

Occult HBV infection among a cohort of Nigerian adults

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Abstract

Objective: To determine markers of HBV infection and detect the presence of its occult infection in serum of a cohort of adult Nigerians.

Methodology: The study involved 28 adult Nigerians with viral hepatitis (Group 1) and 28 apparently healthy adult Nigerians as controls (Group 2). Their sera were assayed for HBsAg, HBeAg, anti-HBe, anti-HBc, anti-HBs, and anti-HCV, while HBV DNA was determined in 15 patients with chronic hepatitis. Significance of differences between the patients and control subjects was assessed using Chi-square test at a 95% confidence level.

Results: Sero-detection of HBsAg, HBeAg, anti-HBe and anti-HBc was higher among the patients compared to the controls. HBV infection was diagnosed by HBsAg (89%) and a duo of HBsAg and anti-HBc (100%) among the patients. Similarly, eleven and four types of different patterns of HBV markers were observed among the respective groups. Anti-HBe (9.5%), anti-HBc (14.3%), and anti-HBs (9.5%) were detected among all the subjects who were sero-negative for HBsAg. HBV DNA was also detected in 86.7% of the 15 patients with chronic hepatitis, while occult HBV infection was observed in 7.2% of the patients and none (0%) of the controls, $p < 0.05$. Furthermore, HCV infection occurred among subjects with all the different patterns of HBV markers, except those with occult HBV infection and natural immunity to HBV.

Conclusion: This study shows that occult HBV infection is present among Nigerian adults and determination of HBsAg, anti-HBc, anti-HBe, and HBV DNA will assist in its detection.

Key words: occult HBV infection, HCV, Nigerians

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Introduction

Hepatitis B virus (HBV) infection, like other hepatitides of viral origin, manifests not only clinically and in an asymptomatic fashion, but also with unusual serological patterns [1,2,3]. However, detection of HBV infection among Nigerians has mostly been performed with the estimation of only hepatitis B surface antigen (HBsAg) in many studies [4,5]. Hence, many subjects who have been sero-negative for HBsAg have been labeled as not having HBV infection [6]. Furthermore, patients who have been transfused with blood that has been screened as HBsAg sero-negative were later found to have developed post-transfusion hepatitis from HBV infection [7]. This phenomenon has encouraged some researchers to incorporate assays for antibodies to hepatitis B core antigen (anti-HBc), hepatitis B envelope antigen (HBeAg), and/or antibody to HBeAg (anti-HBe)[2, 8] with the aim to determine replication of HBV or the presence of previous HBV

infection in Nigerian subjects that might have been missed by an isolated assay of HBsAg. Similarly, determination of all the serological markers of HBV including HBV DNA assay by Polymerase Chain Reaction (PCR), has made it possible to determine different serological markers that may be exhibited by HBV [1]. The markers occur in different combinations depending on the natural history of the infection. There may be varied or incomplete expression of the serological markers depending on the HBV genotype/serotype as well as the type of the variants/mutants of the virus [1,3,9,10]. Assay of the different serological markers has led to detection of occult HBV infection among subjects previously diagnosed with cryptogenic hepatitis [11]. It has also elucidated proper diagnosis of HBV infection, co-infection with other hepatotropic viruses as well as improved the management of patients with hepatocellular jaundice, especially in monitoring the outcome of anti-HBV therapy [1]. Furthermore, it has

Table 1. Serology in Nigerian adult patients with viral hepatitis and controls.

Group (number)	Percentage						
	HBsAg	HBeAg	Anti-HBe	Anti-HBc	Anti-HBs	HBsAg and or Anti-HBc	Anti-HCV
Viral Hepatitis (28)	89	29	46	82	50	100	21.4
Control (28)	35.8	7	0	3.5	35	35.8	11
p-value	P=0.026	P=0.036	P=0.000	P=0.000	P=0.28	P=0.000	P=0.275
HBsAg+ve(35)	100	28.6	31.4	68.5	68.5	100	17.1
HBsAg -ve(21)	0	0	9.5	14.3	9.5	14.3	33.3

made possible the exclusion of occult HBV infection in blood transfusion services, thus curtailing transfusion-associated HBV infection [12]. Presence of occult HBV infection is usually detected when a subject is sero-negative for HBsAg, but positive for HBV DNA, with or without the presence of HBV antibodies [12]. In view of the above-mentioned issues, we aim to assay the markers of HBV infection, such as HBsAg, HBeAg, anti-HBe, anti-HBc, antibody to HBsAg (anti-HBs) as well as HBV DNA and antibody to hepatitis C virus (anti-HCV) among a cohort of adult Nigerians, and hence define HBV infection and detect the presence of its occult infection among adult Nigerians.

