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Evaluation of Total Bile Acid and Aminotransferases in HIV/AIDS Patients with Coinfection of Hepatitis B and C Viruses

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Abstract Patients confirmed to be seropositive for human immunodeficiency Virus (HIV) infection by Western blot technique attending Baptist Medical Centre, Saki, Oyo state-Nigeria were studied; including 30 males; 30 females; 20HIV mono-infected, 20HIV-HBV co-infected, 20HIV-HCV co-infected subjects aged 16 to 65 years. Age and sex matched apparently healthy HIV, HCV and HBV seronegative, subjects (N=50) consisting of 25 (50%) males and 25 (50%) females were recruited as controls. Plasma ALT, AST and Total Bile Acids were determined in the subjects biochemically by spectrophotometry. Antibody to hepatitis C virus and surface antigen to hepatitis B virus was determined in the subjects immunochemically by ELISA. The result obtained showed a significantly higher mean value of plasma ALT,AST and Total Bile Acids in HIV patients co-infected with hepatitis B or C virus than the values obtained in the control subjects($p<0.01$). There was a significantly higher mean value of AST in the HIV mono-infected than the result obtained from the control subjects with $p<0.01$. There was a significantly higher mean plasma value of ALT in HIV patients co-infected with hepatitis B or C virus compared with the HIV mono-infected patients with $p<0.01$. None of the patients was found to have triple infection. Finally there was no significant difference in the degree of severity of hepatitis B or C or liver disorder in the immunosuppressed HIV patients co-infected with HBV compared with the HIV patients co-infected with HCV considering the alterations in the plasma level of the biochemical parameters.

Keywords Total Bile Acid, Aminotransferases, CD4+ T Cell, Coinfection, Human Immunodeficiency Virus , Hepatitis B, Hepatitis C

1. Introduction

Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) infections are common among HIV positive patients in our environment. Rapid detection and investigation of these co-infections may attract better management to avoid complications such as liver cirrhosis, hepatocellular carcinoma, and thrombocytopenia [1]

Biochemical parameters could serve as pointers for early detection of liver disease in HIV patients. Biochemical parameters such as ALT, AST, Alkaline phosphatase, Creatinine, Urea 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 Lokand McMahon [2]\. Prompt diagnosis of HCV and HBV co-infection in HIV patients has both individual and public health benefits[1]

A significant increase in serum liver marker enzymes (ALAT, ASAT, and Alkaline phosphates) in HIV/AIDS patients had been reported [3][4]. 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 [3]. 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 [5]. 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 [5][6]. A raised mean serum ALT concentration above the acceptable range is a strong predictor of insulin resistance [7] and principally reflects direct hepatocellular damage or liver dysfunction.

Liver enzymes AST, ALT ALP levels have been reported by Obi et al [1] to be significantly higher in co-infection with hepatotropic viruses compared with mono-infection and control group under antiretroviral therapy .Otegbayoet al [8] in south western Nigeria had similar result; Ballahetal[9] in the same environment and Ibehet al[5] 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 [3].A raised mean serum ALT concentration above the acceptable range principally reflects direct hepatocellular damage or liver dysfunction [10].

Serum Total Bile Acids (TBA) increase in patients with acute or chronic hepatitis, hepatic schlerosis, obstetric choleostasis and cancer of the liver. TBA is a useful additional marker to the conventional liver enzymes as it is a sensitive indicator of liver damage.. Early detection of liver disease and liver functionality can help patients get effective therapeutic treatment, prevent disease progress, and save lives.Total Bile Acids are both highly sensitive and specific making them a popular marker of normal liver function.Bile acids aid in fat absorption and modulate cholesterol levels. They are produced from cholesterol in the liver and are stored in the gall bladder. Gall bladder contraction with feeding releases bile acids into the intestine. Bile acids undergo enterohepatic circulation, i.e. they are absorbed in the intestine and taken up by hepatocytes for re-excretion into bile[11].

