



ORIGINAL ARTICLE

Occult HBV Infection in Pregnant Women in Northern Benin

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Abstract

Background and methods: Occult infections (OBIs) due to hepatitis B virus (HBV) are identified by the presence of HBV DNA without surface antigen (HBsAg). The population prevalence of HBsAg is high in Benin, but there are no data concerning the presence of OBIs.

The aim of this study was to evaluate the presence of OBIs using samples taken from 220 pregnant Beninese women for the purposes of a previous study of the prevalence of HBV markers. Twenty-four women were HBsAg positive (group I); of the 196 HBsAg negative women, 164 had other HBV markers (group II) and 32 had no HBV marker at all (group III).

Results: Forty-seven (24.0%) of the HBsAg-negative samples were positive for HBV DNA of which 46 (97.9%) in group II (seropositive OBIs) and 1 (2.1%) in group III (seronegative OBIs). About half of the women in groups II had viremia levels of < 20 IU/mL; the mean viremia levels in the remaining samples were 188.7 IU/mL in group II, although some of the women with OBIs had levels of as high as 1187 IU/mL.

Conclusion: The high prevalence of OBIs increases the number of potentially infected subjects in this area in comparison with the findings of tests based on the determination of HBsAg alone, which significant clinical and management implications for both vertical and horizontal transmission.

Keywords

HBV DNA, Occult hepatitis, Pregnant women, Anti-HBc alone, Occult infections

Introduction

Occult infection (OBI) due to hepatitis B virus (HBV) is best defined as the presence of HBV DNA in the liver (with or without serum HBV DNA) in the absence of detectable serum HBV surface antigen (HBsAg) [1] because viral DNA can be detected in the liver even when it is not found in blood [2]. However, as obtaining liver HBV DNA is difficult in clinical practice and there are still no appropriately standardised tests for detecting it in liver tissue [3], it is more usually defined as the presence of normally low levels (< 200 IU/mL) of serum HBV DNA without any determinable HBsAg [1,3,4].

This situation can be found in the case of: 1) Patients recovering from HBV infection with anti-HBsAg antibodies (anti-HBs) and viral persistence after HBsAg has disappeared [5,6]; 2) Patients with chronic hepatitis with a mutant HBV gene S (encoding for HBsAg) and the production of modified HBsAg that cannot be detected by widely used commercial tests ("false OBI") whose viremic levels are comparable with those observed in patients with normal HBV infection [1,4,7]; 3) Chronic carriers without any marker of HBV infection because of the progressive loss of all anti-HBV antibodies or the absence of antibodies from the beginning [8]; 4) Patients co-infected with hepatitis C virus (HCV) in whom the HCV core protein interferes with the replication of HBV and the synthesis of its proteins [9], or co-infected with human immunodeficiency virus (HIV) due to cell

immunodeficiency (decreased CD4+ cell numbers) [10]; 5) Chronic carriers with undetectable unmutated HBsAg in whom the only serological marker is the presence of antibodies to the antigen of the HBV core (anti-HBc), a situation that is often found in areas where HBV is endemic [11].

Occult infections have been divided into seropositive OBIs (the presence of anti-HBc and/or anti-HBs) and seronegative OBIs (the absence of anti-HBc and anti-HBs) on the basis of serum HBV antibody profiles [12]. About 20% of all OBIs are seronegative, 50% are anti-HBc seropositive (with or without anti-HBs), and 35% are anti-HBs seropositive [13].

The prevalence of OBIs varies widely [12] as it depends on factors such as geographical area (endemicity), the characteristics of the patients, including co-morbidities such as HCV and/or HIV, and the sensitivity of the diagnostic technique [1,14] It may also depend on the nature of the tested biological material as the liver is more likely to be positive than serum [15]. In geographical terms, it has been reported that the population prevalence of OBI is as high as up to 25% [16-21] in highly endemic areas such as Africa and Asia, where 41-95% of the inhabitants have been exposed to HBV, and lower in areas such as North America or Europe, where only 5-20% of the inhabitants have been exposed [22].

