



**Bayero Journal of Pure and Applied Sciences, 14(2): 77 - 82**

Received: May, 2021

Accepted: October, 2021

ISSN 2006 – 6996

## **MOLECULAR DETECTION OF HEPATITIS B VIRUS AMONG BLOOD DONORS FROM NATIONAL BLOOD TRANSFUSION SERVICE CENTRE IN KADUNA, NIGERIA**

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### **ABSTRACT**

*The survey was carried out to determine the molecular detection of Hepatitis B virus markers among blood donors between the age of 18-65 years from National Blood Transfusion Service (NBTS) centre in Kaduna, Nigeria. Blood samples were screened for Hepatitis B virus (HBV) using Diagnostic kit for HBV infection marker in whole blood. Hepatitis B serological markers were determined using polymerase chain reaction and confirmed. The socio-demographic factors associated with HBV infection were determined using structured questionnaires. The total prevalence rates of the five (5) HBV markers were: HBsAg 12(7.3%), HBsAb 5(3.0%), HBeAb 12(7.3%), HBcAb 18(10.9%), and all blood samples were negative for HBeAg. HBVDNA was detected in 3 out of 10 samples. Two out of the three samples were positive for HBsAg, HBeAb and HBcAb whereas one sample was positive for HBsAg, HBsAb and HBcAb. The high prevalence of HBcAb (10.9%) among HBsAg<sup>+</sup> (7.3%) and (3.6%) among HBsAg<sup>-</sup> in this survey indicates that the virus is actively replicating in chronic HBV carriers and consistently increasing the spread within the population in the areas that are targeted.*

**Keywords:** *Molecular detection, Hepatitis B virus (HBV), Blood donors, National Blood Transfusion Service (NBTS).*

### **INTRODUCTION**

One of the most important and vital section of the medical services is blood transfusion. The main concern of the blood transfusion service is to ensure the preservation, intelligibility competence, and efficacy of blood supplies at all stages (Islam, 2009). Hepatitis B virus (HBV) a double stranded circular DNA virus, which belong to the family of Hepadnaviridae (Prescott *et al.*, 2008). It possesses a complicated structure and is amongst the most contagious diseases worldwide (Blattacharya *et al.*, 2007), emerging in fatal chronic liver diseases. Based on WHO, present universal burden of HBV infection has been predicted about two billion, from whom three hundred and sixty million have chronic infection by end of 2016, whereas six hundred and twenty thousand dies yearly (Wiktor and Hutin, 2016). HBV infection is among the most frequent cause of liver disease and is a serious health challenge in Nigeria (Musa *et al.*, 2015), if an individual is not immunized they can easily be passed on with hepatitis B virus, through contact with contaminated blood or body fluids like

semen, vaginal discharge, saliva, menstrual fluid and blood. Hence they are acquired through contaminated blood transfusion, intercourse specifically sex without condoms, exchanging sharp infected items like syringes, scalpel, as well as personal contact in congested environment (Rubin and Strayer, 2015). It can also be passed on from an infectious mother to the fetus via the placental barrier. Many people are asymptomatic at the time of initial infection but few results in quick outset of the illness along with dark urine, throwing up, fatigue, abdominal pain and yellowish skin (Rubin and Strayer, 2015). This might require 30 to 180 days for signs to arise. HBV infection may result into either acute or chronic hepatitis (Gerlich, 2013). Hepatitis B surface antigen has the ability to produce viral particles which have a complicated surface antigen and is extensively disseminated in the bloodstream of infected individuals. Thereby comes a period when HBsAg can't be detected within the bloodstream, even though HBV infection still exists in the bloodstream.

During the window phase recognized antibody produced in response to HBcAg functions like an effective serological marker for HBV infection (Bharath and Krishnan, 2016). HBcAb IgG is helpful for detecting chronic HBV infection, HBcAb IgM is helpful in detecting recent HBV infection. Developing the sensitive assays to identify HBV-DNA display that HBcAb positive who are healthy HBsAg negative donors may have an occult Hepatitis B virus infection (OBIs), also they retain HBV-DNA sequence in their blood and liver. This indicates the potential origin of HBV transmission (Cabrerizo *et al.*, 2000; Zaaijer *et al.*, 2008; Said, 2011). OBI arises when the HBV DNA is detected, whereas HBsAg remains undetectable. This study is concerned primarily with the molecular detection of hepatitis B virus among blood donors from National Blood Transfusion Service centre in Kaduna, Nigeria.

