Hepatitis B Virus DNA in Patients With HBsAg in South Western Nigeria

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There are about 400 million people with chronic hepatitis B virus (HBV) infection worldwide with a potential of adverse sequelae including hepatocellular carcinoma. Recent data have shown that the level of HBV DNA in serum or plasma of an infected person probably reflects more accurately the replicative activity of the virus and therefore may serve as a better maker for management of the infection. This study was designed to determine the rate of detection of HBV DNA in blood samples of patients with HBsAg positive in Nigeria in comparison with the HBe and anti-HBe used widely as serological markers of infectivity. Plasma samples from 105 patients with HBsAg positive were tested for the presence of HBeAg and anti-HBe using a commercial enzyme-linked immunosorbent assay while plasma HBV DNA was quantified using the COBAS Amplicor HBV Monitor assay. Of the 105 HBsAg samples, 17 (16.2%) and 85 (81%) were positive for HBeAg and anti-HBe, respectively, while 8 (7.6%) were negative for both HBeAg and anti-HBe. HBV DNA was detected in 86 (81.9%) of the samples, out of which 15 (18.1%) and 67 (80.7%) were positive for HBeAg and anti-HBe, respectively. HBV DNA was detected in 78.4% of the HBeAg negative samples and in all the eight samples that were negative for both HBeAg and anti-HBe. The implication of these findings in the management of patients with HBV infection is compelling. ***J. Med. Virol.***
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INTRODUCTION

Hepatitis B virus (HBV) infection is an important public health problem globally with adverse sequelae including hepatocellular carcinoma (HCC) [Lavanchy, 2004; Goldstein et al., 2005; Stein et al., 2007]. The World Health Organization (WHO) estimates that 300–400 million people suffer from chronic HBV infection [WHO, 2008; Chao et al., 2009]. While most persons who have been infected with this virus are unaware of their infection, there is the possibility of developing debilitating or fatal liver disease and unknowingly transmitting the infection to others [Carey, 2009]. Further, about 600,000 deaths are attributed to HBV infections annually [WHO, 2008]. Infection with this virus is associated with a wide spectrum of clinical manifestations, ranging from acute to fulminant hepatitis and various forms of chronic infection including asymptomatic carrier state, chronic hepatitis, cirrhosis of the liver and HCC [Olubuyide et al., 1997; Wright, 2006]. In combination, HBV and hepatitis C virus infections are the leading cause of liver cancer worldwide, accounting for 78% of cases [Lavanchy, 2004; Chao et al., 2009].

The prevalence of HBsAg varies within and between regions as well as countries. The prevalence of chronic HBV infection worldwide has been categorized as high, intermediate, and low endemicity [Carey, 2009]. It is lowest in Western Europe and the USA and highest in South-East Asia, China, sub-Saharan Africa, and the Amazon Basin, where at least 8% of the population are chronic carriers of HBV. In Nigeria, varying rates of HBV infection have been reported, ranging from 2.4% to 13% among blood donors and the general population [Odaibo et al., 2003; Ajayi et al., 2007; Olukoba et al., 2009; Pennap et al., 2010] to over 50% among patients with HCC and cirrhosis of the liver [Olubuyide et al., 1997; Mustapha et al., 2007].

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While the presence of HBsAg in the blood indicates infection, it does not provide information on the replicative activity of the virus [Zaaijer et al., 1994]. Conventionally, the detection of HBV e antigen (HBeAg) is used as a viral marker for active viral replication. In the treatment of chronic hepatitis B, the presence or absence of HBeAg is considered to represent a high or low replicative state of HBV, respectively [Zaaijer et al., 1994]. However, HBV mutants, which do not produce HBeAg, irrespective of their rate of replication, have been described [Brunetto et al., 1989, 1990; Carman et al., 1989]. It was shown recently that the level of HBV DNA in the serum or plasma of an infected person reflects more accurately the replicative activity of HBV in the hepatocytes [Shao et al., 2007]. This study was therefore carried out to determine the rate of detection of HBV DNA from blood samples of patients with HBsAg positive in Nigeria in relation to their HBeAg and anti-HBe status.

MATERIALS AND METHODS

The study was carried out at the Department of Virology, University College Hospital, Ibadan, Nigeria. The hospital is the premier tertiary referral hospital located in Ibadan city in southwestern region of the country. The samples were collected from patients diagnosed with clinical hepatitis at the gastroenterology and hepatology clinic. Although the catchment area of the hospital is the entire country, most of the referral cases are from within the southwestern region. The study patients included HBsAg positive individuals selected at random who consented to participate in the study. Demographic information including age, gender, and occupation were recorded at the point of sample collection.

Ethical Review Committee

Ethical clearance for the study was obtained from the University of Ibadan/University College Hospital ethical review committee.

Sample Collection and Processing

A total of 105 samples were collected over 2 years, from March 2009 to February 2011. About 5 ml of blood sample was collected from each individual, centrifuged at $447.2 \times g$ for 20 min, the plasma separated into cryovials and stored at $-20°C$ until tested. Samples were tested for the presence of HBeAg and anti-HBe using a commercial enzyme linked immunosorbent assay (ELISA) while HBV DNA in all the plasma samples was detected and quantified using the Roche COBAS Amplicor HBV Monitor assay.

