Seroprevalence and molecular identification of Hepatitis B Virus (HBV) serotypes among HIV patients attending a selected public hospital in Kano, Nigeria

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Abstract

Hepatitis B Virus (HBV) is a problem which causes liver disease worldwide and accounts for over 360 million cases of chronic hepatitis and more than one million deaths per year. HBV is a public health concern, especially among HIV-positive patients for its contribution to increasing the progression of Acquired Immunodeficiency Syndrome (AIDS).

This cross-sectional study aimed to determine the seroprevalence of HBV infection and identify the HBV serotypes among HIV-positive individuals in a tertiary health care centre in Nigeria.

Blood samples of One hundred seventy-eight (178) HIV-positive patients were screened for Hepatitis B Surface Antigen using Enzyme-Linked Immunosorbent Assay (ELISA). Socio-demographic data and risk factors data for HBV infection were collected from the study population using structured questionnaires. Multiplex Polymerase Chain Reaction (PCR) was used for molecular identification of HBV using primers for all four major serotypes under study (ayw), (adw), (adr), and (ayr). The data was analyzed using the SPSS 20.0 version.

Findings showed that out of the (178) samples only Eleven (11) samples were positive for Hepatitis B Surface Antigen with a prevalence of 6.2%. Out of the 11 positive samples, 4 samples were positive for *ayw* serotype and none for other serotypes using Multiplex PCR with a prevalence of just 2% and the

remaining samples were negative for adw,adr and ayr after completing the gene identification test signifying the presence of other minor serotypes in the population. There was no presence of false positives All 11 positive samples come from the female population. Ages (31-40) have the highest positive Hep B of up to 60 %, of the positives (11). similarly, those with non-formal education have a higher prevalence. All the risk factors considered such as marital status, self-manicure, tattoo, smoking, alcohol intake, blood transfusion, sharing of cloth, intravenous drug use, sharing of utensils and tribal marks in the study were found not to be associated with the HBV infection.

The study established a prevalence of 6.2% for HBV in the study area and also identified ayw as the most prevalent serotype of HBV detected. Due to the endemic nature of HBV infection among HIV patients that was found in this study, there is a need for preventive measures such as awareness and vaccination of HIV patients with the HBV vaccine to avoid the HIV/HBV co-infection burden. Serotypes adw, adr, and ayr

Keywords: Hepatitis B Virus, Polymerase Chain Reaction, ELISA, HIV/AIDS

Introduction

The hepatitis B Virus is a potential life-threatening liver infection caused by the hepatitis B virus and accounts for over 360 million cases of chronic hepatitis and more than one million deaths per year (WHO, 2016). Hepatitis B virus (HBV) infection affects 7.0%-10.0% of HIV infected patients, and is associated with an increased frequency of AIDS-related and non-AIDS-related clinical endpoints, such as end-stage liver diseases including cirrhosis and hepatocellular carcinoma (Dayyab*et al*., 2023). HBV co-infection among HIV-positive persons is common; in some e4settings more than two-thirds of HIV infected persons have markers of past exposure to HBV (Fransisci *et al.,* 1995). Other viral infections such as Covid-19 were found to cause more severe symptoms when associated with HBV co-infection (Dayyab *et al*., 2023).

Globally, approximately 35.3 million people are living with HIV worldwide and 4 million HIV positive individuals are co-infected with Hepatitis B Virus (HBV) worldwide as a chronic infection (Soriano *et al*., 2006). The burden of co-infection is greatest in Southeast Asia and Sub-Saharan Africa (WHO, 2015). HBV/HIV Co-infection in Nigeria has been reported to have a prevalence ranging between 10.0%-70.0% (Owolabi *et al*., 2014).

HIV/HBV co-infected patients have higher levels of HBV replication, lower rates of spontaneous resolution of the HBV infection, and higher risk of reactivation of previous infections(Dayyab *et al*., 2023).).