## MATERIALS AND METHODS

### Subjects

The study included apparently healthy blood donors both males and females who came to donate blood in national blood transfusion service (NBTS) centre in Kaduna, Nigeria between July to September 2019.

The sample size was determined using Cochran's formula,

$$n = \frac{t^2 \times p(1 - p)}{m^2}$$

(Cochran, 1977).

Where n is the required sample size, t represent confidence level at 95% (standard value of 1.96), p represent 12% (Edia-Asuke *et al.*, 2016), average prevalence of HBsAg+ in Nigeria from previous studies, m equaled 5% (standard value of 0.05) margin of error. A total of 165 donor samples were taken. Therefore, 165 blood samples were collected from prospective blood donors. All the Institutional and ministry of health ethical clearance for this study was given by the department of Microbiology, Kaduna State University of Science and Ministry of Health Kaduna (MOH/ADM/744/VOL.1/747). All the participants gave their written informed consents before blood samples were collected from them.

### Sample Collection

Five milliliter (5ml) of blood was collected through the vein into the EDTA container. The cassette was kept to the right side matching to HBsAg, HBsAb, HBeAg, HBeAb HBcAb. With a small pipette the whole blood sample was taken and added into the five (5) wells of the cassette by dropping (25µl per well or one drop), Then one drop of the buffer was added into the five (5) sample well as well. The interpretation of test results was performed according to

manufacturer's description. The plasma for positive blood samples was separated through centrifugation at 5000rpm for 15 minutes. The serum was then collected using a pipette and stored in an aliquot bottle below - 20°C.

### Detection of Hepatitis B Markers

For hepatitis B virus, a diagnostic kit for HBV infection marker in whole blood manufactured by micro point technologies Inc U.S.A was used in a stepwise order for the detection of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb respectively in the blood. This method which is immunochromatographic and qualitative in nature, detects the presence of five markers of hepatitis B virus in human blood and can be read in-vitro having more than 99.9 % sensitivity and 99.75 % specificity (BIONEER, 2020).

### Detection of HBV DNA

#### BIONEER Accuprep® genomic DNA extraction kit(K-3032).

All HBsAg positive samples were tested for HBV DNA. Started by adding 20 µl of Proteinase K to a clean 1.5 ml tube after which 200 µl of whole blood, buffy coat or 10<sup>4</sup>~10<sup>8</sup> cultured cells to the tube containing proteinase K. 200 µl of Binding buffer (GC) were added to the sample and mixed instantly by vortex mixer. The solution after was incubated at 60°C for ten minutes. Hundred microliter (100 µl) of Isopropanol was added and mixed well by pipetting. The lysate was cautiously transferred into the upper reservoir of the Binding column tube (fin a 2 ml tube) without wetting the rim. The tube was placed in a centrifuge and spun at eight thousand (8,000) rpm for one minute. The tube was opened and the Binding column tube was transferred to a new two ml tube for filtration. 500 µl of Washing buffer 1 (W1) was then added without wetting the rim after which the tube was closed and centrifuged at 8,000 rpm for 1 min. Tube was then opened and the solution was poured from the 2 ml tube into a disposal bottle. 500 µl of Washing buffer 2 (W2) was added without wetting the rim and then the tube was closed and centrifuged at eight thousand (8,000) rpm for one minute again.

It was centrifuged once more time at twelve thousand (12,000) rpm for one minute to completely remove ethanol, and verified that there were no droplets clinging to the bottom of Binding column tube. The Binding column tube was transferred to a new 1.5 ml tube for elution and two hundred microliters (200 µl) of Elution buffer (EL, or nuclease-free water) was added onto Binding column tube, and then allowed to stand for one minute at RT (15~25°C) until EL was completely absorbed into the glass fibre of Binding column tube. Finally, centrifuged at eight thousand (8,000) rpm for one minute to elute(BIONEER,2020).

