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Recommended Citation
DOI: 10.7454/mss.v24i3.1204
Available at: https://scholarhub.ui.ac.id/science/vol24/iss3/1

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Hepatitis B Surface Antigen and Viral DNA Detection and Prevalence in Nigeria

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Received March 28, 2019 | Accepted June 22, 2020

Abstract

Hepatitis B infection is a major public health issue with a high mortality rate. It is caused by hepatitis B virus (HBV), a small incomplete hepadnavirus with several open reading frames (ORF). High mutation rates of the HBV ORFs due to the virus’ replicating strategy are associated with the development and emergence of mutant strains, which may result in occult infections that are undetectable by conventional diagnostic assays. In this study, we first investigated the presence and prevalence of hepatitis B surface antigen (HBsAg) and HBV DNA among 204 patients visiting the specialist hospital in Osogbo, Nigeria, by using the Acumen diagnostic test strip kit and PCR amplification of the surface antigen gene. Then, we compared the positive results detected by both assays. The mean age of the participants was 30.5 years. Twenty-two (10.78%) cases were positive in the HBsAg test, and 69 (33.82%) cases were positive for HBV DNA. Fifty-four false-negatives (26.47%) and four false-positives (1.96%) were detected by HBsAg. The rapid diagnostic test kit is less sensitivity and has lower detection capability compared with the PCR-based assay, which indicates the presence of mutant HBV strain(s). The results of this work demonstrate that HBsAg is endemic in Nigeria and that HBV DNA is highly prevalent among the population. Our findings highlight the need for immediate action to prevent the further spread of infection.

Keywords: False-negative result, HBV DNA, Hepatitis B surface antigen, prevalence, Nigeria

Introduction

Hepatitis B virus (HBV) is a small enveloped hepatotropic virus that possesses a partially double-stranded relaxed circular DNA genome [1]. It belongs to the Hepadnaviridae family and can cause both acute and chronic infections [2]. As many as 250 million people worldwide are chronically infected with the virus, and 0.62 million deaths resulting from the long-term complications of the disease are estimated to occur annually. Thus, HBV remains a major global public health challenge despite the wide availability of effective vaccines against the virus [2–4]. The prevalence rate of HBV in different parts of the world varies to some extent; for example, prevalence rates of 6.2% and 6.1% have been reported for the Western Pacific and African regions, respectively. Prevalence rates of 3.3% for the Eastern Mediterranean, 2% for Southeast Asia, 1.6% for the European region, and 0.7% for the Americas have also been recorded [5].

HBV can be transmitted by sexual contact with an infected individual, parenteral exposure to infected body fluids, especially blood, during blood transfusion, organ transplant, and transmission from carrier mothers to their babies during the perinatal period. The symptoms of HBV infection include jaundice, dark urine, fatigue, nausea, vomiting, and abdominal pain. The disease is an important occupational hazard for health workers [5, 6].

Implementing preventive measures against the spread of HBV infection is vital for global health safety. Hence, the World Health Organization (WHO) [5] recommends that all blood donations be tested for HBV to ensure blood safety and avoid accidental transmission to persons receiving blood products as a preventive measure against viral infection. The hallmark of this testing is the detection of hepatitis B surface antigen (HBsAg), a serological marker that is present in the acute and chronic phases of the disease [7]. This antigen appears in the serum within 1–10 weeks after exposure to the virus, and its persistence for over 6 months indicates chronic HBV infection [8]. However, mere detection of HBsAg presence is inadequate to establish HBV infectivity [9].

Chronic hepatitis B is the most frequent cause of chronic liver diseases, including cirrhosis, and hepatocellular carcinoma (HCC) worldwide [6, 10]. Current approach-
es to manage this infection, therefore, focus on improving long-term survival by slowing progression to cirrhosis and HCC [5]. However, the definition of a chronic HBV carrier has been complicated by the finding that some individuals have HBV DNA in their circulation despite the absence of detectable HBsAg in their serum or plasma [11, 12]. This discordant serological and viral nucleic acid profile is called occult hepatitis B infection (OBI) and has been defined as the long-lasting persistence of HBV DNA in the liver of patients with a HBsAg-negative status with or without antibodies to HBsAg and/or antibodies to hepatitis B core antigen, which are serological markers of previous exposure. These HBV blood markers are used to diagnose and identify the infection stage (previous or present infection) of patients [13, 14].

