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Missed Serologic Diagnosis of Hepatitis B Virus Infection among Blood Donors in Benue State University Teaching Hospital, Makurdi, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author NIS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OS managed the literature searches and analysis of the study. Author UP assisted in the statistical analysis. Authors EO, NA and EBC read and approved the final manuscript.

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ABSTRACT

Background: Serologic undetected Hepatitis B Virus infections have posed a significant global threat in blood transfusion with attendant active liver disease.
Objective: To detect occult Hepatitis B Virus infection (OBI) in subjects with Hepatitis B surface antigen negative-sera.

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Methodology: One hundred and seventy (170) serum samples were randomly collected from leftovers of the hepatitis B surface antigen (HBsAg) seronegative tested blood samples belonging to anonymous blood donors in the blood bank unit of Benue State University Teaching Hospital, Makurdi. Fifty HBsAg positive samples were employed as controls. The serum samples were tested for Hepatitis B Virus serologic profiles such as an anti-HBs antibody, anti- HB core antibody and anti-HBe antibody by HBV combination immune-chromatographic rapid kits manufactured by Acumen Diagnostics Incorporated (Lot SAG91108; expiry date 12/2018) and compared with results detected from nested polymerase chain reaction carried out.
Results: Hepatitis B Viral DNA (<50 copies/ml) was detected in 5.9% (n=10/170) total HBsAg negative samples as OBI; 71.0% (n=120/170) of total HBsAg negative samples was positive for anti-HBs antibody; 15.3% (n=26/170) was positive for anti- HBe antibody; 8.2% (n=14/170) was sero-positive for Hepatitis Bcore antibody (anti-HBcore). Out of the 10 OBI positive samples, 100.0% (n=10/10) was sero-positive for Hepatitis B core antibody (anti-HBcore); 60.0% (n=6/10) positive for anti-HBs antibody and 30.0% (n=3/10)HBeAg.
Conclusion: Our findings showed a 5.9% prevalence rate of Occult Hepatitis B Virus infection (OBI) among blood donors in Benue State University Teaching Hospital, Makurdi, Nigeria.
However, Hepatitis B Virus core antibody (anti- HBc) serologic screening test could have unmasked this hidden diagnosis. Hence anti-HBc antibody serologic screening test should be made a mandatory serologic test for every subject with HBsAg negative sample serum wishing to donate blood in low economic settings where Nucleic acid testing is not easily affordable.

Keywords: HBV; OBI; blood donors; Nigeria.

1. INTRODUCTION

Occult Hepatitis B Virus infection(OBI) can be defined as the presence of HBV DNA in the absence of serologic detectable HBsAg with or without anti- HBV antibodies [1,2]. Post-transcriptional effect of the mutation on HBsAg expression might be responsible for the escaped mutants [3,4,5]. OBI poses a significant threat to humanity for so many reasons such as HBV infection spread through blood and blood products in blood transfusion infections and organ transplants [6,7]; sexual transmission. It is a health risk to children born to carrier mothers [8,9] and immune escape to current vaccines [10,11].

A proportion of HBsAg seronegativity in HBV endemic population may test positive for anti – HBcore antibody, this phenomenon is otherwise described as ‘ Isolated ‘ anti HBcore hepatitis [12,13].

Before the advent of HBsAg obstetric screening and active/passive immunoprophylaxis of newborn babies of HBsAg-positive mothers, vertical transmission of HBV was one of the main routes of HBV transmission [8,9]. Sexual transmission of HBV infection also had an important role, due to the infrequent use of condoms in unsafe sexual intercourse [10,11,12]. Parenteral transmission of the virus was also frequent in household contact with an HBsAg chronic carrier, in the use of improperly sterilized medical and surgical instruments, blood transfusions, IDU, and for men shaving at a barber’s shop, cosmetic treatment with percutaneous exposure (piercing, tattooing, manicure, pedicure, and acupuncture) dental therapy and promiscuous sexual activity [13].

