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Original Research Paper

Evaluation of Biochemical indices among HIV Positive Patients with Hepatitis B and C Co-infection

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ABSTRACT:
Human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) are blood borne pathogens that have become global threats. Co-infection with hepatitis B or C among HIV positive patients has further exposed the patients to liver infections and osmotic imbalance which if not properly handled can lead to death. This study was aimed at investigating the effect of HBV and HCV on the biochemical indices (AST, ALT and electrolytes) among HIV positive patients from the three geopolitical zones in Rivers State. A total of three hundred and fifteen (375) persons comprising of two hundred and fifty (250) HIV positive patients from the antiretroviral (ART) clinic and one hundred and twenty-five (125) HIV negative controls from medical out-patient department (MOPD) participated in this study. Patients of both sexes and within the age bracket of (10-69) years were included, socio-demographic information was collected by the use of consent forms and questionnaires. Five millilitres (5mls) of blood sample was aseptically drawn from each participant following vein puncture and dispensed aseptically into plain and lithium heparin bottles respectively for the HBV/HCV screening, AST, ALT and electrolytes estimations. The study population comprised of 151 (40.3%) males and 224 (59.7%) females. Comparing the biochemical indices of the participants, the liver enzymes were found to be higher ($p < 0.05$) in the HBV, HCV, HBV/HCV co-infected and HIV mono-infected when compared with the HIV-negative controls for AST (IU/L) (16.15±2.30, 15.88±2.64, 18.33±0.58, 16.81±6.41, 3.57±1.07), ALT (IU/L) 14.57±2.73, 14.75±3.19, 19.33±4.04, 15.10±3.38, 3.88±3.17). This work showed significant increase for some electrolytes when the HBV, HCV, and HBV/HCV co-infected patients were compared with the HIV-mono-infected and the control individuals (($p < 0.05$)), potassium (mmol/l) (3.63±0.65, 3.91±3.67, 3.44±0.66), biocarbonate (mmol/l) (13.19±6.71. 16.52±8.17, 23.57±4.68) and chloride (mmol/l) (92.92±8.93, 96.67±17.36, 98.47±8.28). A constant increase in the levels of these enzymes or electrolytes can adversely affect the liver and kidney of these HIV patients with either HBV or HCV infections respectively.

Keywords: Antiretroviral, Co-infection, Hepatitis B Virus, Hepatitis C Virus, Human Immunodeficiency Virus

INTRODUCTION:

The Human Immunodeficiency Virus (HIV) has continued to pose a serious threat to global health HIV is a virus that has a strong affinity for the immune cells of the body particularly, the CD4+ T cells which aid the body to fight against foreign agents. When these cells are depleted, the immune system of the body is impaired and the virus multiplies. This predisposes the individual to invasion by opportunistic pathogens which could be viral, bacterial, protozoan or fungal. It is necessary to note that HIV cannot be eliminated from the body completely despite antiretroviral therapy, every carrier lives with it for life. When appropriate therapeutic attention and care is given to the patient, there will be viral suppression and the patient can live a normal life, [1,2]. Co-infection of Hepatitis B virus (HBV) and Hepatitis C virus (HCV) among HIV positive patients has now become a major cause of illness due to liver disease globally for the past 20 years. Co-infection of HBV and HCV are very common in HIV positive patients because the three viruses have the same transmission routes. Morbidity and mortality is now greatly reduced as a result of the introduction of the highly-active antiretroviral therapy (HAART) and the rate of survival has been increased for the HIV positive patients[3,4], although co-morbidities like chronic liver disease as a result of HBV and HCV infection is still a problem. The prevalence of mono-infection for hepatitis B infection in the general population ranges from 9% - 39%, the

prevalence rate of HCV in Nigeria is also considerably high, 5.8% - 12.3%. The endemic nature of these viruses in the sub-Saharan region and the shared routes of transmission, had made co-infections of HIV-HBV or HIV-HCV or even the three viruses (HIV-HBV-HCV) very common.

MATERIAL AND METHODS:

Study Area:
This research was conducted in Ahoada, Bori, which are Zonal Hospitals and Rivers State University Teaching Hospital, a referral centre, all in Rivers state.