Detection of HBeAg and Anti-HBe

The presence of HBeAg and HBe antibody was tested with a commercially available ELISA (DIA-PRO, Rome, Italy) using 100 μl of plasma for HBeAg detection and 50 μl for anti-HBe detection. The intensity of the color of change of the reaction product was measured at dual wavelength of 450 and 630 nm and the presence of antigen and antibodies in each sample determined using the cut-off value recommended by the manufacturer.

COBAS Amplicor HBV Monitor Assay

DNA was extracted from each plasma sample as described by the manufacturer. Briefly, 100 μl of plasma was extracted using polyethylene glycol precipitation and alkaline lysis, after which 100 μl of elution buffer was added yielding a final volume of 100 μl of eluted DNA sample. Of DNA extract from patients sample, 50 μl was used to perform the PCR using the reagents provided in the Amplicor kit. Amplification, detection, and quantitation were performed by using a semi-automated COBAS Amplicor instrument. The lower and upper detection limits of the COBAS Amplicor HBV Monitor assay were 316 and 199,880 DNA copies/ml, respectively. Samples with 200,000 HBV DNA copies/ml in the Amplicor assay were diluted 1:10, 1:100, and 1:1,000, as necessary, in HBV DNA-negative normal human serum, and the diluted material was reextracted, amplified, and detected to obtain a result within the analytical measurement range of the Amplicor assay. Final results for diluted samples were determined by multiplying the HBV DNA copies/milliliter obtained in the Amplicor assay by the dilution factor for each sample. Results were reported as HBV DNA copies/milliliter by the COBAS Amplicor instrument.

RESULTS

Study Patients

Of the 105 patients included in the study, 78 (74.3) were male and 27 (25.7) female individuals. The age of the participants ranged from 20 to 61 years with a median of 36 years.

HBV Serology

A total of 105 HBsAg positive samples were tested for the presence of HBeAg and antibodies to hepatitis B e antigen (anti-HBeAb). Only 16.2% (17/105) of the samples were positive for HBeAg while 81.0% (85/105) were positive for the presence of HBeAb (Fig. 1). Eight (7.6%) of the samples were neither HBeAg nor anti-HBe positive.

HBV DNA

Out of the 105 samples analyzed, HBV DNA was detected in 86, giving a prevalence rate of 81.9% of HBV DNA among HBsAg positive individuals. Out of the 86 DNA positive samples, 15 (18.1%) and 67 (80.7%) were positive for HBeAg and anti-HBe, respectively (Table I). On the other hand, among the 88 HBeAg negative samples, HBV DNA was detected in 69 (78.4%). In addition, DNA was detected in the

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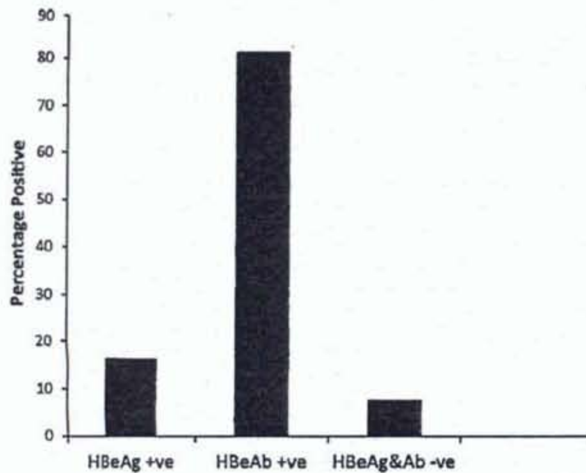

Fig. 1. Rate of detection of HBeAg and HBeAb in HBsAg positive samples.

eight samples that were negative for both HBeAg and anti-HBe. Seven samples had detectable DNA level of over six logs, and out of the seven samples with high HBV DNA load only two (28.6%) were positive for HBeAg while four were positive for anti-HBe and one was negative for both HBeAg and anti-HBe (Table II).

DISCUSSION

In most developing countries, treatment of HBV infection is guided by the presence or absence of HBeAg in the blood sample of a patient with hepatitis [Lavanchy, 2004; Tong et al., 2008]. Detection of HBeAg until recently was considered as the main serological marker of active virus replication while anti-HBe served as an indicator of good prognosis for control of active replication of HBV. However, recent studies have shown that some mutants of HBV do not produce HBeAg and so active replication may not be detected if it is based only on presence of HBeAg. In this study, HBV DNA was detected in 78.4% of the HBeAg negative and 80.7% of anti-HBe positive samples. The implication of this result in the management of patients with HBV infection in Nigeria cannot be overemphasized. The results of this study of 83.8% HBeAg negative hepatitis B is in agreement with some previous observations [Funk et al., 2002]. Both anti-HBe and HBeAg were not detected in 7.6% of the HBsAg positive samples. Although the reason for this condition is not clear, it is possible that these patients were in the early phase of the infection during which HBeAg was yet to develop or at the interface of HBeAg disappearing and development of anti-HBe. The presence of HBV DNA in all eight samples that were negative for HBeAg and anti-HBe is an indication of active replication of HBV in these patients.

