Occult Hepatitis B Virus Infection in Previously Screened Blood from Blood Banks in Kaduna Metropolis

[1,2]Jolayemi Bashir* A.D., [1]Abdulsalami, M.S. and [1]Egbe N.E.
[1]Department of Biological sciences, Nigeria Defence Academy, PMB 2109, Kaduna
[2]National Ear Care Center, Kaduna

Abstract: Hepatitis refers to an inflammatory condition of the liver. It is commonly caused by a viral infection, but there are other possible causes of hepatitis. This study sought to determine the prevalence of Occult hepatitis B virus infection in previously screened blood from blood banks in Kaduna metropolis. The method involved collection of 2.0 ml of blood from donor's pilot sample and re-testing the blood by another rapid test kit, Lab ACON (Hangzhou Biotest Biotech Co. Ltd. China). Those found negative were then tested for HBV-DNA by PCR. The PCR product was Sequenced using Sanger's method to determine its variant. Structured questionnaire was also administered to blood bank facilities for the study. The result showed that all blood banks used employed rapid test kit method only for donors screening. There was a prevalence of 0.5% Occult hepatitis B virus infection in previously screened blood from blood banks in Kaduna metropolis. Statistical measure using Chi – square to compare the difference between use of Rapid test strip method and the PCR method shows that there is no significant difference at $P < 0.01$, but the PCR method is more sensitive than the rapid test strip method. Blast of the sequence shows 92% identity to Hepatitis B V2. Occult HBV infection exists in previously screened blood from blood banks in Kaduna metropolis and the use of rapid test kit alone for screening prospective donors will not eliminate the risk of HBV transmission in blood transfusion. Blood donor samples in Kaduna state should be tested for OBI by Nucleic acid testing (NAT) prior to transfusion to minimize the HBV infection risk.

Keywords: Blood banks, Hepatitis B virus, Kaduna, NAT, Occult, Rapid test

INTRODUCTION

Hepatitis refers to an inflammatory condition of the liver. It is commonly caused by a viral infection, but there are other possible causes of hepatitis. These include autoimmune hepatitis and hepatitis that occurs as a secondary result of medications, drugs, toxins, and alcohol. Autoimmune hepatitis is a disease that occurs when the body makes antibodies against the liver tissue.

There are several hepatitis viruses; they have been named types A, B, C, D, E, F (not confirmed), and G. However, of all these causes, hepatitis B virus infection is considered most important as it is known to be 100 times more infectious than Human Immunodeficiency Virus (HIV) (Okonkwo *et al.,* 2017). The virus causes persistent infection, chronic hepatitis, liver cirrhosis, hepatocellular carcinoma and immune complex disease (Farber *et al*., 1987; Mahoney and Mark, 1999; Strauss and Ellen, 2002).

[1*]Corresponding author Email: jolayemibashir@gmail.com

The diagnoses is by Physical examination, ultrasound (imaging), blood test–which may be antigen based, or antibody based, liver enzyme and liver biopsy (Friedman *et al.,* 2015). In many developing countries including Nigeria screening of donor's blood for transfusion is still based on HBsAg antibodies detection methods only (Salawu and Murainah, 2006).

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 (Raimondo *et al.,* 2008). Occult Hepatitis B infection also technically is defined as the presence of low HBV DNA in serum (<200 IU/mL) and or presence of <10 copies of HBV DNA per test (Torbenson and Thomas, 2002; Raimondo *et al*., 2008). This phenomenon is becoming increasingly recognized in several clinical settings worldwide (Brechot *et al.,* 2001). Studies on a large set of blood donors using NAT confirmed this phenomenon of OBI and formed the basis of mandatory NAT for transfused blood units in many developed countries (Panhotra *et al.,* 2005; Prati *et al.,* 2006).