Identification of HBV co-infection in HIV positive individuals is important because of its major public health concern relating to its potential role in increasing the rates of progression to acquired immune deficiency syndrome. The knowledge of the prevalence of HBV infection among HIV positive patients is a necessary tool for the establishment of control measures as knowledge of the genes can help with prospects in gene therapy among others in the area of genetic engineering. However, without these small strides, the African region will not benefit from the advancement of the Western world which is far more advanced in molecular technology. This we hope will go a long way in helping to reduce the morbidity and mortality caused by the virus. Sub-typing of Hepatitis B Virus (HBV) strains is important as it will provide information about circulating serotypes prevailing in different parts of Kano State. As a result, we chose from the four major serotypes such as ayw, adw, adr, and ayr worldwide (Sumi *et al*., 2003) this is because no research was found to be done in our environment. It can also assist in tracing the route of infection. Adw and adr are more common in Asia, ayw is more prevalent in Europe, the Mediterranean region and Africa (here). Ayr is less common globally. Therefore, this cross-sectional study determines the seroprevalence of HBV infection and also identifies the HBV serotypes among HIV-positive individuals in a tertiary health care centre in Nigeria will be able to bridge the gap and provide information in our visinity.

Material and methods

Study Design and Population:

This study was a hospital-based cross-sectional study. The study population was HIV patients receiving antiretroviral therapy at a Selected Public Hospital, Kano from which Hep B test was carried out to screen those that have co-infection with Hep B infection. Furthermore, gene test was done to determine the most prevalent serotype in the (True Positive) population. All false positives will not be included.

Inclusion and Exclusion Criteria:

All Male and Female HIV-patients of all ages who consented were included in the study and non-HIV individuals as well as the HIV-patients who declined to participate were excluded from this study.

Sample Size Determination:

The sample size used in the present study was determined using $n = \frac{Z^2pq}{L^2}$ (Cochran, 1977). Where n=number of samples, Z = standard normal deviate at 95% confident interval = 1.96, P = previous prevalence = 12.5% (A previous study conducted in Kano by Hamza *et al*. (2013); reported 12.5% as the prevalence rate). p=0.125, q=1-p=0.875, L= allowable error of 5%=0.05, $n = \frac{1.96^2 \text{ x}0.125\text{x}0.875}{0.0025} = 168$.

The sample size was increased to 178 to allow for more precision.

Sample collection and Processing:

Five (5ml) of blood was collected from each participant by venipuncture using sterile needles and syringes into EDTA container. The blood samples were centrifuged at 4000g for 5minutes to obtain the serum. The serum was transferred into cry - pure container using pipette and was stored at -20^{0}C until use.

Detection of anti-HBV using Enzymes Link Immunosorbent Assay Test:

The blood samples were subjected to Hepatitis B surface Antigen using ELISA kit to detect anti-HBV. The procedure was carried out according to the manufacturer's instructions (Biolabs Inc., 2015). The HBsAg Rapid Test Strip is a qualitative, solid phase, two-site sandwich immunoassay for the detection of HBsAg in serum or plasma. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the strip. During testing the plasma specimen reacts with the particle coated with anti-HBsAg antibodies and the membrane and generates a bands. The presence of these bands in the test region indicates a positive result, while its absence indicates a negative result (answer)

PCR Detection of Hepatitis B Virus Serotypes

Hepatitis B Virus DNA Extraction:

DNA extraction was done using a conventional method (Sambrook *et al*., 2001). Fifty microlitres (50 µ L) of samples was diluted with (350µ L) of TE buffer and incubated at 56^{o}C for 2 hours in the presence of proteinase K (1mg/ml) and SDS (0.66%). The solution was brought to room temperature, followed by protein precipitation by addition of NH_4OAc (Fc, 3.4M). The mixture was vortexed and centrifuged at 13,000rpm for 15minutes to extract the DNA from the proteins. The DNA was precipitated and stored until further use.