Most of the patients, who were considered previously to have non-replicative HBV infection because of absence of HBeAg, have been shown to have detectable HBV DNA and HBV replication that persist throughout [Brunetto et al., 1990; Knoll et al., 1999; Hadziyannis and Vassilopoulos, 2001]. This change in the natural history of HBV has been revealed with the use of sensitive PCR-based assays that detect HBV DNA in plasma [Hadziyannis and Vassilopoulos, 2001]. The prevalence of HBV DNA of 81.9% obtained in this study is similar to previous reports from Nigeria as well as some other countries. Ola et al. (2009) reported a prevalence rate of 86.7% HBV DNA detection among patients with chronic hepatitis in Nigeria. Similarly, Zaaijer et al. [1994] reported a DNA detection rate of 90% in the Netherlands.

The results of the study also showed that the rate of HBeAg among HBsAg positive individuals to be only 16.2%. Some previous studies have also indicated a low rate of HBeAg among patients with chronic HBV infection, especially adults [Lok et al., 1987; Hadziyannis and Vassilopoulos, 2001]. The participants in this study were adults with a median age of 36 years, which probably explains the low rate since HBeAg has been shown to be higher among children [Hadziyannis and Vassilopoulos, 2001; Carey, 2009]. Hepatitis "e" antigen is a peptide that is detectable in the blood when HBV is actively replicating and its presence may indicate a higher probability of liver damage. The relatively low rate of HBeAg among patients with HBsAg suggests that the virus in most of the patients may not be actively replicating, however, mutant strains of HBV that replicate without producing HBeAg has been well documented [Brunetto et al., 1989, 1990; Carman et al., 1989; Hadziyannis and Vassilopoulos, 2001] which may account for the findings in this study. Reports of limited phylogenetic studies of HBV in Nigeria have shown that genotype E of the virus with limited production of e antigen is more preponderant in the country [Odemuyiwa et al., 2001]. It is also important to note that the disease is usually more aggressive in individuals with this

TABLE I. Detection of HBV Serological Markers and DNA in HBsAg Positive Individuals

	HBV DNA		HBeAg		Anti-HBe		
Positive	+	−	+	−	+	−	No. of samples tested
HBV DNA +ve	86	00	15	69	67	18	105
HBeAg +ve	14	2	17	00	00	80	105
Anti-HBe +ve	67	17	00	12	85	00	105

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TABLE II. HBeAg and Anti-HBe Status of the Sevens Samples With Very High HBV DNA Copies

Serial no.	HBV DNA (copies/ml)	HBeAg	Anti-HBe
1	10,400,000	−	−
2	15,200,000	−	+
3	10,700,000	−	+
4	6,060,000	+	−
5	2,100,000	−	+
6	2,630,000	+	−
7	14,000,000	−	+

mutant strains [Chisari and Ferrari, 1995; Zhong et al., 2000].

The rate of the HBeAg obtained in this study is lower than 34% and 28.6% previously reported by Olubuyide et al. [1997] and Ola et al. [2009] in adult populations in Nigeria. The reasons for this difference is not clear, however, the consistent reduction in the rate of HBeAg over the years as revealed by these studies in Nigerian is also consistent with reports from other parts of the world that the prevalence HBeAg negative hepatitis is on the increase [Rizzetto et al., 2000; Hadziyannis and Vassilopoulos, 2001].

The prevalence of antibodies to HBeAg (anti-HBe) found in this study was high (81.0%). The detection of anti-HBe is usually assumed to indicate a favorable prognosis because it is always produced after HBeAg is no longer detectable [Marcellin et al., 2004]. The estimated 400 million people with chronic HBV infection globally were diagnosed based on their positivity to HBsAg. However, studies have shown that most of them are HBeAg negative and anti-HBe positive. The detection of HBV DNA in HBeAg negative and anti-HBe positive individuals, a condition referred to as "HBeAg negative Chronic Hepatitis B" by Hadziyannis [1995] has also been reported by other investigators [Lavanchy, 2004; Lok and McMahon, 2007; Shao et al., 2007]. The rate of the condition ranges from 70% to 100% in community based studies [Madzime et al., 1997; Minuk et al., 2000; Hadziyannis and Vassilopoulos, 2001].

Detection of high DNA levels of over 10^6 in five of the HBeAg negative samples supports further the need for HBV DNA testing for patients with HBsAg. Patients with such high level of HBV DNA are known to have higher risk of developing cirrhosis of the liver and HCC [Wright, 2006] and transmission of this virus. The use of HBV DNA as a maker for proper management of HBV infection in Nigeria is therefore strongly recommended. However, a major challenge is the fact that only few centers have introduced partially HBV DNA testing in the country partly because the test is relatively expensive.

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