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Impact of Single and Concomitant Hepatitis B Virus and Human Immunodeficiency Virus infections on Alanine aminotransferase and Bilirubin in Out Patient Departments, Benue State, Nigeria.

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ABSTRACT

This study was to determine the impact of single and concomitant HBV/HIV infections on alanine aminotransferase and bilirubin as well as the impact of Highly Active Antiretroviral Therapy (HAART) on the natural history of HBV in HIV infected individuals. Cross sectional study was carried out in three senatorial districts of Benue State, Nigeria, between April and July, 2013. A total of 219 blood samples were collected randomly from volunteered consented participants and analyzed for HBV markers (HBsAg, HBsAb, HBcAb and HBeAg) using Enzyme Linked Immunosorbent Assay (ELISA). Serum alanine aminotransferase (ALT), direct bilirubin and total bilirubin levels were quantified. Categorical variables were compared using chi square test while Univariate analysis of variance (UNIANOVA) was used to compare numerical variables in groups. Thirty (13.7%) had HBV current infection with 10.0% (3/30) carrying antigens of infectivity (HBeAg). HBsAg was lowest among HIV positive individuals on HAART (3.2%) but was highest among HIV negative subjects (16.9%). Univariate multiple comparisons shows that concomitant HBV-HIV infected individuals significantly had higher ALT compared with HIV mono-infection ($P=0.00$), HBV mono-infection ($P=0.01$), HIV positive individuals on HAART ($P=0.01$) and the control subjects ($P=0.00$). Direct bilirubin significantly had higher elevation among individuals with HBV mono-infection compared with HIV positive individuals on HAART ($P=0.03$) and non HAART ($P=0.01$). There was no individual with severe or life threatening serum elevation of ALT but one case of HBV infected male with severe elevation of total bilirubin was recorded. Hence, HBV-HIV concomitant infection significantly impact serum ALT elevation and HAART is of positive predictive impact on the history of HBV among HIV positive individuals.

Keywords: Alanine aminotransferase, Bilirubin, HBV, HIV, Impact, Liver

INTRODUCTION

Hepatitis B virus (HBV) and human immunodeficiency virus (HIV) are devastating disease agents particularly in high prevalence areas such as Sub-Saharan Africa. Concomitant HBV and HIV infections are reportedly common^[1] and have been associated with increased liver related morbidity and mortality, increased HIV viral loads, immune reconstitution to HBV in the setting of antiretroviral therapy and hepatotoxicity from antiretroviral drugs.^{2,3} Highly active antiretroviral therapy (HAART) has transformed HIV/AIDS from a fatal illness into a manageable chronic infection and has been shown to restore CD4+ T-cells in HIV infected patients.^{4,3} In addition, all currently used regimens of HAART are known to contain lamivudine (LAM) or tenofovir disoproxil fumarate (TDF), which has significant anti-HBV activity.⁵ However, studies on the prophylactic effect of antiretroviral therapy on the natural history of HBV infection in HIV in comparison with HIV negative subjects in Nigeria are rare.

In Nigeria, co-infection have been reported in 10 – 70% of HIV infected individuals,³ and their consequences on the liver, in most studies, are indirectly estimated by the quantification of alanine aminotransferases (ALT) in serum.^{6,7,1} But there is lack of information on the impact of single and concomitant HBV/HIV infections on serum bilirubin of infected individuals. Furthermore, studies on HBV in HIV positive subjects mostly make use of several groups such as blood donors as control for the study, but rarely have any made use of control that are unexposed to any HBV marker and HIV. Hence this study was designed to determine the influence of single and concomitant HBV/HIV infections on serum ALT, bilirubin and the prophylactic influence of HAART on the natural history of HBV in HIV infected individuals.

MATERIALS AND METHODS

Study Area

The study was conducted in 3 locations Benue State of Nigeria; Katsina Ala from Benue East Senatorial District, Makurdi from North West District and Adoka in Otukpo Local Government area of Benue South senatorial district. Benue State is situated between longitude 7°40' and 10°00' E, latitude 6°30' and 8°24' N, with a population of 4,253,641 by the 2006 population census figures,^[8] with a land mass of 31,400sq kilometres.

