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Molecular Detection and Seroprevalence of HBV infections among people living with HIV/AIDS attending some ART facilities in Jigawa State Nigeria

Imran Umar Ujih [1], Nura Muhammad Sani [2], Bashir Sajo Mienda [3]
Research Student, Department of Microbiology and Biotechnology, Federal University Dutse, Jigawa State, Nigeria
Professor, Department of Microbiology and Biotechnology, Federal University Dutse, Jigawa State, Nigeria
Associate Professor, Department of Microbiology and Biotechnology Federal University Dutse, Jigawa State, Nigeria

* Corresponding Author: **Imran Umar Ujih**

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Abstract
Introduction: Global estimates indicates that about 5% – 10% of people living with HIV (PLWHIV) are co-infected with Hepatitis B virus (HBV), of 36.7 million HIV patients globally the highest proportion of all PLWHIV (about 70%) are in sub-Saharan Africa; this research aims to determine the prevalence of HBV/HIV co-infection in Jigawa state Nigeria.
Method: Method was cross-sectional, consenting HIV patients aged from 0 to 80 years were recruited from 3 different health facilities within Jigawa state between the period of January 2020 to April 2020, information about their risk behaviors and social demography were collected using close-ended questionnaires, Patients' medical files were examined to get their ART regimens and viral load. Rapid test was used to determine HBsAg, nested polymerase chain reaction PCR was carried out using specific HBV S-gene primers, a characteristic 756bp band was seen in the gel electrophoresis.
Results: 300 HIV patients consisting of 203 females and 97 males participated in the study, research concluded with the overall prevalence of 7.7% HBV/HIV co-infection (n=23/300). Results obtained from the study revealed higher occurrence in men than women 13% and 4% respectively, widows/widower, patients with a low level of education are observed to have a higher prevalence of HBV/HIV. This research also revealed patronage of local commercial pedicures/manicures as one of the factors associated with HBV/HIV co-infection. Others included vaccination. There was a statistical association between blood transfusion and HBV/HIV co-infection as obtained in the study. Statistical analyses were carried out using SPSS version 13 at a confidence of 95% and 5% margin error.

Keywords: Co-infection, prevalence, molecular detection

Introduction

Studies have shown that HBV is a leading cause of non-AIDS–related deaths among PLHIV in settings where HIV-suppressive antiretroviral therapy (ART) is widely available (Lara *et al.*, 2015) [1]. During co-infection, HIV significantly regulates the natural course of HBV infection. Compared with individuals that are only infected with HBV, the course of chronic HBV infection in HIV co-infected patients is more aggressive resulting in lower transaminase elevation, increased HBV DNA levels, decreased inflammatory activity, and a higher prevalence of cirrhosis and hepatocellular carcinoma (Sarkar *et al.*, 2016) [2]. HIV and HBV share common risk factors, and many generalized HIV epidemics occur in populations with higher HBV prevalence, leading to an increased risk for HBV co-infection (Lara *et al.*, 2015) [1].

Mortality among HIV/HBV co-infected persons is substantially higher than among HIV mono-infected persons, studies have also shown that those with HIV/HBV co-infection are 8 times more likely to die from liver disease than those with HIV mono-infection and 19 times more likely to die from liver disease than the HBV mono-infected individuals (Thio *et al.,* 2010) [3]. Hepatitis B virus (HBV) belongs to the family *Hepadnaviridae* and is known to be highly transmissible (Isa *et al.,* 2015). It is a hepatotropic DNA virus that also includes duck hepatitis B virus (DHBV) and woodchuck hepatitis B virus (WHBV) (Zhang *et al.,* 2016) [4].

The human immunodeficiency virus (HIV) is a retrovirus that causes infection and over time acquired immunodeficiency syndrome (AIDS) (Weiss, 1993 Douek *et.al.,* 2009) [5, 6]. AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment average survival time after infection with HIV is estimated to be 9 to 11 years. Depending on the HIV subtype (UNAIDS; WHO, 2007). In most cases, HIV is a sexually transmitted infection and occurs in contact with or transfer of blood, pre-ejaculates, semen, and vaginal fluids. Non-sexual transmission can occur from an infected mother to her infant through breast milk (Mabuka *et. al.,* 2012) [9]. An HIV-positive mother can transmit HIV to her baby both during pregnancy and childbirth due to exposure to her blood or vaginal fluid (Kumari, 2015) [10].

