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Case report

Occult Hepatitis B Blood Donor Certified Fit for Donation at a Tertiary Hospital in Nigeria: A Case Report

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ABSTRACT

Occult hepatitis B virus infection (OBI), characterized by detection of HBV DNA (≤ 200 copies/µl) in the serum or tissues of subjects who have negative test for HBsAg has become a challenge to blood transfusion services. We present a 24-year old, male, repeat blood donor at the University of Abuja Teaching Hospital, Abuja, Nigeria with viral load of 31379 copies/ul but negative to hepatitis B surface antigen. Hematological and biochemical parameters of the blood donor showed acceptable ranges except abnormally low hemoglobin levels. Serologic markers of HBV infection showed that the donor was positive for hepatitis B surface antibodies only. Gene sequencing and phylogenetic studies showed that the isolate belonged to the HBV genotype E with gene sequences similar to an isolate from Sudan. The study hereby recommends vigilant blood donors selection/recruitment, adequate screening as well as vaccination of populace with HBV vaccines.

Keywords: Occult HBV infection, Blood donors, Screening

INTRODUCTION

Hepatitis B Virus (HBV) infection is a global, public health issue of immense importance. It occurs worldwide and up to two billion people, approximately 30% of the world's population has been infected [1]. Current prevalence rates of viral hepatitis infection in Nigeria are reflective of the global disease burden involving hundreds of millions of persons [2]. In Nigeria, HBV infection is endemic with 11% prevalence rate recorded [2]. Occult hepatitis B virus infection (OBI) is defined as the presence of HBV-DNA in the absence of detectable HBsAg with or without anti-HBV antibodies [3]. The prevalence of occult hepatitis B viral infection in Nigeria has been documented with prevalence rates of 5% to 17% recorded depending on the geographical area [4-6].

Blood transfusion could be an important route for the transmission of infection especially when donated blood is not adequately screened for HBV and other transfusion transmissible infections [1]. Screening of donated blood units for hepatitis B surface Antigen (HBsAg) was introduced in the 1970s and this has greatly reduced HBV transmission through blood transfusions as blood found to be HBsAg positive was not transfused [7]. In many developing countries including Nigeria, screening of blood donors or donated blood units, for HBsAg alone, is still the only practice on which the prevention of HBV transmission during blood transfusion is based [8].

Several scientific papers have highlighted the presence of HBV infection in some individuals negative for HBsAg but having detectable HBV DNA in the liver or blood and some of these publications have documented a viral load less than 100 IU/ml [4-6]. HBV transmission resulting from transfusion of blood units found to be HBsAg negative has been reported by [9]. We describe the first case of occult hepatitis B virus infection in Nigeria with high hepatitis B viral load in a healthy blood donor that has been screened and certified fit for donation.

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CASE DESCRIPTION

This case was discovered at the University of Abuja Teaching Hospital Blood bank. The University of Abuja Teaching Hospital (UATH) is situated in Gwagwalada, Abuja, in the Federal Capital Territory (FCT), Nigeria. Twenty four years old apparently healthy blood donor came for screening and donation of blood for the relative that needed blood transfusion. With the aid of a structured questionnaire, relevant Socio demographic information and HBV risk factors were obtained from the blood donor as done in the hospital.

Ten milliliters of venous blood was collected from the blood donor and the sample was dispensed aseptically into a specimen bottle containing potassium ethylene diaminetetra acetate (K +EDTA) anticoagulant and into a plain tube to obtained serum specimen. The sample was centrifuged at room temperature at 3500 rpm for 10 min within an hour of specimen collection. The plasma was separated into two aliquots of 1000 ul: one of which was stored at -80°C for PCR assay. The other one was used for serological tests such as: HBsAg, HCV antibodies, HIV antibodies and Syphilis antibodies as routinely done for blood donor to ascertain eligibility for donation. Besides, HBV serological markers, CD4 cell count, hematological parameters and HBV DNA studies including gene sequencing and phylogenetic analysis were done. Tests were performed by standard methods following kit manufacturers' instructions. Gene sequencing using Sanger's sequencing method [10] and phylogenetic analysis of nucleotide sequences was done to identify the relationship of the isolate to other isolates deposited at Gen Bank.