**Amplification of HBV DNA using Polymerase Chain Reaction (PCR).**

Ten samples from DNA extraction method that is, BIONEER *Accuprep*® genomic DNA extraction kit(K-3032) protocol were randomly selected for molecular testing. In rapid cycler PCR, ten µl from each sample of extracted DNA was used in nested polymerase chain reaction for hepatitis B virus genomic DNA. Amplification was performed by means of the primer set to the sequence of HBV DNA polymerase gene primer (BIONEER, 2020). For reaction set-up, the templates, specific primers and water were added to the premix. The primers used were hepBP<sub>1</sub> (F- 5' TCACCATATTCTTGGAACAAGA3', and hepBS<sub>1-2</sub> (R 5' CGAACCACTGAACAAATG GC 3', with nucleotide position of 1063) (Abushady *et al.*, 2011). After an initial denaturation step for (5min at 95°C), DNA was amplified using 30 cycles at 94°C for 20sec, 55°C for 20sec, 72°C for 1min, followed by a final extension of 72°C for 5min.

**Gel Electrophoresis**

There are a various agarose products available in the market (they have unlike melting properties) but 1.5%, for three gram of agarose was used. Three gram of agarose was added to TAE buffer and heated the solution in a microwave till agarose got completely liquefied. It was then cool down in a water bath set at 55. Gel casting tray prepared by sealing ends of gel chamber with tape and then placed with suitable number of combs in gel tray. Five microliter of ethidium

bromide was added to cooled gel and pour into gel tray. It was allowed to cool down for fifteen to thirty minutes at room temperature. The comb was then removed and placed in electrophoresis chamber and covered with TAE buffer. The DNA and standard (Ladder) was loaded onto gel. Electrophoresed for one hour. The DNA bands were visualized using ultraviolet light box (BIONEER, 2020).

**Socio-demographic Profile**

A structured questionnaire was used to obtain information on socio-demographic factors (gender, age and occupation).

**Statistical Analysis**

The data was subjected to statistical analysis and chi square test was carried out using SPSS computer software version 23.0 for windows to determine any significant relationship between age, gender and occupation for the different markers of hepatitis B virus.

**RESULTS**

**The Prevalence of Hepatitis B Markers among Blood Donors**

One hundred and sixty-five blood donors took part in this study at the NBTS centre Kaduna. Of the 165 blood samples tested, HBsAg was detected in 12(7.3 %), HBsAb 5(3.0 %), HBeAb 12(7.3 %), and HBcAb 18(10.9 %). All blood samples were negative for HBeAg. The total prevalence of positive and negative markers detected were 7.13 % and 94.3 % respectively (Table 1).

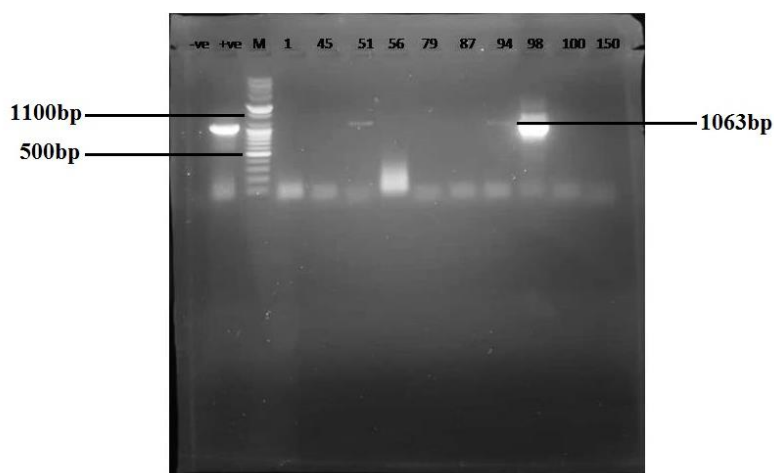
**Table 1:** The Prevalence of Hepatitis B Markers among Blood Donors

HBV Markers	Total samples	Total positive %	Total negative %
HBsAg	165	12(7.3)	153(92.7)
HBsAb	165	5(3.0)	160(97.0)
HBeAb	165	12(7.3)	153(92.7)
HBcAb	165	18(10.9)	147(89.1)

**Agarose Gel Electrophoresis(1.5%) of the Reaction Products of Conventional PCR and DNA Markers**

This plate shows the Agarose gel electrophoresis(1.5%) of the reaction products of conventional PCR and DNA markers stained with ethidium bromide. The plate below is a representation of the bands of HBV DNA isolated

from blood samples of blood donors. Lane M represents 100bp molecular marker(ladder). Lane 51, 94 and 98 represent positive PCR result at (1063bp). Lane 1,45,56,79,87,100 and 150 represent negative PCR results while Lane +ve and -ve represents positive and negative control (Plate 1).