HIV/HBV co-infection is a growing concern because apart from increasing the toxicity to antiretroviral medications, 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 and thus, are at an increased risk of developing cirrhosis of the liver (Owolabi *et al.,* 2014) [11].

Methodology

Study design and study area: a cross-sectional study was conducted between the periods of January to April 2020 among HIV seropositive patients attending antiretroviral (ART) clinics of General Hospital Dutse, General Hospital Hadejia and General Hospital Ringim, located in the 3 senatorial districts of Jigawa state. Jigawa States is one of the thirty-six states located in North-western Nigeria situated between latitudes 11.00^0N to 10.15^0E and covering a land area of 322,410 sq km. According to the 2006 general census; Jigawa state's population was 4,361,002. The state's projected population now stands at 5,828,200 (NPC, 2019). Dutse is the Capital city of Jigawa State located in northern Nigeria, it is home to Federal University Dutse. With an estimated population of over 153000 (Stefan, 2007).

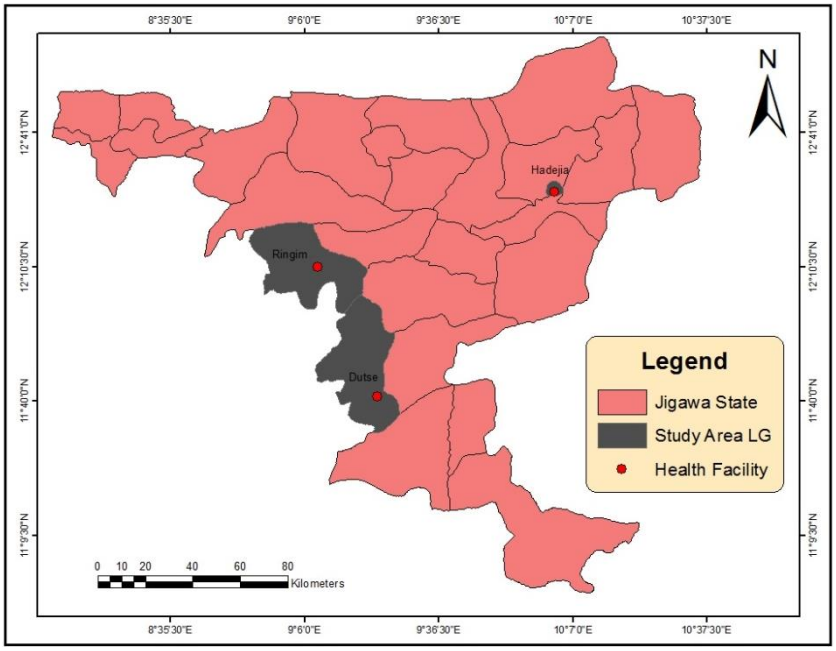


Fig 1: Map of Jigawa State, showing Study Areas

Study population

The study population consisted of 300 consenting PLWHIV with an age range between 0 – 80 years attending ART clinics for routine care. On clinic days which is usually Tuesdays and Thursdays, patients were invited to participate in the study, those who agreed were given consent forms attached with the research questionnaire to respond to; the questionnaire is designed to get their social demographic data, medical history and risk behaviors. Medical files were also reviewed to get information about ART regimens and viral load. The minimum sample size required for this study was calculated using the formula by Thrusfield (1997) [14]: $N= (1.96)^2 \times Pexp (1-Pexp)/ D^2$. N=161.92. 325 the sample size was increased to get more data and ease of statistical analysis. PLWHIV were invited to participate out of which 300 consented. Blood samples were collected from them and allowed to settle then centrifuged at 805 x g for five minutes (WHO, 2019). The samples' screening for HBsAg was done using the ACON USA & KINGSTON rapid test kit per the manufacturer's instructions. The test is shown to have specificity and sensitivity of 99.5 and

