

Occult B Infection in the Brazilian Northeastern Region: A Preliminary Report

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A sentinel study on viral hepatitis is currently being carried out in the village of Cavunge in a semiarid rural region of the state of Bahia, northeastern Brazil. This study has identified individuals in whom anti-HBc IgG was the only serological marker for hepatitis B virus (HBV). This serological pattern may constitute evidence of occult HBV infection. This study investigated the possibility of occult hepatitis B virus infection in individuals in a rural community who tested positive for anti-HBc IgG alone. A cross-sectional population-based study. ELISA III was performed on serum samples to test for serological viral markers, and ultrasensitive PCR (US-PCR) was used to assess viremia. Among the 1,536 serum samples, 3.6% (n=55) were positive for anti-HBc alone. Four years after this first serological survey, 31 of those 55 individuals (56.3%) were retested, and 11 (35.5%) remained anti-HBc positive alone. Two of these 31 (6.5%) were HBV-DNA positive based on US-PCR, with normal aminotransferase levels in both cases. Cases of occult hepatitis B infection were identified in this semiarid rural community of northeastern Brazil, where endemicity of HBV is moderate.

Key-Words: Anti-HBc alone, hepatitis B virus, occult hepatitis B, ultrasensitive PCR.

Approximately 400 million people worldwide are chronic hepatitis B carriers. Despite vaccination, serological tests performed in hemotherapy services, and programs for the prevention of sexually transmitted infections, the number of hepatitis B virus (HBV)-infected individuals remains high, with prevalence ranging from 0.1% to 20% [1].

The serological marker most widely used to detect exposure to HBV is HBcAg IgG; however, this marker is unable to define the status of the HBV carrier [2]. In individuals in whom antibody against the core protein (anti-HBc IgG) is the only positive marker, there are various possible explanations for false-positive test results, including patients with past, resolved infection, in whom the antibody against hepatitis B surface antigen (anti-HBs) is not present, occult HBV infection due to mutation of the S gene, or very low viral replication [3]. In this last hypothesis, analysis for HBV-DNA is considered the most sensitive diagnostic method [4].

Clearance of hepatitis B surface antigen (HBsAg) generally indicates seroconversion to anti-HBs and consequent remission of HBV infection [5]. However, several researchers have shown that seroconversion to anti-HBs is not necessarily indicative of virological cure. In this case, an occult, silent or latent hepatitis B infection may be characterized by presence of viral DNA alone, while the patient tests negative for HBsAg or anti-HBc [6]. A clear example is the diagnosis of this infection in patients with chronic hepatitis of undefined etiology (cryptogenic hepatitis), comorbid hepatitis C virus (HCV), HIV carriers, patients with chronic renal failure or patients with

hepatocellular carcinoma [7,8]. There are also case reports of hepatitis B transmission following blood transfusion or organ donation, despite the absence of serological markers for infection in the donor [9].

Identification of occult hepatitis B is complex, and anti-HBc is not always present. Therefore, viral DNA investigation must be performed, and sensitive techniques must be used since serum levels can be low [10].

In populations in which HBV is endemic, over 30% of individuals who were positive to anti-HBc alone were also found to be positive for HBV-DNA [11]; however, in populations in which endemicity is low, the clinical significance of anti-HBc positivity alone has yet to be fully clarified. We evaluated the occurrence of occult hepatitis B infection in the village of Cavunge (in the district of Ipecaetá, Northeastern Brazil).

Material and Methods

In the first phase of this study, carried out in 1999, blood samples were collected from the inhabitants of the village of Cavunge, which has a population of 2,049. ELISA was carried out to assess HBsAg and the anti-HBV antibodies, anti-HBs and anti-HBc, using commercial kits (Roche Diagnostics, São Paulo, Brazil) [12].

In the second phase of the study, carried out in November 2003, the inhabitants of the village who tested positive for anti-HBc and negative for HbsAg were invited to undergo further serological investigation for these same serological HBV markers. When the second serological tests confirmed positivity for anti-HBc alone, the serum samples were then tested for HBV-DNA using ultrasensitive polymerase chain reactions (US-PCR) [9] at INSERM Unit 271, Lyon, France. Briefly, US-PCR consists of amplifications in two stages (nested or semi-nested PCR), using primers located in well-conserved regions of the viral genome. PCR was performed on one-fifth of the total nucleic acids extracted from 140µl of serum (Quiagen).

