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**Seroprevalence of Hepatitis-B Virus and Evaluation of DNA Extraction Methods from Dried Blood Spot on Filter Paper for Molecular Testing in Kaduna State, Nigeria****Mohammed Ibrahim Tahir<sup>1\*</sup>, Ahmed Babangida Suleiman<sup>2</sup> and Abdurrahman Ahmad El-Fulaty<sup>1</sup>***Department of Medical Laboratory Science, Ahmadu Bello University, Zaria, Nigeria<sup>1</sup>, Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria<sup>2</sup>**Author for Correspondence\*: ghalrash@gmail.com/+234-806-082-2353***Abstract**

Hepatitis B is a potentially life-threatening disease caused by hepatitis B virus. This study was a cross sectional and a total of 300 paired blood samples were collected in tubes and on filter papers from students from three secondary schools located across the three senatorial zones of Kaduna State, Nigeria, from April 2015 to July 2015. Hepatitis B surface antigens were detected in sera by enzyme linked immunosorbent assay (ELISA) and DNA was extracted from DBS to amplify hepatitis B virus polymerase gene by PCR. The overall seroprevalence of Hepatitis B surface antigen was found to be 18.33%. The mean concentrations for Microwave extraction, Phenol-Chloroform and Zymo Research Viral DNA Kit™ methods were 1219.00ng/μl, 528.85 ng/μl and 9.14 ng/μl respectively. Polymerase gene was detected among 40% individuals that showed seropositivity to Hepatitis B surface antigen. This study has demonstrated high prevalence of hepatitis B virus antigenaemia among Secondary School students in Kaduna State, Nigeria. Our study provides evidence that hepatitis B virus infection is highly endemic in the studied area therefore, posing a threat of high incidence of cirrhosis and hepatocellular carcinoma.

**Introduction**

Hepatitis B virus (HBV) is a double-stranded DNA virus belonging to Hepadnaviridae family. Hepatitis B is a potentially life-threatening disease caused by hepatitis B virus. It is a major global health problem and the most serious type of viral hepatitis. It can cause chronic liver disease resulting to death from cirrhosis of the

liver and liver cancer. The primary method of transmission reflects the prevalence of chronic HBV infection in a given area. In low prevalence areas such as United States and Western Europe, where less than 2% of the population is chronically infected, injecting drug by drug abusers and unprotected sex are the primary routes of infection, although other factors such as traditional scarification may also be important (Olatunji *et al.*, 2012). Transmission of hepatitis B virus results from exposure to infectious blood or body fluids. Possible forms of transmission include (but are not limited to) unprotected sexual contact, blood transfusions, re-use of contaminated needles and syringes, and vertical transmission - mother to child (Olatunji *et al.*, 2012).

The aetiological agent (Hepatitis B virus) is a member of the family Hepadnaviridae and the genus *Orthohepadnavirus*. It is a double stranded circular DNA virus composed of an outer envelope containing hepatitis B surface antigen (HBsAg) and an inner nucleocapsid consisting of hepatitis B envelope antigen (HBeAg) and hepatitis B core antigen (HBcAg). Corresponding antibodies to each of these antigens are Hepatitis B surface antibody (anti-HBs or HBsAb), Hepatitis B envelope antibody (anti-HBe or HBeAb) and hepatitis B core IgM and IgG antibody (anti-HBc or HBcAb). The viral core also contains double stranded DNA genome and DNA polymerase. The serological markers of Hepatitis B virus are HBsAg, anti-HBs, HBcAg, anti-HBc (IgM and IgG), HBeAg, anti-HBe and HBV DNA. These are important as they can be used in the diagnosis of the infection

and to determine the severity of the infection (Margaret *et al.*, 2011). Hepatitis B virus deoxyribonucleic acid (DNA) is detectable by Polymerase Chain Reaction (PCR) as soon as one week after initial infection and also detect mutants that escape detection by current serological methods (WHO, 2002).

An alternative to biological tests that require whole-blood samples obtained by venipuncture is dried blood spots (DBS). Dried blood spots can be prepared with whole blood collected from a finger prick, causing the patient less discomfort in the sense of volume of blood collected. Samples do not need to be centrifuged to separate plasma, and serum does not need to be frozen immediately after sampling. Desiccated samples can be stored for transport as non-hazardous material via postal services (Mohamed *et al.*, 2013).