Inhabitants are predominantly farmers, with few civil servants and traders and as it is with most areas the inhabitants engaged in subsistent farming and low income earners. The literacy rate in the area is low; and the ethnic nationalities are Tiv, Idoma and Iggede. The Tiv live in scattered habitations, while the Idoma and the Iggede people live in compounds in clustered patterns.⁹

Subject Selection

A cross sectional random selection of subjects was carried out at the Out Patients Departments (OPD) of General Hospital Katsina-Ala, Adoka Maternity and Clinic Adoka, Otukpo LGA while HIV positive subjects were randomly selected in Bishop Murray Medical Centre Makurdi. HIV positive subjects were categorized into those on Highly Active antiretroviral therapy (HAART⁺) and those that were not (HAART⁻). A total of 219 subjects were sampled, 73 from (Katsina-Ala), 56 from (Makurdi) and 90 from Adoka in Otukpo LGA. There was preliminary counselling by trained medical counsellors; and HBV and/or HIV-positive subjects were given a date to visit the laboratory for a Liver function test. Other information was obtained from the subjects/participants through administration of questionnaires in English language for the educated or local dialect for the illiterate rural dwellers as well as through personal interviews.

Out of the 219 subjects registered, 106(48.4%) volunteered sera for Liver function Test (LFT).

Sample collection and analysis was carried out between April and July, 2013. After counselling on the nature and use of the specimen to be collected, 4-5 mL of venous blood was aseptically taken from each participant by venepuncture and discharged into a vacutainer. The blood was allowed to clot and centrifuged at 1000g for 10 minutes. Serum was aspirated with Pasteur pipettes and was stored in sample vials at 17°C until used for analysis.

Assay of Blood Samples for HBV Serological Markers

All Sera were assayed for three Hepatitis B virus markers Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody (anti-HBs) and Hepatitis B core antibody (anti-HBc) using. Sera that were reactive for HBsAg were further tested for Hepatitis B early antigen (HBeAg).

Two assay techniques were adopted to determine presence and level of these HBV markers in serum using Enzyme Immunosorbent assay (ELISA) kits (Diagnostic Automation /Cortez diagnostics inc. USA). The kits

consisted of a 96-well polystyrene titer-plate, each well pre-coated with monoclonal antibodies or antigen specific for antibodies or antigen. For the test proper approximately 50µL of the patient's serum sample was added to the microwell together with antibody horseradish peroxidase conjugate (HRP conjugate). The experiment was incubated for 60min at 37°C in a microwell incubator (Model: STAT FAX 2200, Awareness Technology Inc, USA).

After incubation the microwells were washed 5 times with an automatic plate washer (Model: STAT FAX 2600, Awareness Technology, USA) to remove excess sample and unbound HRP-conjugate. About 50µL Chromogen solution A containing tetramethylbenzidine (TMB) and 50µL urea peroxide (Chromogen solution B) were added into the wells. After further incubation for 15 min at 37°C, blue colour developed and the reaction was stopped by adding sulphuric acid solution, also provided with the kit. The blue colour turned yellow after stopping the reaction with sulphuric acid.

The absorbance was measured using double filters at 450 and 630nm respectively in an automated micro-plate reader (Model: Stat Fax 2100, Awareness Technology Inc, USA). The cut-off for each batch was calculated using the mean optical densities of the negative control in accordance with the manufacturer's instruction. This cut-off value was accordingly used to calculate the activity index for each sample. Samples with the activity index values higher or equal to those of positive control were considered positive, while those with values below were reported as negative.

Quantitative determination of Serum ALT and Bilirubin

Quantitative determination of serum ALT was carried out using Reflotron Plus kit with the supplied test strips (Roche, Germany). Assay procedure was according to manufacturer's guide and ALT enzyme activity was measured kinetically at 567nm and the result displayed after 140 sec. for a temperature of 25°C.

Normal reference values of the enzyme activity at 25°C were 22u/l for males and 17u/l for females.

Determination of total bilirubin (TBIL) and direct bilirubin (DBIL) was carried out using Randox U.K manual Bilirubin reagent. The test was a colorimetric method in which Direct (conjugated) bilirubin reacted with diazotisedsulphanilic acid in alkaline medium to

form a blue coloured complex. The OD₅₄₆ was read after 15 min incubation at 25°C. Total bilirubin was determined in the presence of caffeine, which releases albumin bound bilirubin by reaction with diazotisedsulphanilic acid and the OD₅₇₈ was read after incubation at 25°C for 15 minutes. All ELISA assays were done in the ELISA laboratory of Biological Sciences Department, Benue State University Makurdi while serum alanine aminotransferase (ALT) and bilirubin quantification were carried out in the Clinical Laboratory of Bishop Murray Medical Centre Makurdi.