Mutations in certain regions of the HBV genome are commonly associated with OBI [22, 15–17]. Mutant strains of HBV have been detected in acute and chronic infections and, in the HBV open reading frames (ORFs), including preS/S, polymerase, precore/core, and X. The preS/S ORF codes for molecules that form HBsAg, which is the antigen recognized for host immunity and aids in the attachment of the virus to hepatocytes. The polymerase ORF codes for the reverse transcriptase domain of the HBV polymerase, and the precore/core ORF codes for the core nucleocapsid and envelope antigen [18]. Different methods for detecting different strains of the HBV polymerase gene include conventional DNA sequencing, PCR-based assays and reverse hybridization [19, 20].

The mechanism of OBI is incompletely understood; however, both viral and host-related factors resulting in sequence variations in HBV genomes and suppression of viral replication, respectively, have been implicated as possible causes of undetectable HBsAg in the presence of circulating HBV DNA [11, 14, 21]. Some studies have shown that the sG145R mutation as well as several other mutations within the “a” determinant of HBsAg decrease the binding affinity of the surface antigen to monoclonal antibodies developed against it, leading to a false-negative HBsAg status [12, 22, 23]. Similarly, mutations in the S region have been implicated in the reduced expression of hepatitis B surface proteins [12, 24]. Other mutations, such as the substitution of guanine with adenine at position 458 of the surface gene, which interferes with the splicing of the S gene mRNA, as demonstrated by Hass et al. [25], as well as therapy-induced amino acid substitution in the HBV polymerase and surface gene, have been associated with OBI [26]. HBsAg mutations could create false-negative HBV results during routine screening and lead to occult HBV infection, thereby posing a significant diagnostic challenge [27].

The prevalence rates of OBI range from 1% to 95% worldwide and are influenced by several factors, such as the endemicity of HBV, various cohort characteristics, including the presence of chronic HCV or other comorbid diseases, and the performance characteristics of laboratory techniques [28]. OBI is generally more common in locations where HBV is endemic, such as Asia and Africa, than in other regions; its prevalence is also higher in patients with hepatitis C virus (HCV) [12].

The clinical significance of OBI is reinforced by the potential risk of HBV transmission through blood transfusion, hemodialysis, and solid organ transplantation; cryptogenic liver disease; liver cirrhosis and HCC; and interference with antiviral treatment for patients with chronic HCV disease [13]. Despite the significant role of OBI in global health safety, however, knowledge of OBI remains limited. Moreover, a testing regimen with suitably high sensitivity and specificity for detecting OBI has yet to be incorporated into the HBV testing algorithms of many resource-limited countries, including Nigeria [29].

Very few studies have reported on the presence and circulation of mutant HBV strains in Nigeria [30]. Thus, the present study aims to detect and determine the prevalence of HBsAg and HBV DNA among female patients visiting the State Specialist Hospital in Osogbo, Osun State, Nigeria.

Materials and Methods

Ethics statement. Ethical clearance (No. HREC/27/04/2015/SSHO/029) for this study was obtained from the institutional review board of the State Specialist Hospital, Osogbo, Nigeria. Written informed consent was obtained from each patient in the presence of at least one witness prior to their participation in this research.

Sample size and sampling period. A total of 204 female patients with ages ranging from 1 year to 50 years and visiting the State Specialist Hospital, Osogbo, Nigeria, during the period of February 2017–April 2018 were recruited to this study. Access to the subjects’ medical histories and other demographic characteristics during the sampling period was not available.

Serological Detection of HBsAg. Approximately 2 mL of whole-blood samples was collected into EDTA bottles from each patient, and serum was obtained from each blood sample. Each serum sample was tested for HBsAg by using a commercial HBsAg rapid test kit (Acumen Diagnostic Test Strip; Acumen Labs and Diagnostic Center, Bangalore, India).

Molecular assay for HBV DNA. Extraction of hepatitis B virus DNA. HBV DNA was extracted from whole-blood samples using ZR viral DNA extraction kits (Zymo Research, USA) according to the manufacturer’s instructions.
Viral gene amplification. Nested PCR was conducted to amplify a 143 bp region of the surface gene (base pairs 303–446) by using the extracted genomic DNA as template DNA. The forward primer (HBV-FP) 5’ACTCACCAAACCTTGTCTT3’ and the reverse primer (HBV-RP) 5’GACAAACGGGCAACATACTT3’, which amplifies both wild- and mutant-type HBV, were used for primary and secondary PCR under the same thermal cycling conditions. Each reaction mixture comprised a total volume of 25 µL, including 12.5 µL of the PCR master mix (2x quick tar master mix Bio research), 1 µL each of the forward and reverse primers (10 µm), 5.5 µL of nuclease-free water, and 5 µL of the extracted DNA sample. Primary amplification was carried out using the extracted DNA template. Secondary PCR was done using the PCR products of the primary amplification. The thermal cycling conditions for the amplifications are as follows: 95 °C for 10 min, 35 cycles of 94 °C for 40 s, 56 °C for 60 s, elongation at 73 °C for 2.5 min followed by 72 °C for 4 min. Negative controls were integrated in all PCR rounds.