Materials and Methods

The study involved 56 adult Nigerians (28 patients with acute or chronic viral hepatitis as Group 1 and 28 apparently healthy subjects as Group 2 or the controls). The subjects in Group 1 were patients with acute (13) and chronic (15) hepatitis. Those in Group 2 (controls) were age and sex matched with a subject in Group 1 but asymptomatic of HBV and HCV infections, as well as without their match subject's risk factors (e.g., use of intravenous drugs, history of blood transfusion or surgery, and scarification). Five milliliters of blood was collected from each subject. Their sera were assayed for HBsAg, HBeAg, anti-HBe, anti-HBc, anti-HBs and anti-HCV using third-generation ELISA Kits (Murex Diagnostics) at the Department of Virology, University College Hospital, Ibadan. In addition, detection of HBV DNA was performed by polymerase chain reaction in 15 patients with chronic hepatitis using the in-house method of a commercial laboratory, Medica (Medizinische Laboratorien Dr F Kaeppli) Zurich Switzerland.

The study was conducted after ethical clearance was obtained from the University of Ibadan's

University College Hospital Institutional Review Committee. The laboratory data were analyzed statistically using Chi square test in investigating the significance of differences between two proportions at 95% confidence level.

Results

The age of the patients and the controls were 34.2 ± 16 and 32.25 ± 11 years respectively. Each group had a male-to-female ratio of 6:1. Table 1 shows that there was no difference in the detection rates of HBsAg compared to anti-HBc among subjects with acute or chronic hepatitis (group1). The proportion of the subjects positive for either HBsAg or anti-HBc was significantly higher than the value for each of the other markers (HBeAg, anti-HBe and anti-HBs). HBV infection was detected in 89% of the patients using only HBsAg but this increased to 100% with the use of the duo of HBsAg and anti-HBc (though not statistically significant), whereas the values were similar among the controls. Similarly, the proportion of subjects positive for each marker was significantly higher in Group 1 than in Group 2 except for anti-HBs. Furthermore, the detection rate of HBsAg was similar to that of anti-HBs among Group 2. Isolated detection of HBsAg was found only among the subjects (3.6%) in Group1. Out of the 56 samples (patients and controls) tested for HBV markers, 9.5%, 14.3% and 9.5% of the HBsAg negative subjects were positive for anti-HBe, anti-HBc and anti-HBs respectively.

Table 2 shows that among the subjects in Group 1, HBeAg was detected with the absence of anti-HBe in six patients (21.4%), while anti-HBe without the presence of HBeAg occurred in 11 patients (39.3%). Similarly, two patients (7.2%) in Group 1 were HBsAg negative but anti-HBc and anti-HBe positive. HCV infection was detected in six patients (21.4%) in Group 1 and three subjects (11%) in Group 2, and

Table 2. Serological patterns in Nigerian adult patients with viral hepatitis and controls.

Groups	HBsAg	HBeAg	Anti-HBe	Anti-HBc	Anti-HBs	HB N=28	Control N=28	Anti-HCV ⁺
Early Incubation period	+	-	-	-	-	1	-	-
	+	-	-	-	+	4	8	2
Late Incubation period	+	+	-	-	+	-	1	-
Acute / Chronic Infection	+	+	-	+	-	2	-	-
	+	+	-	+	+	4	1	1
HBV infection in Convalescence	+	+	+	+	+	2	-	1
	+	-	+	+	-	9	-	1
	+	-	-	+	-	1	-	-
Occult HBV infection	-	-	+	+	+	1	-	-
	-	-	+	+	-	1	-	-
Naturally immuned to HBV	-	-	-	+	+	1	-	-
Carrier	-	-	-	+	+	1	-	-
	+	-	-	+	+	2	-	1
Unexposed	-	-	-	-	-	-	18	3 ^u

^u - Number of HCV infection among the Control N - Number of subjects

these rates were significantly lower than those of HBsAg in group1, $p = 0.00012$ and 2, $p = 0.023$ respectively.

Concerning the different patterns of HBV serological markers observed in both groups, 11 types compared to four types were found among subjects in Groups 1 and 2 respectively. Although HBsAg has the highest detection rate compared to all other HBV markers, all subjects who had exposure to HBV but were HBsAg sero-negative were anti-HBc sero-positive. Furthermore, occult HBV infection was found only in two of the patients (7.2%) who were also anti-HBe⁺ and anti-HBc⁺ with or without anti-HBs⁺, but not (0%) among the controls ($p = 0.04$). HCV infection was observed as well among subjects with all the different patterns of HBV serology with the exception of those having occult HBV infection and those naturally immune to HBV.

HBV DNA was detected in 13 (86.7%) of the 15 patients with chronic hepatitis B by polymerase chain reaction and these were HBeAg⁺ + anti-HBe⁺ while the two patients (13.3%) who were HBsAg carriers and naturally immune to HBV were negative for HBV DNA (Table 3).