Measurement of HBV DNA, otherwise called “viral load,” by a laboratory determines the HBV DNA units found in a millilitre of blood. The result is written in international units per millilitre or IU/ml. High levels of HBV DNA, which can range from thousands up to millions, indicate a high rate of HBV replication. Low or undetectable levels–less than 2,000 IU/ml indicate an "inactive" infection [14]. The technology utilises three oligonucleotides in the PCR mix: a probe labelled with a fluorescent reporter dye and a quenching dye, as well as two primers. Nested PCR approach improves the sensitivity of the PCR assay by avoiding artefacts and non-specific binding associated with the conventional PCR method [14].

Patients with pre-core/core promoter HBV genome mutations have a continued HBV replication and active liver disease with low production of HBeAg [15,16]. Mutations in polymerase gene confer drug resistance to entecavir, dipivoxilentecavir dipivoxil, lamivudine and adenofovir [15]. Hence, some HBV mutants may pose a problem in the diagnosis, pathogenesis and therapy of HBV infection.

Tool for diagnosing HBV infection is either serology or molecular tests. In developing countries, like Nigeria information about HBV is mostly by serology which lacks sensitivity when compared to molecular diagnosis.

Therefore, we intend to identify missed serologic diagnosis of Hepatitis B virus infection through serologic and molecular diagnostic tools, among blood donors in Benue State University Teaching Hospital, Makurdi, Nigeria.

2. METHOD

2.1 Study Area

Benue state is in North Central Nigeria and has a teeming population of about 5 million with its state capital, Makurdi located at the northern part of the state. The state is surrounded by neighbouring states; Enugu state and Kogi state on the East, Taraba state on the West, Cross River state on the South and Nassarawa state on the North. Benue state had a total population of 4,253,641 in 2006 census with an average population density of 99 persons per km² with a land mass of 34,059 km². Agriculture forms the backbone of the Benue State economy, engaging more than 70 per cent of the working population. Makurdi harbours a tertiary medical centre, namely, Benue State University Teaching Hospital with other secondary medical centres.

2.2 Sample Collection

One hundred and seventy (170) serum samples were randomly collected from left-over samples of the hepatitis B surface antigen (HBsAg) seronegative tested blood samples belonging to anonymous blood donors in the blood bank unit of Benue State University Teaching Hospital, Makurdi. Fifty HBsAg positive samples were employed as controls. Each sample was shared into two; a portion was frozen and transported packaged in ice packs (for DNA extraction and viral load Quantification) to either of the two reference laboratories (DNA LABS Ltd. Zaria, Kaduna / Body Affairs diagnostics Abuja, Nigeria) while the second portion was for serologic testing.

2.3 Hepatitis B virus Serologic Profiles

The serum samples were coded and screened for HBV serologic markers namely, Hepatitis B surface antigen(HBsAg), antibody to HBsAg(HBsAb), antibody to HBV core antigen(HBcAb), HBV envelope antigen(HBeAg) and antibody to HBV envelope antigen(HBeAb). HBV combination immuno-chromatographic rapid kits manufactured by Acumen Diagnostics Incorporated (Lot SAG91108; expiry date 11/2018) were used for the analyses following manufacturer's instructions.

2.4 HBV DNA Extraction

QIAamp Blood mini kit (Qiagen, Hilden, Germany) was used according to the manufacturer's instruction. A twenty (20 µl) of the protease was added to 200 µl serum in a 1.5 ml tube. Then, 200 µl of cell lysis solution was added, vortexed and incubated for 10 min at 56°C. The DNA was eluted using 50 µl of elution buffer and stored -20°C until further analysis.

2.5 Nested Polymerase Chain Reaction Procedure

Two sets of published primers were used to amplify the hepatitis B S-gene from the DNA extracted from the serum samples [3] with the first round of PCR using the outer primers, which amplify a 916 bp segment which includes the S-gene, under the following conditions: 30 cycles of 94°C for 5 min, 94°C for 30 sec, 63.8°C for 30sec, and 72°C for 60 sec, followed by a final extension at 72°C for 10 min. The second round of PCR was performed using the inner primers, which amplify the 656bp S-gene amplicon of the surface antigen under the following conditions: 30 cycles of 94°C for 5 min, 94°C for 30 sec, 63.8°C for 30 sec, and 72°C for 60 sec, with a final extension at 72°C for 8 min. A 50 µl reaction mixture containing 1 µl of DNA sample, 25 µl PCR premixed solution (Promega, USA), 1.25 µl of forward and reverse primers(final concentration 0.5 µM), and 21.5 µl of nuclease-free water. This reaction was used for both the first and second rounds of PCR amplification using a thermal cycler (Bio-Rad) as described previously. Genomic hepatitis B DNA and no DNA template control were used as positive and negative controls, respectively. The PCR products were analysed by gel electrophoresis using 1.5% (w/v) agarose gel (Seakem LE, USA) visualised with UV illuminator. The product size was determined by comparison to the DNA molecular marker (GeneDireX). Sample bands corresponded to the size of the positive control bands. To ensure that the primers used in this study specifically amplified the S-gene of HBV, they were tested against a DNA sample from an HBV-positive serum that is positive for anti-Hbc,

