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Prevalence of Hepatitis D Virus (HDV) in HBV/HIV Co-Infected Patients in Ogbomoso, Nigeria

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ABSTRACT

Hepatitis D virus (HDV) requires co-infection with hepatitis B virus (HBV). Human immunodeficiency virus (HIV) shares transmission routes with these viruses. HDV infection increases liver complications compared to HBV alone, especially in HIV-positive people. Although vaccinations and antiviral medications are available, over 1 million individuals worldwide die from HBV-related illnesses each year, which may also co-infect with HDV. Examining HDV infection in HBV/HIV co-infected individuals would provide important epidemiological information for improving healthcare delivery in Nigeria. Using a cross-sectional study, data for this study were collected from 206 consenting individuals receiving treatment for HIV/AIDs at Oyo State General Hospital, Ogbomoso. Also, blood samples were collected from each participant and the plasma was used to determine status of hepatitis B surface antigen (HBsAg) and HDV antibody using ELISA techniques. In this study, 16/206, 7.8% were HBsAg positive among the PWH. While the prevalence of HDV IgG and HDV IgM antibodies among HBV/HIV individual was 7.8% and 0% respectively. The HDV IgG antibodies were found higher among females, individuals between 37-54 years and those with no or little education. The seroprevalence of HDV IgG antibodies at 7.8% indicates that HDV exposure is relatively uncommon in this group, but not insignificant. Although 0% seroprevalence of HDV IgM says no acute or recent HDV infection among the participants as at the time of the study but monitoring for signs of active liver disease or viral reactivation in chronic carriers (IgG positive) is still important. Early and regular HBV screening, monitoring and management among HBV/HIV co-infected individuals are needed as preventing HBV serves as means for HDV prevention.

Keywords: Co-infection, Hepatitis B Virus (HBV), Hepatitis Delta Virus (HDV), Human Immunodeficiency Virus (HIV), Prevalence

INTRODUCTION

Hepatitis D virus (HDV) is a defective subviral agent that needs the presence of hepatitis B virus (HBV) surface antigen (HBsAg) to induce hepatic infection and disease. In recent years, HDV infection was thought to be a quite rate disease due to the widespread implementation of HBV vaccine and the clinical oversight of HDV detection [**1**]. Historically, HDV is ascribed as the most extreme type of viral hepatitis with few available treatments for managing it [**2**]. HDV infection significantly exacerbates the clinical course of HBV-related liver disease, frequently resulting in fast progression to cirrhosis, liver failure, or hepatocellular cancer [**3**].

An estimated 4.5% of the general population who are HBsAg positive have anti-HDV, which translates to around 12 million individuals worldwide [4], and about 20% of instances of liver disease and liver cancer in HBV infected individuals may be attributed to HDV coinfection [5]. Also, HIV affects 35.3 million people worldwide. Globally, 7.4% of HIV-positive people have HBV–HIV co-infection [**6].**

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People Living with HIV (PLWH) who are infected with HBV/HDV have worse medical conditions including cirrhosis, HCC, hepatic flares, decompensation, and higher death rates [7**]**. Sexual contact and intravenous injection are two routes of transmission for HBV and HDV. PLWH are more likely to get HDV due to HIV transmission. Additionally, the progression of HDV-related liver disease in PLWH may be more rapid, impacting both survival rates and quality of life [**8]**.

Abolition of HIV/AIDS, hepatitis, and other STDs is one of the Sustainable Development Goals (SDGs) set for 2030 [**9**]. Despite the availability of HBV vaccination (which is also effective against HDV) in Nigeria's childhood immunisation schedule since 2004, the persistent burden of HBV infection is a major obstacle to public health, as it affects 5-10% of the population [**10**]**,** [**11**]. Therefore, this study aimed to determine HDV seroprevalence (IgM and IgG) among HBV/HIV individuals in Ogbomoso and to identify the risk factors associated with HDV prevalence in HBV/HIV co-infected individuals in the study population. In addition, the prevalence of HBsAg, as the primary requirement will also be determined in HIV individuals receiving treatment for HIV/AIDs at Oyo State General Hospital, Ogbomoso Oyo State, Nigeria.

