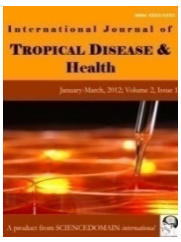

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The Effect of Co-infection of HIV and Hepatotropic Viruses on Selected Biochemical and Haematological Markers of Patients in Northeastern Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SOO and HAB designed the study, wrote the protocol and carried out the experiments. Author SOO wrote the first draft of the manuscript. Authors MMB and GIA supervised the experiments. Author MMB produced the final copy and managed the analyses of the study. Author AB did the statistical analysis of the data. All authors read and approved the final manuscript. Presently, all the authors of this article are actively involved in health research generating information that would promote the health of people in their respective countries.

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ABSTRACT

Background: As deaths caused by HIV declines with the use of HAART, liver disease associated with co-infection of HIV with hepatotropic viruses has become an increasing cause of morbidity and mortality.
Aim: To assess the effect of HIV-mono and co-infections with hepatotropic viruses on haematological and biochemical markers of the patients.
Methodology: 109 HIV patients from tertiary health facilities in northeastern Nigeria were initially screened with Immuno chromatographic kit for HIV antibody and confirmed by western blot prospectively and consecutively. However, Hepatitis B virus surface antigen (HBsAg) and Hepatitis C virus (HCV) antibody were detected on the HIV positive patients

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by ELISA. Blood donors served as control. The study was conducted between January and October 2012.

Results: Of the HIV patients 12.8% and 4.6% had HBsAg and HCV antibody respectively. The prevalence rate of Hepatitis B virus (HBV) infection among males was 12.8% while females had 12.9% but lower rates of HCV were obtained in both males (5.1%) and females (3.3%). However, HIV mono-infections had higher mean baseline values for haemogblobin (Hb), CD4 and platelet counts, protein C (PC) and protein S (PS) in comparison with HIV/ HBV/HCV co-infections ($P<0.05$). In addition, Prothrombin time and partial thromboplastin time were lower in HIV mono- infection in contrast to co-infections ($P<0.05$). Similarly, the mean values of Serum liver enzymes such as Aspartate transaminase (AST), Alanine transaminase (ALT), Akaline Phosphatase (ALP) and creatinine were lower in HIV mono-infection compared with HIV/HBV or HIV/HCV co-infection ($P<0.05$). Total white blood cell count (WBC), total cholesterol (TCH), Random blood sugar (RBS) and potassium ($K^{+)}$ were not significantly different ($P>0.05$) in both groups.

Conclusion: Co-infections of HIV and hepatotropic viruses do occur. Haematological and biochemical parameters serve as pointers for early detection of liver disease in HIV patients. The development of novel therapeutic approaches to impede co-infection of HIV and hepatotropic viruses is encouraged.

Keywords: HIV; hepatitis B virus; hepatitis C virus co-infection; haematological and biochemical markers.

1. INTRODUCTION

Sub Saharan Africa which is the most impoverished regions of the world, bears the highest burden of HIV infection [1,2]. In Eastern and Southern Africa, the number of people living with HIV accessing antiretroviral therapy increased from 625 000 in 2005 to 6.3 million in 2012 with high level coverage in several countries including Botswana, Namibia, Rwanda, Swaziland and Zambia [3]. In West Africa, Ghana has recorded a lowly 1, 37 percent of the population infected with the virus and a significant reductions in new infections among the youth, pregnant women and children [4]. Previous reports have revealed that, the prevalence of HIV in Nigeria has been stabilized within the range of 4.4 and 4.1 from 2005 to 2010 [5]. Other studies had observed HIV/HBV sero-prevalence rate of 5.5% – 12.3% [6-10] and HIV/HCV rates of 0.5 – 0.7% [6,9-11].

