Original Article

The Prevalence of Occult Hepatitis B Infection among Blood Donors in Lagos, Nigeria

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Abstract

Background: In occult hepatitis B virus (HBV) infection, the HBV DNA is present in the blood or liver tissue in patients negative for hepatitis B surface antigen (HBsAg) with or without anti-HBV antibodies. Thus, the absence of HBsAg in the blood only reduces the risk of transmission and is not sufficient enough to ensure the absence of HBV infection. **Aim:** This study was aimed at determining the prevalence of occult HBV infection among blood donors in Lagos. **Study Designs:** A cross-sectional study was done among 101 consenting blood donors at Lagos State University Teaching Hospital, Ikeja, between November 2016 and January 2017. **Materials and Methods:** HBV DNA analysis and viral load were done at the Molecular Laboratory of National Sickle Cell Centre, Idi Araba, Lagos, for all the HBsAg negative blood donors screened by rapid kit at Ikeja. **Results:** The prevalence of occult HBV DNA among the participants was 3% consisting of 3% prevalence of HBV DNA surface antigen and 0% prevalence for precore and core of the HBV DNA. **Conclusion:** The low prevalence (3%) of occult HBV seen in our study does not make it cost-effective to routinely screen blood donors or the general population for HBV infection using DNA polymerase chain reaction.

Keywords: Blood donors, hepatitis B surface antigen, hepatitis B virus DNA

Introduction

Hepatitis B virus (HBV) infection is a major public health problem worldwide and remains the most frequent transfusion-transmitted viral infection.[1-3] The DNA virus was first identified by Blumberg *et al*. in 1965,[4,5] and it is the prototype member of the hepadnaviridae family that causes acute and chronic liver disease, including cirrhosis and hepatocellular carcinoma (HCC). HBV is classified into ten genotypes (A–J) with distinct geographical distribution,[6] with the E genotype being the most prevalent in Nigeria[7] and a prevalence ranging from 9% to 39%.

Routine screening of donor blood for hepatitis B surface antigen (HBsAg)[8] does not preclude transmission of HBV hence the importance of occult HBV infection determination. Thus, the absence of HBsAg in the blood is supposed to reduce the risk of transmission.

In occult HBV infection, the HBV DNA is present in blood or liver tissue in patients negative for HBsAg with or without anti-HBV antibodies.[9,10] Majority of the patients with occult HBV infections are asymptomatic, and detection is usually by viral DNA screening. The chronic occult HBV infection may progress to chronic liver diseases such as liver cirrhosis and HCC.[11]

Furthermore, it may persist in individuals for years before any symptoms of overt HBV infection emerge.[12]

Patients with occult HBV infection have been further subclassified as having "seropositive" or "seronegative" HBV depending on whether they are positive or negative for other HBV markers, most commonly anti-HBc.[13,14] Most of these patients have very low or undetectable serum HBV DNA levels, which account for the failure to detect HBsAg.

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Worldwide, the prevalence of occult HBV is variable and depends on a number of factors, for example, type of assays used in the studies, the population studied as well as the level of disease endemicity in the area studied.[15] Studies in Nigeria have shown differing prevalence rates among blood donors, with a rate of 17% in Oluyinka *et al*.[16] and 5.4% in Ile-Ife.[17]

Occult HBV infection harbors the potential risk of HBV transmission through blood transfusion, hemodialysis, and organ transplantation. It may also affect disease progression and treatment response of chronic hepatitis C.[18]

The diagnosis of occult HBV infection has become possible through the use of molecular biology techniques such as HBV nucleic acid amplification testing. This is a polymerase chain reaction (PCR) technique with detection limits of ten copies of HBV DNA per reaction.[19]

The aim of this study was to determine the prevalence of occult HBV infection among blood donors in order to determine if screening for occult HBV infection in blood donors should be done routinely.

Materials and Methods

Study design

A cross-sectional study was done among consenting blood donors and participants were recruited consecutively.

Study area

Participants' recruitment was at Lagos State University Teaching Hospital (LASUTH), Lagos State. The HBV DNA PCR was done at the Molecular Laboratory of the National Sickle Cell Centre, Idi-Araba, Lagos.

Study period

The study was done over 3 months from November 2016 to January 2017.