Molecular Detection

According to the manufacturer's instructions, HBV DNA was extracted from 200 µl of serum using a QIAampBlood mini kit (Qiagen, Germany). 20 µl of protease was added to the

serum in a 1.5 ml tube. Then, 200 µl of cell lysis solution (AL buffer) was added, DNA was precipitated by adding 200 µL of 100% ethanol vortexed, and incubated for 10 min at 56°C. The DNA was eluted using 50 µl of elution water and stored at -20°C until further analysis. PCR was carried out in a 20 µL final volume comprised of 2µL of the genomic DNA, 10 µL of a GoTaq master mix (Promega, Wisconsin, USA) containing optimized buffer, $MgCl_2$, and dNTP mixes; 1µL each of forward and reverse primers and 6µL ddH_20. Thermocycling conditions were initial denaturation at 95ºC for 1 min, followed by 35 cycles each of 20 sec at 95ºC (denaturation), 20 sec at 57ºC (primer annealing), 1.5 min at 72ºC (extension). This was followed with a 3 min final extension at 72ºC. The second reaction was carried out on the product of the first reaction, 20.5 µL comprised of 2µL of the PCR product, 10µL of master mix (Promega, Wisconsin, USA), 1µL each of forward and reverse primers and 6µL of ddH_20. Amplification and the cycling conditions are the same except for the annealing temperature of 58ºC and extension time of 1min. PCR products were separated in 1.5% agarose gel stained with ethidium bromide and visualized for bands. 5 samples were randomly selected from the 23 HBsAg positive samples for DNA was extraction, the DNA product was amplified using specific universal primers; Forward PolRT-3F (5' - CGAAGACTGGGGACCCTGC-3, nt 129–147) and Reverse HBO2-R (5' - ATATAACCCATAAAGTGTAAGGAATA-3' nt 864–890) for Hepatitis B S-gene, all lanes show a PCR product of expected band size (756bp).

Data analysis

All questionnaire responses were collected and collated. Analysis was carried out using statistical package for social sciences (IBM SPSS) version 23. Descriptive statistics were done to get summaries and frequencies of the data. Chi-square test was done to establish the strength of relationships between variables (HBV, social demography and risk factors) using crosstabs, P-values of < 0.05 are considered statistically significant.

Ethical Approval

Ethical approval for this study was obtained from the Jigawa State Ministry of Health with reference number MOH/SEC.3/S/821/I. all patients recruited signed a consenting letter to be part of the study. Courtesy letters were also written to all the Chief Medical Officers of all the facilities, where directors of clinical services and Heads of ART clinics were consulted before the enrolment of the participant in the studies

Result

The total number of PLWHIV in the study is 300, however, 325 patients were invited to participate in the study giving us a responses rate of 92% and a refusal rate of 7%, some of the reasons for refusal included lack of monetary benefits, lack of time, and for some they are simply not saying what their reasons were. Age range for the participants range between 0 – 80, females (n=203), males (n=97).

