Serological Markers and Polymerase Chain Reaction (PCR) Detection of HBV DNA in HIV Sero-positive Patients in Port-Harcourt

Ayodele, Martins Bamidele Oluseun [1], Frank-Peterside Nnenna [2], Wariso Kennedy Tamunoimiegbam [1]

[1]Department of Medical Microbiology & Parasitology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, P. M. B. 5323, Port Harcourt, Rivers State, Nigeria.
E-mail: ufuomartins@yahoo.com: Tel: +2348037055953
[2]Department of Microbiology, Faculty of Science, University of Port Harcourt, P. M. B. 5323, Port Harcourt, Rivers State, Nigeria.
E-mail: nnenna.frank-peterside@uniport.edu.ng: Tel: +2348033106272

Abstract: This study was carried out to detect the serological markers and DNA of HBV in HIV co-infected patients in Port Harcourt. The immune system of HBV infected individuals normally produces antibodies against viral proteins found in the genome. The measurement or serological testing of such antibodies and antigens is the basis for diagnosis of HBV infection. Detection of viral antigens and nucleic acids in clinical specimens contributed to a better understanding of the pathobiology of HBV infection and disease. Twenty-five, HBsAg sero-positive serum samples from HBV-HIV co-infected patients were screened for HBsAg, HBsAb, HBeAg, HBeAb and HBcAb using the Quick profile HBV panel immunochromatography assay. The HBV DNA detection was done by rapid method using PCR assay with universal outer primers and amplified in 2% agarose gel electrophoresis. Thirteen, 13(52%) were females while 12(48%) were males. The results showed that, 25(100%) were sero-positive to HBsAg, 23(92%) were sero-negative while 2(8%) were sero-positive to HBsAb. Eighteen, 18(72%) were sero-negative while 7(28%) were sero-positive to HbeAg, 16(64%) were sero-negative while 9(36%) were sero-positive to HbeAb, 16(64%) were sero-positive while 9(36%) were sero-negative to HbcAb. The PCR results showed that HBV DNA was detected in 15(60%) while 10(40%) were negative. Sex distribution of HBsAg, HbsAb, HbeAg, HbeAb and HbcAb, sero-positivity in males and females in this study were not statistically significant ($p>0.05$). Detection of HBV DNA and screening of HBV serological markers should be considered as part of routine investigation in HBV infection. Adequate understanding, correct and timely interpretation of such results will assist clinicians in prevention, treatment and progression of HBV infection to liver cirrhosis and hepatocellular carcinoma.
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1. Introduction

Hepatitis B virus (HBV) chronic infection is characterized by the production of antigens antibodies against the virus, which can be detected in the blood by the assay of these substances, to detect either the viral proteins produced by the virus (antigens) or the antibodies produced by the host (WHO, 1999). Serological markers found in the individuals chronically infected by the HBV are, Hepatitis B surface antigen (HBsAg), antibody to Hepatitis B surface antigen (anti-HBs/HBsAb), Hepatitis B e antigen (HBeAg), antibody to Hepatitis B e antigen (anti-HBe/HBeAb), antibody to Hepatitis B core antigen (anti-HBc/HBcAb), and Hepatitis B virus Deoxyribonucleic acid (HBV DNA) (CDC, 2008).

Hepatitis B surface antigen (HBsAg) is the differentiating mark of HBV infection, it is the first serological marker to appear in acute hepatitis and when it persists for more than six months suggests chronicity (Kao, 2008). HBsAg may not be found at the later stage of the infection in some patients, which may be due to gradual clearance in an immunocompetent patients and such a patient will test negative (Liaw et al., 2010). HBeAg appears soon after the appearance of HBsAg in an infected person, it is a strong indication of active replication and enhanced infectivity, but may be absent in some patients due to possible infection by some variants of HBV (Lok and McMahon, 2007). Anti-HBs are produced against HBsAg, if it appears within 1 to 4 weeks after the beginning of symptoms and could indicate clinical recovery and subsequent immunity, because it has neutralizing ability on hepatitis B virus (Gitlin, 1997). After successful vaccination against HBV, the anti-HBs also develop in an individual so vaccinated (CDC, 2008). According to Gitlin (1997), Hepatitis B core antigen is found in HBV infection, essentially as infectious virions and contains an inner "core particle" where the genome of the virus is enclosed. The corresponding antibody to HbcAg is the

anti-HBc, the antibodies belong to the class of IgM and IgG but do not have the ability to neutralize the virus. However, the presence of IgM can be used to identify early infection (Mahoney et al., 1999, Robinson, 1995).