Hepatitis B surface antigen (HBsAg) mutations may give rise to changes in HBsAg antigenicity, resulting in reduced sensitivity and detection capability of current diagnostic tests, and which might present a challenge for HBsAg screening and HBV diagnosis, and ultimately increase the risk of transmission of HBV infection through blood transfusion (Zhang *et al.*, 2013).

Occult HBV infection is defined as the presence of HBV DNA in blood or liver, in the absence of detectable serum HBV surface antigen (HBsAg), and has been classified into seropositive and seronegative infection depending on the presence of the antibody to the HBV core antigen (anti-HBc) and/or HBsAg (anti-HBs) (Brechot *et al.*, 2001). Occult HBV infection is a problem in many parts of the world and affects blood banks, the diagnosis of chronic hepatitis, and immunization (Yong *et al.*, 2009).

Therefore, there is need for molecular based approach for screening of blood for donations and diagnosis of Hepatitis B Virus infection, appreciating its high sensitivity and specificity (Frederick *et al.*, 2003). An efficient unbiased method of DNA extraction that yields pure, high quality DNA is crucial for the success of PCR and sequencing reactions and the subsequent treatment of the disease (Massimiliano *et al.*, 2006).

Forbi *et al.* (2010) highlighted difficulties in liquid sample collection which include storage and transportation of whole blood or separated blood samples in liquid nitrogen. There is also an increased chance of mislabeling due to transfer steps. Many DNA extraction methods have been developed. Whereas a number of DNA extraction kits are commercially available, most were not developed for use with DBS (Zhili *et al.*, 2005).

## **Methodology**

### **Study area**

The study area was Kaduna State, Nigeria. Kaduna State is in the centre of the northern Nigeria and more than 1000km from the Atlantic Ocean. It is located on a latitude of 11° 12' N and a longitude of 07° 37' E. The state has its capital at Kaduna City.

The population of Kaduna State is 6,066,562 (National Population Commission, 2006). It is characterized by a tropical climate with two seasons; a wet season of approximately 210 days (May to October), with a mean annual rainfall of 1092.8mm, and the dry season which spans between November and April. The monthly mean temperature shows a maximum of 39.7°C in June and 13.8°C in December (Ukegbu, 2005). The state has 23 local government area and three senatorial zones. These include Kaduna north, Kaduna central and Kaduna south senatorial zones.

### **Study population**

The study population consisted of apparently healthy students attending some selected Senior Secondary Schools in Kaduna State. One secondary school was selected from each of the three senatorial districts of the state. The secondary schools selected for the study were; Government Secondary School Godo-Godo (Jema'a), Government Secondary School Kurmin Mashi, Kaduna (Kaduna South), and Government Secondary school Hunkuyi (Kudan).