Population and size of study

The population consisted of 101 blood donors.

Determination of hepatitis B virus viral load

It was done at the Molecular Laboratory of the National Sickle Cell Centre, Idi-Araba, Lagos. The included samples viral load was 160 IU/ml.

Inclusion criteria

1. Consenting participants
2. HBsAg negative.

Exclusion criteria

1. HBsAg positive patients (by rapid screening)
2. Intravenous drug users
3. Nonconsenting participants.

Ethical considerations and clearance

Ethical approval was obtained from the Health Research Ethics committee of LASUTH, reference number LREC/10/06/547. Ethical standards and procedures of the committee for human experimentation were adequately followed.

Participant's informed consent

A written informed consent was obtained from each participant, and this was duly signed.

Definition of occult hepatitis B infection[19]

Occult hepatitis B infection

"Presence of HBV DNA in the liver (with detectable or undetectable HBV DNA in the serum) of individuals testing HBsAg negative by currently available assays.

When detectable, the amount of HBV DNA in the serum is usually very low (<200 IU/ml)."

Occult hepatitis B infection (OBI) can be classified on the basis of antibody profile as seropositive, in which anti-HBc and/or anti-HBs are positive or seronegative OBI in which anti-HBc and anti-HBs are negative.

False occult hepatitis B infection

"Serum HBV DNA levels comparable to those who usually detected in the different phases of serologically evident HBV DNA infection, it is usually due to infection by HBV variants with mutations in the S Gene producing a modified HbsAg that is not recognized by some or all commercially available detection assays."

Questionnaire administration and history taking

With the use of a questionnaire administered by the researchers, each participant was interviewed to obtain relevant demographic and history of immunization of HBV in childhood.

Specimen collection

Four milliliters of blood was collected and 2 ml dispensed into a plain sample bottle to obtain serum for HBsAg rapid screening test of all participants, which was done on the same day of collection before inclusion in the study. A volume of 0.5 ml of the serum obtained from each plain sample bottle was aliquoted in cryovials and stored. The other 2 ml of blood was dispensed into ethylenediaminetetraacetic acid (EDTA) sample bottles. The blood sample was properly mixed with the anticoagulant. One milliliter of whole blood and 1 ml of plasma were obtained from the EDTA blood sample and aliquoted in cryovials for DNA analysis. Samples for DNA analysis were transported in a cold chain to the Molecular Laboratory of the National Sickle Cell Centre, Idi-Araba, Lagos. The samples were then stored for DNA extraction and subsequent genetic analysis.

Hepatitis B virus DNA polymerase chain reaction

Plasma DNA isolation was done using QIAamp MinElute Virus Spin Kit (Qiagen Germany). Nested PCR was performed using specific primers derived from the regions coding for HBsAg, hepatitis B core antigen, and pre-C, respectively. Shown in the Table 1 are primer sequences and amplicon sizes. The first-round PCR was carried out in a final volume of 25 μl containing 1 unit of Taq polymerase (Jena Bioscience, Germany), 200 μM dNTP mix, 2.5 μl × 10 Taq polymerase buffer, 15 mM $MgCl_2$ and 10 pmol of each primer (Exiqon,

the Netherlands), molecular grade water (Jena Bioscience, Germany), and 2.5 µl DNA template.

The amplification was carried out for 35 cycles (20 s at 94°C, 30 s at 55°C, and 45 s at 72°C) after initial denaturation for 2 min. A final extension step was performed for 10 min at 72°C. The second-round PCR was carried out using 5 µl of the first PCR product under the same condition as the first-round PCR except that 25 pmol of each internal primer was used. A 15-µl aliquot of the PCR products was electrophoresed on 2% agarose gel at 170V for 30 min and then stained with ethidium bromide (Promega, USA) using ×1 tris boric acid EDTA buffer. Bands of the appropriate size were visualized by gel documentation system (Alpha Imager and Alpha Innotech).

Oligonucleotide primers for the detection of occult HBV DNA through "nested"-PCR amplification are shown in Table 1.

Statistical analysis

Data obtained were analyzed using IBM SPSS (Statistical Package for Social Sciences, Inc.) statistics for windows version 20.0 Armonk, New York, USA. Data were presented in percentages and analyzed using Chi-square test and Pearson's correlation as appropriate, to get the *P* value. The differences were considered to be statistically significant, where $P < 0.05$. Results were also presented in tables and figures.