The results obtained on the preliminary testing as done by the blood bank showed that the blood donor was fit for donation, having tested negative for HIV, HCV, Syphilis antibodies and HBsAg. However, HBV DNA testing showed that the blood donor under review had a high viral load of 31,379 copies/ml. The socio demographic characteristics and HBV risk factors of this blood donor and results of various tests performed are shown in Table 1. The significant characteristics observed were as follow; the blood donor lacked knowledge of HBV/HBV infection and has not been vaccinated with HBV vaccines. It was also seen that this blood donor had abnormal low hemoglobin of 9.3 g/dl and CD4 cell count of 511/µL. Also, this blood donor is a young man of 24 years old student, and a repeat (old) blood donor with anti-HBs positivity.

Table 1: Characteristics of Blood Donor with High Hepatitis B Viral Load.

Characters	Observations
Sociodemographic data	24 Years old, Male, Single, Student
ABO blood group	O+
Donation status	Repeat blood donor/Family replacement
HBV risk factors	No knowledge of HBV, No HBV vaccination, Had domestic accident and visited commercial barber.
HBV markers pattern	HBsAg- HBsAb+ HBcAb- HBeAg- HBeAb- HBcAb IgM-
HBV DNA (viral load)	31379 IU/mL
ALT value	2.9 IU/L (Normal<12.0 IU/L)
AFP level	6.6 ng/ml (Normal<8.5 ng/ml)
HBV genotype	Genotype E
Hematological parameters	WBC: 6.0 × 109/L, PLT:157 ×109/L, HCT 36.6, HGB: 9.3 g/dl, RBC:4.93 × 1012/L
CD4 cell count	511/µL

Note: O+: Blood group O Rhesus positive; HCT: Hematocrit; PLT: Platelets; HGB: Hemoglobin, RBC: Red Blood Cells; WBC: White blood cells; ALT: Alanine Aminotransferase; AFP: Alpha Feto-Protein; -: Negative; +: Positive

Figure 1 presented phylogenetic tree showing relationship between HB1 Isolated from blood donor with OBI and other hepatitis B viruses. The obtained sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The sequence of HB1 showed a percentage similarity to other species at 99%. The evolutionary distances computed using the Jukes-Cantor method [11] were in agreement with the phylogenetic placement of the isolates within the hepatitis viruses and revealed a closely relatedness to hepatitis B virus isolate SDAC_059 (gb: KF170780.1) than other Hepatitis B viruses. This isolate has a close relationship with hepatitis B virus isolated from Sudan. The nucleotide sequence

with accession number H6A- MG562503 has been deposited at NCBI Gen Bank.

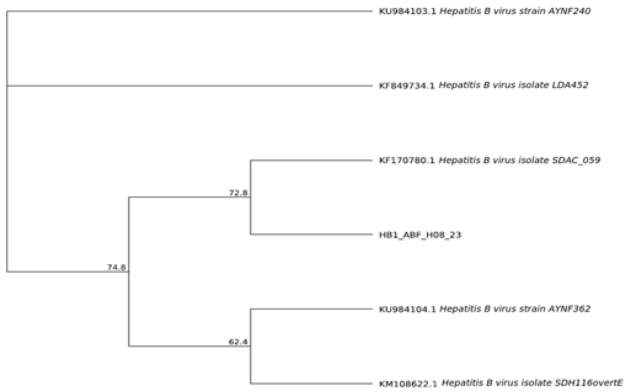

Figure 1: Phylogenetic Tree showing Relationship between HB1 Isolated from Blood Donor with OBI and other Hepatitis B viruses.

Figure 2 showed the nucleotide sequences of hepatitis B virus isolated from apparently healthy blood donor with high viral load. Sequence analysis showed that the isolate had gene sequences similar to HBV isolate from Sudan. This sequence had been deposited with National Centre for Biotechnology Information (NCBI) with Identity number: H6A-MG562503.

TCATCTCGTCATCTTCTCGAGGATTGGGGACCCTGCACCGAACATGGAAAGCACA
ACATCAGGATTCCTAGGACCACTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGA
CAAAAATCCTCACAATACCGCAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTT
TCTAGGGGGAGCTCCCGTGTGTCTTGGCCAAAATTCGCAGTCCCCAATCTCCAGT
CACTCACCAACCTGA

Figure 2: Nucleotide Sequence of HBV isolated from blood donor with Occult HBV.

DISCUSSION

Many studies done in Nigeria have demonstrated that OBI cases had significantly lower HBV-DNA copies compared to HBsAg positive blood donor [4-6]. These studies obtained HBV DNA load less than 100 IU/mL among blood donors with occult HBV infection. The low level of viral load among blood donors with OBI showed almost all OBI cases are infected with replication competent HBV, revealing a strong suppression of replication activity and gene expression, thereby resulting in a reduced viral load [6].