In Africa, the prevalence of HBsAg is 8-20% and that of the markers of exposure to HBV (anti-HBc with or without anti-HBs) is 70-95% [23]. Studies of OBIs in sub-Saharan countries have mainly focused on particular groups of subjects such as HIV-positive patients, among whom their prevalence is 10-23% [24-29] or blood donors, who are characterised by a prevalence of 0.5-17% [30-33] but there are very few studies of pregnant women [31,34].

There are no data concerning OBIs in Benin, although an article concerning asymptomatic pregnant women in a rural area in northern Benin published in 2014 stated that the prevalence of HBsAg was 15.5%, and that of anti-HBc alone was 41.3% [35]. As many of the samples used for that study were still available for HBV DNA research, the aim of this study was to determine the prevalence of occult HBV infection in this group of women.

Methods

In 2011, 283 asymptomatic pregnant women who sequentially attended the Saint Jean de Dieu de Tanguiéta Hospital (located in the rural district of Atacora in the north of Benin) for HIV screening were also examined for markers of HBV and HCV: 15.5% were HBsAg positive, 41.3% anti-HBc positive alone, 7.4% anti-HCV positive, and 3.2% anti-HIV positive [35].

Plasma samples from 220 of these women (mean age 26.2 years, range 15-40) were sterilely separated and frozen, and became subsequently available for

HBV DNA research. On the basis of the results obtained in 2011, the samples were divided into three groups: group I) 24 HBsAg-positive samples (10.9%); group II) 164 HBsAg-negative samples positive for anti-HBc and/or anti-HBs markers of HBV (74.5%); and group III) 32 samples that were negative for HBsAg, anti-HBs and anti-HBc (14.5%). Five of the women (2.3%) were anti-HIV positive (all in group II) and 11 (5.0%) anti-HCV positive (one in group I and ten in group II). There were no statistically significant differences between these 220 women and the initial group of 283 women in terms of age, or HBV, HCV and HIV markers.

The search for HBV DNA was made using a real-time method (AmpliPrep/COBAS TaqMan HBV Test, version 2.0, Roche Diagnostics, Mannheim, Germany), the results of which are linear from 20 IU/mL to 1.7×10^8 IU/mL. The amount of DNA was recorded in IU/mL if it was > 20 IU/mL, "not detectable" if none was detected, or " < 20 IU/mL" if the amount was below the linearity of the test but above the sensitivity stated by the manufacturer (95% for plasma levels of 9 IU/mL). The samples with < 20 IU/mL result were retested.

The HBV DNA-positive samples also underwent a chemiluminescent immunoassay (CLIA) for HBsAg (LIAISON XL MUREX HBsAg Quant, DiaSorin, Saluggia, Italy) that was different from that used in the previous study.

The data were statistically analysed using SPSS software, version 16.0 (SPSS Inc., Chicago, IL), and the chi-squared and Fisher's exact test to evaluate the differences between percentages, and analysis of variance (ANOVA) to evaluate the differences between the quantitative values.

Results

HBV DNA was detected in 68 of the 220 samples (30.9%): 43 with levels of > 20 IU/mL (19.5%) and 25 with repeatedly levels of < 20 IU/mL (11.4%). Among the 196 HBsAg-negative samples (groups II + III), 47 (24.0%; 95% CI: 18.02-29.98) were HBV DNA positive: 26 (13.3% 95% CI: 8.55-18.05) with levels of > 20 IU/mL, and 21 (10.7%; 95% CI: 6.37-15.03) with levels of < 20 IU/mL. Table 1 shows the results by group. The differences in the total percentage of samples with HBV DNA among the three groups were statistically significant ($p < 0.01$). Of the 47 HBV DNA-positive and HBsAg-negative subjects (groups II and III), 1 (2.1%) were in group III (HBsAg/anti-HBs/anti-HBc negative) and 46 (97.9%) in group II (HBsAg negative and anti-HBc and/or anti-HBs positive), including two (4.2%) in subgroup IIa (HBsAg-/anti-HBs+/anti-HBc-), 26 (55.3%) in subgroup IIb (HBsAg-/anti-HBs-/anti-HBc+) and 18 (38.3%) in subgroup IIc (HBsAg-/anti-HBs+/anti-HBc+). Table 2 shows the results by subgroup. There were no statistically significant differences between the three subgroups.