Results

This was a cross-sectional study of 101 participants. A total of 110 participants were initially recruited into the study. Nine participants were excluded because they were found to be HBsAg positive on rapid screening.

Age and sex distribution

The mean age was 30.3 ± 12.0 years and 35.6% were male, while 64.4% were female. The age and gender records of four participants were not captured in the data [Table 2].

Prevalence of occult hepatitis B virus infection

The prevalence of occult HBV DNA among the participants was 3% [Table 3] consisting of 3% prevalence of HBV DNA surface antigen and 0% prevalence for precore and core of the HBV DNA, and the samples' viral load was 160 IU/ml.

The agarose gel electrophoresis of positive and negative control samples is presented in Figure 1.

The three positive HBV DNA samples and the controls are presented in Figure 2.

There was no significant association between childhood immunization in blood donors and occult HBV infection ($\chi^2 = 0.892$, $P = 0.345$). A total of 22 blood donors did not respond as they were not sure if they had childhood immunizations [Table 4].

Discussion

There are four phases of chronic HBV infection. These phases, which are based on the virus-host interaction, include immune tolerance, immune clearance, low or nonreplication, and reactivation, which gives rise to HBsAg negative occult HBV.[20]

Occult HBV infection is a challenging clinical entity, which has been detected in patients with cryptogenic chronic liver disease and may be related to the progression of liver fibrosis and development of HCC.[19]

Patients with occult HBV infection usually do not have active liver disease, but on liver biopsy, variable degrees of fibrosis are present.[21]

The seroprevalence of occult HBV infection in our study was 3%. This is similar to a study done at Ile-Ife, Nigeria, where

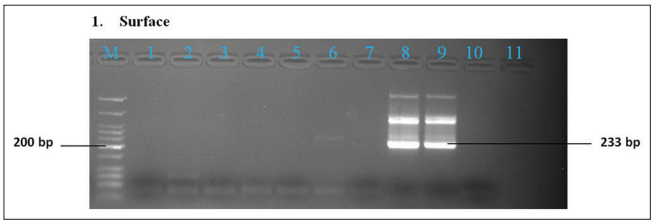

Figure 1: Agarose gel representation of hepatitis B surface antigen showing positive and negative controls and negative test samples. Agarose gel representation of hepatitis B surface antigen showing positive and negative controls and negative test samples: Lane M – Molecular weight marker (low range); Lane 1-7 – negative test samples; Lanes 8 and 9 – Positive control samples; and Lanes 10 and 11 – Negative control samples

Table 1: Occult hepatitis B virus infection primers

	Forward (5'----3')	Reverse (5'---- 3')	Size (bp)
HBsAg			
1st round	TCGTGTTACAGGCGGGGTTT	CGAACCACTGAACAAATGC	513
2nd round	CAAGGTATGTTGCCCGTTTG	GGCACTAGTAAACTGAGCCA	233
HBcAg			
1st round	ACTGTTCAAGCCTCCAAGCT	GGAATACTAACATTGAGATTCCCGAG	600
2nd round	TGCTCTGTATCGGGAGGC	AGTGCGAATCCACACTC	280
Precore/core			
1st round	GCCTTAGAGTCTCCTGAGCA	GTCCAAGGAATACTAAC	442
2nd round	CCTCACCATACTGCACTCA	GAGGGAGTTCTTCTTCTAGG	340

HBsAg – Hepatitis B surface antigen

Table 2: Age and sex distribution

Age (years)	Male	Female	Total
<20	4	14	18
20-30	14	27	41
31-40	11	7	18
41-50	2	8	10
51-59	5	5	10
>59	0	0	0
Total	36	61	97

Table 3: Prevalence of occult hepatitis B virus infection

Occult HBV infection	Blood donors	Percentage
HBV DNA		
Positive surface antigen	3	3
Negative surface antigen	98	97
Total	101	100
Positive core antigen	0	0
Negative core antigen	101	100
Total	101	100
Positive precore antigen	0	0
Negative precore antigen	101	100
Total	101	100