HBV DNA is responsible for the repairing of the region in the genes of the virus that is single stranded and renders them to become fully double stranded molecules and it is infective (Ochei and Kolhatkar, 2000, Chessbrough, 2000). Furthermore, anti-Hbe is released when HbeAg is found in an infected person, the progression of the HBV disease can be predicted based on the seroconversion from e antigen to e antibody, while the presence of anti-Hbe with anti-HBc coupled with the absence of the HBsAg, anti-HBs and core HBV mutants are very good indicators of low ability to transmit the HBV and a good sign of recovery in an infected individuals (Robinson, 1995).

The establishment of the outcome of the detection or assay of antigens or antibodies produced against HBV could be complex (WHO, 1999), and will usually require experts' interpretation.

In the present study, we highlight the profiles of serological markers of HBV and HBV DNA in HIV sero-positive patients in Port Harcourt.

2. Materials and Methods

This study was conducted at the University of Port Harcourt Teaching Hospital and Obio Cottage Hospital, both in Port Harcourt, the capital city of Rivers State South-south, Nigeria between January 2013 and March 2016.

A total of twenty five HBV-HIV co-infected individuals were enrolled in this study. Of which, 12(48.0%) were males and 13(52.0%) were females. Subject were confirmed HIV-infected, mean age was 33 year. The ethical approval was granted by the Ethical Review Committee of the hospitals. Informed consent was obtained and relevant confidentiality was maintained throughout the study.

Twenty five (25) HBV and HIV sero-positive blood samples were collected from co-infected subjects for this study. Venous blood was obtained into non-anticoagulated specimen bottles. The samples were centrifuged at 3000 revolution per minutes (rpm) for 5 minutes to obtain sera. The sera were stored at -20^0C for serologic assay of HIV, HBV and PCR.

Determine HIV1/II screening kit (manufactured by Alere Medical Co. Ltd, Japan) was used in this study. This is a qualitative immunoassay (rapid) method for detection of antibodies specific to HIV I/II simultaneously in serum. 50 µl of the serum sample was applied to the sample pad and after 15 minutes, the results were read. Red colour in the control and patient's windows indicated a positive result while presence of the red colour in the control and its absence in the patient window indicates a negative result.

Each serum sample was screened for HBsAg using a one step rapid immunoassay test (ABON Biopharm, Co., Ltd, China). The test strip was immersed vertically in the serum for 15 seconds, removed and waited for 15 minutes before the results were read. The test line region of the strip had been pre coated with recombinant HBV antigen. The presence of the red colour line indicated a positive result while the absence indicated a negative result.

The Quick profile HBV panel kit (LumiQuick Diagnostics, Inc. Santa Clara, CA, USA) was used in this study. This is a rapid qualitative immunochromatography assay of the markers of HBV including HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in human serum. 100 µl of serum was added into each sample well, results were read after 20 minutes but not more than 30 minutes. For the detection of HBsAg, HBsAb and HBeAg, red colour in the control and patient window indicated a positive result while the presence of red colour in the control and its absence in the patient window indicated a negative result. For HBeAb and HBcAb, red colour in the control window and its absence in the patient window indicated a positive result while red colour both in the control and patient windows indicated a negative result.

HBV DNA was extracted from 100 µl of serum samples using the Quick-gDNA Blood MiniPrep extraction kit (Zymo Research Corporation, USA) according to the manufacturer's instructions.

HBV DNA was detected by amplification of pre-S1 through S genes using universal primers, (P1) sense primer, (S1-2) antisense primer, for detection of all HBV genotypes according to described methods by Naito *et al*., (2001), and adopted by Rashid and Salih, (2014). The total reaction mixture was 40 µL. It was made up of 27.8µL of DEPC-H2O, 1X PCR reaction buffer with 15 mM MgCl2, 250µM concentration of each dNTP, 10 pMol primers, 1U Red hot Tag polymerase (Thermo scientific, UK) and 5 µL of extracted DNA. The thermocycler (GeneAmp PCR system 9700) was programmed to incubate the samples for initial denaturation at 94°C for 5 minutes, followed by 40 cycles consisted of denaturation at 94 °C for 30 seconds, annealing at 55°C for 1 minutes and elongation at 72 °C for 1.5 minutes. The final elongation was at 72 °C for 5 minutes.