anti-HBe, and negative anti-HBs. The absolute detection limit of the nested PCR was determined by a 10-fold dilution of the standard HBV-positive serum and the detection limit was found to 5 copies per µL [3].

2.6 Statistical Analysis

Data were analysed using SPSS version 20 statistical software; chi-square was used to compare the association between proportions and P-values <0.05 was considered significant at 95.0% confidence level.

2.7 Ethical Approval

Ethical approval to carry out this study was obtained from Benue State University Teaching Hospital. Makurdi, Nigeria.

3. RESULTS

Hepatitis B Viral DNA (<50 copies /ml) was detected in 5.9% (n=10/170) total HBsAg negative samples as OBI; 71.0% (n=120/170) of total HBsAg negative samples was positive for anti- HBs antibody; 15.3% (n=26/170) was positive for anti- HBe antibody; 8.2% (n=14/170)was sero-positive for Hepatitis Bcore antibody(anti-HBcore) [Fig. 1]. Out of the 10 OBI positive samples, 100.0% (n=10/10) was sero-positive for Hepatitis B core antibody (anti-HBcore); 60.0%(n=6/10) positive for anti-HBs antibody and 30.0%(n=3/10)HBeAg.

4. DISCUSSION

The present study unravelled a 5.9% prevalence of Occult Hepatitis B infection (OBI) among blood donors in Makurdi, North -Central Nigeria. OBI in the study was slightly lower than the 8% recorded among 100 repeat blood donors in South-Eastern Nigeria [17]. On the other hand, the present finding was much lower than the 17% OBI prevalence recorded in Ekiti, South – Western Nigeria [1]. Owing to high seroprevalence (9% to 39%) of HBV infection in Nigeria, there is a tendency for OBI to be high in our communities [18,1]. The data on OBI prevalence is limited in sub-Saharan Africa. However, among HIV patients in West Africa, records of high OBI prevalence has been seen; 27.8% (Benin), 21.3% (Abidjan, Cote d'Ivoire), 15.1% (Khartoum, Sudan) [19]. Among healthy blood donors, very low prevalence of OBI has been reported; in Egypt (North Africa) 0.5% [20], Taiwan (0.11%), Malaysia (5.5%) [3], other areas of low OBI prevalence include the USA, UK, Italy and Germany [21,3]. On the other hand, OBI prevalence of 33.3%was reported in North East China among healthy subjects [22]. OBI may be due to mutations in the *PreS/S* regions which change HBsAg antigenicity and subsequently inhibiting anti-HBs synthesis. A point mutation at the ‘‘a” determinant (amino acids 124–147, e.g., G145R) of HBsAg can alter the immunologic epitope leading to the reduced release of HBsAg.