Table 1: HBV DNA in pregnant Beninese women divided on the basis of the presence/absence of HBV markers and DNA levels.

Group	No.	HBV Markers	HBV DNA Positivity		
			> 20 IU/mL (95% CI)	< 20 IU/mL (95% CI)	Total (95% CI)
I	24	HBsAg positive	17 (70.8%) (52.60-88.99)	4 (16.7%) (1.78-31.62)	21 (87.5%) (74.27-100)
II	164	HBsAg negative (anti-HBc and/or anti-HBs positive)	25 (15.2%) (9.71-20.69)	21 (12.8%) (7.69-17.91)	46 (28.0%) (21.13-34.87)
III	32	HBsAg/anti-HBs/anti-HBc negative	1 (3.1%) (0.00-9.11)	0 (0%) (0.00-0.00)	1 (3.1%) (0.00-9.11)
Total	220		43 (19.5%) (14.26-24.74)	25 (11.4%) (7.20-15.60)	68 (30.9%) (24.79-37.01)

Table 2: HBV DNA levels in HBsAg-negative pregnant Beninese women divided on the basis of the presence of specific HBV antibodies.

Sub group	No.	HBV Markers	HBV DNA Positivity		
			> 20 IU/mL (95% CI)	< 20 IU/mL (95% CI)	Total (95% CI)
IIa	9	HBsAg-/anti-HBs+/anti-HBc-	1 (11.1%) (0.00-31.62)	1 (11.1%) (0.00-31.62)	2 (22.2%) (0.00-49.35)
IIb	94	HBsAg-/anti-HBs-/anti-HBc+	16 (17.0%) (9.41-24.59)	10 (10.6%) (4.38-16.82)	26 (27.6%) (18.56-36.64)
IIc	61	HBsAg-/anti-HBs+/anti-HBc+	8 (13.1%) (4.63-21.57)	10 (16.4%) (7.11-25.69)	18 (29.5%) (18.06-40.94)
Total	164		25 (15.2%) (9.71-20.69)	21 (12.8%) (7.69-17.91)	46 (28.0%) (21.13-34.87)

Table 3: Relationships between the samples with HBV DNA levels of > 20 and < 20 IU/mL in pregnant Beninese women divided on the basis of the presence/absence of HBV markers.

Group	HBV Markers	HBV DNA Positivity		
		Total	> 20 IU/mL (95% CI)	< 20 IU/mL (95% CI)
I	HBsAg positive	21	17 (81.0%) (64.22-97.78)	4 (19.0%) (2.22-35.78)
II	HBsAg negative (anti-HBc and/or anti-HBs positive)	46	25 (54.3%) (39.90-68.69)	21 (45.7%) (31.30-60.10)
III	HBsAg/anti-HBs/anti-HBc negative	1	1 (100%)	0 (0%)
Total		68	43 (63.2%) (51.74-74.66)	25 (36.8%) (25.34-48.26)

About the viral load, the differences in the percentage of samples with HBV DNA levels of > 20 IU/mL between group I (HBsAg positive) and groups II or III (HBsAg negative) were statistically significant ($p < 0.01$), but not those between groups II and III. Furthermore, there was difference in the percentage of samples with levels of < 20 IU/mL between group I and group III or between group II and group III (< 0.05), but not between groups I and II (Table 1).

The ratios between the samples with HBV DNA

levels of > 20 and < 20 IU/mL is also shown in Table 3 and the difference between group I and group II was statistically significant ($p < 0.05$).