Such a testing regimen has not been incorporated into the testing algorithms of many laboratories in developing countries including Nigeria (Ola *et al.,* 2009).The aim of this study is to screen for the presence of Occult Hepatitis B virus in Blood Banks, and determine the variant of Hepatitis B virus among blood donors in Kaduna metropolis, as there is limited data on the prevalence of OBI in blood donors in this country (Salawu and Murainah, 2006; Salawu *et al.,* 2010). Despite Hepatitis B virus infection being a global health issue of immense importance (Lok and McMahon, 2001; Kao and Chen, 2002; Sorrell *et al.,* 2009). It occurs worldwide and up to two billion people, approximately 30 % of the world's population have been infected globally (Kao and Chen, 2002). Of this, 300 – 400 million people are chronically infected, approximating to about 5 % of the world's population at risk of developing the complications of chronic HBV infection (Maddrey, 2000; Lok and McMahon, 2001; Kao and Chen, 2002; Lavanchy, 2004; Sorrell *et al.,* 2009). Deaths resulting from HBV yearly stand at about 500,000 to 1.2 million worldwide. As such the need to carry out this research on Occult Hepatitis B Virus infection in blood stored in blood banks to determine the phenomenon of OBI and if present to recommend on mandatory NAT testing of blood for transfusion purpose in Kaduna State metropolis.

MATERIALS AND METHODS

Study Area

The study was conducted in Kaduna metropolis which covers about 268,359km, with built up area covering 200,000 hectares. It is within the Guinea savannah ecological zone with wet and dry season (Denwe, 2014). It is in North West Nigeria, located $10^{o}.53$ latitude and $7^{o}.44$ longitudes, situated at elevation 626 meters above sea level.

Figure 1: Map showing Kaduna State metropolis.(https://www.researchgate.net/figure/MAP-OF-KADUNA-METROPOLIS).

Selection of Blood Transfusion Facility

Some of the facilities used in blood transfusion services in Kaduna metropolis were selected for this study, Yusuf-Dansoho Memorial Hospital, Tudun-wada Kaduna (YDMH); Gwamna - Awan General Hospital, Kakuri Kaduna (GAGH) and the National Ear Care Center Kaduna (NECC).

Ethical Approval / Consent

Ethical approval was obtained from the ethical committee of Kaduna State Ministry of Health; certificate number MOH/ADM/744/VOL.1/519, and the National Ear Care Center Kaduna.

Administration of Questionnaires

A structured questionnaire was administered to the Blood transfusion facility for the study. Questions such as counseling of donors before blood donation, method of test employed in HBV diagnosis, method of test used for HBV detection before blood donation, any need for upgrade? were obtained.

Determination of Sample Size

The sample size was calculated based on formula presented by Charan and Biswas, (2013).

$$\text{Sample size} = \frac{Z^2 \times P(1-P)}{d^2} \quad \text{or} \quad \frac{Z^2 P q}{d^2}$$

Where:

Z = is standard normal deviate, usually set at 1.96; corresponds to 95% confidence interval.

P =Expected proportion in population based on previous studies on pilot studies is 8% in Abakaliki South-Eastern Nigeria (Nna *et al.,* 2014).

d = degree of accuracy desired, (absolute error or precision)

q =1.0 - P

Sample size calculation;

$$= \frac{Z^2 \times P(1-P)}{d^2} \quad \frac{1.96^2 \times 0.08 (1 - 0.08)}{0.05^2}$$

$$\frac{3.8416 \times 0.054 \times 0.992}{0.0025}$$

= 121.9477504, the sample size is 122 (Minimum sample size) and was rounded up to 200.

A total of 200 samples from the designated facilities were used for the study.

Sample Collection

Pilot sample (2.0 ml) from donor's pint of blood was collected and centrifuged at 3000 rpm for 5 minutes to harvest the supernatant plasma into a labeled cryovial bottles. The sample was frozen at the respective facility for 0-48 hours before transported to DNA Laboratory where it was stored at -20˚C.

The Rapid Test Strip Method

The sample was thawed and brought to room temperature before testing following manufacturer's procedure.

Polymerase Chain Reaction (PCR)

DNA Extraction

The DNA extraction was carried out using Bioneer Accu Prep Genomic DNA Extraction kit (K-3032) following manufacturers procedure:

Before beginning, a heating block was set at 60˚C. The following were added to 1.5 ml eppendorf tube (20 µl of proteinase K, 200µl of the sample, 200 µl of GB Buffer) and mixed immediately by vortex mixer, then incubated at 60˚C for 10 min. 400µl of absolute ethanol was added and mixed by pippetting. The lysate was carefully transferred into the upper reservoir of the binding column tube and the tube was centrifuged at 8000rpm for 1 min. Then 500 µl of WA1 Buffer (Wash Buffer 1) was added to the collection tube and the tube was centrifuged at 8000 rpm for 1 min. Thereafter, 500 µl of W2 Buffer (Wash Buffer 2) was added and the tube centrifuged at 8000 rpm for 1 min. The collection tube was centrifuged at 13000 rpm for 1 minute to completely remove the ethanol. The binding column tube was transferred to a new 1.5 ml tube for elution by adding 200µl of EA BUFFER (Elution Buffer) onto Binding column tube and waited for about 1 min at room temperature then centrifuged at 8000 rpm for 1 min to elute.