In HIV mono-infections, Cytopenia particularly anaemia (from multiple causes), thrombocytopenia and Thrombotic thrombocytopenic purpura (TTP) are common [12,13-14]. The incidence of thrombosis in HIV patients has increased two-tenfold compared to healthy control population of the same age [15]. In support of that report, Opei [13] observed that, the risk is highest with advanced disease and co-existing infections and malignancies. Furthermore, that report revealed that thrombosis could disrupt the normal balance of coagulation factors, thereby increasing the prothrombotic proteins such as Von Willebrand Factor (VWF). This protein consequently reduces the natural anticoagulant proteins such as protein S and protein C. In another report, 27-73% of HIV patients have increased levels of complement binding protein 4 which attaches to protein S and renders this protein inactive[12,13].

A significant increase in serum liver marker enzymes (ALAT, ASAT, and Alkaline phosphates) in HIV/AIDS patients had been reported [16,17]. Liver transaminases are useful biomarkers of liver injury in individuals with some degree of intact liver function. Most liver diseases cause only mild symptoms initially, but it is vital that these diseases be detected early [16]. In Nigeria, an elevated liver transaminase enzymes (AST: 34.311 U/L, ALT: 38.47 U/L and ALP: 97.31 U/L) among HIV infected patients have been reported [14]. Although the values of the enzymes in that study, were within the normal reference range but the authors advised against prolonged treatment with ritonavir to avoid drug-induced liver injury with elevated hepatic enzymes [14,18]. A raised mean serum ALT concentration above the acceptable range is a strong predictor of insulin resistance [19] and principally reflects direct hepatocellular damage or liver dysfunction. Liver dysfunction secondary to underlying hepatitis C and hepatosteatosis have been demonstrated to be associated with Diabetes Mellitus [20-23]. In addition to liver enzymes, the amount of creatinine in the body can serve as a useful marker to estimate muscle mass. When muscle mass decreases for any reason (HIV/AIDS, muscular dystrophy, paralysis, etc), both urinary and serum creatinine levels decrease [16].

Since the principal routes of HIV and hepatotropic viruses such as HBV and HCV are similar, it is possible that co-infection of these viruses with HIV could occur. As deaths caused by HIV disease are declining due to the introduction of Highly Active Antiretroviral Therapy (HAART), liver disease associated with co-infection of HIV with hepatotropic viruses has become an increasing cause of morbidity and mortality [24]. It has been estimated that one third of deaths in HIV patients are related to liver diseases [25] and HBV/HIV, as well as HCV/HIV co-infections have been associated with reduced survival due to increased risk of progression to cirrhosis and hepato-cellular carcinoma [26]. Infections of HIV/HBV co-infection is linked most often to sexual intercourse while HIV/HCV co-infection has been more frequently associated with non-sexual parenteral route [27]. However, there is paucity of information on the impact of HIV/HBV/HCV co-infections on some baseline pathological markers which could serve as predictors for early detection of liver diseases. Yet, HIV and hepatic disease are independently known to cause a varied range of haematological and biochemical changes. This is partly because of the enormous assault of these viruses on haemopoietic cells/system and the role of the liver in haemopoiesis and coagulation [15]. In addition, hepatotoxicity of Antiretoviral Therapy (ART) administered to AIDS patients, impact negatively on the central role of the liver in many metabolic processes. Consequently, these diseases pose a serious risk on the patient's health [28-29].

With this background, we set out to investigate the implications of these co-infections on some haematological and biochemical biomarkers in patients attending health care facilities in North Eastern Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This was a prospective cohort study conducted between January and October 2012 among HIV positive patients. The study sites were Federal Medical Centre, Ashaka, Gombe State and University of Maiduguri Teaching Hospital (UMTH) Borno State. UMTH is a 560 bedded health facility designated as a Centre of Excellence for infectious disease and immunology. It provides primary, secondary and tertiary health Services for the North Eastern part of Nigeria. It also houses a major HIV care and support Centre and a National blood

transfusion Centre. Neighboring countries (Cameroun, Niger and Chad Republics) avail themselves of services provided by UMTH. Maiduguri the capital of Borno State is the largest settlement in the fringe of Sahara desert and near to the Lake Chad. It is located between longitude 11º8'E and 14ºE and latitude 10º2'N and 1304'N.