Acquired immunodeficiency syndrome (AIDS) is defined in terms of either a CD4+ T cell count below 200 cells per μL or the occurrence of specific diseases in association with an HIV infection. In the absence of specific treatment, around half of people infected with HIV develop AIDS within ten years[12].

This work was therefore designed to compare the degree of severity of liver disorder (hepatitis B and C)through the evaluation of plasma Total bile acid, aminotransferases in patients with CD4 count of less than 150 cells per μL in coinfection of Human Immunodeficiency Virus with hepatitis B or C virus.

2. Materials and Methods

2.1. Study Area

This study was carried out at Baptist Medical Centre, Saki (a referral 300 bedded hospital and HIV/AIDS treatment centre) located at the Northern part of Oyo state -Nigeria which shares border with Kwara state and Burkina Faso(Benin republic). The hospital has three tertiary institution i.e school of Nursing, School of Medical Laboratory Technology and School of Midwifery. It is also a postgraduate training institution for Consultant Family Physicians.

2.2. Sample Size

The appropriate sample size for a population-based survey is determined largely by three factors: (i) the estimated prevalence of the variable of interest –HIV infection in Nigeria in this instance, (ii) the desired level of confidence and (iii) the acceptable margin of error. The prevalence of HIV infection in Nigeria was reported as follow:

a) As of 2012 in Nigeria, the HIV prevalence rate among adults ages 15–49 was 3.1 % [13].Nigeria has the second-largest number of people living with HIV[14].
b) 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 [15]. Other studies had observed HIV/HBV sero-prevalence rate of 5.5% – 12.3% [16][9][18] and HIV/HCV rates of 0.5 – 0.7% [8][16][9][18].
c) Considering the above reports the prevalence of HIV infectionused to determine the sample size is : 4.0

2.3. Calculation of Sample Size

The following formula was used to arrive at the size of the sample which include:
$n = Z^2 \times P(1-P) \div d^2$where n = sample size,
Z = Z statistic for a level of confidence, = 1.96
P = expected prevalence or proportion = 4% = 0.04(in proportion of one; if 20%, P = 0.2), and
d = precision(in proportion of one; if 5%, d = 0.05). =0.05
Z statistic (Z): For the level of confidence of95%, which is conventional, Z value is 1.96.
n= 1.96 x1.96 x 0.04(1 -0.04)/0.05 x 0.05 = 59

2.4. Study Population

Patients confirmed to be seropositive for human immunodeficiency Virus (HIV) infection by Western blot technique were recruited for the study and will include 30 (50%) males; 30 (50%) females; 20(33.3%)HIV mono-infected, 20(33.3%)HIV-HBV coinfected, 20(33.3%)HIV-HCV co-infected subjects aged 16 to 65 years. Age and sex matched apparently healthy HIV, HCV and HBV seronagative subjects (N=50) consisting of 25 (50%) males and 25 (50%) females were recruited as controls. The patients were counseled pre and post -test.

2.5. Inclusion Criteria

Test subjects include HIV monoinfected and coinfected HIV patients with HBV and HCV that were yet to initiate antiretroviral therapy with CD4+ T cell count below 150 cells per μL were included in the study. Both sexes within the age of 16 – 65 years were studied.

2.6. Exclusion Criteria

Patients who are seronegative to HIV regardless of whether they are anti-HCV or HBsAg seropositive were not studied as test. Patients under antiretroviral therapy were not recruited for the study.HIV mono-infected and those that were co-infected with CD4+ T cell count ≥ 150 cells per μL and also Icteric patients were not recruited for the work.

2.7. Sample Collection

Five milliliter(5ml) volume of venous blood was obtained from each of the control subjects and also from each of the test subjects after an overnight fasting before the initiation of antiretroviral therapy into lithium heparinized bottle for the extraction of plasma for viral serology and biochemical assay. After sampling the HIV positive patients were allowed to be placed on highly active antiretroviral therapy (HAART) regimes of the APIN-BMC program of the institution.

