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Prevalence, Profiling and Molecular Identification of Hepatitis B Virus Infection among the Tertiary Students within Birnin Kebbi Metropolis, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A cross sectional study aim the prevalence, profiling and molecular identification of hepatitis B virus infection from the tertiary institutions students in Birnin kebbi metropolis. 329 blood samples were analyzed across the two (2) institutions for HBV using rapid diagnostic kits positive samples were

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taken for profiling using 5panel cassetes for serological markers and polymerase chain reaction to confirm. The social demographical factors of HBV infection were determine using a structured questionnaire. The prevalence rate of HBV markers at WUFPBK were HBsAg 25(41.25%), HBsAb 3(4.95%), HBeAg 5(8.25%), HBeAb 6(9.9%) HBcAb 15(24.75%) and FUBK: HBsAg 18(29.52%), HBsAb 0(0%), HBeAg 2(3.28%), HBeAb 1(1.64%) and HBcAb 6(9.8%). Seven (7) positives sample indicating HBeAg and HBeAb were used to detected HBV DNA, this indicates the virus replicating in chronic HBV carriers and spread through risks factors associated with HBV.

Keywords: Prevalence; HBV profiling; molecular detection; tertiary institutions; Birnin Kebbi metropolis.

1. INTRODUCTION

"Hepatitis B Virus (HBV) infection is a vaccine-preventable liver infection that can be transmitted through contact with the body excretes such as blood, saliva, sweat, urine and fecal matter of an infected person" (Sondlane et al., 2016). "It is generally acquired early in life and develops to chronic Hepatitis B which leads to life threatening diseases like inflammation of the liver, liver cancers and scaring of the liver" (Robotin, Kansil, Porwal, Penman, & George, 2014). "Hepatitis B viral infection is considered a major public health burden with about 250 million people being chronic carriers, of which an estimated 700,000 people die each year globally" (Mueller et al., 2015). "Hepatitis B virus is 50-100 times more infectious than HIV and 10 times more infectious than hepatitis C virus and because it replicates profusely and produces high titer in the blood (108-1010 virions/mL) any parenteral or mucosal exposure to infected blood poses a high risk of HBV acquisition" (Pennap et al., 2011). "Although literatures on HBV infection in Nigeria are growing, yet, there is paucity of information among the youths who are known to be a group that is highly at risk because of their sexually active stage. In addition, they also form the bulk of the group that is usually required when there is need for blood donation. Hepatitis B virus infection is widely referred to as a silent killer because many carriers do not realize they are carrying the virus within the sexually" (Pennap et al., 2011) and hence fail to seek appropriate medical attention. Studies have shown that the prevalence of HBV infection in antenatal population is a reliable indicator of HBV prevalence rate in the general population (Ahizechukwu et al., 2011). "Screening students for HBsAg can also give a reliable prevalence of the disease in a population, since they fall within the sexually active group and are prone to the risks factors. This work was therefore aimed at determining the seroprevalence and risk factors associated with hepatitis B viral infection among students of Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Hepatitis B virus has been described as a major public health problem or risk, occurring endemically, in all areas of the world. Approximately 1 million persons die each year (2.7% of all death) from viral hepatitis related causes (WHO, 2010). An estimated 57% of cases of liver cirrhosis and 78% of cases of 1% liver cancer results from HBV or HCV infection" (WHO, 2010). However, 80% of countries identified hepatitis as an urgent public health problem (WHO, 2010). "It has been estimated that about 2 billion people have been infected with hepatitis B virus and 350 million have chronic lifelong infection. About 50million people are chronic carriers of HBV in Africa with the carrier rate ranging from 9 to 20% in sub-Saharan Africa" (Ballah et al., 2012). "Studies done in Nigeria showed HBV carriage rate in the range of 9 to 39% (Emechebe et al., 2009). A study in Nigeria showed that the hepatitis B vaccine coverage rate is 36.2% in those that received full coverage of three doses, while 64.5% had received at least one dose of HBV vaccine" (Ogoina et al., 2014). "The consequences of the problems of low pick up rate of HBVinfection due to poor screening and the low vaccination rate are that vertical transmission of the virus has become the major route of transmission of the virus in Nigeria in addition to heterosexual relationship" (Ahizechukwu et al., 2011).