2.2 Study Population

Patients and blood donors confirmed to be seropositive for human immunodeficiency Virus (HIV) infection by Western blot technique were used for the study. Males were 78 (72.2%) and females 31 (27.7%).The ages of the patients ranged from 16 to 59 years with a mean age 36±20 years for both sexes. Age and sex matched apparently healthy subjects who served as controls (blood donors: N=100) consisting of 78 (78%) males and 22 (22%) females were also recruited.

The confirmed HIV positive patients were on highly active antiretroviral therapy (HAART) regimes of the APIN PEPFAR program of the institution. The choice of drug combination administered to each patient was within the jurisdiction of the clinician and all the patients were followed up for a minimium of six months. Worthy of note is that different drug combination was administered to patients co-infected with either HBV or HCV. Confirmed HIV patients were also screened for hepatitis B virus (HBV) and hepatitis C virus (HCV). Information concerning sexual behavior, blood transfusion, intravenous drug use and demographic data were obtained through a brief structured questionnaire and laboratory records. Informed consent and pre-test counseling were also instituted. One hundred apparently healthy blood donors who were seronegative for HIV, HBV and HCV served as control.

2.3 Sample Collection and Analysis for Haematological and Biochemical Paremeters

10 ml volume of venous blood was obtained from each participant; 2ml was dispensed into EDTA containers for Haemoglobin (Hb), white blood cell count (WBC) and platelet (PLT) count. Haematological counts were performed using automated blood analyzer, Haematology analyser –model, Sysmex Kx-21 S/N A8893 (Sysmex corporation Kobe-Japan) and its dedicated reagent kits, Sysmex Europe GMBIH (Bornbarchl Nordergledt). 4ml of blood was collected into plastic bottle containing sodium citrate (0.11molar solution) to give a final blood/citrate ratio of 9:1. Platelet poor plasma obtained by centrifuging immediately at 3000g was used for prothrombin time, partial thromboplastin (PTT) time, protein C (PC) and protein S (PS) assay through an automated Automated Coagulometer-Model, Sysmex 560 S/N 1016 (Sysmex corporation kobe Japan) using reagent acquired from Reagent kits from Siemens Health care diagnostics products GMbH 36041 [Marburg, Germany]. A 4ml volume of blood was allowed to clot in a plane blood bottle and serum separated immediately and used for Aspartate transammase (ASP) Alanine transaminase (ALT), Alkaline phosphatase (ALP), Creatinine (creat), total cholestroral (TCH), random blood sugar (RBS) and Potassium (k+). The analysis was carried out using Biochemistry autoanalyser-Model, Hitachi-cobas C311 luems S/N0 46-02 (Cobas Roche GmbH-D68298 Mannheum) and Reagent kits from Roche GmbH-D68298 (Mannheum).

2.4 Screening for HIV antibodies

HIV screening was carried out using Immuno chromatographic kit (Chembio HIV 1 and 2 STAT-PAK). Positive samples were further confirmed by Western blotting (Qualicode^{TM} HIV 1 and 2 kit).

2.5 Screening for HBsAg by Enzyme- linked Immunosorbent Assay (ELISA)

The ELISA kit from BIORAD Monolisa HBsAg ULTRA EIA92430 Marnes-La-Coqutte- France was used. The instruction of the manufacturer was strictly adhered to. Briefly, HBsAg in the serum was captured by monoclonal antibodies coated to the micro titre plate. Reaction was actualized by an enzyme peroxidase labeled with conjugated monoclonal antibody from mouse and polyclonal antibody from goat directed against the captured HBsAg. After incubation, the unbound antigen was washed off. The antigen-antibody complex conjugated to the enzyme hydrolyzed the substrate in the subsequent step resulting in a color change. The Optical density OD was read at 450/620 to 700 nanometre. In each plate four negative (Tris HCl buffer containing BSA) and one positive control were added. The cut off value was determined by the mean of negative control + 0.05 (0.08). The test was valid if all values of negative control were lower or equal to 0.08 and Positive control was over 0.08 or equal to 1.0. A test sample was considered negative if the ratio value of sample: cut off value was lower than 1.0 and positive if equal to or greater than 1.0.