**Plate 1:** Agarose gel electrophoresis(1.5%) of the reaction products of conventional PCR and DNA markers stained with ethidium bromide.

Key

M: PCR markers(1200, 1000, 900, 800,700, 600,500, 400,300,200 and 100bp) (BIONEER, 2020).

Positive PCR results: 51, 94 and 98, Negative PCR results: 1,45,56,79,87,100 and 150

+ve: Positive control, -ve: Negative control

## DISCUSSION

In this study, 7.3 % prevalence of HBsAg detected in 165 blood samples analyzed is an indication of the occurrence of HBV among blood donors from NBTS centre in Kaduna, Nigeria. This moreover indicates that HBV infection is endemic in the region of the study, and this might be ascribed to not receiving the complete doses of the vaccine and also due to individual behavior and practices in the studied area. The prevalence of HBsAg reported in this study is lower than 4.87 % as stated in a previous study which was 12 % as reported by Edia-Asuke *et al.* (2016). Also the prevalence of 7.3 % stated in this study is 0.4 % lower than the reported 7.7 % carried out by Ikerionwu (2018).

Safe blood is of uppermost importance and the National Blood Transfusion Service are responsible and all units in both private and public health organizations to assure that safe blood is made accessible for the purpose of transfusion to patients. Hepatitis B is a major health problem globally and is linked with life-threatening complications. Therefore, it is recommended that each blood unit of blood has to be tested for hepatitis B infection (Jadeja *et al.*, 2014). Research during the 70's among healthy Iraqi population reported that the prevalence of HBsAg in blood donors and military personnel blood donors was 3.6% and in normal population it was 3.3% while in the 80's it was 4.3% in normal population and 4.1% in blood donors (Atallah, 1987). Hence comparing these values with the data obtained from this survey, it is clear that much-needed to be done to prevent complications that may arise from patients receiving "screened blood" from the hospitals

(Odiabara *et al.*, 2020). It is well-known that maintained high levels of HBV DNA are linked with progressive liver disease. Serum DNA levels are a prognostic factor and they help to define the phase of chronic hepatitis B infection, the treatment indication, and allow an assessment of the efficacy of antiviral therapy. High levels of HBV DNA are an independent risk factor for cirrhosis (Fattovich *et al.*, 2008). Research from different areas of Nigeria have documented varying occurrence rate of HBsAg within blood donors nonetheless; DNA analysis of all gathered samples is not reasonable due to the cost of executing the test in Nigeria as a result of which information on other markers of HBV is rare (Agbesor *et al.*, 2016).

The result of PCR showing (30%) of the blood donors with seropositivity to HBsAg indicated active replicative infection among the HBV DNA positive blood donors. The blood donors harbouring the active virus, this could progress to chronic infection and finally develop liver disease and cancer unless not treated. This could be due to the genes were identified as early as one week after initial infection. This is in line with WHO report of WHO (2002).

## CONCLUSION

Currently, hepatitis B viruses are detected by classical assay like biochemical assays, rapid tests and modern molecular assays. Rapid tests have low sensitivity and specificity while molecular assays are rapid, sensitive and specific but costly. Still Hepatitis B virus is a big problem and affect large segment of the population world-wide. A rapid sensitive, specific, and cost effective assay for HBV will surely be useful for better patient

management and to curb the infection in early stages. There is still need for the development of some specific therapeutic agent for Hepatitis B Virus. Hence, the cost of an assay is a significant factor in controlling a disease in developing countries.

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## RECOMMENDATION

Government must assure proper enforcement of blood banking system for safe blood transfusion services. This must include testing HBV DNA to eliminate occult hepatitis B virus infection before transfusing a donor's blood and also to ensure that no old premix is used for HBV DNA detection because it causes smearing during gel electrophoresis.

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