Statistical Analysis

Data generated were entered into a computer software; Statistical Package for Social Sciences (IBM SPSS version 20.0). ALT and direct bilirubin were graded according to standard Adults AIDS Clinical Trials Group (ACTG) definition.¹⁰ Comparisons between groups were made using Chi square (χ^2) test. Univariate analysis of variance (UNIANOVA) was carried out to test the differences in ALT and Direct Bilirubin levels between individuals with HBV, HBV/HIV, HIV on HAART, other HIV subjects and the Control group. Multiple comparisons between these subgroups were made so as to ascertain group(s) with the least significant difference (LSD). Probability values (P values) less than or equal to 0.05 were considered significant.

RESULTS

Out of 219 that participated in the study, 30(13.7%) had HBV current infections, of which 3(10.0%) were positive for HBsAg, an antigen of infectivity. HBsAg was 16.9% and was higher among HIV negative individuals (23/136) compared to 8.4% (7/83) HIV positive subjects (P=0.08). Antibodies of immunity (HBsAb) were found in 55(25.1%) of the individuals with or without HBV vaccination. Eighty seven (38.8%) had HBcAb alone (indeterminate), while 49(22.4) had no evidence of exposure to HBV (Table 1). Only one subject (3.2%) among those on HAART had HBV current infection, 2(8.0%) of HIV positive individuals that were monitored but had not started receiving HAART were currently infected with HBV while HBV highest infection (16.9%) were among HIV negative individuals (Table 1).

Though HIV-positive males had slightly higher HBV

Table 1: Hepatitis B virus Infection Status of Subjects in Benue State n(%)

Subjects	Number Tested	Currently infected	Immune	Indeterminate	Unexposed
HAART ⁺ HIV ⁺	31	1(3.2)	8(25.8)	6(19.4)	16(51.6)
HAART ⁻ HIV ⁺	25	2(8.0)	4(16.0)	8(32.0)	11(44.0)
HIV ⁺ singly	27	4(14.8)	9(33.3)	12(44.4)	2(7.4)
HIV ⁻ singly	136	23(16.9)	34(25.0)	59(43.4)	24(14.7)
TOTAL	136	30(13.7)	55(25.1)	85(38.8)	49(22.4)

infection (11.8%) than HIV-negative males (10.5%), HIV-positive females had significantly ($P=0.020$) lower HBV prevalence than their HIV-negative counterparts (Table 2).

On the whole, HBV infection was higher in Adoka

Table 2: Prevalence of HBsAg among HIV positive and HIV Negative Subject According to Sex

Sex	HIV Test	Total number Tested	HBsAg+ (%)	P value
Male	Positive	17	2(11.8)	0.89
	Negative	57	6(10.5)	
	Total	74	8(10.8)	
Female	Positive	66	5(7.6)	0.02*
	Negative	79	17(21.5)	
	Total	145	22(15.2)	

*significant

(18.9%) than in Katsina-Ala (16.0%) but lowest in Makurdi (5.4%). There was no difference in HBV infection rates between HIV-positive and HIV-negative individuals in Katsina-Ala ($P=0.68$) and Adoka ($P=0.49$). However, in Katsina-Ala, HIV-positive subjects had apparently higher HBV infection rate (16.0%) than HIV-negative individuals. None of the 2 HIV-positive subjects in Adoka was infected with HBV.

Of the 106 individuals that made available their sera for the Liver function Test (LFT), 45 (42.5%) were HIV-positive, 22 (20.8%) were singly infected with HBV while 6(5.7%) were concomitantly infected with HBV and HIV. Out of the 45 HIV singly infected, 21(19.8%) were on HAART (HIV⁺HAART⁺) while the remainder 24(22.6%) were either not on HAART or their ART status was unknown but were grouped as those not on HAART (HIV⁺HAART⁻). Twelve (11.3%) of the

participants were neither positive for HBV or HIV and served as the control group.