PCR reaction set up:

To the PCR tube containing premix, the following was added; 1µl of forward (sense) primer,1µl of reverse (antisense) primer, 2µl of the DNA sample and 16µl of deionised distilled water.

One pair of oligonucleotide primers specific for 1063 bp (Pre-S through the S gene) region of the hepatitis B virus with the sequences from 5′to 3′

P1 Universal sense 5′ - TCACCATATTCTTGGGAACAAGA - 3′

S1-2 Universal anti sense 5′ - CGAACCACTGAACAAATGGC - 3′ was used (Eman *et al.,* 2018).

For the positive control, 2 µl of known HBsAg positive DNA sample was used

PCR Condition

The thermocycler was set to the following parameters. Pre-Denaturation temperature of 95˚C for 5 minutes, Denaturation temperature of 94˚C for 20 seconds, Annealing temperature of 55˚C for 20 seconds, Extension temperature of 72˚C for 1 minute and Final extension temperature of 72˚C for 5 minutes.

Agarose gel Electrophoresis

PCR product was separated by gel electrophoresis (1.5% agarose, 1X Tris Acetate EDTA (TAE) buffer, and 0.5µg/ml ethidium bromide). PCR products were loaded in the gel as follows: 8µl of PCR product, 3µl of 6X sample loading buffer and 1000bp ladder. Gel was run at voltage 120V for 1 hour in 1X TAE buffer, bands were visualized under a UV trans illuminator and photographed.

Dye terminator cycle sequencing with quick start kit.

The sequencing was carried out in DNA Labs. Kaduna using the instrument AB1310 Prism. Sequencing reaction was prepared in a 2.0ml tube. All reagents were kept on ice while preparing the sequencing reactions and added in the order listed below:

dH_2O 5µl, DNA template 5 µl, Primers 2.0 µl, DTCS Quick start master mix 8.0µl. The sequencing reaction was set up in the PCR machine as below:

Thermal cycling program: 96˚C for 20sec to denature the DNA, 50˚C for 20sec to anneal the DNA with the dNTP, 60˚C for 4min to elongate the DNA strands, This was set for 30 cycles followed by holding at 4˚C

Bioinformatic Analysis

The ensued sequence in the fasta format form was compared with data in the GenBank database using BLASTn (Altschul *et al.,* 1990; Zhang *et al.,* 2000) and the best match recorded

Statistical Analysis

Data obtained from the laboratory procedures and the questionnaires was analyzed using simple descriptive statistics involving frequency, percentages and chi square methods.

RESULTS

Table 1 shows numbers of blood screened by Lab-ACON test kit from different blood banks, comprising of 150 samples from Yusuf Dansoho Memorial Hospital (YDMH), 30 samples from Gwamna Awan General Hospital (GAGH), 20 samples from National Ear Care (NECC). All 200 sera still remained negative for HBsAg.

Table 1: HBsAg screening with Lab ACON rapid test strip method

	YDMH	GAGH	NECC	TOTAL	PERCENTAGE
Number screened	150	30	20	200	100
Number positive	00	00	00	00	00
Number negative	150	30	20	200	100

The PCR result of the various samples already tested by the rapid test method from different blood banks is presented in Table 2. Out of the 150 samples from YDMH, 01 was Positive and 149 remained Negative. The 30 samples tested from GAGH shows 00 Positive and 30 Negative. 20 samples tested from NECC also showed 00 Positive and 20 Negative. Total number of Positive sample was 01 while 199 was Negative. Percentage positive was 0.5% and 99.5% was negative.

Table 2: The PCR result of the various samples

PCR assays for HBV DNA of the 200 HBsAg Negative Specimens Previously Screened with Rapid Test Method.

Blood Bank	YDMH	GAGH	NECC	TOTAL	PERCENTAGE
Number tested	150	30	20	200	
Number positive	01	00	00	01	0.5%
Number negative	149	30	20	199	99.5%

PCR Results

PCR results of the gel electrophoresis are presented in Plate 1 and 2. Plate 1 indicates a Negative result while Plate 2 indicates a Positive result.