2. MATERIALS AND METHODS

The study was conducted in Birnin Kebbi, Kebbi State that is located in the North-Western part of Nigeria. Birnin Kebbi, is situated between latitudes 100 8I N and 130 15I N and longitudes 30 30I E and 60 02IE. "The State is bordered by Sokoto and Zamfara States to the East, Niger State to the South, Benin Republic to the West and Niger Republic to the North. Birnin Kebbi, Kebbi State occupies an area of about 37,699 square kilometers

out of which 36.46% is made up of farmland. The State has an estimated population of about 5,563,900 people" (NPC, 2006) Kebbi State has tropical weather conditions with three seasons: rainy, dry and hot. The annual rainfall is variable and declining, being 600mm to 850mm with an average of 650mm. The monthly temperature in the region ranges from 25^{0}C to 45^{0}C. "The State possessed two important Agricultural lands namely: dry land (arid-prolonged dryness) and Fadama (floodplain-significant alluvial clay particles). These two lands remained the key source of income to millions of people in the State" (Usman *et al*., 2016). "Agriculture is the most important economic activity, with riverine floodplains producing crops like groundnuts, cotton, rice, millet, sorghum and vegetables such as tomato, onions etc. most of the land in the State is used for grazing cattle, goat and sheep. The most populated ethnic groups in the State include Fulani, Hausa, Lel'na (Dakarkari) and Kambari" (Amy, 2019). Kebbi state has Nine (9) Government runned tertiary institutions, in Birnin Kebbi there are Three (3) Government runned tertiary institutions.