2.8. Methods

Plasma ALT, AST were Carried Out Using Cobas C111 Auto-Chemistry Analyzer Using the Reagent Kit of Roche Diagonostics, Gmbh Sandhofer straße, 116, D-68305, Mannheim.www.roche.com

Total Bile Acid was Determined in the Subjects Using Enzymatic Colorimetric Method and Reagent Kit of Randox Principle

In the presence of Thio-NAD, the enzyme 3-α hydroxysteroid dehydrogenase (3-α HSD) converts bile acids to 3-keto steroids and Thio-NADH. The reaction is reversible and 3-α HSD can convert 3-keto steroids and Thio-NADH to bile acids and Thio-NAD. In the presence of excess NADH, the enzyme cycling occurs efficiently and the rate of formation of Thio-NADH is determined by measuring specific change of absorbance at 405nm.

2.8.1. 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 (Immunoetics Qualicode TM HIV 1 and 2 kit)

2.8.2. Screening for Hbsagby Enzyme- Linked Immunosorbent Assay (ELISA)

The ELISA kit from BIORAD Monolisa HBsAg ULTRA EIA92430 Marnes-La-Coqutte- France was used. ELISA was done according to the manufactures instruction. The Optical density OD was read at 450/620 to 700 nanometre. The cut off value was determined by the mean of negative control + 0.05 (0.08). The test is valid if all values of negative control are lower or equal to 0.08 and Positive control was over 0.08 or equal to 1.0. A test sample is considered negative if the ratio value of sample: cut off value is lower than 1.0 and positive if equal to or greater than 1.0.

2.8.3. Screening for HCV Antibody by ELISA

ELISA kit from DIA PRO Diagnostic Bioprobes 20099 Sesto San Giovanni (Milano)-Italy was used. ELISA was done according to the manufactures instruction The Optical density OD is read at 450/620 to 700 nanometre. The cut –off value is 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 is positive.

2.8.4. Statistical Analysis

Statistical Analysis: the data was subjected to statistical analysis to determine the mean values, standard deviation and student's' test, for t value , p value and level of significant at 0.05 using online Student T-Test Calculator for 2 Independent Means.

CD4 Count was Carried out by Cytoflometry Using the Reagent Kit of Partec and Partec CD4 Machine

2.8.5. Ethical Consideration

The proposal for this study was reviewed and approved by the Research and Ethical Committee of the Baptist Medical Centre, Saki -, Nigeria. Only patients that consented and volunteered themselves for the study were recruited.

3. Result

The result obtained showed a significantly higher mean value of plasma ALT,AST and Total Bile Acids in HIV patients co-infected with hepatitis B and C virus than the values obtained in the control subjects($p<0.01$). However there was no-statically significant difference in the plasma value of ALT and Total Bile Acids in the HIV mono-infected patients compared with the control subject($p>0.01$). There was a significantly higher mean value of AST in the HIV mono-infected than the result obtained from the control subjects with $p<0.01$. (Table 1 & 2).There was no-statically significant difference in the plasma value of AST, ALT and Total Bile Acids in the HIV patients co-infected with hepatitis B virus compared with the result obtained from HIV patients co-infected with hepatitis C virus with $p>0.01$(Tables 1&2).

Table 1. Mean and Standard Deviation (SD) of Plasma ALT, AST and Total Bile Acid in the test and control

	Control N=50	HIV monoinfected N=20	HIV/HCV Coinfected patients n=20	HIV/HBV Coinfected patients n=20
ALT (Mean ±SD)U/L	26±1.5	31±2.3	68±2.8	70±1.0
Total Bile Acid (Mean ±SD)µmol/L	5±1.2	9.0 ±1.2	23±2.1	22±1.0
AST (Mean ±SD)U/L	18.0 ±1.8	48.0 ±2.2	55.0 ±2.1	58.0 ± 2.8
CD4+ T cell count in cells per µL(mean±SD)	700±12.5	140±2.5	140±3.0	146±3.0