Study Population:
This study was carried out among the attendees of the three Hospitals with majority of them from the ART Clinic. Patients of both sexes and within the age bracket of (10-69) years were included. A total of 375 participants were used for this study, comprising of 125 HIV positive naïve patients, 125 HIV patients on ART and 125 HIV negative controls from the three hospitals.

Determination of Sample Size:
The minimum sample size was calculated using the formula by [5] at 95% confidence level and the prevalence of HIV in Rivers State for the year 2018 which was 3.8% [6].

$$N = \frac{Z^2 Pq}{d^2}$$

Where N = Sample size
Z = Statistic corresponding to level of confidence level, 1.96
P = Expected prevalence 3.8% (0.038)
d = Level of significance (allowable error) = 5% (0.05)
q = 1 – P

$$N = \frac{(1.96)^2 \times 0.038 \times (1-0.038)}{0.05^2}$$

$$= \frac{3.8416 \times 0.038 \times (0.962)}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.038 \times 0.962}{0.0025}$$

= 56.2 (minimum sample size).

Sample Collections:
Samples were collected from patients of both sexes within the ages of 10-69 years from either the ART clinic or the MOPD. Five milliliters (5mls) of blood was drawn from each patient through vein puncture [6,7]. About 2ml of blood was dispensed into plain bottle for HIV, HBV and HCV tests and the remaining 3ml was transferred into Lithium heparin bottle for Biochemical analysis.

Quality Assurance:
Quality control samples were ran alongside with the test samples to ensure that the kits, analyzers and procedures were alright.

HIV Screening:
The National algorithm for HIV screening which involves the use of three test kits was employed. Two for parallel testing and one for a tie-breaker was adopted. The three kits were Determine- HIV 1/2 (Abbott Japan Co. Ltd. Germany), Uni-Gold- HIV ½ (Trinity Biotec, France) and Stat-pak Dipstick (Chembio Diagnostic System Inc. The Manufacturers' Standard Operating Procedures (SOP) were followed. HIV sero-positivity was defined as a reactive result on two of the test kits. Non-reactive subjects were considered sero-negative.

Determine HIV 1/2 Test Principle and Procedure:
The Abbott Determine HIV ½ is an in vitro, visually read, rapid immune-chromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2 in human serum, plasma or whole blood. It involves applying 50µl of the test sample to the test pad (marked by arrow symbol) and then waiting for the sample to migrate through the conjugate pad. As the sample migrates, it reconstitutes and mixes with the selenium colloid antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient widow site. If antibodies to HIV-1 and /or HIV-2 are present in the sample, the antibodies bind to the antigen – Selenium colloid and to the antigen at the patient window, forming a red line at the patient window site. If antibodies to HIV-1 and /or HIV 2 are absent in the sample, the antigen-selenium colloid flows past the patient window and no red line is formed at the patient window site. To ensure assay validity, a procedural control is incorporated in the device and is labeled "control". If the control bar does not turn red at the completion of the assay, the test result is invalid and the sample must be retested.

Screening for Hepatitis -B Surface Antigen (HBsAg):
HBsAg One Step Hepatitis B surface Antigen Test strip test Kit with control band incorporated was used. The method used involved testing the serum of each subject for the presence of Hepatitis B surface Antigen (HBsAg) using commercially available DiaSpot HBsAg test strip, made in USA with a Lot number: HBSAG 20070003 and an expiry date of 2021-11 as a means of diagnosing hepatitis B infection. The DiaSpot One Step HBsAg Test strip is a rapid chromatographic immuno assay for the quantitative

dictation of HBsAg in serum or plasma. It employs a specific monoclonal and polyclonal antibodies directed towards HBsAg by double sandwich principle in a solid phase membrane. Chromogen embedded moves by diffusion to bring about color to be read visually at test band region where there is antibody-antigen-antibody (Ab-Ag-Ab) complex. Simultaneously, human immunoglobulins present in the serum will be captured by anti-human globulin antibodies on the control region, also colored by chromogen after Ag-Ab reaction.