Oligonucleotide Primers

The Primer pairs for the major serotypes world-wide were used for this research work which are Serotypes ayw, adw, adr, and ayr it

was adopted from the work of Duanthanorm *et al*., (1994)

Serotypes	Primers	Size	PCR cycle
adw	F CAACCTCTTGTCCTCCAAT		
	R CAGGAATCGTGCAGGTCTT	119bp	45
ayw	F CTAGGGGGAGCACCCAC		
	R AAAAGAAAATTGGTAAGAGA	198bp	45
adr	F CAACCTCTTGTCCTCCAAC		
	R CAGGAATCGTGCAGGTTCT	128bp	45
ayr	F CTAGGGGGAACACCCGT		
	R AAAAGAAAATTGGTAAGAGG	538bp	45

Cycling Procedure (Okamato *et al*., 1988)

Program No	**Cycling Procedure**
1	45 cycles (95^0C, 15 minutes; 94^0C, 1 minute; 62^0C, 1 minute, 72^0C, 1 minute)
2	45 cycles (95^0C, 15 minutes; 94^0C, 1 minute; 62^0C, 1minute, 72^0C, 1 minute)
3	45 cycles (95^0C, 15 minutes; 94^0C, 1 minute; 47^oC, 1minute; 66^oC 1 minute)
4	45 cycles (95^0C, 15 minutes; 94^0C,1 minute; 47^0C, 1 minute; 66^0C,1minute)

PCR Amplification of the target gene

In a reaction mixture containing, 5µl of DNA template, 12.5µL of master mix, 5.5µL of (MH_20), 0.5µL and 0.5µL of reverse and forward primer for (ayw, adw, adr, and ayr) and 0.5µL and 0.5µL reverse and forward primer for (adw).Twenty (20µL) of the reaction mixture was transferred into PCR

tube, followed by centrifugation at 5000g for 30seconds and the tube was covered. The reaction was performed in a programmable DNA thermo cycler for 45cycles. One amplification cycle consists of initial denaturation for 15minutes at 95^{o}C, 45 cycles of denaturation at 94^{o}C for 1minute, annealing for 1minute at 62^{o}C and extension/elongation at 72^{o}C for 1minute.While annealing with the primers P3 for (adr) and primers P4 for (ayr) was performed at 47^{o}C and extension/elongation was made at 66^{o}C.

Agarose gel electrophoresis procedure:

One and a half (1.5gram) of Agarose powder was weighed using sensitive weighing balance into a beaker, one hundred and fifty milliliter (150ml) of Tris Borate Ether buffer was measured and poured into a beaker containing the Agarose powder. The mixture was heated using microwave for 30 seconds and (10µL) of ethidium bromide was added and swirled. The gel casting trays were assembled with the combs in place and the gel was poured into the casting tray and then allowed to solidify. The combs were carefully removed from the gel trays and the tank filled to 500ml gauge mark with the 1X TBE running buffers. The first well was loaded with (4µL) of the DNA Ladder, positive control was also added into the second well and the remaining wells were loaded with 2µL 6X loading dye mixed with 5uL of the amplicons. The voltage was set at 80V and allowed to run for 40minutes. The gel tray was removed and transferred into a gel Documentation which is connected to the computer according to manufacturer's instructions of the gel doc).

Statistical Analysis:

The data obtained was analyzed using the statistical package for social science statistical software (SPSS) version 20.0. A Chi-square and odd ratio test was performed to determine the association between variables(hepatitis B and Hiv CO infection). A $\leq$0.05 P-value significance level was considered.