2.6 Screening for HCV Antibody by ELISA

ELISA kit from DIA PRO Diagnostic Bioprobes 20099 Sesto San Giovanni (Milano)-Italy was used. Microtitre plates were coated with HCV-specific antigens derived from 'Core' and 'NS' region encoding for conservative and immune-dominant antigenic determinants (Solid phase). The solid phase was first treated with the diluted serum sample and the HCV antibody were captured by the antigens. After Incubation and washing to remove the unbound antibody, polyclonal antibody conjugated to peroxidase was added to the plate. The antigen-antibody complex with the enzyme hydrolyzed the substrate in subsequent reaction resulting in color change. The Optical density OD was read at 450/620 to 700 nanometre. The cut –off value was calculated as follows:

NC (negative control) +350= cut-off (C), Calibrator mean value=0.540, S/C=1.4 (where S= sample and C- cut off). S/C = higher than 1.1. Any sample with a ratio value of sample /cut off less than 0.9 was considered negative and if higher than 1.1 was positive.

3. STATISTICAL ANALYSIS

Statistical package for social science (SPSS) version 17 was used. Statistical comparison between HIV mono-infections, co-infections and each haematological and biochemical parameters were performed by ANOVA (one way). Data was presented as mean (x) and standard deviation (SD). The level of significance was taken at 95% confidence interval and P value less than 0.05 was considered significant.

4. RESULT

A total of 109 HIV positive patients were enlisted for the study. Males were 78 (72.2%) and females 31 (27.7%). The mean age of both sexes was 36±20 (16 – 59) years. Age and sex

matched apparently healthy subjects (blood donors: N=100) consisting of 78 (78%) males and 22 (22%) females were also recruited. These served as controls against the HIV infected individuals. The number in the control group should have been similar to the test (109) but due to both financial and logistic constrains, only 100 subjects were recruited. All the patients studied attested to having heterosexual relationship but had neither blood transfusion nor intravenous drug use. Out of 109 HIV positive patients, 14 (12.84%) were positive for HBsAg and 5 (4.6%) had HCV antibody.

Table 1 represents the age distribution of HIV mono-infections, co-infections of HIV/HBV and HIV/HCV. The prevalence rate of HIV mono-infections appeared to decrease with increase in age with the least (3.7%) at ages 10-19 and 50-59years. Although a similar trend was observed in HIV/HBV co-infection, those co-infected with HCV showed no defined pattern. However, more HIV/HCV co-infections were detected in those aged 40-49 years compared with other age groups. There was no age related differences between or within groups (*P=05*).

Table 1. Age distribution of patients with HIV mono-infections, HIV/HBV and HIV/HCV co-infections

HIV mono-infections			HIV/HBsAg			HIV/HCV		
Age group	**No Tested**	**% positive**	**Age group**	**No Tested**	**% positive**	**Age group**	**No Tested**	**% positive**
10 – 19	14	3.7	14	0	0	14	1	0.9
20 - 29	43	39.4	43	9	8.3	43	0	0
30 – 39	42	38.5	42	5	4.6	42	0	0
40 – 49	16	14.7	16	0	0	16	3	2.8
50 – 59	4	3.7	4	0	0	4	1	0.9
Total	109	100	109	14	12.9	109	5	4.6

The gender of patients with HIV mono and co-infections is presented on Table 2. A total of 14 (12.96%) of 109 HIV patients were positive for HBsAg, 10 (12.82%) males and 4 (12.90%) females. HIV/ HCV co-infection was positive for 5 (4.6%); males, 4 (5.1%) and female 1 (3.3%). A similar prevalence rate of HIV/HBV was observed among males and females but in HIV/HCV co-infection, more males were infected than females. The controls (blood donors) neither had HIV nor the hepatotropic virus antibodies.