### **Ethical Clearance**

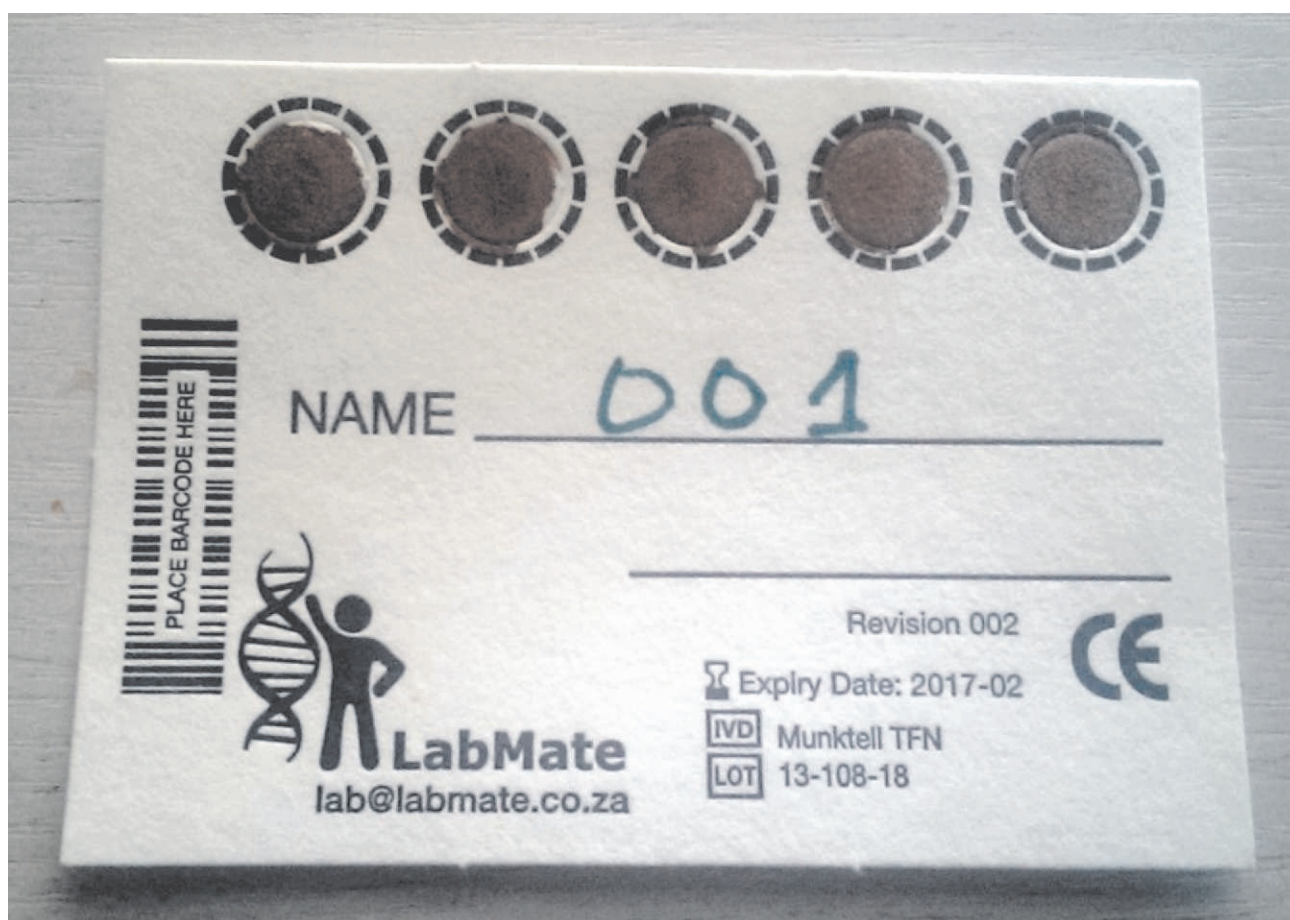
Ethical approval to carry out this research was obtained from the ethical committee of Ministry of Health, Kaduna State, Nigeria. Prior to sample

collection, a structured questionnaire was used to obtain information on sociodemographic and risk factors from each student.

#### **Sample collection storage and transportation**

Written informed consent was sought from the volunteers prior to sample collection. This was accompanied with questionnaires to capture basic demographic data, history of illness, risk factors and clinical information. A drop (approximately fifty microliters 50 $\mu$ l) of venepunctue whole blood drawn in syringe was inoculated onto the 12-mm pre-printed disks on the dried blood spot (DBS) card (Whatman cards

903, Lasec South Africa) (Fig. 1) while the remaining blood dispensed into EDTA-anticoagulated tube. The DBS cards were left to dry for 4 hours at room temperature and placed in individual sealable plastic bags containing silica desiccant. The paired DBS card and the separated plasma were assigned a unique number for identification. The blood was transported in ice pack to Department of Microbiology, Ahmadu Bello University, Zaria and centrifuged at 3500 rpm for plasma separation. The plasma samples were stored at -20°C and the DBS cards at room temperature for further processing.



**Figure 1: DBS on Whatman filter paper 903**

#### **Laboratory analysis**

##### **Serological assay for Hepatitis B virus Surface antigen**

All plasma samples processed for serological testing were assayed using enzyme-linked immuno-sorbent assay for Hepatitis B virus Surface antigen (HBsAg).

#### **Procedure**

1. One blank, two positives and two negatives controls were set and 0.05ml plasma sample, positive and negative sera were added into the coated wells.
2. One drop (approximately 0.05ml) of enzyme conjugant was added into the same coated wells (the blank well was omitted), mixed thoroughly and incubated for 60

minutes at 37°C.