Procedure:

The test device was removed from the pouch. The test device was dipped into fresh serum specimen for 3 seconds with the arrow end pointing down. The device was laid on a clean, dry, non-absorbent surface at the work-top bench.

The result was read within ten minutes.

Hepatitis C Virus Screening:

This was done using the DiaSpot HCV Rapid Test Strip which is a rapid chromatographic immunoassay for the qualitative detection of antibody to HCV in serum or plasma. The HCV test strip is a quantitative, membrane based immunoassay for the detection of antibody to HCV in serum or plasma. The membrane is pre-coated with recombinant HCV antigen on the test line region of the strip. During testing the serum or plasma specimen reacts with recombinant HCV antigen conjugated colloid gold. The mixture migrates upward to the membrane and generate a colored line. The presence of this colored line indicates positivity, while its absence shows negativity. To serve as control, a colored control line will always appear at the control line region.

Test Procedure:

The pouch was brought to room temperature and the test strip removed from it. The test device was dipped into fresh serum specimen for 3 seconds with the arrow end pointing down. The device was later laid on a clean, dry, non-absorbent surface at the work-top bench. The result was read within ten minutes. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) AST and ALT were analysed using a colorimeter and RANDOX reagent. The principles for the determination of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in serum or plasma is based on the AST catalyzed transfer of amino group of L-aspartate to alpha-ketoglutrate to form L-glutamate and Alanine aminotransferase (ALT) catalyzed transfer of amino group of L-alanine to alpha-ketoglutarate to give L-glutamate[8,9].

Procedure:

Tubes were labelled for blank and sample and 0.5ml of reagent loaded into each tube. Into the blank tube, 0.1ml of distilled water was added while 0.1 ml of sample was added to the sample tube. The mixture was mixed and incubated at 37^{o}C for 30 minutes. Also, 0.5ml of reagent 2 was added to both tubes, mixed and allowed to stand 20mins at 20 – 25^{o}C. Finally, 0.5 ml of Sodium Hydroxide was added to both tubes, mixed and absorbance of the sample read against the blank after 5min.

Electrolytes:

In assessing the functionality of the kidney, the electrolytes, urea and creatinine (EUC) levels were determined**.**

The analysis was done using AUDICOM (model AC9900) made in China, electrolyte analyse which is an automated chemistry analyser capable of analyzing different ions found in plasma, serum and urine.

Principle of Ion Selective Electrode Method:

Ion selective electrode method is an electrochemical sensor. It can transfer the charge of ionic activity in solution into the change of electrode potential. There is a linear relationship between the logarithm of the ionic activity in solution and electrode potential. In a kind of electrolytic solution, most salts exist in the form of ions. The electro-switching reaction occurs between selective electrode and the relative ion. The potential of the ion selective electrode changes as the ion concentration in the sample changes but, the potential of the reference electrode is always constant thus, forming a potential difference between the ion selective electrode and the reference electrode. The potential difference is measured and the corresponding ion concentration can be calculated. The AC9900 electrolyte analyser measures K, Na, Cl and CO_3^- in samples with two- point method. That is to measure the solution of two known concentration: A calibration reagent and B standard reagent. The potentials of two solutions are measured by electrode. The calibration curve line is established in instrument through two potentials and then the potential in sample of unknown concentration is measured. The ionic concentration in sample is calculated on the calibration curve line established [10].

Procedure:

The ‘on-button’ on the analyser was pressed, followed by ‘flush’, loading activation reagent. The electrodes were activated and system calibration allowed to go to completion. Samples were then loaded in sample cups, ‘analyse sample’ pressed, sample ID imputed and the sample ran. Results were imputed and automatically printed.

Statistical Analysis:

Data generated from this work were analysed using SPSS version 22 and Excel Data were presented as mean ± SD, Student t-test was used to determine significance between the test and control samples. Tables and graphs were used for data representations and p–value <0.05 was considered statistically significant [11].