Table 2. Gender distribution of patients with HIV/ HBV and HIV/ HCV antibodies

Gender	**No Tested**	**HIV positive (%)**	**HIV/HBV positive (%)**	**HIV/HCV positive (%)**
HIV patients				
Males	78	78	10 (12.82)	4 (5.1)
Females	31	31	4 (12.9)	1 (3.2)
Controls				
Males	0	0	0 (0)	0 (0)
Females	0	0	0 (0)	0 (0)
Total	**209**	**109 (52.1)**	**14 (6.7)**	**5 (4.6)**

Table 3 depicts the haemotological parameters of HIV mono-infection, co-infected patients and the controls. The mean values of all the hematological parameters used were significantly lower in both mono and co-infections compared to the control groups. Low CD4 and Platelet count (PLT) values were common in the three group of infections studied (P<.001). Similarly, low values of CD4, PLT, and Protein C (PC) were common in both co-infections (P<.001). However, protein S (PS) was more affected (P<.001) in HBV than HCV co-infection (P=.000) while Haemoglobin (Hb) was significantly lower (P<.001) in HCV than HBV co-infection (P=000). Overall, lower mean values of Hb, PS, PC, prothrombin time (PT), and white Blood Cells (WBC) were significantly lower in co -infections compared to HIV mono-infection infection.

Table 3. The haemotological parameters between HIV-mono and co-infected patients

Parameters	HIV Mono-infection	HIV /HBV	HIV/HCV	Controls
Hb g/dl	11.29±1.89	10.02±1.19	9.060±1.02	13.7±0.91
Wbc ($x10^9$/L)	5.46±1.56	4.14±1.08	3.72±0.62	5.30±1.38
CD4 (cell/µL)	217.28±47.56	109.89±49.40	156.40±24.17	504.12±213.89
PLT ($X10^9$/L)	198.13±60.64	111.21±31.78	153.80±58.45	274.60±85.24
PT (Seconds)	13.00±11.98	14.70±0.97	14.30±1.20	12.4±2.14
PTT (Seconds)	34.88±14.99	49.64±9.06	46.40±9.12	31.90±5.74
PC (%Acitivity)	82.02±10.32	67.79±8.44	74.00±13.02	94.22±13.50
PS (%Activity)	80.07±10.93	68.14±14	54.060	95.71±17.06

**Hb=Hemaglobin, WBC=White Blood Cells, CD4=Cluster of Differentiation 4, PLT=Platelet, PT=Prothrombin Time, PTT=Partial Thromboplastin Time, PC=Protein C, Protein S.*

Table 4 displays the mean values of some biochemical markers of the HIV mono-infection and co-infection with hepatotropic viruses. The mean values of Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), and creatinine were significantly lower in the three groups compared to the control. Low mean values of AST were common in both HIV mono and co-infections. However, ALT (P=.001) and creatinine (P<.001) were significantly lower in both co-infections than mono-infections. Total Cholesterol (TCH), Random blood (RBS) and Potassium (K+) were not significantly different in the three groups of infection.

