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Research Article

Occult Hepatitis B Virus Infection among Sickle Cell Anaemia Subjects in Lagos, Nigeria

Badiru M.A.[1], Akinbami A.A.[2], Uche E.I.[2], Ibrahim I.N.[3], Bamiro R.A.[4], Adelekan O.O.[5], Benjamin A.[3], Suleiman A.M.[3], Ogbenna A.A.[6], Adeyemi I.O.[4], Anaduaka C.D.[4]

[1]Department of Pathology, National Orthopaedic Hospital, Igbobi, Lagos. Nigeria.
[2]Department of Haematology and Blood Transfusion, Lagos State University College of Medicine, Lagos. Nigeria.
[3]Department of Haematology and Blood Transfusion, Ahmadu Bello University, Zaria. Kaduna State. Nigeria
[4]Department of Haematology and Blood Transfusion, Lagos State University Teaching Hospital, Ikeja
[5]Public Health Advisor Canadian Red Cross. [6]Department of Haematology and Blood Transfusion, College of Medicine, University of Lagos

[*]***Author for Correspondence:***
Badiru M. A.
Department of Pathology, National Orthopaedic Hospital, Igbobi, Lagos
Email: mulkahlav@gmail.com

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ABSTRACT

Objective: Hepatitis B virus (HBV) infection remains a major public health problem worldwide. HBV is one of the transfusion transmissible infections. Occult HBV infection (OBI) is the presence of HBV DNA in blood or liver tissue in hepatitis B surface antigen (HBsAg) sero-negative subjects. Thus, the absence of HBsAg in the blood is inadequate to determine the presence of occult HBV infection. This study aimed to determine the seroprevalence of occult HBV infection among sickle cell anaemia (HbSS) subjects in Lagos, Nigeria.

Methods: This was a cross sectional study among 100 consenting adult HbSS patients attending the Haematology out-patient clinic at the Lagos State University Teaching Hospital, Ikeja (LASUTH). All participants were screened for HbSAg using SD Biolin HBsAg rapid kit and those with negative results had HBV DNA Polymerase Chain Reaction (PCR). Data were analyzed by statistical package for social science (SPSS) version 23, $p \leq 0.05$ was considered significant.

Results: The prevalence of occult HBV infection among the HbSS participants was 1%, consisting of 1% prevalence for the surface antigen and 0% prevalence of pre-core and core antigens of the HBVDNA.

Conclusion: The low prevalence (1%) of occult HBV seen in our study shows that it may not be cost effective to routinely screen HbSS subjects for Occult HBV infection using PCR.

INTRODUCTION

Sickle cell anaemia (SCA) is a common haemoglobinopathy in Nigeria affecting about 2% of the population.[1] HbSS is characterized by; Chronic haemolytic anaemia with intermittent hyperhaemolytic crisis, increased susceptibility to infections and vaso-occlusive crisis.[2] HBV infection is of high interest in HbSS patients because they are chronic blood transfusion recipients as a result of their frequent anaemia and as such stand the risk of acquiring HBV infection. Therefore, the need to determine the prevalence of occult HBV infection amongst SCA patient cannot be underestimated. Liver and biliary tract dysfunctions are also common complications of SCA.[3]

Hepatitis B Virus

Hepatitis B virus (HBV) infection remains a major public health problem worldwide.[4] HBV is the only DNA virus with a human only reservoir.[5] It was first identified by Blumberg and colleagues in 1965 as a circular DNA molecule of about 3,200 bases encased within viral specific proteins.[6-8] HBV is the prototype member of the Hepadnaviridae family that causes acute and chronic liver disease including cirrhosis and primary liver cell carcinoma. There are 10 known genotypes (A-J) of HBV with distinct geographical distribution.[9] HBV E genotype is the most prevalent in Nigeria with a prevalence range of 9% - 39% [10,11] HBV is a transfusion- transmissible infection that can occur despite serum HBsAg negativity.[12,13] Hence, the term occult HBV infection.