174 out of the 300 participants have no formal Education, Primary Education (n=51), Secondary Education (n=42) and Tertiary Education (n=33). Single (n=47), married (n=197), divorced (n=32) and Widows/widowers (n=24). 162 of the patients live in a rural setting while 138 live in urban settings. 29 of the participants are students, 44 are farmers, 36 are employed, 64 are unemployed and 127 are self-employed. The study recorded a higher occurrence of HBV/HIV co-infection among males than females 13% and 4.9% respectively. HIV is mostly transmitted through sexual transmission, occurrence in relation n to marital status from the study showed HBV prevalence of 6% among Single patients, 7.6% among married patients, 16.6% among widows/widowers and 3% among divorced. As established; HBV and HIV share common route of transmission, risk factors examined for HBV/HIV co-infection shows the following percentages in the study population; 4% positive among patients who have had blood transfusion and 8% among patients who never had transfusion. 6.5% among patients who have multiple Sexual partners and 7.7% among those who don't have multiple sexual partners. As high as 16.6% among patients who consume Alcohol while 7% among people who don't consume alcohol. 28% among patients with Tattoos and 7% among the patients who don't have Tattoos. 13% among patients who smoke and 6.8% among those who don't smoke. 15.2% among patients who engage in the service of commercial manicure and 6% among those who do not. 9% among patients who share clothing with other people and 6.8% among those who do not. 9.7% among patients who do not share unsterilized objects, 8% among patients who have never been vaccinated against HBV and lastly 3% among patients who have had surgery. All 5 samples randomly selected for molecular investigation are positive for HBV DNA. The Overall Prevalence of HBV/HIV co-infection recorded in the study is 7.7% (n=23/300). This study also establishes a statistical relationship between Gender, Education and Pedicure to HBV/HIV co-infection. Viral load of HBV/HIV co-infected patients doesn't have an irregular pattern from patients who are HIV mono-infected patients, of the 23 HBV/HIV co-infected patients; 1 have undetectable level of HI-virus in serum, 13 patients have (1-<20 cp/ml), 5 patients (21 – 500cp/ml), 3 patients (501 – 1000cp/ml) and 1 patient (>1000cp/ml). All patients in the study are already placed on ART, only 3 out of the 23 HBV/HIV patients are on a non-TDF/3TC-based ART regimen.

Discussion

The prevalence of HBV/HIV co-infection in this study was 7.7% among patients aged from 0 – 80. This is lower than 12.3% previously reported by (Hamza *et al.*, 2013), but it is consistent with similar studies conducted in other parts of the country; Alaku and others reported a prevalence of 7.5% among HIV patients in a tertiary facility in north-central Nigeria. 12.5% prevalence was reported by Pennap *et al.*, 2017 [18] among HIV patients accessing healthcare in FMC Keffi. Okeke *et al.*, 2017 [19] and Nwolisa *et al.*, 2013 [20] reported 5.8% in Imo and 6.3% in Anambra State among HIV-infected children. Other studies around Nigeria have reported higher prevalence like 35% prevalence among HIV patients in Plateau State (Onwuliri *et al.*, 2014) [21], 16.4% in Delta State (Avwioro *et al.*, 2014) [20], 15.5% in Benin City (Ojide *et al.*, 2010), 13.2% in Niger (Omosigho *et al.*, 2010) [24] and 12.8% in Gombe State (Obi *et al.*, 2014) [25], 11.2% in Ekiti (Opaleye *et al.*, 2014) [26]. This variation in would be largely due peculiarity of the people, location and their culture, Nigeria is a highly diverse country with more than 250/300 languages and ethnicities. Studies around Africa have also reported 7.1% among PLWHIV in south-Africa and 7.0% among women attending CTC care (Boyles and Cohen,

2011, Matthews *et al.*, 2015) [27, 28]. 16% prevalence was recorded in Uganda (Baseke *et al.*, 2015) [29]. It was observed that none of the 23 HBV/HIV patients were vaccinated, this also means that vaccination remains the best practice to prevent HBV infection, most often what is obtainable in ART clinics in Nigeria is that routing tests for HIV patients doesn't include hepatitis and this has increased over the years the risk of non-HIV related deaths among HIV patients and by extension for those already infected with HBV; the risk of 3TC-drug resistance may begin to set in. studies like that of Benhamou *et al* (1999) [30] and Gu *et al* (2015) [31] have shown that if emtricitabine (FTC) or lamivudine (3TC) are used as the only HBV-active agents in ART among HIV/HBV co-infected patients, there is a higher risk of developing HBV resistance.