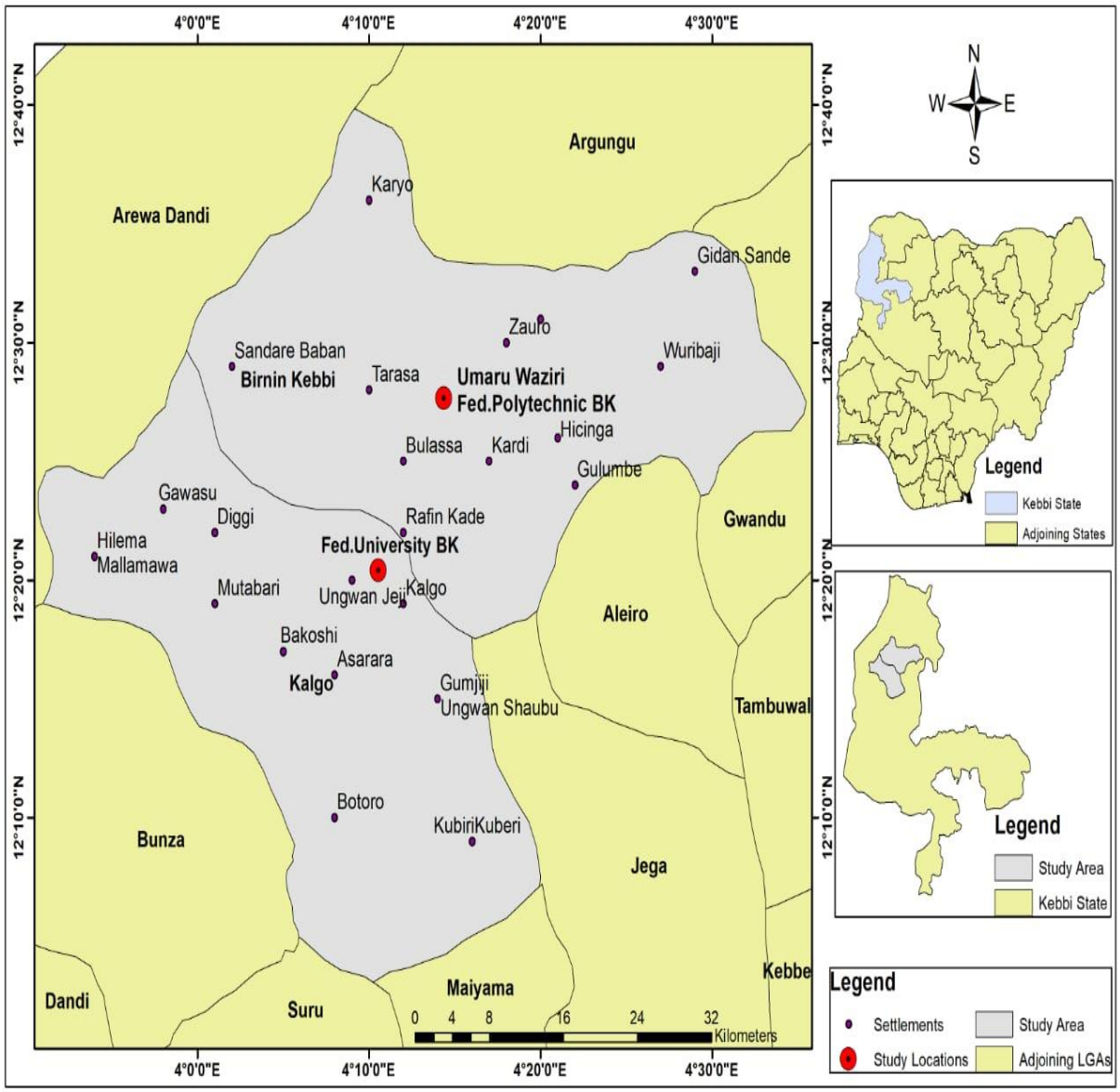


Fig. 1. Map of Kebbi State showing the study location

2.1 Study Design

This is cross sectional study includes both male and female students of tertiary institutions within Birnin Kebbi, which comprise of Federal University Birnin Kebbi and Waziri Umaru Federal Polytechnic Birnin Kebbi. The participants consent was sought and their blood samples taken for analysis. The collecting point was institutional health center. A structured questionnaire used to obtain socio-demographic features, risks factors for hepatitis B virus infections.

2.2 Sample Size Determination

Institutional-based cross-sectional study design was used to examine the prevalence and factors associated with hepatitis B and C infection among students of tertiary institutions within Birnin Kebbi metropolis. The sample size was calculated based on a single population proportion formula;

$n_0 = z^2pq$

e^2 (Singh and Masuku, 2012).

Where:

Z = standard normal deviate which is 1.96 at 95% confidence interval
P = the proportion of the population estimated to be at risk (0.311)
q = the proportion of the population not at risk (1-0.311=0.689)
e= the desired level of precision set at 5% (0.05)
n_0= final sample size calculated (329)
$n_0 =$ $(1.96)^2$ x 0.311 x 0.689
$(0.05)^2$
$n_0 =$ 0.8231742064
0.005
n_0= 329

Exactly Three hundred and twenty nine (329) Samples were collected from both institutions, one hundred and sixty five (165) samples were collected from Waziri Umaru Federal Polytechnic Birnin Kebbi and one hundred and sixty four (164) were collected from Federal University Birnin Kebbi.

2.3 Inclusion Criteria

Consenting male and female students, at the age of 18-60years infected and uninfected from various departments and level of programs within the Tertiary institutions who were willing to partake in the exercise.

2.4 Exclusion Criteria

Non-consenting students on medication, below age 18 and above 60, students who are not from the institutions and who are not interested.

2.5 Determination of Socio-Demographic Information of the Study Participants

A structural questionnaire to each participant was designed to capture the socio-demographic information of the participants including the institution, age, sex, marital status, geopolitical zone, tribe, religion, family type, educational level and occupation**.**

2.6 Sample Collection for Seroprevalence

Five milliliters (5ml/l) of venous blood was aseptically collected from consented participant using sterile syringe then transferred into sample bottles. The blood is allowed to stand for 30 minutes, centrifuged at 2,500 rpm for five minutes. The serum obtained is used for profiling using 5 panel cassette.

2.7 Detection of HBV Using Rapid Diagnostic Kit

The sample were collected as instructed by the manufacturer protocol on the slip/leaflet, this was done under safe condition; by tying a tourniquet on the upper arm of the participants, a clean swab with spirit was used to clean the site of venipuncture and whole blood was drawn. The sample was transferred in-to a red top collection tube that has no anticoagulant, the sample was allowed to stand for 20-30minutes in a vertical position to clot, the sample was then Centrifuged at 1500rpm for 3minutes to obtained serum, the kits was dipped half way for serum to run over in the kits and then place lying on the packed, the result is read after 15minutes.