Gel electrophoresis. The PCR products were assessed by electrophoresis with 2% agarose gel with a 50 bp DNA ladder at 90 V. Electrophoresis was conducted for 45 min, and the band patterns were observed under ultraviolet light.

Statistical analysis. Descriptive statistics of the participants’ ages were obtained using Microsoft Excel 2010, and the frequencies of HBsAg- and HBV DNA-positive results between the serological (HBsAg) and molecular (nucleic acid) methods was compared among participants. Finally, the HB detection accuracy of the two methods was assessed by calculating the frequency of HB false-positive and -negative results.

Results

A total of 204 participants were included in this study. All of the participants were female, and their mean age was 30.5 years. HBsAg and HBV DNA detection was carried out in all 204 cases. Twenty-two (10.78%) cases were positive and 182 (89.22%) cases were negative for HBsAg; by comparison, 69 (33.82%) cases were positive for HBV DNA (Figure 1). The frequencies of HBsAg- and HBV DNA-positive results among age groups are shown in Figure 2. Figure 3 compares the total number of samples and proportions of seropositivity and seronegativity for both assays. No individual in the age group 11–12 years revealed a positive HBsAg or HBV DNA result. Fifty-four (26.47%) cases yielded false-negative results, while four (1.96%) cases yielded false-positive results (Table 1). The agarose gel electrophoresis results of some samples are shown in Figure 4.

The distribution of cases according to age and HBsAg- and HBV DNA-positivity was not evenly distributed; for example, more cases were positive for HBsAg and HBV DNA in the age groups 21–30 years and 31–40 years than in the other age groups. Individuals within the age group 21–30 years, followed by those in the age group 31–40 years, showed the highest incidence of seropositive results for both HBsAg and HBV DNA (Figure 2).

Figure 1. Comparison of Positive Results Detected by Molecular Assay and Rapid Testing
Molecular assay (PCR) revealed a greater number of positive results than the rapid kit assay.

Figure 2. Comparison of Numbers of Persons (in Age Groups) Positive for HBsAg and HBV DNA
The age groups 21–30 years and 31–40 years showed the highest incidence of seropositivity for both HBsAg and HBV DNA.

Figure 3. Distribution of HBsAg and HBV DNA Seropositivity and Seronegativity According to Age Group
Positive HBV DNA and HBsAg was recorded among the age groups 21–30 years and 31–40 years but not among the age group 11–20 years. HBV DNA but not HBsAg was detected among the age groups 1–10 years and 41–50 years.
Discussion

The detection and prevalence of HBsAg and HBV DNA in a portion of the female population of Osogbo, Osun State, Nigeria, is reported in this work. The results confirm the inclusion of Nigeria in the HBV crescent, as reported by Faleye [30]. HBV infection is widely spread and constitutes a major public health problem in sub-Saharan Africa. According to the WHO, a high endemicity of HBV infection is defined as a HBsAg prevalence greater than 7% [31]. In this report, 10.6% HBsAg prevalence was detected using the rapid kit and 34.6% HBV DNA prevalence was revealed by the PCR molecular-based assay. These results confirm the endemicity of HBV infection in the study population. Obi [32], Olokoba [33], and Eke [34] reported the high prevalence of HBV among pregnant women in different parts of the country. The high prevalence of HBsAg in the study population may be due to poor awareness, weak vaccination programs, and other risk factors that could promote the spread of HBV infection. In this study, the groups with the highest numbers of HBsAg-positive patients were those including sexually active and fertile persons. Thus, sexual activity and fertility may be contributing factors to the high prevalence of HBV infection recorded. A consequence of HBV infection in females of this age group (21–30 years and 31–40 years) is the high risk of vertical transmission (i.e., mother to child); the HBV-contaminated newborn may develop as an asymptomatic chronic carrier and progress to liver cirrhosis and HCC. Our results also showed significant differences in the numbers of positive HBsAg and HBV DNA cases detected in each age group.