Discussion

Occult HBV infection is present when an individual is sero-negative for HBsAg, but positive for HBV DNA with or without the presence of HBV antibodies [12]. Our study reveals varying percentages of detection rates of HBV markers with the highest rate for HBsAg in subjects with exposure to HBV. The result is similar to earlier observations

reported among Nigerian patients with acute icteric hepatitis [4] and chronic hepatitis [2], as well as among doctors, and dentists [8]. The absence of the detailed assay for HBV markers among the subjects involved in earlier studies may have caused their sero-negative status to be falsely diagnosed as having non-HBV infection. This is shown by the observation from our study that anti-HBc could be sero-positive in HBsAg sero-negative subjects; hence assay of both HBsAg and anti-HBc will be useful in the detection of subjects with exposure to HBV. Furthermore, our present study has provided useful information on the detection of patients who are anti-HBc, anti-HBe and/or without anti-HBs sero-positive but HBsAg negative. This reveals under-diagnosis of HBV infection with the use of only HBsAg as its surrogate marker [13]. Furthermore, the assay of HBeAg and anti-HBe in addition to HBsAg has shown evidence of HBV replication in the HBV infected subjects, which is valuable information of epidemiological importance in monitoring the disease's progression, as well as the patient's response to antiviral therapy [1]. The observation of the high number of HBeAg sero-negative but anti-HBe sero-positive patients agrees with previous reports among Africans [3,9,10] and suggests the need for correct diagnosis of patients with HBV infection [10]. The detection of other HBV markers among both HBsAg sero-positive and sero-negative Nigerians is significant. It calls for an exhaustive and extensive serological investigation in the detection of HBV infection and management of every patient with the infection [14]. The different

Table 3. Serological patterns in Nigerian adult patients with chronic hepatitis.

Groups of Infection	HBsAg	HBeAg	Anti-HBe	Anti-HBc	Anti-HBs	Number =15	
						Anti-HCV	HBV DNA
HBeAg positive	+	+	-	+	-	-	2
HBeAg negative	+	-	+	+	-	1	9
Occult HBV	-	-	+	+	+	-	1
	-	-	+	+	-	-	1
Natural immunity	-	-	-	+	+	-	-
Carrier	+	-	-	+	-	-	-

serological markers of HBV infection and their patterns observed among our subjects follow the natural history of the disease and they show the study subjects exposed to HBV in different stages of infection, ranging from the early phase (incubation period) to the late recovery stage of the development of natural immunity against HBV, as well as the HBsAg carrier stage [1,15]. Detection of HBV DNA by PCR in some of our patients with chronic hepatitis has confirmed the clinical significance of each of the other markers and help in the detection of the viral-replicating activities in the patients' serum [14]. This molecular assay of HBV markers has led to the detection of occult HBV infection among our subjects [12,16]. It is important to note that our subjects with the occult HBV infection were only patients and not representative of a cross-section of the population; all were anti-HBe, anti-HBc and HBV DNA positive but HBsAg and HBeAg negative. This is especially important in blood transfusion services where blood is usually screened for HBV infection by routine assay of only HBsAg prior to blood transfusion. This study shows that there is need for detecting the presence of anti-HBc in addition to HBsAg prior to transfusion or organ/tissue donation in order to detect blood harboring occult HBV infection and thus prevent post-transfusion HBV infection [12]. The detection of occult HBV infection (which may be due to the presence of mutant viruses having a strong suppression of viral replication and gene expression) among our subjects further supports the presence of mutant HBV [16] which coexists with the wild type. Hence, there is need for the diagnosis of occult HBV infection among Nigerian patients with high clinical indices of viral hepatitis but HBsAg sero-negative by detecting the presence of anti-HBc, anti-HBeAg and/or anti-HBs by ELISA prior assay of HBV DNA by PCR. Furthermore, detection of HBeAg by

ELISA would be useful for the management of patients with HBV infection. In addition, the assay of anti-HBs will reveal full recovery from HBV infection with development of natural immunity as well as its protective level after the completion of a vaccination schedule against HBV [1].

It is also significant that HCV infection was absent only in the subjects who had an HBV serological pattern suggestive of natural immunity and occult HBV infection although there is no cross-protection against HBV and HCV. On the other hand, the co-occurrence of the different HBV serological patterns with HCV points to the similarity of the routes of acquisition of the two viruses.

In conclusion, although the sample size of the studied population was limited by the available number of kits for serological markers of HBV and HCV infections because of their high costs, the study has shown the importance of well-elucidated assay of the different HBV markers in determining how HBV infection may manifest among Nigerians exposed to the virus. In addition, the study shows that occult HBV infection occurs among Nigerians, and its detection is necessary. With availability of funds, further study involving a larger sample size will be needed to corroborate our findings.

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