3. The liquid in the coated wells was discarded and brought to dry. The wells were filled with wash solution, discarded and brought to dry. This step was repeated five times.
4. One drop (approximately 0.05ml) of substrate A and B were added respectively to each well and incubated for ten minutes at 37°C.
5. A drop (approximately 0.05ml) of stop solution was added into each well, mixed thoroughly and absorbance was measured at 450nm against the blank.

### DNA extraction methods

Only DBS cards with seropositive Hepatitis B virus Surface antigen were used for molecular testing.

#### Processing the DBS

Nine (9) 3mm-disks were punched out of the dried blood spots with a sterile punch and placed into cryovials and 800µl of 1x PBS was added and incubated at room temperature for 10 minutes. The vial was vortexed for 30 seconds and 200µl was transferred to microcentrifuge tubes for further analysis. This preparation was carried out for each extraction method.

#### A: Microwave protocol

The protocol employed by Bhagwati *et al.* (1992) was modified as follows:

The reconstituted sample in the microcentrifuge tubes were subjected to radiation in a microwave oven at full power for 10 min. The pellets were dissolved in 200µl of Tri-EDTA (TE) (10 Mm TrisCl, 1mM EDTA pH 8) buffer and extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) for 15 min. The aqueous phase was recovered by centrifugation for 20 min and precipitated with ethanol (Omar *et al.*, 2014). The pellet was air dried and suspended in 20µl of TE.

#### B: Phenol-chloroform protocol

Sadat and Ali's (2010) Phenol-chloroform extraction procedure was adopted with little modification to suit DNA extraction from DBS. 200µl of lysis buffer (25mM EDTA, 200mM Tris-HCl pH 7.5, 250mM NaCl, 1%SDS ) was added and incubated at room temperature for 60

min. and 400µl equilibrated phenol pH 7.8 was added, decanted and incubated for 10 min at room temperature. Tubes were centrifuged at 12000g for 5 min. Then mix with an adequate volume of chloroform: isoamyl alcohol (24:1). Then was precipitated with 3M sodium acetate pH 5.2 and 96% ethanol and incubated at -20°C for overnight. After centrifugation for 15 min at 4°C at 16000g, the pellet was washed with 70% cold ethanol and centrifuged at 16000g at 4°C. The pellet was air-dried and suspended in 20µl of TE by vortexing, and then stored at -20°C until using (Sadat and Ali, 2010).

#### C: Zymo Research Viral DNA Kit™ protocol

Commercial DNA extraction kit was used to extract DNA from DBS sample. Using 200µl reconstituted sample, the manufacturer's instruction was followed as below:

1. In a 1.5 ml microcentrifuge tube, 4 volumes of **ZR Viral DNA Buffer** was added to each volume of sample (e.g., 800µl **ZR Viral DNA Buffer** to 200µl serum). It was mixed briefly by vortexing and Allowed to stand at room temperature for 10 minutes.
2. The mixture was transferred to a **Zymo-Spin™ IC Column** in a **Collection Tube** and Centrifuged for 1 minute. The flow through was discarded from the **Collection Tube**.
3. 300µl **DNA Wash Buffer** Was Added to the column centrifuged for 1 minute. The flow through was Discarded and the step Repeated.
4. The **Zymo-Spin™ IC Column** was placed into a microcentrifuge tube. 10µl **DNA Elution Buffer** was added directly to the column matrix. It was allowed to stand at room temperature for 1 minute and centrifuged for 1 minute to elute the DNA (Zymo Research, 2014).

#### Determination of concentration and purity of extracted DNA using NanoDrop ND- 100 Spectrophotometry

1. The equipment was switched on.
2. Program was opened by clicking on the ND-1000 icon.
3. Nucleic Acid icon was clicked.
4. The spectrophotometer was initialized by

placing 1µl clean water onto the lower optic surface, the lever arm was lowered, and “initialize” was selected in the NanoDrop software. Once the initialization was completed, the optical surfaces were cleaned with tissue.

5. A blank measurement was performed by loading 1µl deionized water or buffer and “blank” was selected. Both optical surfaces were cleaned once the blank was complete.
6. Appropriate sample type (DNA) was chosen.
7. TE buffer was used as blank, by adding 1.2µl to pedestal; the blank reading gave 0.0 ng/µl.
8. Sample identification was typed in the box for reference. Pedestals were cleaned with a dry tissue. The sample was mixed well and 1.2µl added to the pedestal and lid closed.