Table 4 shows the mean, median and range of HBV DNA levels in the samples with levels of > 20 IU/mL. The difference between group I and II ($p < 0.05$) was statistically significant.

In terms of co-infections, none of the HIV-positive women and only one of the HCV-positive women was

Table 4: Mean, median and range of HBV DNA levels in the samples with levels of > 20 IU/mL divided on the basis of the presence/absence of HBV markers.

Group	HBV Markers	HBV DNA Positivity			
		No.	Mean (IU/mL)	Median (IU/mL)	Range (IU/mL)
I	HBsAg positive	17	122,086.30	405.5	31-881,539
II	HBsAg negative (anti-HBc and/or anti-HBs positive)	25	188.7	91	24-1187
III	HBsAg/anti-HBs/anti-HBc negative	1	47	47	
Total		43	46,622	116.5	24-881,539

Table 5: HBV DNA results by HBV markers and age.

Group	No.	HBV Markers	Positive for HBV DNA		Negative for HBV DNA	
			No.	Mean age in years (range)	No.	Mean age in years (range)
I	24	HBsAg positive	21	27.5 (18-38)	3	27.7 (25-32)
II	164	HBsAg negative (anti-HBc and/or anti-HBs positive)	46	26.9 (15-40)	118	26.2 (17-40)
III	32	HBsAg/anti-HBs/anti-HBc negative	1	31.0	31	25.1 (18-37)
Total	220		68	27.1 (15-40)	152	26.0 (17-40)

positive for HBV DNA (and HBsAg negative) in group II.

With the aim of confirming the HBsAg results of the previous study, all samples, which were negative for HBsAg and positive for HBV-DNA, were retested with the CLIA test (that was different from that used in the previous study). All these samples were HBsAg negative with the CLIA test.

Table 5 shows the HBV results by age and the presence/absence of HBV markers. The between-group differences in age and the differences in age between the women with and without HBV DNA were not statistically significant. There was also no significant difference in age between the women with > 20 IU/mL and those with < 20 IU/mL in any of the groups.

Discussion

HBV infection is highly endemic in Benin: a study of pregnant women carried out in 2011 found that 15.5% were HBsAg positive but 41.3% were positive for anti-HBc [35]. As the presence of anti-HBc alone can identify chronic HBV carriers in highly endemic areas even if they are HBsAg negative [11], the suspicion that OBI may also be highly prevalent in Benin led us to look for HBV DNA in the blood samples still available from the 2011 study. Our findings suggest that 24% of the samples indicated OBI as they showed the presence of HBV DNA in the absence of HBsAg. This figure is similar or slightly higher than that found in other studies carried out in Africa, which have reported OBI prevalence rates of 10-23% among HIV-positive subjects [24,26-29], 17% among blood donors [32], but much higher than that found in pregnant women with prevalence of about

2% [31,34]. On the other hand, one study carried out in Nigeria (which borders on Benin) found a prevalence of 36% among patients attending a hospital Emergency Department [36]. However, the Nigerian study found that about 60% of the patients were co-infected with HIV, whereas the prevalence of co-infection in the Benin study was only 2.3%, which reflects the low prevalence of HIV infection in the general population of Benin at the time the blood samples were taken [35].

The high prevalence of OBI in countries such as Benin where HBV is endemic may be related to the fact that most of these infections are contracted perinatally or during early childhood and, as such infections are often chronic, there is a correspondingly proportion of adults with chronic infection, including some without determinable HBsAg levels [37]. In countries where the disease is less endemic, transmission is mainly horizontal [38].

Almost all the OBIs identified in this study (97.9%) were seropositive OBIs associated with markers of HBV, especially isolated anti-HBc (55.3%) and there was no age-related difference in the presence or absence of viremia.