Occult HBV

Occult HBV infection is the presence of HBVDNA in blood or liver tissue of HBsAg sero-negative patients [14,15]. Occult HBV infection is manifested by the presence of very low levels (< 200IU/ml) of Hepatitis B Viral DNA (HBV DNA) in the blood or the liver while exhibiting undetectable HBV Surface Antigen (HBsAg) [16]. Most occult HBV infections are asymptomatic and would only be detected by viral screening for HBVDNA. Occult HBV infection may persist in individuals for years before any symptom of overt

HBV infection emerges.[17] The long asymptomatic nature of chronic occult HBV infection in patients poses the risk of developing severe, long term liver damage such as liver cirrhosis and Hepatocellular carcinoma, which may lead to premature deaths.

Occult HBV infection has been found to be more prevalent among subjects at high risk for HBV infection and with liver disease.[18] Occult HBV may be due to HCV co-infection and genetic mutations in the viral surface gene.[19, 20] In sickle cell anaemic patients, the Relative Risk (RR) of blood transmitted HBV infection has been found to be 5.8% with an attributable risk (AR) of 22%, despite existing screening modalities.[21]

Worldwide the prevalence of occult HBV infection is quite variable, depending on the level of the disease endemicity, the assay technique used, and the different populations studied.[22] The gold standard for diagnosing occult HBV infection is the use of HBV Nucleic acid amplification (NAT) which is a PCR technique with detection limits of 10 copies of HBV DNA per reaction.[23] In Nigeria, HBV infection studies have shown a 17% prevalence rate of occult HBV infection in Ogbomoso,[24] 5.4% in Ile-Ife[25] among blood donors and 14.6% among end stage renal disease patients on haemodialysis in Lagos.[26]

The aim of this study was to determine the prevalence of occult HBV infection among HbSS patients so as to ascertain if routine screening for occult HBV in sickle cell anaemic patients isnecessary as part of their management protocol in order to aid the early diagnosis of HBsAg negative but occult HBV positive HbSS patients.

MATERIALS AND METHODS

Study Location

Patients' recruitment was at Haematology out-patient clinic of LASUTH. The HBVDNA PCR was done at the molecular laboratory of the National Sickle Cell Centre, Idi-araba, Lagos.

Study Design

This was a cross-sectional study done among consenting adult HbSS patients attending the adult Haematology Clinic of the Lagos State University Teaching Hospital. Ikeja. Nigeria.

Study Period

The study was done over a period of 3 months, from November 2016 to January 2017.

Population and Study Size

The population consisted of 100 consenting HbSS patients.

Inclusion Criteria

1. Consenting HbSS subjects only
2. HBsAg negative using SD Biolin HBsAg rapid kit.

Exclusion Criteria

1. HBsAg positive
2. Other Hb phenotypes (e.g. SC, SD)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 duly signed informed consent was obtained from each consenting HbSS patient

Specimen Collection

Five (5mls) of blood was collected, out of which 2mls was dispensed into a plain sample bottle and the remaining 3mls into an EDTA sample bottle. About 1ml of serum from the plain sample bottle was used for HBsAg rapid screening. A total of 0.5mls of the serum obtained from each plain sample bottle was aliquoted in cryovials and stored. About 1ml of plasma was obtained from the whole blood EDTA sample bottle, aliquoted in cryovials and transported in a cold chain for subsequent storage for a maximum of three months at -20oC before the HBV DNA extraction and genetic analysis were done at the molecular laboratory of the National Sickle Cell Centre, Idi- araba, Lagos. The remaining 2mls of whole blood was used for complete blood count and Haemoglobin Electrophoresis of each HbSS participants.

HBV DNA Polymerase Chain Reaction [27]

Plasma DNA isolation was done using Q/Armp MinElute Virus spin kit (Qiagen Germany) Nested PCR was performed using specific primers derived from the regions coding for HBsAg, hepatitis B core antigen (HBcAg) and pre-C respectively. Primer sequences and amplicon sizes are shown below in table 1. The first round PCR was carried out in a taq master mix volume of 25µlcontaining 1unit of Taq polymerase (Jena Bioscience, Germany), 200µM dNTP mix, 2.5µ1 10x Taq polymerase buffer, 15mM, and 10 pmol of each primer (Exiqon, Netherlands), molecular grade water (Jena Bioscience, Germany), and 2.5µ1 eluted DNA template.