The Measure icon was clicked. A concentration was given in ng/µl.

9. The print on the graph and the 260/280 value were given automatically.
10. Pedestal and top were wiped with a dry tissue between each sample (Frederick *et al.*, 2003).

#### Amplification of HBV DNA by PCR

Only samples from DNA extraction method with best purity that is, **Zymo Research Viral DNA Kit™ protocol** were used for molecular testing. In rapid cyclers PCR, 10µl from each sample of extracted DNA was used in nested PCR for HBV genomic DNA using PCR master mix kit (Qiagen GMBH, Hilden, Germany). Amplification was performed using the primer set to the sequence of HBV DNA polymerase gene primer as presented in Table 1.

**Table 1: Primer set to the sequence of HBV DNA polymerase gene**

Gene	Primer	Reference
<b>First run primer set</b>		
HBV381	Forward primer 5'-TGCGGCGTTTTATCATCTTCCT -3'	(Ma <i>et al.</i> , 2011)
HBV840	Reverse primer 5'-GTTTAAATGTATACCC AAAGAC-3'	
<b>Second run primer set</b>		
HBV381	Forward primer 5'-TGCGGCGTTTTATCATCTTCCT -3'	
HBV801	Reverse primer 5'-CAGCGGCATAAAGGGACTCAAG -3'	

#### Assay Procedure

The fragment of the polymerase gene was amplified by semi-nested PCR with two rounds of amplification. The reaction volume was 20µl. The first round of amplification was performed with an initial 5 min denaturing step at 94°C, followed by 30 cycles of denaturing for 45 s at 94°C, annealing for 30 s at 50°C, and elongation for 90 s at 72°C, with a final extension period of 10 min at 72°C using primers HBV381 and HBV840 (Table 1). The second round of amplification was performed with an initial 5 min denaturing step at 94°C, followed by 30 cycles of denaturing for 45 s at 94°C, annealing for 30 s at 55°C, and elongation for 60 s at 72°C, with a final extension period of 10 min at 72°C using primers HBV381 and HBV801. The reaction products of the semi-nested PCR were run on a 2% agarose gel stained with ethidium bromide (Ma *et al.*, 2011).

#### Running Agarose Gel

To 40ml 1x Tris Acetate EDTA Buffer (pH 7.9) in a small glass bottle was added 0.6g agarose and boiled in a microwave oven for 30mins. After boiling, the volume was made up to 50mls with distilled water and cooled to about 50°C. Two microliter (µl) of ethidium bromide was added, swirled and the gel poured in to gel cassette with a comb in place. The gel was allowed to solidify at room temperature for 30mins and the comb removed. The cassette tray was submerged beneath TAE buffer in an electrophoresis apparatus. About 7ul of each sample, positive and negative controls, and a 100-1000 base pairs (bp) molecular size marker was mixed with bromophenol blue (BPB) and loaded into separate wells of the gel. The first well of the gel was loaded with molecular size marker. The ten samples were loaded and then positive and negative controls respectively. The gel was

electrophoresed at 90V for 30mins at room temperature until the BPB reached the bottom of the vertical gel. The gel was removed and viewed under UV light and photographed. The Amplified PCR products (amplicons) of four hundred and fifteen (415) nucleotide sequence were determined based on their sizes

## Results

### HBsAg test result

Plasma sample were obtained from 300 secondary school students aged between 12 to 25 years, with an average of 16.83 years. The overall seroprevalence of hepatitis B surface antigen (HBsAg) among the studied population was found to be 18.33% (55/300).

Result analysis based on sex of the participants indicated males with seropositive of 21.76% (42/151) and females with seropositive of 12.15% (13/94). This result showed significant difference of seroprevalence of male greater than that of female ( $\chi^2=4.2479$ ,  $df=1$ ,  $p=0.0393$ ). Among the studied population, males were two times more likely to be infected with hepatitis B virus than their female counterpart (OR = 2.0112, 95% CI= 1.0258-3.9432).

Age group 21-25 had highest prevalence of 25% (3/12), age group 10-15 had least prevalence of 16.35% (17/104) while age group 16-20 had prevalence of 19.02% (35/184). The differences seen among the prevalence have no significant different ( $\chi^2=0.6885$ ,  $df=2$ ,  $p=0.7087$ ).