RESULTS:

A total of three hundred and seventy-five (375) patients from three hospitals; Rivers State University Teaching Hospital (RSUTH), Zonal Hospital Bori (ZHB) and Zonal Hospital Ahoada (ZHA). Demographic information of the patients were also taken and various analysis were carried out on the collected blood samples to determine the effect of Hepatitis B and C on the liver enzymes and electrolytes. The number of patients from each group; those on ART 125 (33.3% each), Naive 125 (33.3% each) and control 125(33.3%) Males constituted 40.3% of the total population while the females constituted 59.7%. Finally the age interval of subjects are also shown on the table with 20-29 years having the highest percentage (25.3%), followed by 30-39years (24.3%) and 60-69 years having the least (6.7%) (Table 1).

The mean standard deviation of biochemical indices among HIV patients showed significant increase ($p<0.05$) when AST (IU/L) (19.10±3.86, 15.49±2.60, 3.21±1.04) and ALT (IU/L) (16.41±2.73, 19.29±6.04, 3.57±1.07) were compared for those on ART, the ART-naive and HIV-negative control. There was also significant increase ($p<0.05$) in the mean concentrations of potassium (Mmol/l) 3.63±0.65, (3.91±3.67, 3.44±0.66) AST (IU/L) (19.10±3.86, 15.49±2.60, 3.21±1.04) and ALT (IU/L) (13.83±0.65, 13.91±3.67, 3.44±0.66) and a decrease in biocarbonate (Mmol/l) (13.19±6.71. 16.52±8.17, 23.57±4.68) and chloride (Mmol/l) (92.92±8.93, 96.67±17.36, 98.47±8.28) as shown in Table 2.

For the mean standard deviation of biochemical indices according to gender (ART**),** only AST (IU/L) showed a significant increase ($p<0.05$) with males (17.00±3.04) having higher mean concentration than the females (16.03±2.47) as seen in Table 3.

The biochemical indices among co-infected patients revealed significant increase ($p<0.05$) for AST (IU/L) (16.15±2.30, 15.88±1.64, 18.33±0.58, 16.81±6.41, 3.57±1.07), ALT (IU/L) 14.57±2.73, 14.75±3.19, 19.33±4.04, 15.10±3.38, 3.88±3.17) and Bicarbonate (Mmol//) 16.46±4.68, 14.25±6.18, 16.33±4.04, 14.61±8.09, 13.57±4.68) when comparing the mean ± SD of the two liver enzymes and the electrolytes for HBV, HCV, HBV/HCV co-infected, HIV mono-infected and the HIV-negative control as shown on Table 4.

Table 1: Demographic and clinical characteristics of the participants from the three hospitals

Characteristics	Total No of subjects (%) (N=375)	No. of subjects on ART (%) (N=125)	No. of ART - naive subjects (%) (N=125)	No. of control subjects (%) (N=125)
Gender				
Male	151 (40.3)	50 (40.0)	53 (42.4)	48 (38.4)
Female	224 (59.7)	75 (60.0)	72 (57.6)	77 (61.6)
Age group (yrs)				
10-19	26 (6.9)	10 (8.0)	9 (7.2)	7 (5.6)
20-29	95 (25.3)	32 (25.6)	33 (26.6)	30 (24.0)
30-39	91 (24.3)	29 (23.2)	30 (24.0)	32 (25.6)
40-49	77 (20.5)	26 (20.8)	24 (19.2)	27 (21.6)
50-59	61 (16.3)	18 (14.4)	21 (16.8)	22 (17.6)
60-69	25 (6.7)	10 (8.0)	8 (6.4)	7 (5.6)
Hospital				
RSUTH	126 (33.6)	42 (33.6)	42 (33.6)	42 (33.6)
AZH	126 (33.6)	42 (33.6)	42 (33.6)	42 (33.6)
BZH	123 (32.8)	41 (32.8)	41 (32.8)	41 (32.8)

Legend: Antiretroviral therapy (ART), Rivers State University Teaching Hospital (RSUTH), Bori Zonal Hospital (BZH) and Ahoada Zonal Hospital (AZH).