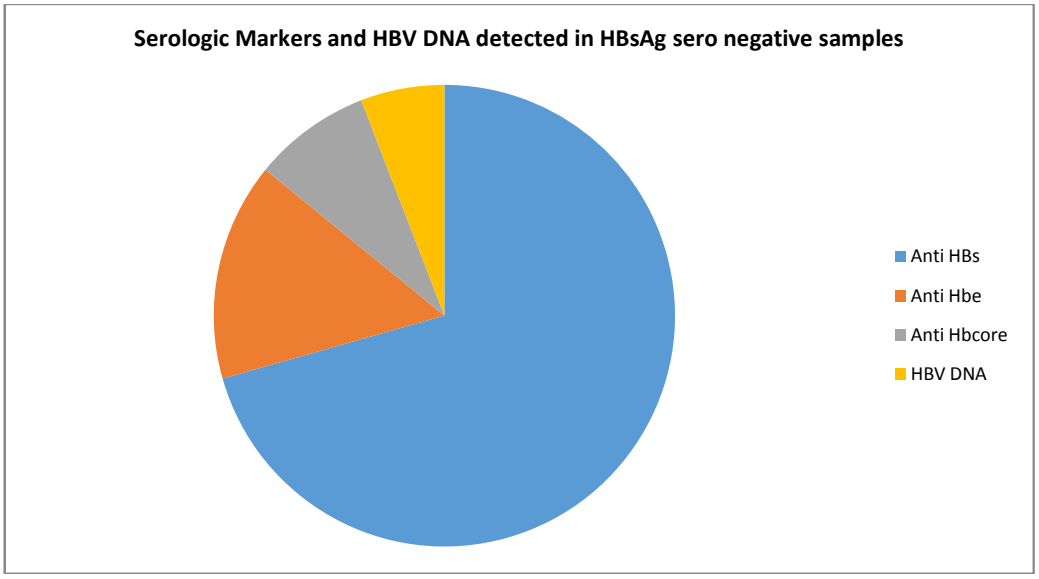

Fig. 1. Percentage serological markers and molecular detection of HBV DNA among the HBsAg seronegative blood samples of Blood donors in BSUTH

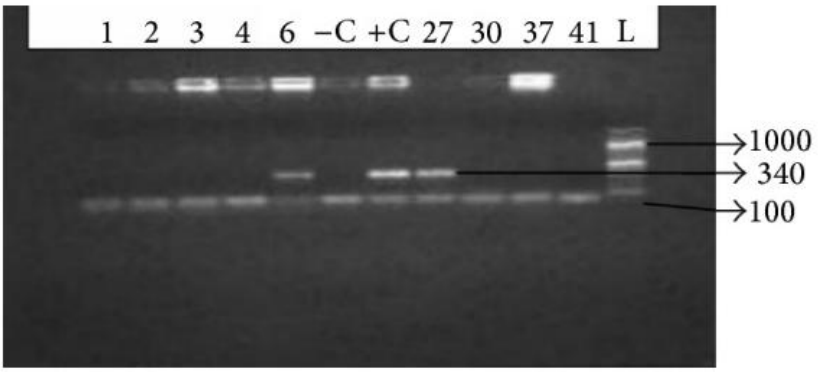

Fig. 2. PCR detection of HBV DNA in HBsAg negative patients. Agarose gel electrophoresis of PCR products shows the 0.7% agarose gel picture showing a 340 base pairs amplicon. Lanes 6 and 27 were positive for HBVDNA. Lanes 1, 2, 3, 4, 37, and 42 were negative for HBVDNA. +C: positive control, −C: negative control and L: molecular weight size marker

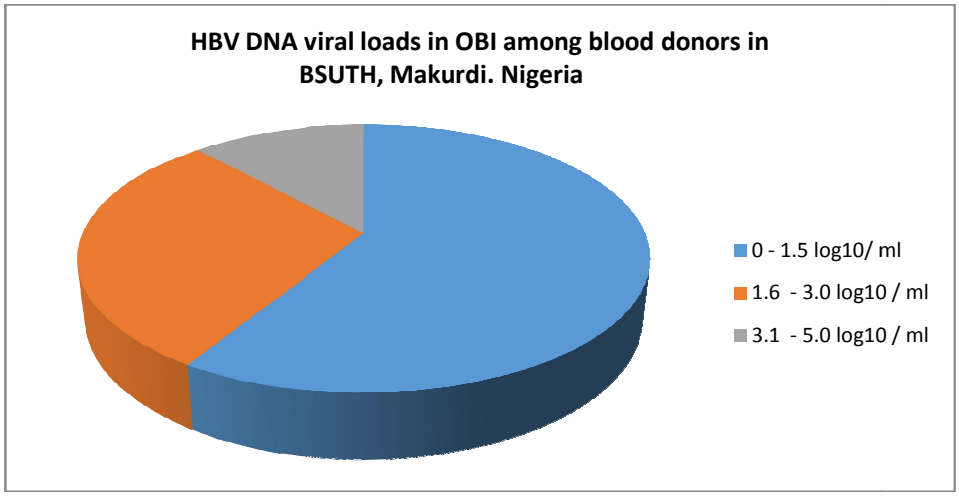

Fig. 3. Among the subjects with OBI with positive HBV DNA, 60.0% (n=6/10) had viral loads of less than 1.5 Log10 copies/ml while 30.0% (n=3/10) had between 1.6 and 3.0 Log10 copies / ml, and the remaining 10.0% (n=1/10) had viral load of between 3.0 and 5.0 Log10 copies/ml