In a Univariate analysis ALT level in serum was significantly associated with the type of subject under the study ($P=0.01$). Multiple comparisons show that concomitantly HBV/HIV infected individuals significantly had higher ALT compared with HIV single infection ($P=0.00$), HBV single infection ($P=0.01$), HIV⁺HAART⁺ individuals ($P=0.01$) and the Control subjects ($P=0.00$). There was a significant difference between ALT of the Control group and that of HBV positive subjects ($P=0.06$), but no difference between ALT of Control and HIV⁺HAART⁺ individuals ($P=0.08$) as well as HIV⁺HAART⁻ subjects ($P=0.10$) (Figure 1). HIV individuals who were on HAART for less than 2 years had higher ALT levels compared to those 2 years and above but the difference was not significant ($P=0.75$).

Similarly, there was a significant association between Direct Bilirubin (DBIL) expressed in sera and the groups in a univariate analysis ($P=0.02$). Multiple comparisons shows significantly higher DBIL elevation among individuals with HBV infection compared with HIV⁺HAART⁺ ($P=0.03$) and HIV⁺HAART⁻ subjects ($P=0.01$) but, there was no significant difference in DBIL elevation between HBV subjects and the control group ($P=0.83$). The control group also had higher DBIL elevations compared with HIV ($P=0.04$) as well as HIV⁺HAART⁺ individuals ($P=0.02$) (Figure 1).

Indirect estimation of the severity of the infections shows

Figure 1: Mean Alanine Aminotransferase (ALT) and Total Bilirubin (TBIL) Expressed in Sera of the Studied Subjects in Benue State

that no individual had Grade 3 (severe) and Grade 4 (Life threatening) elevation of ALT, but one case of HBV infected male with Grade 3 elevation of total Bilirubin was recorded (Table 3).

Table 3: Infection Severity Due to ALT and TBIL Serum Expression among Subjects in Benue State

Parameter	Sex	Total tested	Normal	Mild	Moderate	Severe	P value
ALT	Male	24	19(79.2)	5(20.8)	0(0.0)	-	0.40
	Female	82	72(87.8)	9(11.1)	1(1.2)	-	
	Total	106	9(85.8)	14(13.2)	1(0.9)	-	
T Bil	Male	23	16(69.6)	4(17.4)	2(8.7)	1(4.3)	0.01*
	Female	82	73(89.0)	9(11.0)	0(0.0)	0(0.0)	
	Total	105	89(84.8)	13(12.4)	2(1.9)	1(1.0)	

*significant

DISCUSSION

Seroprevalence of HBsAg of 13.75 observed in this study among HIV positive and HIV negative individuals exceeds the upper limit (8.0%) of WHO classification for HBV intermediate endemicity areas.¹¹ HBV infection rate of 8.45 among HIV positive individuals in this study reveals a decline when compared with 27.8%, 25.0% and 16.7% reported in Keffi⁴, and in Jos,^{12,3} respectively in North central Nigeria. The finding is also lower compared with 13.4% and 12.3% reported in South Eastern Nigeria,^[13,14] while 26.5% and 12.1% were documented in Gombe,¹⁵ and in Maiduguri⁷ in North Eastern Nigeria, respectively. Most of these authors studied these subjects on their first visit either as Outpatient with symptoms suggestive of HIV^[15, 12, 4] or as pre-ART HIV confirmed patients to be enrolled for care and HAART.³ Conversely, we investigated a cross section of outpatients whose HBV/HIV status were unknown, confirmed HIV-positive patients on HAART (HIV⁺HAART⁺) and HIV⁺HAART⁻ individuals who were closely monitored. Hence, a sharp decline in the prevalence of HBV among HIV positive individuals in this study is suggestive of the dual effect of currently used HAART regimen on both HBV and HI Reports have shown that, currently used regimen of H A A R T c o n t a i n s l a m i v u d i n e o r tenofovir disoproxil fumarate which has significant anti-HBV activity.^{16,5}

This may explain the 3.2% HBV prevalence among HIV positive individuals on HAART compared with 8.0% non HAART, 14.8% HIV positive of unknown care records

and 16.9% recorded among HIV negative individuals (Table 1). The HBV infection rate of 3.2% among HIV⁺HAART⁺ subjects in this study is similar to 3.2% (51/1800) also reported among adults enrolled for care in Irrua Specialist Teaching Hospital, Edo State in Nigeria.¹⁷ In addition, 8.4% recorded among individuals enrolled for care in our study is comparable to 7.8%, 7.0%, and 7.7%, respectively reported among children on HAART in APIN Makurdi,¹⁸ HIV antiretroviral treatment naïve patients in APIN Lagos,¹⁹ and among children on an antiretroviral treatment programme in Benin, Nigeria.²⁰ These further buttress the dual effect of HAART on HIV and natural history of HBV in a region where HAART usage is rapidly expanding. However, this study suggests a prospective cohort study involving a larger population to further assess the impact of the current HAART regimen on the natural history of HBV in Nigeria.