HBV – Hepatitis B virus

Table 4: Childhood hepatitis B virus immunization

	HBV DNA +VE	HBV DNA -VE	Total
Immunization in childhood	1	49	50
No immunization in childhood	0	29	29
Total	1	78	79

22 individuals did not remember whether they had immunization during childhood. HBV – Hepatitis B virus

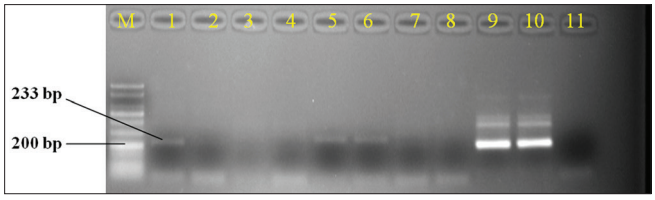

Figure 2: Agarose gel representation of hepatitis B surface antigen showing three positive samples with controls. Agarose gel representation of hepatitis B surface antigen showing three positive samples with controls: Lane M – Molecular weight marker (low range); Lanes 1, 5, and 6 – positive samples; Lanes 2–4 and 7–8 are negative test samples; Lanes 9 and 10 – Positive control samples; and Lane 11 – Negative control sample

Olotu *et al*. documented a prevalence rate of 5.4% in their study done among 504 blood donors.[17]

In contrast, similar studies done among blood donors in other parts of Nigeria have reported higher prevalence rates, ranging from 8% found by Nna *et al*. in the Southeastern part of Nigeria[22] to 18% among 429 blood donors documented by Oluyinka *et al*. in southwestern Nigeria.[16]

The difference in the prevalence of occult HBV in these studies may be as a result of varying sample sizes used, methodology of the assays as well as the different burdens of chronic HBV infection seen in different geographical locations.

The exact pathogenesis of occult HBV infection is not yet fully understood, and various hypotheses have been suggested. Both host immunity and viral factors are important in ensuring that viral replication is sustained at very low levels.[23] Host factors which may play a role in the pathogenesis include immunosuppression.[24]

There is evidence that occult HBV infection is a veritable source of contamination in blood and organ donations; it may also act as a reservoir from which full-blown hepatitis can arise. Therefore, the clinical implications of occult HBV infection in transfusion and transplantation medicine cannot be overemphasized.[10]

Transfusion-transmitted HBV infection is still a possibility in Nigeria despite the use of enzyme-linked immunosorbent assay (ELISA) for HBsAg screening; there is also a risk of transfusion of HBV-infected blood through donors with occult HBV infections.

Occult HBV infection has clinical implications in the setting of immunosuppression, radiotherapy, immunotherapy, or chemotherapy as it could flare up.[18] OBI detection is also clinically important in the cases of orthotopic liver transplantation from an OBI-seropositive donor to seronegative recipient who may require prophylactic use of lamivudine.[25,26]

HBV infection is commonly transmitted either vertically from a chronically infected mother to her child or horizontally, and HBsAg positivity rates are as high as 15% in Nigeria.[27] Therefore, universal vaccination against HBV as well as postexposure prophylaxis with hepatitis B immunoglobulin remains the most effective tools available to reduce the incidence of HBV infection and occult HBV infection in Nigeria.

Due to the high prevalence of HBV infection in Nigeria, a diagnosis of occult HBV infection should be considered in the differential diagnosis of patients with apparent cryptogenic chronic liver disease who are HBsAg negative. However, the suitability or otherwise of HBV DNA as a screening tool can be based on the World Health Organization (WHO)[28] criteria for evaluating population-wide screening tests, which may help decide whether or not to have such a test. The WHO criteria include the following:

- Screening should be done only for diseases with serious consequences so that screening tests could potentially have clear benefits to people's health
- The test must be reliable enough, and not harmful in itself
- There must be an effective treatment for the disease when detected at an early stage, and there has to be scientific proof that that treatment is more effective when started before symptoms arise

- Neutral information should be made available to the public, to help people decide for themselves whether or not to have a screening test.

Conclusion

There is a low prevalence (3%) of occult HBV in our study.

Study limitations

This research was self-funded, and cost was a major limiting factor. Use of ELISA as a screening tool instead of the rapid kit for the HBsAg would have been more appropriate. A small sample size for a population study is also a limitation.

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Conflicts of interest

There are no conflicts of interest.

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