Therefore there is a need for routine and periodic screening of HBsAg among PLWHIV since individuals found to be HBsAg-positive will need ART regimens that include two drugs that also target the hepatitis B virus (WHO, 2016). It is recommended that for HIV/HBV co-infection, a combination of tenofovir (TDF) with either 3TC or FTC in the ART regimen should serve as the first line of treatment (Konopnicki *et al.*, 2005) [33]. As mentioned earlier only 3 out of the 23 HBV/HIV patients are on a non-TDF/3TC-based ART regimen, which means that the remaining 20 are possibly resistant to 3TC, or it could be due to inconsistency in taking their drugs. It is important to also immunize all HBV negative PLWHIV as this would help significantly in reducing the disease morbidity. This found statistical association between HBsAg and local pedicure, this would be evident as this practice is highly risky and often highly patronized in Jigawa state, we feel if a more or larger sample is studied over a relatively long period, we would explore and understand better the association between the disease and its risk factors in peculiarity to the variation in PLWHIV and their location. Some of the limitations of the study few associations between HBsAg and several risk factors was shown, other HBV serological markers such as HBsAb, HBeAg, HBeAb, anti-HBc and IgM, were not examined. This is largely due to limited resources available. We suggest that future studies recruit more clients and possibly secure a research grant to address this shortcoming.

Table 1: Showing the Prevalence of HBsAg in relation to Socio-demographic factors among HIV seropositive patients in Jigawa State., Nigeria

Socio-demographic factors	No. examined	No. Positive (%)	Chi-Square (X^2)	P-value
Gender				
Male	97	13(13)	6.661	0.010
Female	203	10(4.9)		
Total	300	23(7.7)		
Age (years)				
0-10	7	1(14.0)	4.205	0.756
11-20	18			
21-30	75	6(8.0)		
31-40	112	9(8.0)		
41-50	57	6(10.0)		
51-60	15			
61-70	12	1(8.3)		
71-80	4			
>80	0			
Total	300	23(7.7)		
Educational Status				
None	174	9(5.1)	8.890	0.031
Primary	51	7(13.7)		
Secondary	42	6(14.2)		
Tertiary	33	1(3.0)		
Total	300	23(7.7)		
Marital Status				
Single	47	3(6.0)	3.789	0.285
Married	194	15(7.6)		
Widow(er)	24	4(16.6)		
Divorced	35	1(3.1)		
Total	300	23(7.7)		
Occupation				
Employed	36	3(8.0)	3.209	0.524
Unemployed	64	4(6.0)		
Self-employed	127	9(7.0)		
Farmers	44	6(13.0)		
Student	29	1(3.0)		
Total	300	23(7.7)		
Residence				
Urban	138	9(6.0)	0.064	0.801
Rural	162	14(8.0)		
Total	300	23(7.7)		

Table 2: Showing the Prevalence of HBsAg in relation to associated risk factors among HIV Seropositive patients in Jigawa State, Nigeria

Risk Factors	No. examined	No. Positive (%)	Chi-Square (X^2)	P-value
HBV vaccine			1.496	0.221
Yes	17	0(0.0)		
No	289	23(7.7)		
Total	300	23(7.7)		
Tattoo			4.425	0.35
Yes	7	2(28.0)		
No	293	21(7.0)		
Total	300	23(7.7)		
Blood Transfusion			1.020	0.313
Yes	64	3(4.6)		
No	236	20(8.0)		
Total	300	23(7.7)		
Pedicure/Manicure			9.044	0.003
Yes	46	7(15.2)		
No	254	16(6.0)		
Total	300	23(7.7)		
Use Unsterilized objects			0.770	0.380
Yes	9	0(0.0)		
No	281	23(7.7)		
Total	300	23(7.7)		
Multiple sex			0.019	0.891
Partners				
Yes	46	3(6.5)		
No	254	20(7.5)		
Total	300	23(7.7)		
Clothes/Beddings			0.526	0.468
Yes	97	9(9.2)		
No	203	14(6.8)		
Total	300	23(7.7)		
Smoking			1.853	0.173
Yes	38	5(13.0)		
No	268	18(6.8)		
Total	300	23(7.7)		
Alcohol			0.321	0.571
Yes	12	2(16.0)		
No	288	21(7.0)		
Total	300	23(7.7)		
History of Surgery			0.156	0.693
Yes	9	1(11.1)		
No	291	22(7.6)		
Total	300	23(7.7)		

Table 3: Prevalence of HBV infection in the study

HBsAg Status		Frequency	Percent (%)
	Positive	23	7.7
	Negative	277	92.3
	Total	300	100.0