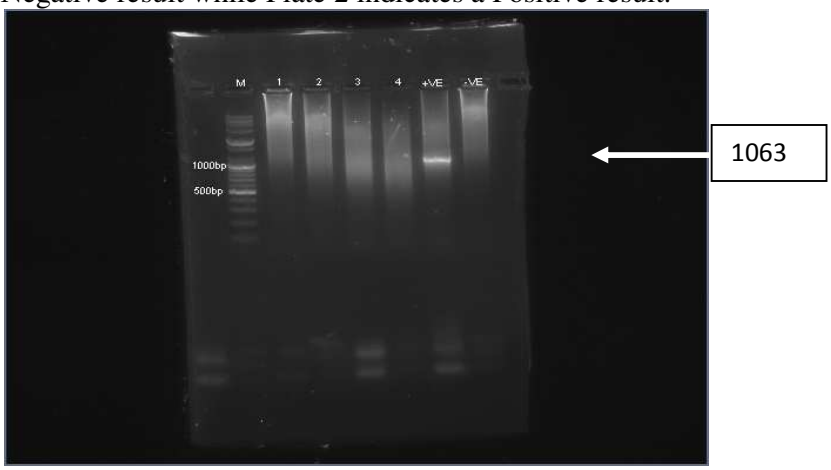

Plate 1: Gel electrophoresis showing some Negative runs, M - (1000) base molecular weight marker, lane 1-4 shows Negative PCR samples, lane 5 Positive control(1063bp), lane 6 Negative control

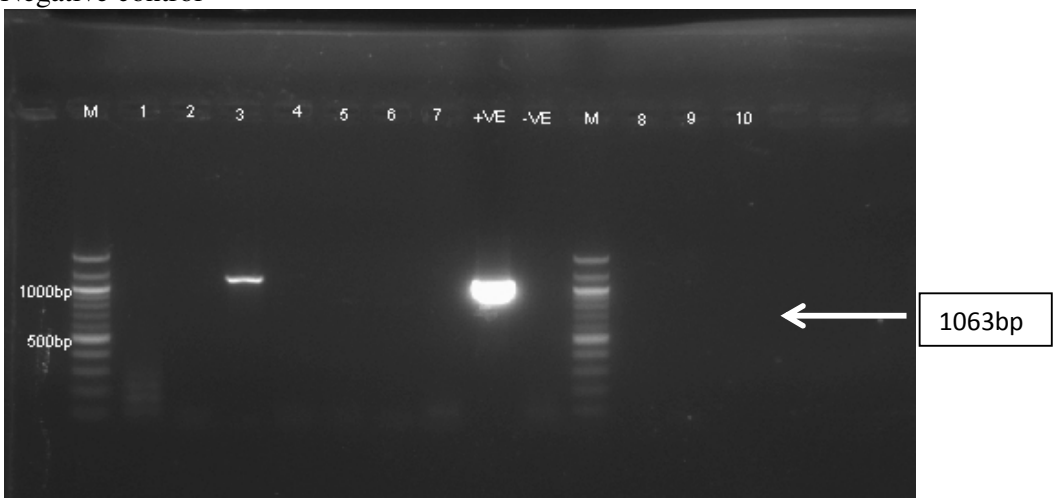

Plate 2: Gel electrophoresis of the HBsAg Positive amplicon. M - (1000 base) Molecular weight marker, lane 1, 2, 4, 5, 6, 7 are Negative, lane 3 shows a Positive Sample (1063bp), lane 8 Positive Control (1063 bp), lane 9 Negative Control, lane 10 - Marker (1000 bp), lane 11-13 shows Negative samples

The string of letters below represents sequence for the Hepatitis B Virus obtained after sequencing of the purified PCR product in fasta format, while Table 3 shows the result of the BLAST search.

TGTAACAGCGGTTTTTTCTTGGTTGACRAKAATCCTCACAATACCACGAACTATACA
TGTGGGGGGTTGGTTTAATATTCCAAGGGAAATTGCGTGGGGATGGGGAAATTCGG
AACCGGATCCCTGCCTCAGAGTTGGGCACTCATCTCGTCGTCTGTGAGGGTTGGGAC
CTGCACGAAATGGAAACTTATCGATCTAGACCTGCTCTGGTCGGGGGGTTTTTTTTG
TTGAAAAATATTCATAGAAATATATCSGGGGG

Table 3. Blast result of the detected sequence (Product of PCR) showing significant Alignment.