The amplified products were resolved in a 2% agarose gel at 120 V for 20 minutes and visualized in a UV transilluminator alongside 91kb molecular marker.

3. Results

Twenty-five (25) HBV-HIV co-infected serum samples were analysed. Of which 12(48.0%) were from males while 13(52.0%) were from females. Figure 1 showed that 25(100.0%) were sero-positive to HBsAg while 0(0%) were sero-negative, 2(8.0%0 were sero-positive to HbsAb while 23(92.0%) were sero-negative, 7(28.0%) were sero-positive to HBeAg while 18(72.0%) were sero-negative, 9(36.0%) were sero-positive to HBeAb while 16(64.0%) were sero-negative, 16(64.0%) were sero-positive to HBcAb while 9(36.0%) were sero-negative, HBV DNA was detected in 15(60.0%) but not detected in 10(40.0%).

The age specific prevalence of the patients in this study is as shown in Table 2. It showed that age group 31-40 years had higher frequency of 12(48.0%) than the other age groups.

Table 1. Sex Distribution of HBV-HIV Co-infected Patients

Variables	Frequency	(%)
Sex		
Females	13	52.0
Males	12	48.0
Total	25	100.0

Table 2. Age Distribution among HBV-HIV Co-infected Patients

Variables	Frequency	%
Age in years		
20-30	8	32.0
31-40	12	48.0
41-50	5	20.0
Total	25	100.0

The sex specific prevalence of the serological markers and HBV DNA were as shown in Table 3. It revealed that 13(52.0%) females and 12(48.0%) males were sero-positive for HBsAg while 0(0%) of both sexes were sero-negative. Of the tota number 12(48.0%) females and 11(44.0%) males were sero-negative to HBsAb while, 1(4.0%) each of males and females were sero-positive. 9(36.0%) each of males and females were sero-negative to HBeAg while, 3(12.0%) males and 4(16.0%) females were sero-positive. 10(40.0%) females and 6((24.0%) males were sero-negative to HBeAb, while 3(12.0%) females and 6(24.0%) males were sero-positive. 8(32.0%) females and 4(16.0%) males were sero-negative to HBcAb while 8(32.0%) each of males and females were sero-positive. HBV DNA was not detected in 5(20.0%) each of females and males, but was detected in 8(32.0%) females and 7(28.0%) males.

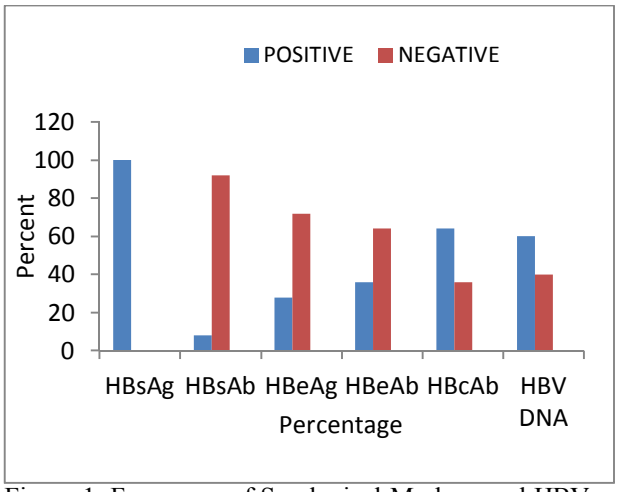

Figure 1. Frequency of Serological Markers and HBV DNA among HBV-HIV Co-infected Patient

Table 3. Sex Distribution of Serological Markers and HBV DNA among HBV-HIV Co-infected Patients