MATERIALS AND METHODS

Study Design

This cross-sectional study, was carried out among HIV/AIDs positive individuals of all ages receiving treatment at Anti-Retroviral Therapy clinic, Oyo State General Hospital Ogbomoso, Oyo State, Nigeria. The sample size for this study is 206. This was calculated using the formula; $N=Z^2PQ/E^2$ Where: N = Desired sample size, Z = the standard normal deviate at 5% significance level (1.96) P = Prevalence rate of HBsAg in HIV individual taken as 16%(from previous research done in southwest, Nigeria, Opaleye et al.,), Q = 1-P and E is the level of precision (allowable error) = 5%.

Ethical approval was obtained as part of the prerequisites before research of this nature can be carried out on human participants from the ethical committee of Oyo State Ministry of Health. Each respondent gave informed consent after being clearly explained the study, and questionnaires were self-administered.

Participant Inclusion criteria and Exclusion criteria

All consented patients of all ages that have a confirmed diagnosis of HIV infection and are receiving treatment for HIV/AIDS at the Oyo State General Hospital, Ogbomoso. For the underage, consent was taken from their parents or guidance. Exclusion criteria adopted are for individuals who did not give their consent to take part in the research.

Sample Collection and Blood Sample Analysis

Blood samples were taken from 206 consenting PLWH using syringe and needle into EDTA bottles, centrifuged at 2000rpm for 15 mins and the plasma separated and stored at -80^0C analysed. All samples were screened for HBsAg using PROMED HBsAg rapid test kit, and those positive for HBsAg were further analysis for HDV specific IgG and IgM antibodies using ELISA (Diagnostic Automation/ cortez Diagnostics, Inc. 21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA).

For detection of HDV IgG and IgM antibody, HDV IgG and IgM ELISA use the "INDIRECT" ELISA technique, where HDV antigens are pre-coated onto polystyrene microwell strips. Patient serum is combined in the microwell with anti-human IgG coupled to the enzyme horseradish peroxidase (the HRP-conjugate). The solid phase is coated with the particular immunocomplex that develops during incubation if there is HDV IgG in the sample. Wells are supplemented with chromogen solutions comprising tetramethyl-benzidine (TMB) and urea peroxide after washing to eliminate sample serum proteins and unbound HRP-conjugate. The attached HRP-conjugate hydrolyses the colourless chromogens to produce a blue-colored product when the antibody-antigen complex is present. When the reaction with sulphuric acid is stopped, the blue colour changes to yellow. It is possible to quantify the colour intensity, which is equivalent to the quantity of antibody present in the sample and the quantity of antibody caught in the wells [**12**]**,** [**13**].

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For the quantitative analysis of HDV IgG and IgM, the entire specimen and kit reagents were equilibrated at room temperature and carefully mixed. The washed buffer was diluted (20X) as indicated in the instruction for washing. The miocroplate's wells were formatted for control and patient specimen to be assayed. 100ul of the sample diluents were introduced into their corresponding wells, with the exception the blank and control wells (2 positive and 2 negative control wells). After covering the plate, it was incubated at 37°C for 30 minutes. Once the incubation period concluded, the plate cover was taken off and disposed. Following this, diluted wash buffer was used to wash each well five times. To get rid of any leftover material, the plate was placed on a blotting paper after the last round of washing [**13**].

After carefully pressing the plate to blend the 100ul of conjugate that had been poured to all well excluding the blank, the plate was covered with the plate cover once again and incubated for 30 minutes at 37°C. Following the incubation period, the plate cover was taken off and disposed away, and each well was given five washes with diluted wash buffer. To get rid of any leftover material, the plate was placed on a blotting paper after the last round of washing. Each well received 50 ul of substrate solutions A and B, which were then incubated for 15 minutes at 37 °C without exposure to light. Each well was carefully stirred after 50 ul of stop solution was introduced using a pipette. After calibration, the absorbance was taken at 450 nm using the plate reader [**13**].

All laboratory analysis was carried out at Center for Emerging and Re-emerging Infectious Diseases (CERID) Laboratory, Ladoke Akintola University Ogbomoso Nigeria.

Data Analysis

Statistical Package for Social Sciences (SPSS) version 24 was used to analyse all of the data. The seroprevalence for HDV was expressed as a percentage for the entire study group, with the P-value set at ($P<0.05$) for significance at the 95% confidence interval.