2.8 Interpretation of Results

Negative: absence of a band in the test region (T) on the test kit showed, and a pink band appear in the control region (C) on the test kit, indicates that no HBsAg have been detected.

Positive: the appearance of the band in the control region (C) of the test kit, another pink

band appeared on the test region (T) of the test kit. This shows that the sample have HBsAg.

Invalid: absence of band in the control (C) region of the test kit, regardless of the presence or absence of line in the test region (T) of the kit, indicates an error which may occur during the test. In this situation the tests were repeated according to AGARY pharmaceutical limited procedure.

2.9 Determination of HBV Profile for Core Antigen/ Antibodies Using 5 Parameter Rapid Test Cassette: Interpretation Assay result

Samples that were positive for HBV/HCV rapid diagnostic test were subjected to HBV core antigen/ antibody profiling. The protocol established by the manufacturer as contain on the leaflet of the kit were adopted, 5µl of serum/plasma sample were dispense into each 5wells of the cassette and allow to pass for about 15seconds. Do not pass the MAX line on the kit. Start the timer, the result should be read after 15minutes. According to the manufacturers protocol.

Negative: the presence of band at C region is developed on the HBsAg, HBeAg strip or both C and T bands are developed on either the HBeAg or the HBcAb strip, the test indicates a negative result on the parameter being tested.

Positive: presence of band at C and T bands are developed on the HBsAg/ HBsAb/HBeAg kit or only the C band is developed on HBeAb/HBcAb strip, the test indicates positive of the parameter being tested.

Invalid: absence of C band is developed, the assay on the strip is invalid regardless of color development on the T band as indicated below. Repeat the assay with a new device. According to Combo 5 panel cassette for HBV Rapid test pharmaceutical industry procedure.

2.10 Molecular Identification of HBV (Extraction and Purification of DNA)

Genomic DNA extraction was prepared for seven samples; 140µl of sample into DNA tube, 500 µl of lysis buffer was put into sample DNA tube and then mixed thoroughly (lysis method), incubate into water bath for 10-15mins at 60^{o}c, precipited 560µl of absolute ethanol into DNA tube was spinned at 13rpm for 1min precipited was discarded supernatant was washed using pipited 500µl of 70% ethanol was spin at 13rpm for1min and then dry spinned at the same speed. Elusion stage he washed tubes was introduced into DNA extraction tube, 30µl of elusion buffer was introduced into washed tubes was allowed to stand for 3-5mins was then spinned at 13rpm for 1min to get the DNA extract of HBV Wang *et al*. (2011).

2.11 Hepatitis B Virus DNA Detection

Primers; DNA extraction protocol described by Wang *et al*., (2011) the extracted DNA were quantified using Nano Drop Spectrophotomer and extracts with concentration above 20ng/ul were used for PCR. The DNA was then tested for HBV DNA using primers that targets the Pre-S gene of the virus. The Pre-S gene of HBV was amplified in a semi-nested PCR protocol. The first round PCR was performed in a 25µl reaction containing 5µl of red load PCR mix by Jena Biosciences (Jena Germany), and 2µl each of primers 979 (5'CAAAAGAC CCACAATTCTTTG ACATACTTTCCAA3') and SF (5'GTGTCTTG GCC AAAATTCGCAGT3') with 5µl of DNA and 11µl of nuclease-free water. The amplification was done in ABS 7000 thermal cycler following an initial denaturation of 950 C for 5 minutes, followed by 35 cycles of 950 C for 30 seconds, 620 C for 45secs and 720 C for 45 seconds and a final elongation of 720 C for 10 minutes. The amplified product of the first round PCR was used as a template for the second round PCR in a similar 25µl reaction volume Using primers 979 and MC-F (5'TCGGATCCGGTATGTTGCCCG TTTGTC3'and the same cycling condition. The amplified product was detected using agarose gel electrophoresis with 2% agarose and TBE used as running buffer, the product was then visualized in LED transilluminator.