Table 4. Biochemical parameters between HIV mono and co-infections

S/No	Biochemical Parameters	HIVmono-infections	HIV/HBV	HIV/HCV	Controls
1	AST (u/l)	23.66±7.57	66.93±20.74	66.80±15.64	14.7±11.56
2	ALAT (u/l)	24.89±8.14	46.00±15.63	36.60±9.29	25.0±11.56
3	ALKP (u/l)	65.30±22.37	94.36±13.608	102.80±9.71	73.30±29.63
4	CREAT (µmol/l)	59.75±26.95	89.5±24.80	91.60±17.69	40.00±31.39
5	TCH (mmol/l)	5.23±4.41	5.057±1.57	4.12±0.69	5.91±1.31
6	RBS (mmol/l)	4.70±1.64	4.60±1.18	3.78±1.25	5.0±1.96
7	K^+ (mmol/l)	3.84±1.30	4.27±1.01	3.20±0.61	3.44±0.76

**AST=Aspartate transaminase, ALT=Alanine transaminase, ALP=Alkaline phosphatase, Creat=Creatinine, TCH=Total Cholesterol, RBS=Random blood sugar, K+=Potassium*

5. DISCUSSION

Diverse degree of immunopathogenesis in HIV infected patients carries enormous haematological and biochemical consequences [14]. In this study, most of those studied

were heterosexually active with a substantial number having multiple sex partners. None of the study population received blood transfusion or engaged in intravenous drug use (IDU) but all had heterosexual relationships in agreement with previous reports [27,30]. This may be responsible for the low prevalence rate of HIV/HCV in this study in support of the report that HCV is not efficiently transmitted by sexual routes [6,10,30] but by percutaneous exposure to blood/products and IDU in particular [9,31]. It has been reported that IDU is the most efficient route for triple infection with HIV/HBV/HCV in an urban population [30,32,33]. This may explain why none of the patients in this study presented with triple infection. However, this study recorded HIV/HBV co-infection prevalence rate of 12.8% in agreement with Otegbayo et al. [11] who reported a similar rate in South Western part of Nigeria.

In this study, the mean values of CD4, Hb, PLT, PC, PS, WBC, PT and PTT were significantly different in HIV mono and co-infections from the control group. These patients were receiving HAART at the time of sample collection. The limitation of this study lies in the fact that, we did not know the drug combination administered to HIV patients and the duration of drug administration at the time of sample collection. Nevertheless, our finding compares favorably with the reports that HIV disease impacts adversely on haematological profile *o*f a patient due to the enormous assault of the virus on haemopoietic cells/system [34,35]. This occurs in addition to hepatotoxicity and nephrotoxicity of anti- retroviral drugs (ARD) administered to AIDS patients [34,35]. In this study, lower mean values of Hb, PS, PC, PT and WBC were observed in co-infections than mono-infection. Although, cytopenia particularly anaemia (indicated by low mean Hb values) are common in HIV infected patients [12-14], the mean value was lowest in HIV/HCV co-infection. This finding compared favorably with a report that Hemoglobin concentration was significantly lower in the HIV/HCV co-infected patients compared with the HCV mono-infected control subjects [36]. These authors speculated that the resultant effect of HIV/HCV co-infection could be due to an additive or synergistic effect of the two infections. Although there are multiple of possible causes of anemia in HIV infections, but it is commonly attributed to bone marrow failure, peripheral destruction and opportunistic infections and HAART therapy [12-13]. Erythropoietin therapy at doses 100-200 IU/Kg three times weekly for a total of 12 weeks or until haematocrit ≥38% is achieved had been recommended [10].

In this study, the mean platelet count was significantly lower in both mono and co-infections compared with the control group. Contrary to our finding, significantly lower mean platelet count was obtained in HIV/HCV co-infected patients compared with the HCV mono-infected group [36]. Probably the discrepancy could be attributed to the fact the platelet count in HIV/HCV co-infection was compared with HCV mono-infection instead of HIV as in this study. The mechanisms involved in low platelet count include accelerated platelet clearance due to immune complex disease [37-39], anti-platelet glycoprotein antibodies [40-41] and/or anti- HIV antibodies that cross react with platelet membrane glycoprotein commonly known as antigenic mimicry [42-43]. McMillan et al [44] defined Thrombocytopenia as any disorder in which there is an abnormally low amount of platelets which may result from immune system malfunction. Therefore if these infections are not properly managed to increase the platelet count, prolonged infections could result in thrombocytopenia and /or Thrombotic thrombocytopenic purpura (TTP). Nonetheless, an escalated and uncontrolled platelet count may indicate disease progression and may sometimes be associated with abnormal bleeding [14]. There is need for caution in managing these infections so as to maintain the normal range of platelet count. Antiretroviral therapy has been reported to improve platelet counts in most patients [12]. Patients with moderate thrombocytopenia (platelet count >50,000 cells /µl) require no treatment.