The viral load in the women with seropositive OBIs was very low (< 20 IU/mL in almost half of the cases), and significantly different from that observed in the HBsAg-positive women in line with published data [1,3,4]. The method used by our centre has a declared sensitivity of 9 IU/mL for plasma (although the linearity of the test starts from 20 IU/mL), and thus approaches the HBV DNA detection limit of 5 IU/mL suggested as indicating OBI [3]. It can therefore be assumed that about half of

the OBIs identified in this study were characterised by HBV DNA levels of between 9 and 19 IU/mL, and this sensitivity may explain the high prevalence of OBI in comparison with other studies. On the other hand, it is necessary to bear in mind that HBV DNA levels may fluctuate over time [39,40], and so patients should really be evaluated serially in order to determine whether they have a chronic infection with a progressive loss of all anti-HBV antibodies, or whether they were devoid of antibodies from the beginning [8]. Unfortunately, we do not have any subsequent data: The women who had been screened for HIV were no longer traceable and, as the women were asymptomatic for liver disease at the time of HIV screening and the HBV marker prevalence study was performed retrospectively, there are no biochemical liver data that can be associated with OBI.

In an attempt to explain the presence of chronic HBV carriers without HBsAg, we investigated the possibilities that HCV may have interfered with HBV replication [9] or that HIV may have caused cellular immunodeficiency [10]. However, none of the woman with OBI had HIV infection and only one had HCV infection, so the presence of OBI therefore did not seem to have been due to viral interference.

It has been suggested that viremia may be partially kept under control as a result of a cytotoxic response by T lymphocytes after the resolution of an acute infection [41]. It is not known why the eradication is not complete, but it has been hypothesised that the occurrence of mutants in the S region may explain the persistence of viremia, although it has also been reported that more than half of the subjects without apparent mutations in the S gene have OBIs [36]. We re-tested our HBV DNA-positive and HBsAg-negative samples using a different test from that used in the previous study that is capable of detecting the most frequent mutants of HBV [42] because it has been suggested that HBsAg should be tested in this way [2]. With this test all samples were negative according to OBIs are characterised by undetectable HBV DNA and non-mutated HBsAg levels [11]. The presence of other possible mutations that may generate escape-mutants was not investigated because it was impossible to sequence the samples. Other immunological factors that may be involved include a non-cytolytic T lymphocyte response to specific HBsAg [43].

It has been suggested that different HBV genotypes may at least partially explain the existence of patients with or without HBsAg [44], but only a few studies [45,46] have provided a complete genomic sequence, not least because of the difficulty of amplifying a complete genome in samples with low levels of HBV DNA [4]. It has been possible to detect sufficient levels of viremia to determine the genotypes of Beninese women with positive HBsAg [35] but this was not possible in the case of our women with OBIs because the remaining samples were too small.

Another limitation of this study is that we have no information concerning the children of these women that would make it possible to estimate the rate of vertical/perinatal transmission. Animal-based studies have shown maternal-fetal transmission in marmots with maternal occult infection [47], leading to the production of persistent, seronegative and asymptomatic infection in their offspring. Vertical transmission has also been postulated in humans [3], and it has been reported that a baby of a mother with OBI was positive for HBV DNA at birth [34]. Some authors believe that neonatal transmission through cord blood from mothers with OBI is not a clinical problem because of the low levels of HBV DNA [48], but some of our women with OBI had HBV DNA levels that were as high as 1187 IU/mL, which could be sufficient for HBV transmission.

In conclusion, this study found a high prevalence of both seropositive and seronegative OBIs in Benin that has various clinical, organisational and epidemiological implications. The significant proportion of OBIs increases the number of HBV carriers who may transmit the virus vertically/perinatally (screening for maternal HBsAg may be insufficient and should perhaps be supported by neonatal immunoprophylaxis), but also horizontally (for example, a negative HBsAg test does not guarantee a sufficient level of safety for transfusion services) [43]. A vaccination campaign was started in Benin in 1999 [49], but the administration of immunoglobulins to newborns in order to prevent perinatal infection may also be required [49,50].

Conflict of interest

The Authors declare NO financial or other relationships that may involve conflicts of interests.

Experimental Ethics

The study was approved by the Hôpital Saint Jean de Dieu medical direction and informed consent was obtained for patients.

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