This case report showed that the blood donor had high value of HBV DNA to the tune of 31379 IU/mL. This high value has not been reported among blood donors with occult HBV infection in Nigeria. In a recent study by in Burkina Faso, more than two-thirds of the subjects with HBV DNA (40/56) and HBsAg negative had a viral load of 200 to 13.6 × 106 IU/mL) [12]. Another study reported a viral load between undetectable and 3,670 IU/mL in "OBI" cases among blood donors in Southeast Asia [13]. A possible reason for this high HBV DNA load could be the virus was released from the hepatocytes to blood circulation at the point of bleeding the donor for screening hence the high viral load recorded. This could also be attributed to escape mutations that can lead to a change in the immunologic epitope thus inhibiting HBsAg secretion [14]. Another reason for this high viral load could be reactivation of latent virus. Reactivation of HBV is an abrupt increase of HBV replication in a patient with inactive or resolved hepatitis B and can occur spontaneously [15]. Also, individuals who undergo chemotherapy have a higher risk of viral reactivation than people who are not on medication [15]. This is because chemotherapy could lead to immunosuppression with resultant viral multiplication. Surprisingly, it was observed that this blood donor with high viral load has a CD4 cell count of 511/ul which is slightly low and a mark of immunosuppression. Also this blood donor is positive for anti-HBs. We were unable to determine the titre of Anti-HBs marker. This is because an antibody titre<100 IU/ml is an indication that the blood donor is infectious and could transmit the virus to blood recipient [7]. This calls for further study. Also, it would have been interesting to have a longitudinal data for serologic markers and HBV DNA load of the blood donor to evaluate viral dynamics but the blood donor could not be reached.

In 2008, the statements from the Taormina expert meeting on occult hepatitis B virus infection had clarified the definition of OBI in establishing a threshold value of serum HBV DNA<200 IU/mL [3]. Furthermore, it also clarified the confusion between a cleared infection of HBV and a "false OBI". Thus, cases with serum HBV DNA levels comparable to those usually detected in the different phases of serologically evident (overt) HBV infection have to be considered as "false OBI" and are usually due to infection by HBV variants [3]. These become in fact chronic hepatitis B cases. Whatever may be the case whether occult or "false OBI", the blood donor under review and other individuals with high viral load and surface antigen negative poses a risk to blood transfusion as most blood transfusion centers in Nigeria screen blood donors for HBsAg only and bleed donors based on its negativity. Although most hematological parameters e.g. Hematocrit (HCT) appears normal, hemoglobin content of the red blood cells was low. Also, biochemical markers of liver injury such Alanine Aminotransferase (ALT) and Alpha Feto-protein (AFP) were normal at this point. However, continuous viral replication and multiplication could lead to destruction of liver cells and alteration of hematological profile with resultant manifestation of liver Cirrhosis and Hepatocellular Carcinoma [7].

Sociodemographic data of this case showed that the blood donor under review is a repeat (old) blood donor. This means he has been donating blood in the past to recipients and possibly infecting them with HBV. It is necessary to track the blood donor and ensure he is counseled and treated to prevent further transmission and spread of HBV infection. Hepatitis B virus risk factors assessment showed that this blood donor had no knowledge of HBV Infection, have not received HBV vaccination, and had needle prick injury in the past. These probably could be the sources of the HBV infection. Besides, this donor is 24-years old. This age bracket is the most active sexual age. A study has revealed that most HIV infection and other sexual transmitted infection such HBV infection occurred within age bracket of 19-30 years old [7].

CONCLUSION

This study had shown that screening blood donors with HBsAg only is inadequate as some blood donors negative for HBsAg could be OBI with high HBV DNA load. The study hereby recommends adequate screening of blood donors with ELISA kit for HBsAg and HBV serologic markers since DNA testing for the blood donors is not cost effective in a resource constraint economy like Nigeria. There is need for mass vaccination of people in the community with potent HBV vaccines to ensure protection of people against HBV infection.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicting interests.

AUTHORS' CONTRIBUTIONS

AIO conceived the study. AIO, BMM, IE and MT undertook laboratory analysis. AIO and NRA prepared the manuscript while MOI, IE and MT provided ideas and comments during the manuscript preparation. All authors have read through and approved the final manuscript.

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