Description	Max.Score	Quary Cover	E-value	Identity	Accenssion number
Hepatitis B virus Isolate V2 Complete genome	57.2%	14%	2e-04	92%	KM392071.1
Hepatitis B virus Isolate CHB-127-SS Protein(S) gene Partial cds.	55.4	14%	9e-04	92%	KY428757.1
Hepatitis B virus Isolate B070 2010 C polymerase(P) gene Partial cds	55.4	18%	9e-04	88%	KX372128.1
Hepatitis B virus Isolate 5093.complete Genome	55.4	18%	9e-04	88%	KU695745.1
Hepatitis B virus Isolate 5081.complete genome	55.4	18%	9e-04	88%	KU695745.1

Table 4. Blood transfusion facilities responses to questionnaire

BLOOD BANK FACILITY METHOD	TEST	YDMH	GAGH	NECC
Diagnostic system used for HBV test by the facilities	RAPID TEST	YES	YES	YES
	ELISA	NO	NO	NO
	PCR	NO	NO	NO
Method used for HBV Detection during Donor selection	RAPID TEST	YES	YES	YES
	ELISA	NO	NO	NO
	PCR	NO	NO	NO
Method preferred by responder (facility)	RAPID TEST	NO	NO	NO
	ELISA	YES	YES	NO
	PCR	NO	NO	YES

The rapid test method was the method used for HBV detection during blood donor's selection and in diagnosis. YDMH and GAGH preferred use of Elisa method while NECC preferred PCR method as presented in table 4.

KEY:
YDMH = Yusuf Dansoho Memorial Hospital, GAGH = Gwamna Awan General Hospital and NECC = National Ear Care Center

DISCUSSION

The rapid test method shows that the entire 200 samples tested remained Negative using lab ACON rapid test strip. This is similar to the work of Oluyinka *et al.,* (2015) where all the 429 serum samples of blood donors investigated still remained Negative for HBsAg when re-tested by a second Elisa method in another laboratory. However, in a similar study carried out at Obafemi Awolowo University Teaching Hospital (OAUTHC) and the Seventh Day Adventist Hospital (SDAH) in Ile-Ife by Amadin *et al*., (2016), the 507 blood donors tested at both OAUTHC and SDAH blood banks for HBsAg using immunochromatographic rapid test kit and found negative were tested for HBsAg using Elisa and five of them were found to be HBsAg positive.

This study found an OBI prevalence of 0.5% among 200 blood samples in blood bank in Kaduna metropolis that had previously been screened with rapid test kit and declared HBsAg negative.

This finding support the work of Fong *et al.,* (1993) who stated that HBsAg is often present at very low level and may go undetected, the concomitant presence of hepatitis B surface antibodies which form immune complex with HBsAg can also be a problem with the antibody detection method (Hsu *et al.,* 2004). Issue of serological window period, different genotypes and mutation are also factors of concern (Chu and Loc, 2002; Weber, 2005).

Although published data on OBI in blood donors in Nigeria is sparse, Nna *et al.* (2014) found a prevalence of 8% in Abakaliki, South-Eastern Nigeria among 100 blood donors, Opaleye *et al*. (2014) found a very high prevalence of 36% among 429 donors while Oluyinka *et al.* (2015) found a prevalence of 17 % in South Western Nigeria among 429 donors. No data capture has been reported in North- Western part of Nigeria. These prevalence values are all higher than that found in this study which is also significant at $P > 0.01$ using Chi square. Meanwhile there may be difference in prevalence of OBI among blood donors from one part of the country to another reflecting differences in the prevalence of overt HBV infection which exist from one part of the country to the other (Jombo *et al*., 2005; Forbi *et al.,* 2010). The prevalence in this study is lower than the 1.7 % found in Ghana by Zahn *et al.* (2008) and the prevalence of 3.3% found in Brazil, (Silver *et al.,* 2005). The prevalence found in this study is in agreement with what has been found in the USA and some other Western countries where only 0.1%-2.4% of HBsAg Negative blood donors were found to have HBV-DNA (Hollinger and Sood, 2010; Larrubia, 2011). In North Africa, a study conducted among 1026 Egyptian blood donors sample revealed 0.5% were Positive for HBV-DNA (Antar *et al.,* 2010) which is in agreement with our study. Blast of the HBV-DNA Sequence shows that our amplicom was Hepatitis B virus complete genome V2 with 92% identity and Ascension number KM392071.1