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All participants admitted to the study had to have had a fixed residence for more than six months prior to admission, and all signed an informed consent form. Patients with chronic mental disease and/or were unable to comprehend the objectives of the study were excluded from the study irrespective of their age if no legal guardian was available.

The Statistical Package for Social Science (SPSS) software program, version 9.0 (SPSS Inc, Chicago, Illinois) was used for data analysis. Means, medians and standard deviations were calculated for the continuous variables, while categorical variables were described as absolute numbers and proportions.

Results

A total of 1,476 blood samples from residents of the village of Cavunge were analyzed for HBsAg, and 1,548 samples for anti-HBc. In this first phase of the study, carried out in 1999, HBsAg positivity was found in 2.6% of the participants (n=38), while 3.5% (n=55) of the individuals studied tested positive for anti-HBc IgG alone. The majority of individuals in the sample (62%) were male.

In the second phase of the study, carried out in 2003, 31 (56.3%) of the 55 individuals who had tested positive for anti-HBc IgG alone in the first phase, were retested. The remaining subjects (n=24) were not retested because they had moved to another part of the country, could not be found at home or because they refused to provide another blood sample. During the entire study period, which extended from 1999 to 2003, no deaths were recorded in this group.

Among the 31 individuals who were retested, 4/31 (13.0%) had low anti-HBs titers (cutoff = 0.455; low cutoff <1); 14/31 (45.1%) were positive for anti-HBs (cutoff > 1); and 3/31 (9.6%) were negative for all HBV serological markers. Only 11/31 subjects (35.5%) were positive for anti-HBc alone.

Among the 11 individuals who were positive for anti-HBc IgG alone, 9 (82%) were male, with ages ranging from 36 to 80 years (mean 50.5 ± 13.6 years). In addition, 9/11 (82%) tested negative for HBV-DNA (US-PCR), while 2/11 subjects (18%), both males, tested positive despite normal aminotransferase levels. No cases of comorbidity with HCV were found.

Discussion

The seroprevalence of HBsAg (2.6%) found in the first phase of this study confirms that HBV infection is moderately endemic in this rural area. In Brazil, few epidemiological studies have been carried out on hepatotropic viruses in rural areas [13]; most studies describing the prevalence of anti-HBc have been restricted to blood donors living in urban centers [14,15].

The role of anti-HBc positivity alone has been investigated more thoroughly in some specific subpopulations [16]; however, it is probably related to occult HBV infection with very low viral replication [6]. Little is known about the significance of positivity to this serological marker alone in populations with low HBV endemicity and in asymptomatic individuals.

One finding that is relevant is the seroconversion to anti-HBs observed in 58% of the cases (n=18) within the four year interval. It is possible that anti-HBs was already present at undetectable levels when the measurements were made of the first serum samples. However, the possibility of late seroconversion or recent acute infection at the time of the first sampling cannot be ruled out.

Clearance of all serological markers was observed in about 10% of the patients who tested positive for anti-HBc alone. This spontaneous viral clearance may occur in adult individuals [17]; however, it is possible that some of these cases are a consequence of false-positive results obtained in the ELISA performed on the first samples.

Except for the normal aminotransferase levels found in patients who tested positive to both anti-HBc alone and to HBV-DNA by US-PCR, no abnormal findings were detected during physical examination. Therefore, it is reasonable to speculate that the presence of this serological marker has no clinical implications, particularly considering that the high sensitivity of this method could overestimate the frequency of infection. Further studies conducted on different groups of infected individuals, such as subjects who are anti-HBs positive, would provide a more accurate evaluation of the applicability of this method. So far, no large population study has assessed this possibility; however, long-term follow-up studies would be required, including sequencing of the HBV S and X regions, where deletions and mutations associated with cases of occult HBV infection can occur [18].

In light of these results, questions about the duration of follow-up of these patients and the clinical importance of PCR HBV-DNA in this specific subpopulation must be raised. Meanwhile, we need to know more about the potential progression of occult hepatitis B, as well as its transmission, carcinogenesis [19] and association with hepatitis C [20].

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