RESULTS

Socio-demographic Characteristics of the Participants

The social and demographic characteristics of the participants are shown in the table 1 below. Most of the participants are between the age of 37-54 (45.1%). This was followed by those between 19-36 years (34.0%). The ones that are less than 18 years and 55 years and above accounted for 11.2% and 9.7% respectfully. There are more female participants (67.0%) than male participants (33.0%) in the study sample. Also, majority of them are married (67.0%) while others are single (20.9%), widowed (9.7%) and divorced/separated (2.4%). The participants with only SSCE (38.1%) constitute the majority. This was followed by those with primary school leaving certificate (30.1%). Other educational qualifications are OND/NCE (15%), HND/BSc. (6.8%) and Masters/PhD (0.5%). Those without formal education are 9.2%. The occupation of the participants showed that majority of them are self-employed (61.7%) while 18.4% are unemployed. Civil servants accounted for 8.7% while those that are involved in any other kind of occupation like farming and business accounted for 11.2%. From the ethnicity of the participants, majority of them are Yoruba (92.2%) while Hausa and Igbo accounted for 3.9% each. Religious wise, there are more Christians (69.9%) than Muslims (29.1%) and Traditional African Religion (1.0%).

Table 1: Socio-demographic characteristics of the Participants

Characteristics		Number	Percentage (%)
Age (years)	Less than 18	23	11.2
	19 – 36	70	34.0
	37 – 54	93	45.1
	55 years and above	20	9.7

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Gender	Male	68	33.0
	Female	138	67.0
Marital status	Single	43	20.9
	Married	138	67.0
	Divorced/separated	5	2.4
	Widowed	20	9.7
Educational qualification	No formal education	19	9.2
	Primary sch. Leaving cert.	62	30.1
	SSCE	79	38.3
	OND/NCE	31	15.0
	HND/BSc.	14	6.8
	Masters/PhD.	1	0.5
Occupation	Unemployed	38	18.4
	Self-employed	127	61.7
	Civil servant	18	8.7
	Others	23	11.2
Ethnicity	Yoruba	190	92.2
	Hausa	8	3.9
	Igbo	8	3.9
Religion	Christianity	144	69.9
	Islam	60	29.1
	Traditional African Religion	2	1.0

Prevalence of HBV

From the table 2, the HBsAg test revealed that 16 (7.8%) of the HIV positive individuals tested positive to HBV while 190 (92.2%) tested negative to HBV.

Table 2: Prevalence of HBV

HBsAg test	Number	Percentage (%)
Positive	16	7.8%
Negative	190	92.2%

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Prevalence of HDV

The prevalence of HDV is shown in table 3. 16 individuals that tested positive for HBsAg were tested for HDV IgG in which all of the results came out positive. Upon further test for IgM, all the results came out negative. The HDV IgG indicates that the infection is in the past whereas the HDV IgM indicates the infection is chronic. HDV IgG have a significant relationship with HDV IgM (X^2 = 206.000, df = 1, p = 0.00).

Table 3: Prevalence of HDV

HDV Prevalence		Number	Percentage (%)	Pearson Chi-Square (□^{2})	Df	p-value
HDV IgG result	Positive	16	7.8%	206.000[a]	1	0.00
	Negative	-	-			
	Not tested	190	92.2%			
HDV IgM result	Positive	-	-			
	Negative	16	7.8%			
	Not tested	190	92.2%			

Risk Factors of HBV and HDV in HBV/HIV

co-infection

The risk factors of HDV in HBV/HIV co-infection is given in the table 4. The factors considered include taking of sharing of sharp objects, engagement in unprotected sex, number of sexual partners, intravenous drug user and blood transfusion. From the table, 85.5% of the participants do not share sharp objects while 14.1% do share sharp objects. Also, 88.3% do not engage in unprotected sex while 11.7% engages in unprotected sex. The participants with only one sexual partner constitute the majority and they accounted for 78.6%. those with two and more than two sexual partners accounted for 2.4% and 1.0% respectfully. 18.0% of the participants are with no sexual partners. The participants that do not engage in intravenous drug user accounted for 97.6% while 2.4% engages in it. The participants that have not received blood transfusion are also accounted for 76.2% while 23.8% have received blood transfusion. Generally, from the foregoing, it can be seen that majority of the participants are not engaged in activities that are categorised as risk factors of HDV in HBV/HIV co-infection.