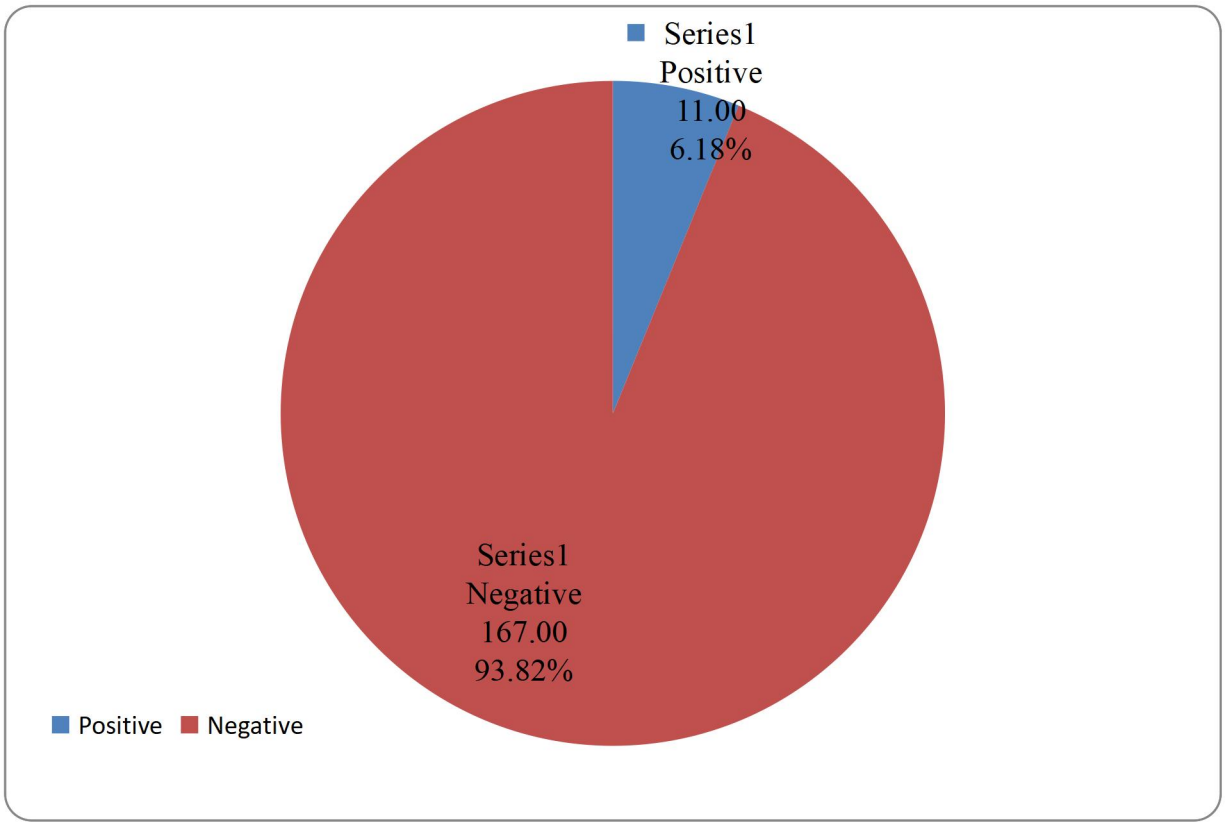
Ethical consideration and consent

Ethical approval was obtained from the Ministry of Health Kano State, Nigeria, while the patients' consent was obtained before enrollment in the study.

Ethical clearance No; MOH/off/797/T.I/694

Results

Out of the 178 HIV patients recruited in the present study, eleven (11) were positive for HBV using HBsAg with overall seroprevalence of 6.2% (Figure1)



Positive = 11, Negative = 167

Figure 1: Prevalence of HBsAg among HIV Patients attending a Selected Public Hospital, Kano State, Nigeria

Seroprevalence of HBsAg among HIV patients attending a Selected Public Hospital, Kano State, Nigeria with Respect to Socio-demographic data.

Table 1 shows the prevalence of HBV in relation to marital status, the married patients had a higher prevalence of 6.52%as compared with the single/divorced (5.0%), though the difference was not statistically significant, this could be because single ladies are less likely to engage in sexual intercourse ($P = 0.75$). Based on gender, the result showed that all the patients who were positive for HBsAg were females with a prevalence of 8.15% probably because of the more women we have in the society at present. furthermore, age grouping shows that ages 31-40 years had the highest prevalence of 37% this also shows the age bracket for ecomomic stability as well as maturity although the difference in prevalence based on age group was however not statistically significant ($P = 0.36$).This study observed highest prevalence of HBV among participants with Non-formal education

(15.15%) while those with secondary education had the least prevalence (4.17%). This shows the importance of education in prevention of diseases. The difference was however not statistically significant ($\chi^2 = 7.54$,P= 0.06).

The distribution of HBV among the participants based on occupation showed that the unemployed had a prevalence of 10.00%, patients while those engaged in business had a prevalence of 8.54% and students had 4.08% prevalence. However the difference was not statistically significant.

Table1: Seroprevalence of HBsAg among HIV patients attending a Selected Public Hospital Kano State, Nigeria with Respect to Socio - demographic data.