2.12 Amplification Using PCR

Amplification and detection of HBV Genogroups was performed PCR. Primers targeting specific gene region was employed on positive serum samples. In a PCR cycler, 10µl in each sample of extracted DNA. (BIONEER, 2020).

2.13 Statistical Analysis

Chi-square test shows the association, compare proportions as well to test the relationship among dependent variables with continuous outcome

and the groups (institutions within Birnin Kebbi). P-value of <0.05 is considered significant.

3. RESULTS

The present study investigated the prevalence of Hepatitis B and C virus infection among Students of Tertiary institutions within Birnin Kebbi. Out of 329 students from both institutions, 165 Students from Waziri Umaru Federal Polytechnic Birnin Kebbi and 164 Students from Federal University Birnin Kebbi were screened using rapid serological methods. The prevalence of Hepatitis B Virus Surface antigen (HBsAg) and Hepatitis C virus Infection, in relation to their social demographic characteristics is presented in Table 1. Out of 165 participants screened only 25 were positive for HBsAg and 1 positive for HCV from the students of Waziri Umaru Federal Polytechnic Birnin Kebbi and 164 screened 18 were positive from students of Federal university Birnin Kebbi.

3.1 Molecular Identification of Hepatitis B Virus

The positive samples for HBeAg and HBeAb from both institutions were carried for molecular identification as it was the key markers for determine the core antigen stage of hepatitis B among the students.

3.2 Genomic DNA Isolation

The result showed that the full amount of DNA obtained (Plate 1) using this protocol was efficient for DNA extraction for Hepatitis B virus, since good yield of genomic DNA was obtained.

Table 1. Prevalence of HBV infection among the students of tertiary institutions within Birnin Kebbi metropolis

Sample area	No. of sample Collected		No. of Positive samples		F	%
	M	F	M	F		
WUFPBK	133	32	18	7	190	41.25
FUBK	118	46	16	2	182	29.52
TOTAL	329		43		372	70.77

Table 2. Prevalence of HCV infection among the students of tertiary institutions within Birnin Kebbi metropolis

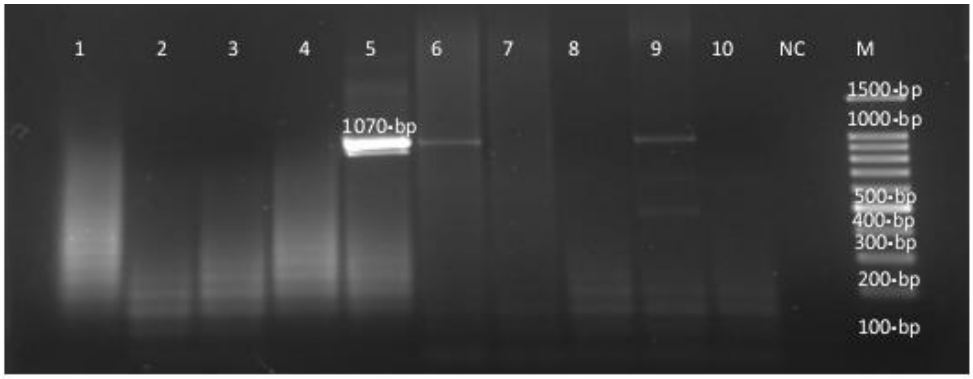
Sample area	No. of sample Collected		No. of Positive samples		F	%
	M	F	M	F		
WUFPBK	133	32	1	0	166	1.64
FUBK	118	46	0	0	164	0
TOTAL	329		1		330	1.64

Table 3. Profiling positive HBV samples from the students of tertiary institutions within Birnin Kebbi metropolis

MARKERS	WUFP BK	FUBK
HBsAg	25(41.25%)	18(29.52%)
HBsAb	3(4.95%)	0(0%)
HBeAg	5(8.25%)	2(3.28%)
HBeAb	6(9.9%)	1(1.64%)
HBcAb	15(24.75%)	6(9.8%)

KEY:
HBsAg – Hepatitis B surface Antigen HBsAb – Hepatitis B surface Antibody
HBeAg – Hepatitis B envelop Antigen HBeAb – Hepatitis B Antibody
HBcAb – Hepatitis B core Antibody
WUFPBK – Waziri Umaru Federal Polytechnic Birnin Kebbi
FUBK – Federal University Birnin Kebbi