Knowledge of hepatitis B virus infection among subjects was analyzed and 18.31% (13/71) of those that had the knowledge had the surface antigen while 18.34% (42/229) of those without its knowledge were seropositive to the antigen. There was no significant difference between the prevalence observed ( $\chi^2=0.0288$ ,  $df=1$ ,  $p=0.9953$ ). The knowledge of hepatitis B virus infection did not increase or decrease chances of infection with the virus (OR = 1.0021, 95% CI= 0.5035-1.9944).

Observed seroprevalence of HBsAg among subjects with history of any form of liver disease was 25% (6/24) and higher than that among subjects without any history of any form of liver

disease. However, there was no statistically significant different between the two prevalence ( $\chi^2=0.7585$ ,  $df=1$ ,  $p=0.3838$ ) (OR = 1.5374, 95% CI= 0.5804-4.0727).

There was no significant difference between prevalence of individuals that never received full hepatitis B virus vaccine and those that were fully vaccinated ( $\chi^2=0.1002$ ,  $df=1$ ,  $p=0.7516$ ). Although subjects with full vaccination status had higher prevalence of 21.05% (4/19) than those that never received full vaccine with prevalence of 18.15% (51/281), there was reduced chance of being infected with the virus among vaccinated subjects (OR = 0.8315, 95% CI= 0.2649-2.6102).

Indiscriminate unprotected sex as a risk factor for acquiring hepatitis B virus was analysed among the studied population. Prevalence among individuals with the habit was 28.57% (4/14) while those without the habit recorded prevalence of 17.89% (51/286). No statistically significant difference was detected between the observed prevalence ( $\chi^2=0.5426$ ,  $df=1$ ,  $p=0.3106$ ). In this study there was no increased chance of acquiring the disease because of indiscriminate unprotected sex (OR = 0.5426, 95% CI= 0.1637-1.7985).

There was high prevalence among those that were ever been transfused with blood than individuals that never been transfused but this did not account for any statistical significance between the two prevalence ( $\chi^2=0.0293$ ,  $df=1$ ,  $p=0.8641$ ). The prevalence among those ever transfused and those never been transfused were 20% (3/15) and 18.25% (52/285) respectively. There was no increased chance of being infected due blood transfusion (OR = 0.8927, 95% CI= 0.2432-3.2769).

There was no association between prevalence of the virus and scarification ( $\chi^2=0.5466$ ,  $df=1$ ,  $p=0.4597$ ). The chance of acquiring the disease is almost equal between subjects with scarification and those without it (OR = 1.2923, 95% CI= 0.6540-2.5535). Prevalence among those with scarification is 15.66% (13/83) and lower than those without it which is 19.35% (42/217).