Variable	Number Examined	Positive (%)	Chisquare	P value	OR	CI
Marital status						
Married	138	9(6.52)	0.12	0.75	1.32	0.27-6.39
Single/Divorced	40	2(5.0)				
Total	178	11(6.18)				
Gender						
Female	135	11(8.15)	3.74	0.05		
Male	43	0 (0.0)				
Total	178	11 (6.18)				
Age (Years)						
0-10	9	2(22.22)	8.75	0.12	1.04	0.27 - 4.11
11-20	9	0(0.0)				
21-30	50	3(6.00)				
31-40	64	6(9.38)				

41-50	25	0(0.0)				
>50	21	0(0.0)				
Total	178	11 (6.18)				
Level of Education						
Non-formal	33	5 (15.15)	7.54	0.06	1.71	0.36 - 8.22
Primary	58	4(6.90)				
Tertiary	39	0(0.0)				
Secondary	48	2(4.17)				
Total	178	11 (6.18)				
Occupation						
Civil servant	27	0(0.0)	3.44	0.33	1.76	0.37 - 8.46
Business	82	7(8.54)				
Student	49	2(4.08)				
Unemployed	20	2(10.00)				
Total	178	11 (6.18)				

OR, odd ratio, Cl: Confidence Interval.

Seroprevalence of HBsAg among the study paticipants concerning Risk factors is presented in Table 2. From the Table above those patients that practised self-manicure had a higher prevalence of hep B infection up to 6.75%, compared to those who didn't with (0.0%) signifying inappropriate usage as a cause. About tattooing, a higher prevalence of 8.33% was recorded among those who had tattoos and 6.02% among those without tattoo. However, the association is not statistically significant ($\mathrm{OR} = 1.42, \mathrm{CI} = 0.17 - 12.11$). Participants that were non smokers had a prevalence of 6.36%, while smokers had a 0.0%.Hepatitis B infection was not found to be statistically associated with smoking

(P = 0.56). Based on Alcohol intake, those who did not consume alcohol had a higher prevalence of 6.22% while those who consumed had 0.0%. there was however no association between alcohol intake and HBV infection (P= 0.79).

Those patients that had not received blood transfusion had a higher prevalence of 7.29%, while those with history of blood transfusion had a prevalence of (0.0%) signifying improvement in screening services in the region , though the difference was not statistically significant (P = 0.15). As shown in Table2, those participants who shared clothes had a prevalence of 13.04% while those that did not share had a prevalence of 5.16% indicating sharing of cloths as a risky behavior. The difference was statistically not significant (P= 0.14).

As shown in Table2, ironically non-intravenous drug users had the prevalence of 6.25% while those that involved in intravenous drug had prevalence of (0.0%), although the difference was not statistically significant (P= 0.72).

Those participants who shared utensils had a prevalence of 9.26%, while those that did not share had a prevalence of 4.84% showing possible implication of saliva as a cause of hep B infection. The association was not significant between sharing of utensils and the HBV infection.

Results presented in Table 2, showed that, participants who had tribal marks had a prevalence of 6.25% while those that did not have tribal marks had a prevalence of 6.14% there was no association between having tribal mark and the HBV infection (= 0.26)

Table 2 Seroprevalence of HBsAg among HIV patients Attending a Selected Public Hospital, Kano State, Nigeria with Respect to Risk factors

Variable	Number Examined	Positive (%)	Chisqu are	P value	OR	CI
Self-manicure						
yes	163	11(6.75)	1.08	0.29		
No	15	0(0.0)				

Total	178	11 (6.18)				
Tattoo						
Yes	12	1(8.33)	0.10	0.75	1.42	0.17 - 12.11
No	166	10(6.02)				
Total	178	11 (6.18)				
Smoking						
Yes	5	0(0.0)	0.34	0.56		
No	173	11(6.36)				
Total	178	11 (6.18)				
Alcohol intake						
Yes	1	0(0.0)	0.07	0.79		
No	177	11(6.22)				
Total	178	11 (6.18)				
Blood transfusion						
Yes	27	0(0.0)	2.09	0.15		
No	151	11(7.29)				
Total	178	11(6.18)				
Sharing of cloths						
Yes	23	3(13.04)	2.15	0.14	2.76	0.68 – 11.25
No	155	8(5.16)				
Total	178	11 (6.18)				