Table 4: Risk factors of HBV/HDV in HBV/HIV co-infection

Factors		Number	Percentage (%)
Sharing of sharp objects	Yes	29	14.1
	No	177	85.9
Engagement in unprotected sex	Yes	24	11.7
	No	182	88.3
Number of sexual partners	One	162	78.6
	Two	5	2.4

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	More than two	2	1.0
	None	37	18.0
Intravenous Drug User	Yes	5	2.4
	No	201	97.6
Blood transfusion	Yes	49	23.8
	No	157	76.2

Relationship between socio-demographic characteristics and HDV prevalence in HBV/HIV co-infection

Table 5 presents the relationship between socio-demographic characteristics and HDV prevalence in HBV/HIV co-infection. 11 (5.3%) out of the 7.8% of the HBV/HDV positive individuals are between the age of 37-54 years. Other age categories are 19-36 years and less than 18 years with 4 (1.9%) and 1 (0.5%) respectfully. However, age has no significant relationship with HBV/HDV infection (X^2 = 4.612, df = 3, p = 0.202). The gender of HBV/HDV positive individuals revealed that 12 (5.8%) are females while 4 (1.9%) are males. Similar to age, gender has no significant relationship with HBV/HDV infection (X^2 = 0.503, df = 1, p = 0.478). Marital status of the HBV/HDV positive individuals revealed that 6.3% (13) are married while 3 (1.5%) are single. Marital status also does not have any significant relationship with HBV/HDV infection (X^2 = 2.669, df = 3, p = 0.445). SSCE, primary school leaving certificate, OND/NCE, and HND/BSc are the educational qualifications of the HBV/HDV positive persons, and they accounted for 6 (2.9%), 5 (2.4%), 2 (1.0%), and 2 (1.0%) of the total. Only 1(0.5%) HBV/HDV positive individuals is with no formal education. Similar to the aforementioned, the relationship between educational qualification and HBV/HDV infection is not significant (X^2 = 1.167, df = 5, p = 0.948). For the occupation of the HBV/HDV positive individuals, 9 (4.4%) are self-employed, 3 (1.5%) are in a form of occupation such as business and farming, 2 (1.0%) are civil servants and 2 (1.0%) are unemployed. However, there is no significant relationship between occupation and HBV/HDV infection (X^2 = 1.589, df = 3, p = 0.662). The ethnicity of the HDV positive individuals are Yoruba and Hausa with 15 (7.3%) and 1 (0.5%) respectively. The relationship between ethnicity and HBV/HDV infection is not significant (X^2 = 0.928, df = 2, p = 0.629). The religion of the HBV/HDV positive individuals are Christianity and Islam with 12 (5.8%) and 4 (1.9%) respectively. Similar to the other demographic characteristics, there is no relationship between religion and HBV/HDV infection (X^2 = 0.334, df = 2, p = 0.846).

Table 5: Relationship between socio-demographic characteristics and HDV prevalence in HBV/HIV co-infection

Characteristics		HBV/HDV infection		Pearson Chi Square (χ^2)	Df	p-value
		Positive N(%)	Negative N(%)			
Age (years)	Less than 18	1(0.5)	22(10.7)	4.612	3	0.202
	19 – 36	4(1.9)	66(32.0)			
	37 – 54	11(5.3)	82(39.8)			
	55 years and above	-	-			
Gender	Male	4(1.9)	64(31.1)	0.503	1	0.478
	Female	12(5.8)	126(61.2)			

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Marital status	Single	3(1.5)	40(19.4)	2.669	3	0.445
	Married	13(6.3)	125(60.7)			
	Divorced/separated	-	2.4			
	Widowed	-	9.7			
Educational qualification	No formal education	1(0.5)	18(8.7)	1.167	5	0.948
	Pry. sch. leaving cert.	5(2.4)	57(27.7)			
	SSCE	6(2.9)	73(35.4)			
	OND/NCE	2(1.0)	29(14.1)			
	HND/BSc.	2(1.0)	12(5.8)			
	Masters/PhD.	-	1(0.5)			
Occupation	Unemployed	2(1.0)	18.4	1.589	3	0.662
	Self-employed	9(4.4)	61.7			
	Civil servant	2(1.0)	8.7			
	Others	3(1.5)	11.2			
Ethnicity	Yoruba	15(7.3)	175(85.0)	0.928	2	0.629
	Hausa	1(0.5)	7(3.4)			
	Igbo	-	8(3.9)			
Religion	Christianity	12(5.8)	132(64.1)	0.334	2	0.846
	Islam	4(1.9)	56(27.2)			
	Traditional	-	2(1.0)			