Table 4: Showing Viral Load distribution in the study population

Viral load Cp/ml		Frequency	Percent (%)
	<20	152	50.7
	21-500	88	29.3
	501-1000	13	4.3
	>1000	27	9.0
	Target Not detected	20	6.7
	Total	300	100.0

Table 5: Showing ART regiment distribution in the study population

ART combination	Frequency	Percent
Tenofovir/Lamivudine+ Dolutegravir (TDF+3TC+DTG)	230	76.7
Tenofovir/Lamivudine+ Atazanavir/Retonavir (TDF+3TC+ATV/r)	28	9.3
Tenofovir/Lamivudine+ Lopenavir/Retonavir (TDF+3TC+LPV/r)	13	4.3
Zidovudine/Lamivudine+ Lopenavir/Retonavir (AZT+3TC+LPV/r)	19	6.3
Abacavir/Lamivudine+ Atazanavir/Retonavir (ABC+3TC+ATV/r)	10	3.3
Total	300	100.0

Keys: ART = antiretroviral therapy, TDF = Tenofovir disoproxil fumerate, 3TC = (−)-L-2′, 3′-dideoxy-3′-thiacytidine, DTG = dolutegravir, ATV = Atazanavir, r = Retonavir, LPV = Lopenavir, AZT = Azidothymidine, ABC = Abacavir.

Table 6: ART Combination, Age group and Gender Distribution among HBV/HIV co-infected patients

ART Regimen	Age group (years	Gender
Tenofovir/Lamivudine + Dolutegravir (TDF+3TC+DTG)	21-30	Female
	31-40	Female
	21-30	Female
	31-40	Female
	31-40	Female
	31-40	Male
	41-50	Male
	31-40	Male
	21-30	Male
	61-70	Male
	41-50	Male
	31-40	Male
	31-40	Male
	41-50	Male
	41-50	Male
	31-40	Male
	41-50	Male
Tenofovir/Lamivudine+ Atazanavir/Retonavir (TDF+3TC+ATV/r)	21-30	Female
	21-30	Female
	41-50	Female
Tenofovir/Lamivudine+ Lopenavir/Retonavir (TDF+3TC+LPV/r)	-	-
Zidovudine/Lamivudine +Lopenavir/Retonavir (AZT+3TC+LPV/r)	21-30	Female
	0-10	Male
Abacavir/Lamivudine+ Atazanavir/Retonavir (ABC+3TC+ATV/r)	31-40	Female
Total	23	

Keys: ART = antiretroviral therapy, TDF = Tenofovir disoproxil fumerate, 3TC = (−)-L-2′, 3′-dideoxy-3′-thiacytidine, DTG = dolutegravir, ATV = Atazanavir, r = Retonavir, LPV = Lopenavir, AZT = Azidothymidine, ABC = Abacavir.

Table 7: Viral load, Age group and Gender Distribution among HBV/HIV co-infected patients

Viral load Cp/ml	Age-group (years)	Gender
Not Detected	41-50	Male
< 20	31-40	Male
	21-30	Female
	31-40	Female
	61-70	Male
	21-30	Female
	41-50	Male
	21-30	Female
	31-40	Female
	31-40	Male
	41-50	Male
	31-40	Male
	31-40	Female
	41-50	Male
21-500	31-40	Male
	21-30	Male
	31-40	Female
	31-40	Male
	0-10	Male
501-1000	21-30	Female
	41-50	Male
	41-50	Female
>1000	21-30	Female
Total	23	

Keys: Viral loads measured in copies per milliliter of Serum (cp/mL). Not detected (no viral particles detected), < 20 (less than 20cp/mL), 21-500 (between 21-500cp/mL), 501-1000 (between 501-1000cp/mL) >1000 (above 1000cp/mL).