3.3 PCR Analysis



M= Marker (100-bp), NC=Negative Control, 1to 10= Sample wells

Plate 1. 1500-bp PCR products of HBV DNA were identified in all sample using 1g pulsed field certified agarose gel electrophoresis. Ladder 5, 6 and 9 were various samples of Hepatitis B virus

4. DISCUSSION

In this study out of 329, 165 students tested 41.25% from Waziri Umaru Federal Polytechnic Birnin Kebbi and 164 students 29.52% from Federal University Birnin kebbi. Butt et.al (2021) recorded “7.3 % prevalence of HBsAg detected in 165 blood samples analyzed, indicate the prevalence of Hepatitis B (HBV) among blood donors varies depending on the study and the population being studied NBTS Centre in Kaduna, Nigeria. If the prevalence of Hepatitis B (HBV) infection is higher than reported in a study, it could be due to a number of factors, this might be ascribed not receiving the complete doses of the vaccine”. The prevalence of HBsAg reported in some studies in Nigeria is higher than 4.8% by Edia-Asuke et al. (2016). 0.4% lower than the reported study 7.7 % carried out by Ikerionwu (2018). “National Blood Transfusion Service cared for all units in both private and public health Hospitals assures that many efforts to make safe blood more accessible, Blood transfusions are used to treat a variety of conditions, HBV is a major global health problem that can lead to life-threating complications. it is recommended that all blood donations are tested for hepatitis B to ensure blood safety and prevent accidental transmission. Blood and organ donors are carefully screened for markers of hepatitis infection” (Jadeja et. al., 2014). “Research during the 70’s among healthy Iraq population the prevalence of HBsAg in blood donors and military personnel blood donors was 3.6% in the 1970s and 4.1% in the 1980s, while in the normal population it was 3.3% in the 1970s and 4.3% in the 1980” (Atallah,1987). Study results were compared with those of other researchers, it is much-needed to be done in other to avoid further complications from students receiving/donating “screened blood” from the hospitals (Odiabara et al., 2020). “It is well-known that maintained high levels of HBV profiling are linked with progressive liver disease. Serum DNA levels, also known as viral load are prognostic factors for chronic hepatitis B and can help determine treatment and assess treatments efficacy HBV DNA levels are an independent risk factor for cirrhosis, even when accounting for other factors like hepatitis B e-antigen status and serum alanine transaminase level” (Fattovich et al., 2008). “Research from different areas of Nigeria has shown varying prevalence rates of HBsAg among blood donors; DNA analysis for Hepatitis B (HBV) is not always feasible in Nigeria due to the cost of the test, and other markers are used to detect HBV” (Agbesor et al., 2016). PCR result showing (30%) active replicative infection in blood donors who are seropositive to HBsAg indicates that the donors have HBV DNA and are actively replicating the virus. If left untreated, this infection can progress to chronic hepatitis B, liver disease, and cancer. The chronic occult HBV infection may progress to chronic liver diseases such as liver cirrhosis. This is due to the genes were identified as early week initial infection. This is in line with WHO report of WHO (2002).

5. CONCLUSION

Hepatitis B virus is presents among the tertiary institution within Birnin kebbi and HBV are

identified, the different types of assays used to test for Hepatitis B virus (HBV) have different characteristics, and the best one to use depends on the situation, a rapid, sensitive, specific, and cost-effective assay for hepatitis B virus (HBV) can be useful for patient management and to curb early stages of infection. There is need for new developments of some specific therapeutic agent against Hepatitis B Virus. Moreover the cost implications of an assay is a noticeably affects a situation in controlling a disease in developed worlds.

6. RECOMMENDATION

Tertiary institutions should assure proper enforcement of screening for HBV and to those students detected positive for HBV, profiling should be carried out to determine the core antigen/ antibody, also public health awareness should be carried out across all the departments to have knowledge of its effects against the internal organ and its risk factors.

ETHICAL APPROVAL

Ethical clearance for this study was collected from Kebbi State Ministry of Health Birnin Kebbi, Kebbi State Nigeria. With Registration number: KSHREC 107:046/2023.

CONSENT

The participants consent was sought and their blood samples taken for analysis.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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