Intravenous drug use						
Yes	2	0(0.0)	0.13	0.72		
No	176	11(6.25)				
Total	178	11(6.18)				
Sharing utensil						
Yes	54	5(9.26)	1.27	0.26	2.01	0.59 - 6.88
No	124	6(4.84)				
Total	178	11(6.18)				
Tribal marks						
Yes	64	4(6.25)	0.0008	0.98	1.02	0.29 – 3.62
No	114	7(6.14)				
Total	178	11(6.18)				

Key:OR, odd ratio, Cl: Confidence Interval

Agarose-gel profiles of the Polymerase Chain Reaction (PCR) products for primers p1 - p2

Lane M is the 100bp ladder with standard molecular weights. Lane PC is the positive control. Lane 2, 5, 7 and 9 are positive for ayw serotype of HBV with the amplicons size of 198bp. Lane NC (nuclease free water) is negative control.

Agarose-gel profiles of the Polymerase Chain Reaction (PCR) products for primers p3 - p4

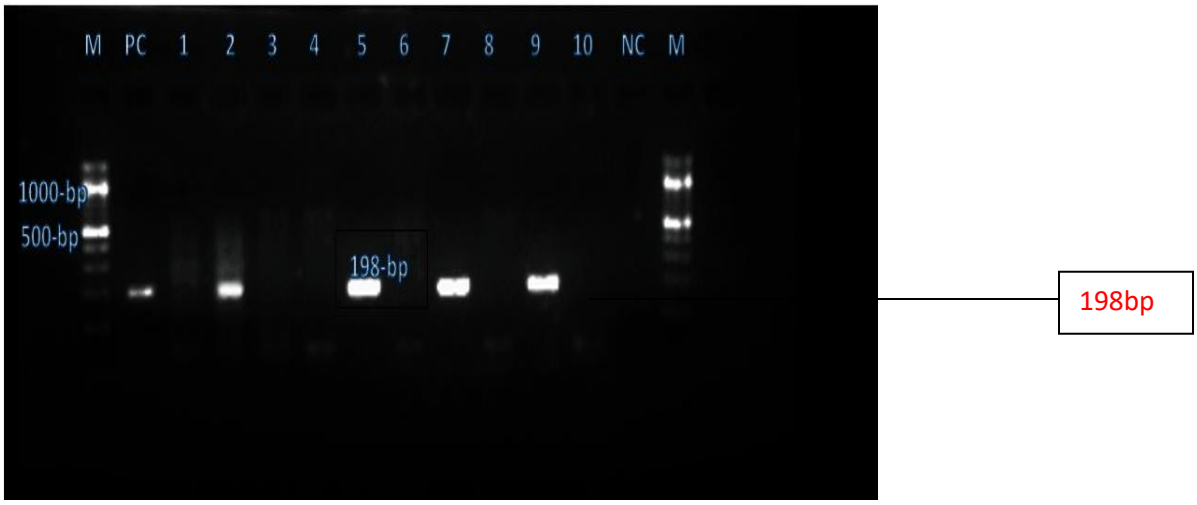
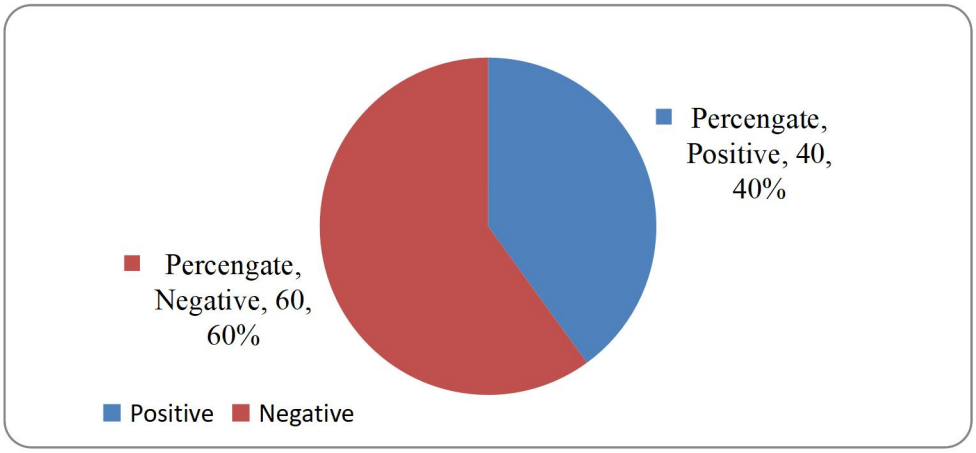