Serological Markers	Male				Female			
	Positive		Negative		Positive		Negative	
	Frequency	%	Frequency	%	Frequency	%	Frequency	%
HBsAg	12	48.0	0	0.0	13	52.0	0	0.0
HBsAb	1	4.0	11	44.0	1	4.0	12	48.0
HBeAg	3	12.0	9	36.0	4	16.0	9	36.0
HBeAb	6	24.0	6	24.0	3	12.0	10	40.0
HBcAb	8	32.0	4	16.0	8	32.0	5	20.0
HBV DNA	7	28.0	5	20.0	8	32.0	5	20.0

4. Discussion

This study highlighted the patterns of serological markers and HBV DNA among HIV co-infected patients in Port Harcourt. HBsAg was found in 25(100%) of the patients while 0(0%) were negative. The prevalence of HBsAg as a marker of HBV have been studied and prevalence of 6.67% by Frank-Peterside and Neenwi (2010), 7.8% by Anigilaje and Olutola (2013), 9.7% by Ejele et al., (2004), 12.5% by Ijarotimi et al., (2015), 51.9 by Iwalokun et al., (2006) and 70.5% by Nwokedi et al., (2006) have been documented, but most of the studies except Ijarotimi

et al., (2015) did not highlighted the serological markers, and HBV DNA detection was not done.

HBsAb was detected in 2(8.0%) of the patients while 23(92.0%) were sero-negative. The significance of presence of this antibody suggested clinical recovery and subsequent immunity (Gitlin, 1997) or successful vaccination against HBV (CDC, 2008). It therefore implies that 23(92.0%) of the patients who are sero-negative to HBsAb are chronically infected. Out of the 25 patients studied, 18(72.0%) were sero-negative to HBeAg and 7(28.0%) were sero-positive, the positivity ratio in these patients is an indication of active replication and enhanced infectivity (Lok and McMahon, 2007). The HBeAb profile showed that 9(36.0%) were sero-positive while 16(64.0%) were sero-negative. The antibody is present only in patients who have successfully cleared the HBV infection and can no longer transmit the virus to another person coupled with absence of viral replication (Robinson, 1995). The antibody to the core antigen, HBcAb was recorded as sero-positive in 16(64.0%) while it was sero-negative in 9(36.0%) of the patients. The appearance of HBcAb in 16(64.0%) of these patients could be the only serological evidence that HBV infection still exist in them both in early and late infection particularly where HBsAg is not detected (Liaw et al., 2010).

HBV DNA was detected in 15(60.0%) of the patients while it was not found in 10(40.0%). Irrespective of the presence or absence of the other serological markers in HBV infected person, the detection of HBV DNA is a more accurate index and can be used to confirm the accuracy or otherwise of the occurrence of the other serological markers. When it is no more detectable in an infection, it indicates complete clearance but its presence can be used to monitor treatment or to detect mutant that may escape current detection methods.

5. Conclusion

HBsAg was found in all patients examined in this study, but other serological markers were found in different ratios that were inconsistent with the sero-positivity of the HBsAg. Generally, HBsAg is the first detectable antigen in HBV infection and the most frequently used serological index in the diagnosis of HBV infection in Nigeria. However, findings in this study showed that other serological markers such as HBsAb, HBeAg, HBeAb and HBcAb and HBV DNA could exists simultaneously with this serological marker. This study is the first to report the serological markers and HBV DNA detection among HBV co-infected HIV patients in Port Harcourt, Nigeria.

The interpretation of the results of the serological markers' screening and HBV DNA detection is important to determine the infective status of the individual patients and possible commencement of treatment. It is then very imperative that the full complements of the serological status of every HBV infected patient is established before commencing treatment for HBV infection especially in HIV co-infected individuals. Furthermore, confirmation of infection using the detection of HBV DNA in such patients gives a more accurate diagnostic index than the use of serological markers only.

We therefore recommend that screening of serological markers and detection of HBV DNA should become part of routine investigations in the diagnosis and management of HBV infections.

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Correspondence to:
Ayodele, Martins B. O.
Dept of Medical Microbiology & Parasitology,
Faculty of Basic Medical Sciences,
College of Health Sciences,
University of Port Harcourt,
PMB 5323 Choba, East-West Road,
Port Harcourt, Rivers State, Nigeria;
ufuomartins@yahoo.com,
tuntun2503@gmail.com
Tel.: +2348037055953

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