaire that gathered epidemiological, clinical and therapeutical data was completed by clinicians. This project was approved by the Iranian Society for Support Patients with Infectious Diseases Ethics Committee and informed consent was obtained from patients prior to their enrollment.

Blood samples were collected from all patients and plasmas were stored at -80ºC. All samples were tested for HBsAg, anti-HBs, hepatitis C antibody (anti-HCV) and anti-HBc by ELISA. The commercial enzyme immunoassay kits used were as follows: HBsAg and anti-HBs (Hepanosticka Biomerieux, Boxtel, The Netherlands), anti-HBc (Dia.Pro Diagnostic BioProbes, Milan, Italy), anti-HCV (Biorad, Segrate, Italy). Recombinant immunoblot assay (RIBA Innogenetics, Ghent, Belgium) was employed to confirm anti-HCV reactivity. Alanin aminotransferase (ALT) was also determined in all of the patients.

Human immunodeficiency virus antibody (anti-HIV) was determined by ELISA (MP Biomedicals, Illkirch, France); with positive tests confirmed by Western blot assay (Diaplus, San Francisco, USA). All assay protocols, cutoffs and result interpretations were carried out according to the manufacturers' instructions.

HBV-DNA was extracted using High Pure Viral Nucleic Acid kit (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's instructions. HBV-DNA was determined quantitatively by real-time PCR using the artus HBV RG PCR kit (QIAGEN, Hamburg, Germany) on the Rotor-Gene 3000 real-time thermal cycler (Corbett Research, Sydney, Australia). The analytical detection limit of the kit is 20 IU/ml according to the user manual.

The Mann-whitney U, Chi-square and $t^2$-tests were used with the SPSS 16 Package program for statistical analysis (Chicago, IL, USA). Data are presented as mean ± SD or, when indicated, as an absolute number and percentage. The 95% confidence interval (95% CI) was calculated.

## Results

A total of 395 patients including 289 patients on chronic HD and 106 HIV infected subjects were enrolled in this study.

The mean age of HD patients was 55±16 (range: 15-89) years. Sixty percent (60%) of them were male. The duration of HD was 5.2±5.1 years. The mean ALT levels in them was 16.4±9.4 IU/l. HBsAg, anti-HBs, anti-HCV and anti-HIV were found in 2.8%, 77.5%, 3.1% and 0.34% of these patients, respectively.

The mean age of HIV positive patients was 36.6±9.6 (range: 23-60) years. 74.5% of patients were male and 25.5% were female. The mean CD4 count was

349.08±181.07 (2-940) cells/mm$^3$. The mean $\log_{10}$ HIV viral load was 1.97±2.03. The mean ALT levels was 32.4±20.1 IU/l. The possible routes of HIV transmission were intravenous drug use (52.8%), infected husband (24.5%), heterosexual contact (3.8%), infected blood and blood products transfusion (4.8%), vertical transmission (3.8%), tattooing (1%), heterosexual contact and intravenous drug use (5.6%) and in 3.7% the route of HIV acquisition was not identified. Co-infection with HCV and HBV occurred in 67% and 3.8% of patients, respectively.

Of the 395 patients, 40 (18 HD and 22 HIV infected patients) had isolated anti-HBc (10.13%, 95% CI, 7.1%-13.1%). There was not any significant difference between patients with and without isolated anti-HBc regarding age, sex, length of time on dialysis, ALT levels and treatment with antiretroviral drugs.

HBV-DNA was detectable in 12 out of 40 patients (30%, 95% CI, 15.8%-44.2%) who had isolated anti-HBc. Of these 12 patients, 9 of them were HD pateints and 3 of them were HIV infected subjects. There was not any significant difference between HBV-DNA positive and negative patients regarding age, sex, ALT levels, length of time on dialysis and treatment with antiretroviral drugs.

## Discussion

In this study the significance of anti-HBc as the only marker of HBV infection was assessed. HBV-DNA was detectable in 30% of subjects with isolated anti-HBc. Our survey showed that occult HBV infection was relatively common in patients with isolated anti-HBc regardless of age, sex and ALT levels.

Occult HBV infection can occur in different clinical scenarios. Typically, seroclearance of HBsAg is followed by development of anti-HBs with coexisting anti-HBc. If anti-HBs remain undetectable, anti-HBc serves as the only marker indicating past HBV infection (14,15). The latter may be seen in three different situations : a resolving acute HBV infection after HBsAg disappeared, but anti-HBs remains undetectable (window period); a past HBV infection, especially an infection which occurred long time ago and anti-HBs has fallen to an undetectable level and a true occult HBV infection with low level of HBV replication (2,8,14-17) .

The frequency of detection of occult HBV infection depends on the relative sensitivity of both HBsAg and HBV-DNA assays. It also depends on the prevalence of HBV infection in the population (2).

In published studies the prevalence of occult HBV infection in HIV-seropositive populations ranged from 0% to 10% by standard PCR assays (18-20) to 35–89% by more sensitive methods (21,22). The prevalence of occult HBV infection in renal dialysis patients ranges between 0 and 58% in published reports (23-27). In a study conducted by Yildirim *et al.* (28) on 45 patients with isolated anti-HBc, 24.4% of them were HBV-DNA positive. In our study, 10.13% of patients exhibited isolated anti-HBc. Approximately 30% of this group was HBV-DNA positive. As mentioned before these discrepancies in the rate of occult HBV infection may reflect the diverse prevalence of HBV infection in different countries, sensitivity of molecular biology techniques and size and virological features of the patient groups.

The role of occult HBV infection in the etiology of liver disease is still unclear. However, there is clear evidence that transmission of HBV by HBsAg-negative material occurs (29). In a study, flares of hepatic transaminases were found to occur more frequently in patients with occult HBV infection than in patients who tested HBV-DNA negative (30). However, there are also conflicting data found in an English study (31) which showed detection of HBV-DNA was not associated with elevated ALT levels. We also did not find any relationship between biochemical liver test and occult HBV infection in our study population.

The failure of demographic findings to suggest the presence of occult HBV is not unexpected. Detectable HBV-DNA in serum was not predicted by demographic parameters (32) and occult infections have been described in both high and low prevalence regions and various ethnic populations with no age or gender predilections (8,28) like our study.

## Conclusion

Our study showed that detection of isolated anti-HBc could reflect unrecognized HBV infection; hence screening of these patients is useful to preventing of HBV transmission.

## Acknowledgement

The authors are grateful to Iranian Society for Support Patients with Infectious Diseases for financial support of this study. The authors declare that there is no conflict of interests.

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