The amplification was carried out for 35 cycles (20 seconds at 94^0C, 30 seconds at 55^0C, 45 seconds at 72^0C) after initial denaturation for 2min. A final extension step was performed for 10 min at 72^0C. The second round PCR was carried out using 5µ1 of the PCR product under the same conditions 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 30mins and then stained with ethidium bromide (Promega, USA) using 1X Tris boric acid EDTA (TBE) buffer. Bands of the appropriate size was visualised by gel documentation system (Alpha Imager, Alpha Innotech) and an Olympus camera which is linked to a computer software was used to improve the quality of the image.

Statistical Analysis

Data obtained were analyzed using statistical package for social science (SPSS version 23). The data were presented in percentages and analyzed using chi-square test to get the P-value. The differences were considered to be statistically significant where the P-value obtained was ≤ 0.05. Results were also presented in tables and figures.

RESULTS

This was a cross-sectional study involving 100 HBsAg

seronegative HbSS patients. Initially, a total of 111 were recruited into the study. Seven of the participants were excluded because they were HBsAg positive on rapid screening and four others were excluded because they had the HbSC phenotype.

Age and sex distribution

The mean age of the participants was 24.4 + 7.9years, consisting of 44% males and 56% females; as shown in Table II.

Prevalence of occult Hepatitis B Virus Infection

The fast result, readily accessible SD Biolin HBsAg rapid kit, was used for this study as against the cumbersome ELISA screening because SD Biolin HBsAg rapid kit equally has a high sensitivity of >99% and specificity of >98% which is very close to a routine ELISA screening. Moreover, SD Biolin HBsAg rapid kit has a very low rate (0.5%) of false negative results [28]

The prevalence of occult HBV DNA among the HBsAg seronegative HbSS participants was 1% (Table III) consisting of 0% prevalence for HBV DNA pre-core and core antigens and 1% prevalence of HBV DNA surface antigen. The sample's viral load was 180IU/ml. The agarose gel electrophoresis of the positive and negative control samples is presented in figure 1. The only positive HBV DNA test sample with positive and negative control sample is shown in figure 2.

Table 1: OBI Primers

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

Oligonucleotide primers for detection of occult HBV DNA through nested PCR amplification (Table 1)

Table 2: Age and sex distribution

Age in years	Male	Female	Total
<20	15	15	30
20-30	23	29	52
31-40	5	9	14
41-50	1	3	4
Total	44	56	100

Table 3: Prevalence of Occult HBV Infection in Hbss

HBV DNA Codon	HBsAg	HB pre-core Ag	HB core Ag
Positive (%)	1	0	0
Negative (%)	99	100	100
Total (%)	100	100	100

1. SURFACE

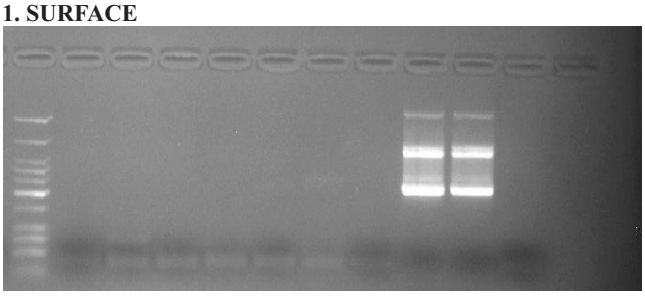

Figure 1: Agarose Gel representation of HBsAg showing Positive and Negative Controls and Negative Test Samples.

Agarose gel representation of HBsAg showing Positive and negative controls and negative test samples:
Lane M = Molecular weight marker (Low range) in the DNA Ladder for comparison
Lane 1 – 7 = Negative test samples not containing HBsAg DNA codon.
Lane 8 – 9 = Positive control samples containing HBsAg DNA codon
Lane 10 – 11 = Negative control samples not containing HBsAg DNA codon. On the positive control samples,
Lower positive band = HBsAg DNA Codon Middle positive band = HB core Ag DNA Codon
Upper positive band = HB pre-core Ag DNA Codon

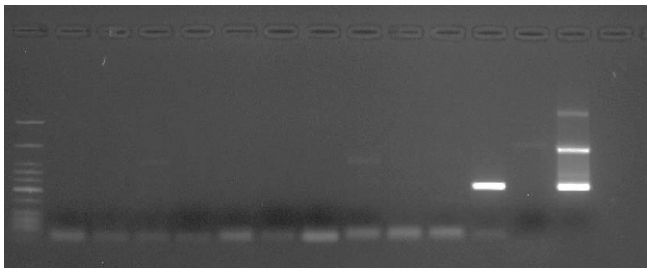

Figure 2: Agarose Gel representation of HBsAg showing one Positive Test Sample with Controls

Agarose gel representation of HBsAg showing one positive test sample with controls: Lane M = Molecular weight marker (Low range); Lanes 1-10 and 12 = negative test samples; Lane 11 = positive test sample; Lane 13 = Positive control sample; Lane 14 = negative control sample.