Table 2: Mean Standard Deviation of Biochemical Indices among HIV Patients

Biochemical Indices	ART N=150	Non-ART N=135	Control N=30	p-value	F-value	
AST(IU/L)	19.10 ±3.86[c]	15.49. ±2.60[b]	3.21± 1.04[a]	<0.001	45.51*	
ALT(IU/L)	16.41 ±2.73[b]	19.29 ±6.04[c]	3.57 ±1.07[a]	<0.001	21.19*	
Na(Mmol/l)	131.76 ±7.70	132.50 ±16.31	130.60±10.92	0.72	0.33	

K(Mmol/l)	3.83 ±0.65[ac]	3.91 ±3.67[a]	3.44 ±0.66[c]	<0.001	7.14*	
HCO^-_3(Mmol/l)	13.19 ±6.71[a]	16.52 ±8.17[b]	23.57 ±4.68[c]	<0.001	27.64*	
Cl(Mmol/l)	92.92 ±8.93[a]	96.67 ±17.36[b]	98.47 ±8.28[ab]	0.019	3.99*	
Urea(Mmol/l)	2.74 ±1.55	3.09 ±1.11	2.48 ±0.39	0.11	2.27	
Creatinine(µmol/l	80.84 ±2.82	84.36 ±6.56	82.43 ±15.98	0.77	0.26	

Legend: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Sodium (Na^+), Potassium (K^+), Biocarbonate (HCO_3^-), Chloride (Cl^-) Note: Values with different subscripts are significantly different (P<0.05).

Table 3: Mean Standard Deviationof Biochemical indices According to Gender (ART)

Gender	ALT (IU/L)	AST (IU/L)	Na^+ (Mmol/L)	K (Mmol/L)	$HCO3^-$ (Mmol/L)	CL^- (Mmol/L)	Creat. (µmol/L)	Urea (Mmol/L)
MALE	13.36±1.07	17.00±3.04[b]	134.26±7.52	3.72±0.64	13.17±6.86	91.83±8.95	80.57±13.69	2.92±2.4
Female	13.11±1.01	16.03±2.47[a]	130.22±7.43	3.96±1.77	13.21±6.66	93.61±8.73	81.01±12.31	2.62±0.5
p.value	0.16	0.034*	0.38	0.27	0.99	0.75	0.18	0.17
T-Value	1.97	4.56	0.77	1.22	<0.001	0.11	1.83	1.91

Legend: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Sodium (Na^+), Potassium (K^+), Biocarbonate (HCO_3^-), Chloride (Cl^-), Creatinine (Creat).
Note: Values with different subscripts are significantly different (P<0.05).

Table 4: Biochemical indices among Co-infected Patients

Biochemical Indices	HIV/HBV	HIV/HCV	HIV/HBV/HCV	HIV	Control	P-Value	F-Value
ALT (IU/L)	14.57±2.73[b]	14.75±3.19[b]	19.33±4.04[d]	15.10±3.38[c]	3.88±3.17[a]	<0.001*	7.36
AST (IU/L)	16.15±2.30[c]	15.88±1.64[b]	18.33±0.58[c]	16.81±6.41[b]	3.57±1.07[a]	<0.001*	6.02
Na^+ (Mmol/L)	128.92±9.61	132.13±14.14	127.67±17.04	131.76±14.52	130.60±10.92	0.93	0.22
K^+ (Mmol/L)	6.49±5.76	5.13±3.63	7.07±6.01	4.92±3.33	3.44±0.65	0.19	1.56
HCO^-_3 (Mmol/L)	16.46±4.68[c]	14.25±6.18[b]	16.33±4.04[c]	14.61±8.09[b]	13.57±4.68[a]	<0.001*	9.27
Cl^- (Mmol/L)	97.38±7.05	91.13±12.56	102.33±8.74	96.71±15.50	98.47±8.28	0.71	4.54
CREAT (µmol/L)	84.15±14.05	80.13±15.30	89.00±14.79	84.20±13.65	82.43±15.98	0.99	0.20
UREA (Mmol/L)	2.72±0.57	2.55±0.50	2.80±0.56	2.15±1.29	2.88±0.39	0.48	0.88

Legend: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Sodium (Na), Potassium (K), Biocarbonate (HCO_3), Chloride (Cl). Note: Values with different superscripts are significantly different (P<0.05).

