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Occult hepatitis B virus in liver tissue of individuals without hepatic disease☆

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***Background/Aims*: While many data are available concerning occult hepatitis B virus (HBV) infection in patients with hepatic disorders, there is little information about this cryptic infection in individuals without liver disease. The aim of this study was to investigate the prevalence of occult HBV in the general population by examining liver specimens from a large series of HBV-surface-antigen negative individuals with no clinical and biochemical evidence of liver disease.**

***Methods*: The presence of HBV DNA was evaluated by testing, through polymerase chain reaction techniques, DNA extracts from 98 liver-disease-free individuals who underwent liver resection or needle biopsy during abdominal surgery. Sixteen of them were anti-HBV-core antigen (anti-HBc) positive and 82 were HBV serum-marker negative. All patients were negative for antibody to hepatitis C virus.**

***Results*: Occult HBV infection was revealed in 16 of the 98 cases (16.3%). In particular, 10/16 anti-HBc positive (62.5%) versus 6/82 (7.3%) HBV-seronegative individuals were occult carriers ($p < 0.0001$).**

***Conclusions*: This study revealed that about 1/6 of the Italian general population might be carriers of occult HBV infection, and this condition is significantly associated with the anti-HBc positive status.**

***Keywords*: Occult HBV; HBV DNA; Normal liver; Anti-HBc; HBV-seronegative**

1. Introduction

Occult hepatitis B virus (HBV) infection is characterized by the persistence of HBV DNA in the liver of individuals negative for HBV-surface-antigen (HBsAg) [1–3]. Recent data demonstrated that occult HBV may exist in the hepatocytes as a free genome, its molecular basis being related to the long-term persistence in the hepatocyte nuclei of the viral covalently-closed-circular DNA (HBVcccDNA), a replicative intermediate that serves as template for gene transcription. Much evidence suggests that occult HBV infection may have a significant impact in several clinical contexts, since it might

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Abbreviations: HBV, hepatitis B virus; HBsAg, HBV-surface-antigen; anti-HBc, antibody to the HBV-core-antigen; anti-HBs, anti HBsAg antibody; HBVcccDNA, viral covalently-closed-circular DNA; OLT, orthotopic liver transplantation; PCR, polymerase chain reaction; SD, standard deviation.

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favour the progression of liver fibrosis and the development of hepatocellular carcinoma in patients with co-existing additional causes of liver damage, such as chronic hepatitis C virus (HCV) infection [4–6]. Moreover, occult HBV may acutely reactivate when an immunosuppressive status occurs, and it may be transmitted in the case of blood transfusion and organ transplantation, causing classic forms of hepatitis B in newly infected individuals (reviewed in [2,3]). Mainly, carriers of occult infection may be a source of HBV transmission in the event of orthotopic liver transplantation (OLT) as an obvious consequence of the fact that the hepatocytes are the site of the virus reservoir [7–9]. At present, however, it is really difficult to predict the frequency of events such as HBV reactivation or its transmission in cases of OLT, also due to the very little information available on occult HBV prevalence in the general population. In fact, since the amount of serum HBV DNA in the occult status is very low (if not totally absent) and the viral cccDNA reservoir is located in the hepatocytes, only the analysis of liver tissue extracts can provide an exact evaluation of occult HBV prevalence in a defined set of individuals [1–3]. In fact, whereas many data are available on occult HBV infection occurring in patients with liver disease and who undergo liver biopsy for clinical/diagnostic reasons, the extent of the occult HBV tissue in individuals without hepatic injury – who obviously represent the vast category of potential liver donors – is still largely unknown.

The aim of this study was to investigate the prevalence of occult HBV infection in a large series of subjects free from liver disease through the analysis of liver DNA extracts.

2. Methods

2.1. Patients

In this prospective study, we examined liver tissue specimens from 98 Italian patients [39 male, 59 female, mean age 54 years, 15.7 standard deviation (SD)] consecutively admitted from 2002 to 2006 to four surgery departments located in hospitals of three different Italian cities (Messina, Bergamo and Palermo). All the patients were negative for HBsAg and anti-HCV; 16 of them were positive for the antibody to the HBV-core-antigen (anti-HBc), 5 of whom were also positive for anti-HBsAg antibody (anti-HBs). HBsAg, anti-HBs and anti-HBc were determined, respectively, by AxSYM HBsAg (V2), AxSYM CORE and AxSYM AUSAB (Abbott Diagnostics, Abbott Park, IL). All cases had normal liver biochemistry and none had a clinical history of liver disease. Moreover, the histological examination of the liver tissue fragments (obtained either through surgical resection or needle biopsy performed during surgery) showed absence of fibrosis and absence or minimal signs of inflammation in all cases, although obese subjects had steatosis of different degrees. Eight individuals underwent surgery for benign liver tumours or liver cysts, 36 for gallstones, 26 for metastases in the liver from tumours of the colon (17 cases), pancreas (3 cases), stomach (4 cases), lung (1 case) and breast (1 case); finally, 28 subjects underwent laparoscopic gastric banding for morbid obesity. Once taken, part of each liver specimen was immediately frozen for subsequent molecular analyses.

2.2. Occult HBV DNA analysis

Frozen liver specimens were tested for occult HBV DNA through the methods detailed in our previous reports [5,6,10]. In particular, DNA extracts from each case were examined for the presence of HBV genomes by performing four different in-house nested-PCR amplification assays to detect preS-S, precore–core, Pol and X HBV genomic regions, respectively. We considered the cases that showed positivity in at least two different viral genomic regions as HBV DNA positive. Appropriate negative and positive controls were included in each PCR experiment. In particular, as negative controls we included in each PCR experiment (a) serum and tissue DNA extracts from subjects known to be negative for HBV infection, (b) DNA-free reaction buffer, (c) water. In addition, to eliminate false negative results the beta-globin was used as a house-keeping gene. Moreover, direct sequencing of all amplified HBV sequences confirmed the specificity of the reactions. The limit of sensitivity of our nested-PCR methods was 2×10^{-4} copy per liver cell.

The study protocol was approved by the Ethics Committee of the Messina University Hospital and informed consent was obtained from all patients. Statistical analyses were evaluated using the Student's *t*-test and the χ square method.

3. Results

HBV DNA sequences were detected in liver tissues from 16 out of the 98 cases examined (16.3%). In particular, among the 10 anti-HBc positive cases six were positive for all four HBV genomic regions examined (S, Core, Pol, X), two cases were positive for three genomic regions (one for Core, Pol, X; one for Pol, S and X) and two were positive for two genomic regions (one for Pol and S; one for Core and Pol); among the six HBV-seronegative individuals, three were positive for all four HBV genomic regions and three were positive for two genomic regions (two for Core, Pol; one for S and X) (Table 1). No statistical association as regards sex and

Table 1
Detection of S, Core, Pol and X viral genomic regions by nested-PCR in liver DNA extracts from 16 occult HBV infected cases according to the anti-HBc status

Cases	HBV genomic region			
	S	Core	Pol	X
Anti-HBc pos				
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	–	+	+	+
8	+	–	+	+
9	+	–	+	–
10	–	+	+	–
Anti-HBc neg				
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	–	+	+	–
5	–	+	+	–
6	+	–	–	+