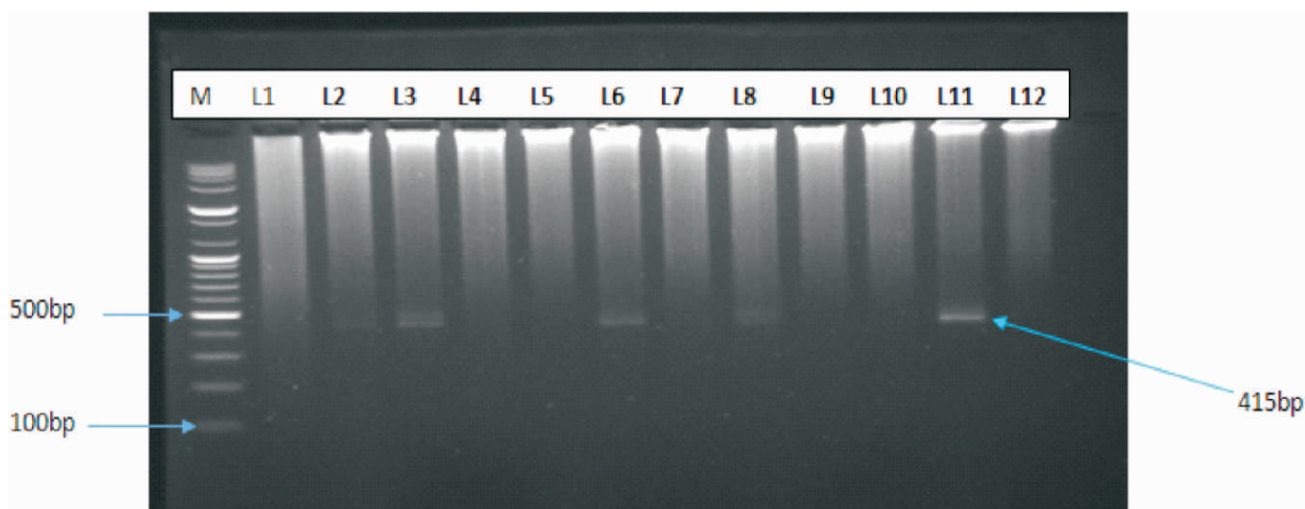
### DNA concentration and purity

The mean concentrations of DNA extracted from DBS by Microwave, Phenol-Chloroform and Zymo Research Kit methods were 1219.00ng/ $\mu$ l, 582.85ng/ $\mu$ l and 9.14 ng/ $\mu$ l respectively. There was significant different in DNA concentration across all the methods employed ( $p=0.000$ ). DNA Purity means were 0.67, 0.71 and 1.61 for Microwave, Phenol-Chloroform and Zymo Research Kit methods respectively. Significant different in purity was detected among all the extraction methods ( $p=0.000$ ). Significant strong positive correlation was detected in

Microwave method between DNA concentration and purity. No significant correlation was seen in Phenol-Chloroform method. Zymo Research extraction kit showed strong negative correlation between the DNA concentration and purity.

### Polymerase Chain Reaction (PCR)

Out of the ten randomly selected HBsAg positive samples, four yielded positive for hepatitis B virus DNA. Therefore, the prevalence of HBV DNA among HBsAg seropositive subjects was 40% (Fig. 2).



**Fig. 2: Agarose gel electrophoresis (2%) of the reaction products of semi-nested PCR and DNA Markers stained with ethidium bromide. M: PCR markers (1000, 750, 500, 250 & 100 bp), L2, L3, L6, L8: positive PCR results, L1, L4, L5, L7, L9, and L10: negative PCR results, L11: positive control, L12: negative control.**

### Discussion

#### Prevalence of hepatitis B surface antigen

Screening asymptomatic people is an important instrument in disease detection, prompt diagnosis and intervention, particularly at an early stage of the disease. This may improve the health outcome as well as better understanding of the transmission pattern of the disease [19].

This study indicated overall prevalence of 18.33% among secondary school students of age range between 12 to 25 years. This prevalence is comparable to report of Ndako *et al.* (2011) where 18.4% was detected in the same Kaduna State among secondary school students between the age ranges of 10 to 24 years. A relatively lower prevalence of 6.0% was reported by Adoga

*et al.* (2010) from the north-central of Nigeria among apparently healthy individuals. Umolu *et al.* (2005) in Benin City, Nigeria reported lower prevalence of 5.8% among voluntary blood donors of age range 18 to 25 years. Higher prevalence as seen in this study can be attributed to endemicity in tropical Africa with range between 8-20% (WHO (2002)). The prevalence rate of this work confirms the WHO (2002) classification of Nigeria as part of high endemicity region.

Musa *et al.* (2015) reported pooled prevalence of 13.6% among Nigerians in a meta-analysis which is slightly lower than prevalence of this study.

Lack of significance different in prevalence among the three schools may be associated with the similarity of lifestyle across the State as risk factors affect disease prevalence (Machado *et al.*, 2013). Male recorded prevalence of 21.76% which is significantly higher than female prevalence of 12.15% moreover; male were two times more likely to be infected than their female counterparts. Analogous relative higher prevalence among male was reported by Ndako *et al.* (2011) with prevalence of 25.5% and 10.9% for male and female respectively. In contrast, Lavanya (2012) reported prevalence with no significant different between the gender. Higher prevalence in male and more chance for being infected could be routed to the fact that more males were sampled than female as girl-child education is limited in some settings. It could still be connected with more promiscuous nature of male which predisposes infection.

The prevalence in relation to age group was 30.91%, 63.64% and 5.45% for 10-15, 16-20 and 21-25 age groups respectively. Although there was no significant different among the age group, the higher prevalence seen in age group 16-20 could be associated to paucity of sample in other group. Lavanya *et al.* (2012) reported similar prevalence of 1.7% among age group 19-25 years. Departure from this was appreciated in work reported by Ndako *et al.* (2011) with age range 10-14, 15-19 and 20-21 having prevalence of 20%, 17.6% and 19.4% respectively.