DISCUSION:

When comparing the mean values of the liver enzymes of the HIV- positive patients with the controls, there was no significant difference (p>0.05). For this research, the liver enzymes were found to be higher in the HBV, HCV, HBV/HCV co-infected and HIV mono-infected when compared with the HIV-negative controls for AST (IU/L) (16.15±2.30, 15.88±1.64,

18.33±0.58, 16.81±6.41, 3.57±1.07), ALT (IU/L) 14.57±2.73, 14.75±3.19, 19.33±4.04, 15.10±3.38, 3.88±3.17) and is consistent with the findings from Kolkata, South India and Northern India [12,13,14]. The findings from this work is in agreement with another study from Ethiopia also showed raised AST and ALT levels for HIV/HBV, HIV/HCV and HIV/HBV/HCV than HIV mono-infection [15]. This is because, both HIV and HBV or/ HCV can cause serious damage to the liver cells with the resultant release of all or some of the liver enzymes such as ALT and AST. The males also showed higher mean value for AST (17.00±3.04) than the female (16.03±2.47) ($p<0.05$), reason may not be far from the fact that males are muscular in nature than females. This statistical increase in the mean levels of the liver enzymes (AST and ALT) was observed between HIV-mono-infected and HIV-viral hepatitis co-infected patients and this is in agreement with another study which was conducted in South Africa with 70% of HIV- viral hepatitis co-infected patients having significantly elevated AST and ALT [16]. The differences in these liver enzyme levels between different studies may be due to differences in study design, the condition of the patient like having chronic alcoholism or other drug induced hepatoxicity and the duration of the viral hepatitis infection. In addition, HIV can also infect the hepatic or kupffer cells that may further contribute to the development of liver fibrosis and raised liver enzyme levels. HIV-positive patients with Hepatitis B or C viral co-infection are more likely to have liver–related complications as shown above [15]. The results of this work showed significant increase for some electrolytes when the HBV, HCV, and HBV/HCV co-infected patients were compared with the HIV-mono-infected and the control individuals, potassium (Mmol/l) (3.63±0.65, 3.91±3.67, 3.44±0.66), biocarbonate (Mmol/l) (13.19±6.71. 16.52±8.17, 23.57±4.68) and chloride (Mmol/l) (92.92±8.93, 96.67±17.36, 98.47±8.28). Several studies had reported different observations with respect to the effect of HIV or other co-infection on plasma electrolytes. Ayodele and associates reported that sodium, potassium, chloride and bicarbonate of HIV and Hepatitis- B co-infected persons were significantly reduced [17]. The result of this work is similar to the findings of Ayodele and associates in Port-Harcourt with a slight difference in sodium. There was no significant difference in sodium when the HIV mono infection and the co-infections were compared with the controls ($P>0.05$) and was also similar to the findings of [15] which suggest that, HIV patients are at risk of kidney disease whether acute or chronic. Increase potassium is a characteristic of renal tubular acidosis type 4 resulting from a defect in the secretion of hydrogen and potassium in the distal tubules rather from the deficiency of aldestorone [17]. This could be the reason for the increased potassium level observed among the HIV and those co-infected with HBV, HCV or both. The finding from this work with respect to the liver enzymes was quite different from a study in Ghana aimed at determining the effect of ARTs on liver enzymes which showed very minimal effect on the toxicity of the hepatocytes. Serum aminotransferase (AST) and alanine aminotransferase are very important in evaluating viral hepatitis biologically. A constant increase for more than six months is a sign of chronic infection and in about 60% of infected persons, elevated liver enzymes progression to fibrotic liver disease may occur, which is different from those with normal transaminases [18].

CONCLUSION:

This study has revealed that co-infection can alter the levels of some biochemical indices like; potassium, bicarbonate and chloride which are important for maintaining osmotic balance in the body and also raise the levels of serum aspartate aminotransferase and alanine aminotransferase which are liver enzymes.

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