Furthermore, HBV infection is reportedly common in HBsAg-negative/HBcAb-positive HIV infected patients and is predicted by undetectable HBsAb.²¹ Hence, 44.4% prevalence of HBcAb in this study with no detectable HBsAb may indicate occult HBV infection which may be associated with lower CD4⁺ T cell counts.^[3] Lower prevalence (19.4%) HBcAb detected alone and highest rate (51.6%) of unexposed individuals to HBV recorded among HIV positive on HAART compared with other HIV subjects in this study may also be attributed to the dual effect of HAART on HIV and HBV. A study on the predictors and kinetics of hepatitis B virus infection in HIV infected persons,²¹ explains that occult HBV in HIV positive individuals could become repeatedly undetectable for HBV-DNA over a median of 19 months in individuals on antiretroviral therapy containing lamivudine or Lamivudine/Tenofovir.

Significantly lower HBV prevalence among HIV positive females compared with their HIV negative counterparts in this study may also be linked to treatment, care and close monitoring that is given to people living with HIV and AIDS in our study area. More so that most unmonitored HBV infected are clinically asymptomatic or are biochemically normal or near normal.²² Thus, explaining highest HBV rate of 18.9% recorded in Adoka, an area with lower HIV prevalence than Katsina- Ala with higher HIV rates, but with a lower HBV prevalence.

Our results demonstrated that individuals with HBV-HIV concomitant infections had significantly higher mean ALT elevation than HIV mono-infection (p=0.00). This has

been reported by other researches within and outside the Nigeria.^{3,7,1}

However, this finding contrasts the report of Anigilaje and Olutola^{1,8} who found no significant impact of HBV-HIV on the mean ALT and hepatotoxicity. The disagreement may be as a result of their sample population being children compared to others who studied mainly adults.

We could not quantify HBV-DNA levels in our study but Bhattacharya *et al.*⁶ dissociated serum ALT elevation from HBV-DNA titre, but higher ALT levels have been associated with high HBV-DNA and HBeAg by Idoko and his colleagues³ in Jos, Nigeria. They also found that elevated baseline ALT is associated with increased risk for hepatotoxicity and recommended close monitoring of ALT levels at ART initiation. Our study could not involve subject at baseline initiation of HAART, but, higher ALT levels found among individuals less than two years on HAART agrees with the recommendation of Idoko *et al.*³

No individual in our study had severe or life threatening ALT elevation, but one case of moderate (Grade 2) ALT elevation in an HIV positive female patient was recorded. Jaundice is associated with Hepatitis B virus infection due to destruction of the infected hepatocytes and intrahepatic cholestasis caused by scars of the healed hepatocytes. This could explain a significant high direct bilirubin levels found in HBV mono-infected individuals compared with HIV⁺HAART⁺ (P= 0.04) and non HAART HIV infected HIV⁺HAART⁺ patients (P = 0.02). The higher direct bilirubin elevation among our control subjects was unexpected. However, this could be due to underlying infections not considered in our study such as HCV or Malaria.

CONCLUSION

This study found that HBV-HIV concomitant infection significantly impact serum ALT elevation and HAART is of positive predictive impact on the history of HBV among HIV positive individuals. However, the study could not associate high levels of serum bilirubin to HBV/HIV infections, when compared with the control subjects.

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Competing Interests

Authors have declared that no competing interests exist.

Authors' Contributions

Author 1* was a Ph.D student supervised by authors 2 and 3 who were part of designing and protocol writing while authors 4 and 5 managed the analyses of the study.

Consent

All authors declare that participants voluntarily signed consent forms either in own handwriting or with thumb prints as proof of willingness to provide samples for the tests. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

Ethical Approval

Ethical clearance was sought and obtained from the ethics committee of Benue state Ministry of Health and Human Services Makurdi. In respect of confidentiality, all data were kept anonymous in accordance with World Medical Association (WMA) declaration of Helsinki.^[23]

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