In this study, antigenaemia was not associated with indiscriminate sex, failure to receive vaccine, inadequate knowledge of hepatitis B virus, history of any form liver disease, transfusion and scarification. It goes the same trend as in the work of Lavanya *et al.* (2012) and Ndako *et al.* (2011), except for later that recorded significant association between antigenaemia and scarification or tattooing.

All the risk factors studied showed no increased risk of being infected with the virus though there was reduced chance of being seropositive among vaccinated individuals.

#### **Extracted DNA concentration and purity**

As molecular testing becomes routine in screening programs around the world, a simple,

low-cost, reliable, and scalable method for extracting amplifiable gDNA from DBS is necessary (Saavedra-Matiz *et al.*, 2013). Significant different was observed in concentration of DNA extracted among the three methods employed. The mean concentrations for Microwave, Phenol-Chloroform and Zymo Research extraction Kit were 1219.00ng/ $\mu$ l, 582.85ng/ $\mu$ l and 9.14ng/ $\mu$ l respectively. The marked difference gotten could be attributed to possible protein impurities as no treatment with proteinase was adopted in Microwave and Phenol-Chloroform methods. This could be true because some protein absorb light at wavelength of 260nm as nucleic acid do (Ausubel *et al.*, 2003). This can also be justified from the result of the DNA purity of this study. The means of DNA purity were 0.67, 0.71 and 1.61 for Microwave, Phenol-Chloroform and Zymo Research extraction Kit respectively.

The quality of DNA isolated in Microwave and Phenol-Chloroform methods was poor, therefore may not be suitable for microarray and qPCR analysis but suffice for DNA detection (Zakaria *et al.*, 2013). Genomic DNA yield has strong significant positive correlation with its purity in case of Microwave method. This demonstrates that sample with less protein concentration tend to yield more DNA concentration because absorbance at 280nm will be less hence, rise in purity index (Ausubel *et al.*, 2003).

Significant negative correlation between DNA concentration and purity in Zymo Research extraction Kit method could be associated with the fact that the method has adequate protocol for clearing protein impurities therefore, as the absorbance at 260nm increase and absorbance at 280nm decrease, so purity decrease and eventually DNA concentration increases.

#### **PCR**

Polymerase gene was detected among 40% individuals that showed seropositivity to HBsAg. This gene can be detected as early as one week after initial infection. This result indicated active replicative infection among the HBV DNA positive individuals (WHO, 2002). Successful extraction and detection of hepatitis B virus DNA from DBS was achieved.



## Conclusion

This study has demonstrated the prevalence of HBV antigenaemia among secondary schools students in Kaduna State, Nigeria. The data were generated from three secondary schools, one from each of the three senatorial region of the state. Our study provides evidence that HBV infection is highly endemic in the studied area therefore, posing a threat of high incidence of HCC and liver cancer. With this high prevalence rate and relatively low vaccine coverage and traditional lifestyle that predisposes acquiring infection; transmission of the virus will probably increase and pose more potential public health concern. Successful extraction of HBV DNA from DBS on filter paper using different methods creates more prospect for selection of economically and conveniently suitable method of choice. This will also be more appropriate in remote settings where conventional blood sample collection for molecular testing is posed with storage and transportation challenge. Detection of HBV polymerase gene among HBsAg carriers indicates need for therapeutic intervention and other baseline laboratory investigation to establish prognosis and also for use as benchmark for treatment. Future work on extraction of DNA from DBS while adopting Phenol-Chloroform and Microwave methods should include proteinase in the protocol to minimize level of protein in the sample and hence raise the purity index of the extracted DNA. More extensive research work should be carried out on HBV genotyping to establish mutants that escape drug or immune reaction. Government should be committed to incorporate HBV vaccination regimen into Expanded Program on Immunization (EPI). This will target pregnant women, newborns, children and adults. There is need for partnership between non-governmental organizations and government to intervene on issues of treatment and surveillance. Government should regulate and enforce blood banking system for safe blood transfusion services. The populace needs to be engaged in health talk to increase awareness on HBV infection. This knowledge will help in curbing the transmission. This campaign will stress issues like dangers of indiscriminate unprotected sex, traditional scarification and tattooing, transmission from mother to child and other crude practices that predispose infection.

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