DISCUSSION

The significance of assessing the prevalence of HBV and HDV in Nigeria, especially among HIV-positive people who may be at risk for various liver complications due to their epidemiological transmission routes, is crucial [**8**]. In this study, the prevalence of HBsAg among HIV positive individual is 7.8% (n=16/206). The prevalence of HBsAg observed in this study is consistent with 7.9% HBV/HIV co-infection reported by Nnakenyi et al., (2020) [14] in Southeast of Nigeria. However, the prevalence exceeds that of other studies conducted in Nigeria. These are: Northern region at 6% [15], South senatorial district at 5.47% [16], and Eastern region at 4% [17]. Compared to earlier publications from Southwestern Nigeria, where the prevalence of HBsAg was 16% [18] and 13.9% [19], this particular study is lower. The 7.8% HBV infection prevalence in the study area indicates a moderate prevalence according to WHO's [20] estimate, that categorised moderate prevalence to be 2-7.9% HBsAg. When compared to the previous reports from Southwestern Nigeria, the 7.8% HBsAg found in the study area indicates a moderate public health concern among PLWH who are on antiretroviral therapy in Ogbomoso.

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According to the study's serological testing, the frequency of HDV IgG among HBV/HIV was 7.8%, suggesting that co-infections between HBV and HDV are widespread among PLWH receiving antiretroviral therapy. Finding HDV IgG antibodies implies past HDV infection. This suggests that those who test positive had been exposed to HDV but developed antibodies. All 16 individuals who tested positive for HBsAg also showed positive results for HDV IgG, this might be due to the fact that those who have HBV are also susceptible to having HDV, due to modes of transmission of both HDV and HBV [2], [8], [18].

The seroprevalence of HDV IgM in HBV/HIV co-infected people in this study is 0 %. All the HBsAg positive individuals tested negative for HDV IgM. The absence of IgM means no active or recent HDV infections in the sampled HIV population. The report is in contrasts to the study of Anejo-okopi *et al.*, [**16**], who has a high prevalence of 13.3% in Northern Nigeria. Since HDV IgM indicates chronic and acute infection [**21**], the absence of HDV IgM indicates resolution of HDV infection which may be due to ability of the body to fight off acute infection [**10**] and not because they were treated for HDV.

In this study the prevalence of HDV IgG antibodies was higher among females (75%) study participants than in males (25%) as well as among study participants that were 37-54 (68.8%) compared to other age groups. While this is consistent with Anejo-okopi *et al.*, [**16**] and Abdulmumini & Hali [**22**] where prevalence was higher in females; for this study, it may be because there are more female participants (67%) than male (33%) and more participants (45.1%) falls within the age group 37-54 years. From the findings, the prevalence of HDV infection to global prevalence and other previous research done in southwest Nigeria is low. This means HDV is not widespread in the study population.

The risk behaviours of HIV was cross-examined with HBV/HDV infection. 37.5% of the study participants have history of blood transfusion while 6.25% have history of sharing sharp objects, even though the association is not statistically significant, but cannot be ruled out as possible cause of risk factor for HDV occurrence in HBV/HIV co-infection because of the common routes (such as sexual and parenteral) of transmission of the infection. This finding is consistent with Argirion et al., [**8**] who established that HDV infection is more common in PLWH due to shared modes of transmission.

CONCLUSION

The result from this study shows that co-infection prevalence of HBV in HIV patients in this population reflects global trends, supporting the idea that HIV-infected individuals are at a similar risk of HBV infection as those in other regions. The seroprevalence of HDV IgG antibodies at 7.8% indicates that HDV exposure is relatively uncommon in this group, but not insignificant. Although 0% seroprevalence of HDV IgM says no acute or recent HDV infection among the participants as at the time of the study but monitoring for signs of active liver disease or viral reactivation in chronic carriers (IgG positive) is still important. The absence of IgM may also suggest that HDV transmission dynamics in the study population are currently low.

To know the severity of the HDV infection, there is a need for further clinical examination like liver functioning assessment for individuals who tested positive to HDV IgG. They should also be monitored for potential late complications related to past HDV exposure, because majority of HBV patients do not exhibit any symptoms and may not do so until the liver is severely damaged [**23**] [**24**]. This finding emphasise the importance of early and regular HBV screening, monitoring and managing HBV among HIV-positive individuals to prevent further complications. HBV vaccination as a critical public health strategy should be promoted, as preventing HBV infection also prevents HDV infection. Additionally, whenever clinically needed (for example, in the event of acute decompensation of chronic liver disease), HBsAg-positive individuals should undergo retesting for anti-HDV antibodies.

Conflict of Interest

There is no conflict of interest.