Low $CD4^+$ cell count, protein S and protein C deficiency as obtained in this study are reported to be strongly associated with two to tenfold increased risk of venous thrombosis (VTE) in HIV infections [15,45-46]. Also, 27-73% of HIV patients have increased levels of complement binding protein 4 which attaches to protein S and renders this protein inactive. Consequently, the risk of VTE in these patients is substantially increased. Furthermore, our findings agree with a report that the risk of VTE is highest with advanced disease and co-existing infections and malignancies [13] because the mean values of PS and PC were significantly lower in HIV/HBV than mono-infection. .It could be speculated in this study that low level of these proteins could advance the course of VTE in HIV co-infections even before the manifestation of AIDS. Treatment of unprovoked thrombosis with anticoagulation for an undefined period of time has been recommended [12-13]. Although the mean values of CD4 in this study was significantly lower than the control group but they were high enough for HAART administration in consonance with Mills et al. [47]. In that report, Starting HAART at CD4 cell counts below 50 cells/µl increases the relative risk of death by approximately 60% when compared with HAART initiation at 150-249 cells/µl. Yet, as evidenced by CD4 count in our study, the degree of immune suppression may increase in HIV co-infection with HBV and HCV in agreement with International Association of Providers of AIDS Care (IAPAC) [48], Otegbayo et al. [11] in the South Western Nigeria and Idoko et al. [49] in North Central Nigeria.

Our findings show that PT and PTT were significantly higher in co-infections especially HIV/HBV than mono-infection. Usually, PT and PTT remain normal till the level of clotting factors (most of which are synthesized by the liver) fall to less than 30-40% [50-51]. In mild liver disease, PT is prolonged while PTT is usually normal until the liver disease becomes advanced in which case PT and PTT become prolonged [51]. In this study, the mean values of PT in co-infections were significantly higher than mono-infection indicating the probability of mild liver disease. Therefore, more tests may be necessary to rule out the involvement of the liver at this stage of the infection. The low mean values of WBC obtained in this study appear advantageous to the three groups of patient because, when it is elevated, it may indicate infection, lack of response to treatment or an abnormality [14].

Liver enzymes AST, ALT ALP levels were significantly higher in co-infection with hepatotropic viruses compared with mono-infection and control group in agreement with Otegbayo et al. [11], in south western Nigeria, Ballah et al [9] in the same environment as this study and Ibeh et al. [14] in Eastern part of Nigeria. The ALT is found in serum and in various bodily tissues, but is most commonly associated with the liver. It catalyzes the transfer of an amino group from alanine to a-ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate [16]. A raised mean serum ALT concentration above the acceptable range is a strong predictor of insulin resistance [19] and principally reflects direct hepatocellular damage or liver dysfunction [52]. Hepatitis C infection is associated with insulin resistance owing to an increased pro-inflammatory state and production of cytokines especially tumour necrosis factor α and interleukin 6. Consequently, transcription of the glucose transporter-4 and peroxisome proliferator – activated receptor γ is inhibited resulting in lipid metabolism and deposition in the hepatocytes and eventually Hepatosteatosis. Hepatitis C co-infection is also associated with autoimmune pancreatic beta cell damage leading to Diabetes Mellitus [20-23]. Usually, the mean value of ALT varies lightly between laboratories [53] but the 95^{th} percentile levels for ALT in healthy weight, metabolically normal, liver disease-free KNHANES has been set as 34 IU/L for men and 25 IU/L for women [54] against 40 IU/L in many hospitals. Although gender was not considered when the subjects were tested for this biomarker, the value (46.00±15.63 u/l in HCV co-infection compared with 24.89±8.14 and 25.0±11.52 in HIV