Infected hepatocytes often contain covalently closed circular DNA with prolonged existence in their nucleus, which leads to difficulty in clearing chronic HBV infection [35]. Antiviral therapies to treat HBV infection include nucleoside/nucleotide analogs (NA), which aim to clear and prevent the progression of liver disease to cirrhosis and HCC. NAs are designed to target one or a combination of synthesis of the short negative DNA strand, DNA-dependent DNA replication or reverse transcription from pregenomic RNA to negative DNA strand [35]. However, some factors, such as HBV genetic variability, enable HBV to persist and resist NAs, which often leads to increases in HBV infection prevalence and the emergence of mutant strains. These factors/conditions may play various roles in promoting the high prevalence rate of HBsAg in the studied population. Non-detection of HBV via normal routine screening leading to occult infections is often caused by mutations in HBsAg [27]; hence, molecular-based assays should be used for HBV detection. In this study, comparison of the molecular assay and rapid test methods revealed false-negative and -positive results. Viral DNA was successfully isolated and amplified from 37 (21.5%) samples that were HBsAg-negative. Our findings demonstrated a high number of false-negative results obtained via the rapid diagnostic kit. This result is indicative of the presence of a large number of HBV mutant strains within the population studied. Mutations in HBsAg have been implicated to cause decreases in the binding affinity of the surface antigen to monoclonal antibodies and the expression of HB surface protein, which produces false-negative HBsAg results [12, 21]. This report is in agreement with Foy [27], who reported on a hemodialysis patient with high levels of HBV DNA and antibody but was HBsAg negative. Our findings also agree with those of Faleye [30] and Erhabor [37], who reported false-negative results among blood donor samples in Northwestern Nigeria.

HBV rapid-screening test kits are inferior compared with enzyme- and molecular-based assays and often associated with false-negative and -positive results. The
diagnostic accuracy, sensitivity, and specificity of HBV rapid-screening test kits are unreliable, and other assay techniques are usually necessary to confirm a diagnosis [30, 36, 37]. HBV rapid diagnostic test kits are known to vary in terms of their ability to detect certain genotypes and variants of HBsAg [27]. The inability of rapid test kits to detect HBsAg in some blood samples despite the detection of viral DNA by the molecular PCR technique may indicate HBV genetic variability among the population or an infection within the window period. The accuracy of PCR-based assay is extremely high, even in cases of low viremia in the samples [9]. The occurrence of mutant strains of HBV has been reported in different parts of the world [20, 27, 30, 38].

PCR assay revealed that some HBsAg-positive samples lack the presence of viral DNA, which indicates a false-positive result. The underlying causes of false-positive results are multifold and include viral mutation, drugs/medication, vaccine-induced HBsAg reactivity, and differences in the specificity of the test kits used [39–41]. PCR-based assays have long been employed to detect and diagnose bacterial, parasites, and viral infections. This method has proven to be reliable and yields higher sensitivity and specificity compared with other methods, such as microscopy or rapid test kits [42–46]. Several studies have evaluated the diagnostic accuracy and superiority of PCR-based assay in diagnosing infections and compared the method with rapid test kits [9, 42, 44, 47–49].

The high prevalence of HBsAg and HBV DNA in the population under study highlights the need for stakeholders to take appropriate measures to prevent the further spread of infection. Because accurate detection of HBV is of great importance, the rapid test kit method cannot be recommended as the sole assay for HBV screening; instead, employment and total incorporation of viral DNA molecular assay in all screenings involving HBV should be considered. This measure may help reduce the incidence of HBV false-negative results reported for blood screening cases and enable accurate and proper diagnosis.

The recruitment of only female patients to this study may have been a limitation to this work and may have introduced bias to the findings in this study.

**Conclusion**

The present study revealed the high prevalence of HBsAg in the studied population. It also showed high levels of false-negative results, which indicates the presence of HBV mutant strains and the limited detection and sensitivity of commercial rapid test kits for detecting genetic variations in HBsAg. PCR molecular-based assay was confirmed to have robust sensitivity and detection capability for detecting viral DNA. The results stress the need for total incorporation of PCR-based HBV diagnostics in all blood screening cases. Further investigations, perhaps with a larger population size, are necessary to ascertain the HBV genotype- and mutation type(s) circulating in the population and obtain a better understanding of the characteristics of HBV infection.

**Acknowledgements**

The authors thank ACEGID Redeemer’s University, Ede, Nigeria, for providing a facility in which the study could be carried out.

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