CONCLUSION

The rapid test strip method is the method employed for testing blood donor before donation; however when the samples were tested by the PCR method it gave a 0.5% positive which is not significant at $P < 0.01$ using Chi square but the PCR method is more sensitive than the rapid test strip method. This shows that residual risk of HBV transmission from blood donors in Kaduna metropolis can be minimized by employing NAT (PCR) assay therefore the introduction of HBV-DNA assay will be a useful tool in the quest to approach near to zero risk of HBV transmission.

RECOMMENDATIONS

1. We propose that blood donors sample in Kaduna metropolis be tested for OBI status by NAT prior to transfusion so as to minimize the risk of acquiring HBV
2. There is need to intensify public awareness campaigns for HBV infection through mass media, just as it is done for the HIV and Polio virus campaign in recent times.

REFERENCES

Altschul S, Gish W, Miller W, Myers E and Lipman D (1990), Basic local alignment search tool. *Journal of Molecular Biology* 215 (3), 403-410.

Amadin A, Adesola O, Lateef S, Rosemary A, Azuka P, and Aaron O. (2016). Occult hepatitis B virus infection in previously screened, blood donors in Ile-Ife Nigeria: implications for blood transfusion and stem cell transplantation. *Virology journal* 13:76

Antar W, El Shokry MH, Abd El Hamid WA, and Helmy MF (2010) Significance of detecting anti-HBc among Egyptian male blood donors negative for HBsAg*. *Transfusion Medicine 20*: 409–413.

Brechot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, and Paterlini-Brechot P. (2001). Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: Clinically significant or purely "occult"? *Hepatology 34: 194–203.*

Charan J and Biswas T. (2013). *Indian Journal of Psychological Medicine*. April; 35 (2):121-6.

Chu C. J., Lok A. S. (2002). Clinical utility in quantifying serum HBV DNA Level using PCR assays. *Journal of Hepatology. 36:pp549-551.*

Denwe, (2014). Comparative Diagnosis of enteric fever, characterization and antibiogram of Salmonella isolated from some patients of selected Hospitals in Kaduna metropolis.

EmanYasin Salih; Walaa Saeed Eltahir Saeed; Maria Mohamed HamadSatti; Gawahir Abbas; Isam Adam Suleiman; Eltahir Awad Gasim Khalil (2018)* Hepatitis B virus genotypic patterns in Sudan reflects population movements. *Global Journal of Virology and immunology vol.3 (1) pp 176-180*

Farber E, Philips F T, and Kaufman (1987). Pathogenesis of liver disease .Los Angeles: Williams andWalkins W A (eds.)

Friedman, Lawrence S. (2015). *"Chapter 16: Liver, Biliary Tract, & Pancreas Disorders". In Papadakis, M; McPhee, SJ; Rabow, MW.Current Medical Diagnosis & Treatment 2016 55e.McGraw Hill.* ISBN 0071845097.

Fong T. L. (1993). Persistence of hepatitis B virus DNA in the liver after loss of HBsAg in chronic hepatitis B.*Hepatology. 8:1313–8.*

Forbi J.C, Vaughan G, Purdy M.A, Campo D.S, and Xia G.l, (2010). Epidemic history and evolutionary dynamics of hepatitis B virus infection in two remote communities in rural Nigeria. *PLoS One. 5(7), e11615. doi:*

Hollinger F.B, Sood G. (2010). Occult hepatitis B virus infection: a covert operation. *Journal of Viral Hepatology. 17:1–15*

Hsu H.Y. (2004). Survey of hepatitis B surface variant infection in children 15 years after a nationwide vaccination programme in Taiwan. *Gut. 53: 1499–1503.*

Jombo G.T, Egah D.Z, and Banwat E.B. (2005). Hepatitis B virus infection in a rural settlement of Northern Nigeria. *Niger Journal of Medicine. 14(4):425–8.*

Kao J.H, Chen D.S. (2002). Global control of hepatitis B virus infection. *Lancet Infectious. Diseases. 2:395–403.*

Larrubia JR. (2011). Occult hepatitis B virus infection: A complex entity with relevant clinical implications. *World Journal of Gastroenterology*. 17(12):1529–30.