Table 1. Biochemical factors in HBV DNA positive and HBV DNA negative

Characteristics	HBV DNA negative (n=160/170)	HBV DNA positive (n= 10/170)
*ALT>40 IU/ L	40%	60%
AST >30 IU/ L	35%	65%

*P>.05; *ALT= Alanine Transaminase enzyme*
AST= Aspartate Transaminase enzyme

The study also revealed a 100.0% (n=10/10) prevalence of Occult Hepatitis B infection in Hepatitis B core antibody seropositive blood donors. An investigation in Malaysia [3] also revealed a 100% prevalence of occult hepatitis in hepatitis B core antibody-positive healthy individuals. Additionally, other studies have reported finding occult hepatitis B virus more frequently in individuals with anti-HBc-positive serology than in those with anti-HBc-negative serology [23]. Thus, Hepatitis B Virus core antibody (anti- HBc) serologic screening test has become an invaluable surrogate marker for OBI. OBI clients positively reactive for anti-HBc, anti-HBs and HBV-DNA may show viral persistence on recovery with a low viral load. However, anti-HBs presence may be low owing to loss of recognition to Anti-HBs antibody, is poorly

neutralizing, thereby giving rise to mutant viruses to evade neutralisation [24].

Sixty per cent of the OBI samples had HBV viral load of fewer than 15 copies/ml while only 30.0% fell in between 15 and 50 copies/ml of the samples examined by PCR in the study. OBI is usually characterised with low HBV viral load finding amplified by PCR before detection and often lack sensitivity to serology tests. Again, rapid immuno-chromatographic kits rather than the gold standard ELISA are commonly used in our low-income settings [25,18]. The low viral load in this study corresponds with findings in other studies [23,24,25], showing that most OBIs result from the replication of incompetent HBV, leading to a reduction of overall replication process and gene expression. However, the risk of transmission of the infection is dependent not only on the viral load but on low anti -HBs in the donor blood, low immune status and poor vaccination history of the recipient. The low HBV replication was associated with mild liver damage as reflected by insignificant ALT and AST elevation in this study. However, reactivation of this condition can lead to active liver disease [25].

The small sample size in the study which was owing to the high cost of serologic and molecular studies might have been a limitation to the research. HBV combination immuno-chromatographic rapid kits used for the serology tests in the study might not be as sensitive as the gold standard ELISA, hence might contribute to some seronegative results and act as a limitation to the study.

Our finding showed a 5.9% prevalence rate of Occult Hepatitis B Virus infection (OBI) among blood donors in Benue State University Teaching Hospital, Makurdi, Nigeria. However, the use of Hepatitis B Virus core antibody (anti- HBc) serologic screening test could have unmasked this hidden diagnosis.

Hence anti- HBc antibody serologic screening test should be made a mandatory serologic test for every subject with HBsAg negative sample serum wishing to donate blood in low economic settings where Nucleic acid testing is not easily affordable.

We, therefore, recommend Hepatitis B Virus core antibody (anti-HBc) serologic testing be made a significant integral part of the testing algorithm in the national guidelines for blood transfusion safety in rural areas to minimise HBV infection transmission risk in Nigeria.

5. CONCLUSION

Our findings showed a 5.9% prevalence rate of Occult Hepatitis B Virus infection (OBI) among blood donors in Benue State University Teaching Hospital, Makurdi, Nigeria.

However, Hepatitis B Virus core antibody (anti-HBc) serologic screening test could have unmasked this hidden diagnosis. Hence anti-HBc antibody serologic screening test should be made a mandatory serologic test for every subject with HBsAg negative sample serum wishing to donate blood in low economic settings where Nucleic acid testing is not easily affordable.

ETHICAL APPROVAL

Ethical approval to carry out this study was obtained from Benue State University Teaching Hospital. Makurdi, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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