Ethical Approval

Ethical approval with NREC ASSIGNED NUMBER: NHREC/OYOSHRIEC/10/11/22 was obtained from the Ethical Review committee of the Oyo State Ministry of Health, Department of Planning, Research and Statistics Division, Ibadan, Oyo State.

Similarly, informed consent was sought from all the participants that are 18 years and above before their participation in this study. Consents was sought from the parents/guardians of the participants that were lesser than 18 years.

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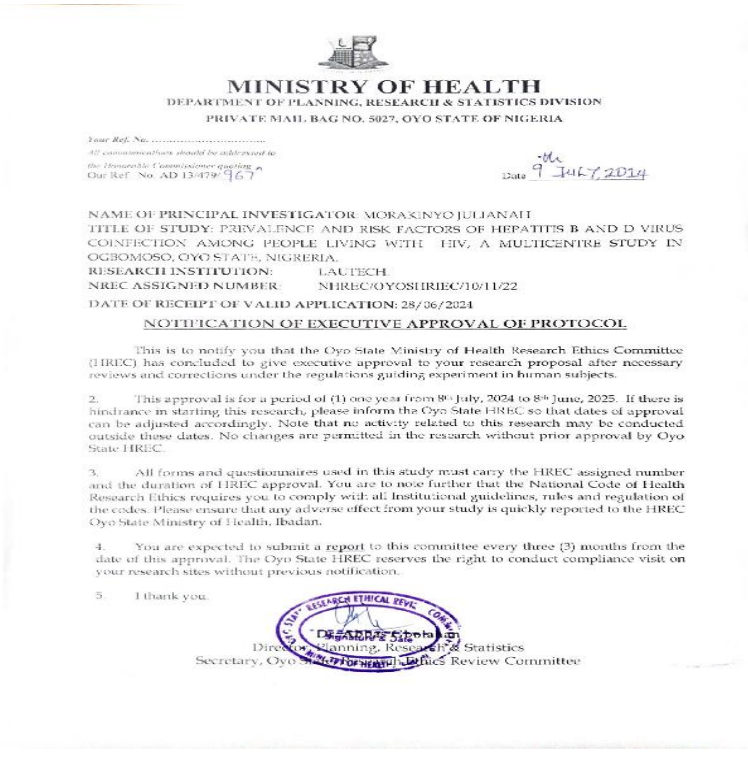

MINISTRY OF HEALTH
DEPARTMENT OF PLANNING, RESEARCH & STATISTICS DIVISION
PRIVATE MAIL BAG NO. 5027, OYO STATE OF NIGERIA

Your Ref. No. .....
All communications should be addressed to the Honorable Commissioner quoting our Ref. No. AD 13/479/967

Date 9th July, 2014

NAME OF PRINCIPAL INVESTIGATOR: MORAKINYO JULIANAH
TITLE OF STUDY: PREVALENCE AND RISK FACTORS OF HEPATITIS B AND D VIRUS COINFECTION AMONG PEOPLE LIVING WITH HIV, A MULTICENTRE STUDY IN OGBOMOSO, OYO STATE, NIGERIA.
RESEARCH INSTITUTION: LAUTECH
NREC ASSIGNED NUMBER: NHREC/OYOSHREC/10/11/22
DATE OF RECEIPT OF VALID APPLICATION: 28/06/2024

NOTIFICATION OF EXECUTIVE APPROVAL OF PROTOCOL

This is to notify you that the Oyo State Ministry of Health Research Ethics Committee (HREC) has concluded to give executive approval to your research proposal after necessary reviews and corrections under the regulations guiding experiment in human subjects.

2. This approval is for a period of (1) one year from 8th July, 2024 to 8th June, 2025. If there is hindrance in starting this research, please inform the Oyo State HREC so that dates of approval can be adjusted accordingly. Note that no activity related to this research may be conducted outside these dates. No changes are permitted in the research without prior approval by Oyo State HREC.

3. All forms and questionnaires used in this study must carry the HREC assigned number and the duration of HREC approval. You are to note further that the National Code of Health Research Ethics requires you to comply with all Institutional guidelines, rules and regulation of the codes. Please ensure that any adverse effect from your study is quickly reported to the HREC Oyo State Ministry of Health, Ibadan.

4. You are expected to submit a report to this committee every three (3) months from the date of this approval. The Oyo State HREC reserves the right to conduct compliance visit on your research sites without previous notification.

5. Thank you.

Director, Planning, Research & Statistics
Secretary, Oyo State Health Services Review Committee