Plate 1: Agarose-gel Image of the PCR for ayw serotype using primers 1 for (adw) and primers 2for (ayw) for Samples 1-10, M is a 100bp ladder, PC is the positive control, NC is the negative control and Lanes 1-10 are the samples with Lanes 2, 5, 7 and 9 showing amplicons.

Prevalence of Hepatitis B Virus Serotype (ayw)

Only four (4) out of the ten (10) HBsAg positive samples presented for PCR were ···/e for the ayw serotype, with a prevalence of 40% as shown in Figure: 3.



Positive = 40, Negative =60%, Total = 10

Figure 3: Prevalence of HBV Serotype ayw among HIV Patients Infected with Hepatitis B Virus

Discussion

The prevalence of HBV Co-infection with HIV was 6.20% in this study. This prevalence is low compared to previous reports (Olatunji and Iseniyi, 2008; Hamza *et al*., 2013) in Nigeria. The variation in prevalence could be as a result of enlightenment/awareness about the transmission routes of Hepatitis B Virus.

Concerning marital status, higher prevalence of HBV/HIV co-infection was observed among the married 6.52%, this finding is in line with the previous report by Okeke *et al.* (2012), where married women recorded higher prevalence compared to other categories of women this could be because intercourse as one of the major causes of transmission is relatively low among the female singles (Dayyab *et al*., 2023). . The Centre for Disease Control (CDC) reported that at least 38% of women infected with HBV are through sexual contact with HIV-positive partners (CDC, 2002). This could be because most of the ladies with the disease have better health seeking behavior compared to the men. Again the stigma associated with the disease means that most men can be able to travel outside their location to access care (Dayyab *et al*., 2023).

Concerning gender, this study showed that the prevalence of HBV/HIV co-infection was higher in females with the prevalence of 8.15% than in males 0.0%. This finding is similar to the report by Okechukwu *et al*. (2014), who found higher prevalence in females (65.6%) than males (34.4%). Study by Olayinka *et al*. (2016) from Ibadan reported higher prevalence of 60.8% among females than males (39.2%).The reason for higher prevalence among females could be because they were more in number than the males among the study group. This present an circumstantial limitation in this study.

Another important factor in the study was the level of education. Participants with non-formal education had the highest prevalence of 15.15%. This is in line with earlier reports, for instance, a study conducted in Ethiopia showed that participants in the non-formal education category had the highest prevalence of 19.4% while 7.1% was recorded among thus with primary level of education and lowest among secondary education 6.1% (Yeshi *et al*., 2016). Another study had also noted that the seroprevalence of HBV infection decreased with the increasing level of education, this might be due to differences in the level of awareness about the transmission of HBV (Dayyab *et al*., 2023).

Highest prevalence was recorded among patients who are unemployed with the prevalence of 10.00%, this could be as a result of lifestyle and possible influence of

education and public enlightenment/awareness about the infection.

Conclusion

The study established a prevalence of 6.2% for HBV in the study area. The study identified ayw as the most prevalent serotype of HBV other serotypes were absent signifying ayw as the dominant serotype in the region. The other negative result shows the need for further research to identy the hidden serotype and their clinical value in comparison the the dominant ayw. Female participants were found to have higher prevalence (8.75%) of HBV in the study due to their better health-seeking behavior.

Recommendation

HIV positive patients should be screened for HBsAg to prevent the risk of developing chronic Hepatitis B virus infection. Patients who are HBV-infected should be informed about the transmission routes and methods to prevent further spread of the virus and HIV positive individuals should be counseled about the risk of becoming infected with HBV infection.

There is need to identify other serotypes by using promers outside the ones used for this study in order to understand the distribution of serotypes and their prevalence

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