Lavanchy D. (2004). Hepatitis B, virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *Journal of Viral Hepatology. 11:97–107.*

Lok A.S, McMahon B.J. (2001). Chronic hepatitis B.*Hepatology. 34(6):1225–41.*

Maddrey W.C. (2000). Hepatitis B: An Important Public Health Issue. *Journal of Medical Virology. 61:362–6.*

Mahoney francis J and Mark Kane (1999). 'Hepatitis B Vaccine' in vaccine, 3rd ed. Stanley A. Plotnick and walter A Orenstein, eds .W B Saunders company; Philadelphia chap.10, pp 159-82.

Nna E, Mbamalu C and Ekejindu I. {2014) Pathogens and Global Health. 108(5):223-8. Okonkwo U ,Ngim O E, Osim H, Inyama M A, Esu M K, Ndoma-Egba R, and Ezedinachi E. (2017).Knowledge of hepatitis B virus infection among traders in.*Niger Journal Clinical Practice 20:415-20*

Ola S.O, Otegbayo J.A, Odaibo G.N, Olaleye D.O, Olubuyide I.O, and Summerton C.B. (2009). Occult HBV infection among a cohort of Nigerian adults. *Journal of Infection in Developing Countries 3: 442–446.*

Oluyinka OO, Tong HV, Bui Tien S, Fagbami AH, Adekanle O, Ojurongbe O (2015). Occult Hepatitis B Virus Infection in Nigerian Blood Donors and Hepatitis B Virus Transmission Risks.*PLoS ONE 10(7): e0131912. https://doi.org/10.1371/journal.pone.0131912*

Opaleye OO, Fagbami AH, Bolaji OS.(2014) Occult hepatitis B virus infection in fully screened blood units in Nigeria. *International Journal of InfectiousDieases (16th ICID Abstracts). 21(Supplement 1):455.*

Panhotra B.R, Bahrani A, Joshi C.S, and Hassan Z.U. (2005). Occult hepatitis B virus infection among anti-HBc positive blood donors: Necessitates substitution of screening by HBV NAT. *Journal of Infection 51: 263.*

Prati D, Gerosa A, Porretti L. (2006). Occult HBV infection and blood transfusion. *Journal Of Hepatology 44: 818.*

Raimondo G, Allain J.P, Brunetto M.R, Buendia M.A, Chen D.S, and Colombo M. (2008). Statements from the Taormina expert meeting on occult hepatitis B virus infection.*Journal of Hepatology. 49: 652–657.*

Salawu L, Murainah H.A. (2006). Pre-donation screening of intending blood donors for antibodies to infectious agents in a Nigerian tertiary health institution: a pilotstudy.*African Journal of Medicine and Medical.Science. 35(4):453–6.*

Salawu L, Bolarinwa R.A, Adegunloye A.B, and Muraina H.A. (2010). HBsAg, anti-HCV, anti-HIV and VDRL in blood donors: prevalence and trends in the last three and a half years in a tertiary health care facility in Ile-Ife, Nigeria. *International Journal of Medicine and Medical Science. 2(11):335–41.*

Silva CMD, Costi C, Costa C, Michelon C, Oravec R, and Ramos AB, *et al.* (2005). Low rate of occult hepatitis B virus infection among anti-HBc positive blood donors living in a low prevalence region in *Brazil Journal of Infection. 51:24–9.*

Sorrell M.F, Belongia E.A, Costa J, Gareen I.F, Grem J.L, and Inadomi J.M, (2009). National Institutes of Health Consensus Development Conference Statement: Managementof hepatitis B. *Annals of International Medicine. 150:104–10.*

Strauss James and Ellen (2002). Viruses and Human diseases.Academic press, San Francisco.The franklin institute inc. *Blood-the human heart, Retrieved 19 march, 2009.*

Torbenson M and Thomas D.L., (2002). "Occult hepatitis B," *Lancet Infectious Diseases*, 2(8):479–486.

Zahn A, Li C, Danso K, Candotti D, Owusu-Ofori S, Temple J, and Allain JP. (2008) Molecular characterization of occult hepatitis B virus in genotype E-infected subjects. *Journal of General Virology*.89:409–18.

Zhang Z, Schwartz S, Wagner L, and Miller W, (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* 7 (1-2) 203-214