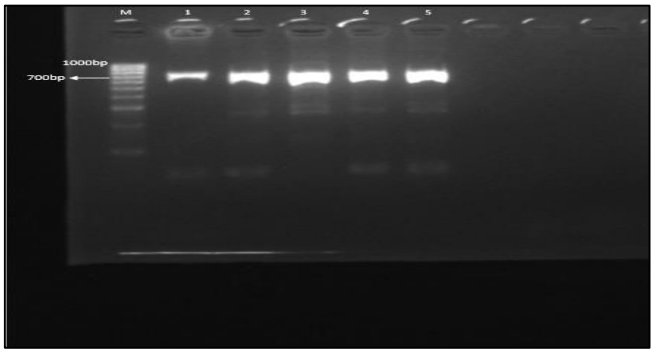
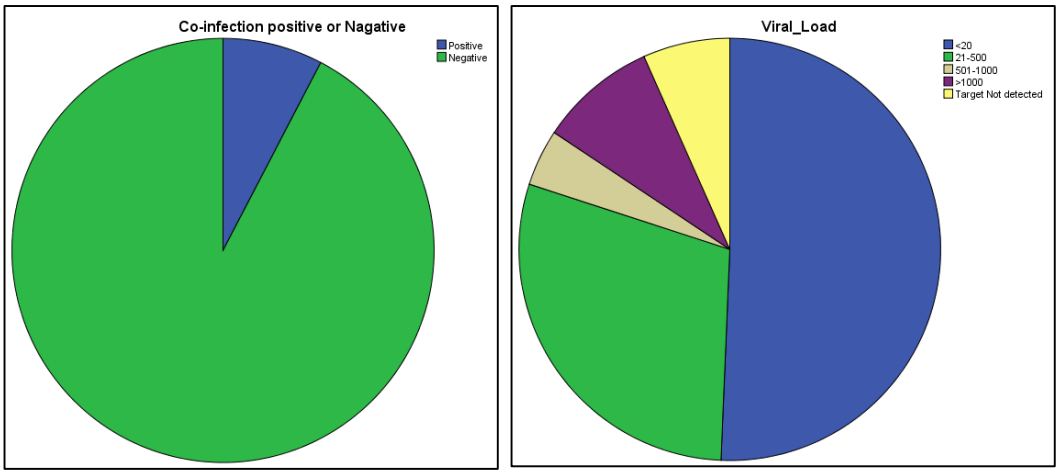
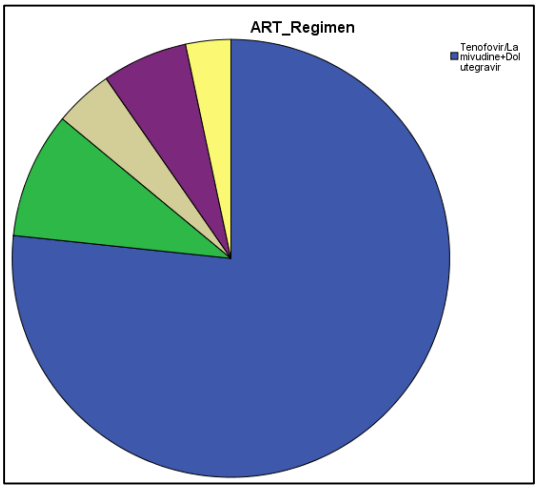


Fig 2: 15% agarose gel electrophoresis, polymerase chain reaction for the detection of HBV. A distinct band of 756bp is evident in lanes 1-5. Lane M: represents Hyperladder Bioline (1013bp).



A B



C

Pie charts A: Prevalence of HBV/HIV co-infection in Jigawa state Nigeria, **B:** Viral load distribution in the study population, **C:** ART regimen distribution in the study population

Conclusion

The study concluded with the prevalence of 7.7% HBV/HIV co-infection. This is a relatively high occurrence rate considering the group under study (PLWHIV). The need for an enhanced treatment plan for co-infected patients cannot be overemphasized as the risk of resistance increases, studies have shown that co-infected individuals must be placed on two (2) antivirals that are effective against HBV hence, proper treatment of PLWHIV would require routine screening for HBsAg should be considered so that co-infected India can be identified in time so as to improvidualve quality of life, reduce morbidity and other related liver complications.

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