mono-infection and control respectively is still above the threshold ascribed for either male or female. The possibility of hepatic disease in the patients studied is supported by a report that HIV modifies the natural history of HCV infection by accelerating the histological progression of HCV infection, leading to cirrhosis and end-stage liver disease in a shorter period of time [55-58]. However, it is worth noting that prolonged treatment with ritonavir could increase the risk of liver injury with elevated hepatic enzymes [14,18]. It is difficult to ascribe the raised ALT in our study to prolonged treatment with HAART or the underlying co-infections with the hepatotropic virus. However, more tests and information on these patients will clear this doubt.

In addition, mean values of Creatinine were significantly higher in HBV and HCV co-infections than mono-infection and the control subjects in agreement with Parboosing et al. [59] and IAPAC [48]. Creatinine is a product of metabolism and it is usually excreted from the body through the kidneys. If the kidney malfunctions, the level of creatinine rises in the blood stream and it is commonly used as a test of renal function. The normal level of creatinine is usually 60 to 110 mmol/L but the range may differ slightly in different laboratories (http://www.netdoctor.co.uk/ate/liverandkidney/203123.html). Low level of blood creatinine indicates efficient and effective pair of kidneys. However, elevated creatinine in this study is still within the normal range but if the co-infections are not probably and rapidly managed, the tendency of this biomarker to rise above the normal range indicating kidney damage is high.

6. CONCLUSION

HBV and HCV infections are common among HIV positive patients in our environment and rapid detection of these co-infections may attract better management to avoid complications such as liver cirrhosis, hepatocellular carcinoma, and thrombocytopenia. Also, HIV co-infections with HBV and HCV could enhance VTE due to depletion of protein S and protein C. All co-infected patients are advised to carry out liver and renal function test to ensure that the liver and kidney are still intact. Further testing is required to exclude VTE and if it is confirmed appropriate treatment should be initiated immediately. Both ALT and HBV DNA should be monitored closely. For HBV co-infection, if the value of HBV DNA exceeds 2,000 iu/ml, treatment becomes inevitable as recommended by Lok et al [60]. Prompt diagnosis of HCV and HBV co-infection in HIV patients has both individual and public health benefits. Proper counseling of all co-infected patients with HIV/ HBV or HIV/HCV on reducing the risk of transmitting HBV or HCV to close household members, sex partners and children with close physical contact through dried blood, open cuts, and shared toothbrushes, needles or razors is a necessity. Those who shared injection drug equipment with the patient should be screened for HBV and vaccinated against these viruses if they are not actively infected. As with HIV prevention, condom use with sex and avoidance of shared needles and other equipment for injection drug use are recommended measures for reducing the risk of HBV transmission. Counseling should include advising the patients against intake of hepatotoxins which include alcohol and high doses of acetaminophen. Early antiretroviral therapy is especially recommended for co-infected patients. Virologically suppressive HIV therapy has been shown to slow the pace of hepatitis C disease progression and improve the prognosis of co-infected patients [61]. All HIV/HBV co-infected patients with an indication for HAART are advised to start HIV treatment that includes effective anti-HBV.

Haematological and biochemical parameters could serve as pointers for early detection of liver disease and renal malfunction in HIV patients. The development of novel therapeutic approaches to impede co-infection of HIV and hepatotropic viruses is encouraged.

CONSENT

All authors hereby declare that informed consent and pre-test counseling were instituted using structured questionnaire.

ETHICAL APPROVAL

The ethical committee of University of Maiduguri Teaching Hospital and Ashaka Cement Medical Centre Gombe, Nigeria gave approval for the study

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DECLARATION OF ORIGINALITY

All authors wish to affirm that this article has not been published in any other journal.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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