DISCUSSION

Occult HBV infection is a challenging clinical entity that has been detected in patients with cryptogenic chronic liver disease and has been found to be contributory to the development of progressive liver fibrosis and Hepatocellular carcinoma. [29,30] Chronic HBV infection occurs in four phases based on the virus-host interaction. The phases are; immune tolerance, immune clearance, low or non-replication and reactivation which gives rise to HBsAg negative occult HBV.[31]

The exact mechanism for the pathogenesis of occult HBV is yet to be fully understood and many hypotheses have been suggested. Host factors such as immunosuppression [32] and viral factors such as the oncogenic potency of the virus [33] may play a role in the pathogenesis of occult HBV. Interactions between these host and viral factors are important in keeping the viral replication at very low levels [34].

Occult HBV may be due to HCV co-infection and genetic mutations in the viral surface gene. [19,20]. The molecular mechanisms involved in HCV co-infection causing occult HBV are not fully understood. Scientific suggestions have it that occult HBV infection can be chronically suppressed by HCV infection with alternate phases of dominance of one virus on the other [35, 36]. Furthermore, occult HBV reactivation may occur once the inhibitory effect of HCV co- infection is removed following HCV treatment [37].

In our study, occult HBV seroprevalence of 1% was found among the 100 HbSS participants. This correlates well with a prevalence of 2.2%.[38] in a UK-based study of haemodialysis patients and 1.26%.[39] obtained by El-Zayadi et al. In contrast, similar studies done in other parts of Nigeria have reported higher prevalence rates, such as 5.4% by Amadin *et al* [25] in Ile-ife among 504blood donors and 14.6% among end stage renal disease patients on haemodialysis in Lagos.[26]. The difference in the prevalence of occult HBV in these studies may be attributed to the larger sample size, varying endemicity of chronic HBV infection in different parts of Nigeria and use of more sensitive Real time PCR as against Nested PCR technique used in this study. Furthermore, the young age in this study may contribute to the low prevalence compared to other studies.

Occult HBV infection can be transmitted through blood, stem cell or organ transplantation causing typical hepatitis B in newly infected individuals or may be induced by immunosuppression.[40] Blood is the most important vehicle for transmission but other body fluids have also been implicated, including semen and saliva.[41] Occult HBV infection is of high interest in HbSS patients because of their increased risk of acquiring HBV infection through therapeutic blood transfusion.

Though, no valid association can be deduced between blood transfusion and occult HBV infection from this study, the clinical implications of occult HBV infection in transfusion medicine, stem cell transplantation and other organ transplantations cannot be undermined. Moreover, evidence has shown that occult HBV infection can be a source of virus contamination in blood and organ donations, as well as act as reservoir from which full-blown hepatitis can arise.[42]

With a 1% prevalence of occult HBV infection from this study it may appear cost ineffective to routinely screen HbSS patients for occult HBV using HBV DNA PCR technique. However, we recommend that blood donors be screened for occult HBV as the donor may be in the seroconversion (window) period. Furthermore, occult HBV infection screening should be considered in patients with apparent cryptogenic liver disease who are HBsAg negative.

CONCLUSION

There is a low prevalence (1%) of occult HBV infection among HbSS patients in Lagos from our study.

Study Limitations

This research was fully self-funded, and cost was a major limiting factor. A larger sample size would have been more appropriate for such population study.

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Competing Interest: The authors declare no competing interest or conflict of interest.

Authors' contributions: Study conceptualization and design was done by the lead author, MB. Data Acquisition: MB, AA, EU

Data Analysis and Interpretation: MB, AA, BH
Manuscript drafting and review: All authors
Final manuscript approval: All authors.

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