Table 2. Comparative analysis of Plasma ALT, Total Bile Acid and AST in the test and control subjects

		Control/HIV-HCV	Control/HIV-HBV	Control/HIV	HIV-HCV/HIV-HBV	HIV-HCV/HIV	HIV-HBV/HIV
ALT	't'	11.7	17.4	1.72	1.7	10.26	17.4
	'p'	0.0073^{s}	0.003^{s}	0.23^{ns}	0.23^{ns}	0.009^{s}	0.003^{s}
	Comment	P<0.01	P<0.01	p>0.01	p>0.01	P<0.01	P<0.01
TOTAL BILE ACID	't'	13.4	12.0	2.83	0.5	6.3	9.19
	'p'	0.006^{s}	0.007^{s}	0.11^{ns}	0.7^{ns}	0.03^{ns}	0.011^{ns}
	Comment	p<0.01	p<0.01	p>0.01	p>0.01	p>0.01	p>0.01
AST	't'	13.8	17.9	10.60	0.83	1.8	2.8
	'p'	0.0053^{s}	0.0031^{s}	0.009^{s}	0.5^{ns}	0.22^{ns}	0.11^{ns}
	Comment	p<0.01	p<0.01	P<0.01	p>0.01	p>0.01	p>0.01

s= significant ; ns =not significant

There was a significantly higher mean plasma value of ALT in HIV patients co-infected with hepatitis B and C virus compared with the HIV mono-infected patients with p<0.01(Tables 1&2). However there was no significant difference in the mean plasma value AST and Total Bile Acids in HIV patients co-infected with hepatitis B and C virus compared with the HIV mono-infected patients with p>0.01(Tables 1&2).

None of the patients was found to have triple infection.

4. Discussions, Conclusions and Recommendations

This work was used to evaluate plasma Total bile acid, ALT and AST in Human Immunodeficiency Virus patients coinfected with hepatitis B or C virus to be able to compare the degree of severity of hepatitis B or C in HIV coinfection.

The significantly higher mean value of plasma ALT,AST and Total Bile Acids in HIV patients co-infected with hepatitis B and C virus than the values obtained in the control subjects could be attributed to the fact that hepatitis B and C viruses are hepatotrophic viruses that elicit immune response that causes liver damage which was revealed by the increase in plasma ALT, AST and Total Bile Acids. 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[5].A significant increase in serum liver marker enzymes (ALAT, ASAT, and Alkaline phosphates) in HIV/AIDS patients has also been reported [3][19] but associated with the hepatotoxic effect of antiretroviral drugs. Patients studied in this work have not initiated antiretroviral therapy but were co-infected with hepatitis B or C virus. 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 [3]

Liver enzymes AST, ALT ALP levels have been reported by Obi et al[1]to be significantly higher in co-infection with hepatotropic viruses compared with mono-infection and control group under antiretroviral therapy . Otegbayoetal[8] in south western Nigeria had similar result; Ballahet al[9] in the same environment and Ibehet al[5] in Eastern part of Nigeria. The ALT is found in serum and in various bodily tissues, but is most commonly associated with the liver. A raised mean serum ALT concentration above the acceptable range principally reflects direct hepatocellular damage or liver dysfunction [10].

The result obtained with reference to Total Bile Acids(TBA) could also be attributed to the fact that TBA is a useful additional marker to the conventional liver enzymes as it is a sensitive indicator of liver damage.. Early detection of liver disease and liver functionality can help patients get effective therapeutic treatment, prevent disease progress, and save lives. Total Bile Acids are both highly sensitive and specific making them a popular marker of normal liver function[11].

There was a significantly higher mean plasma value of ALT in HIV patients co-infected with hepatitis B and C virus compared with the HIV mono-infected patients the same explanation as stated above also holds for this.

A significantly higher mean value of AST obtained in the HIV mono-infected than the result obtained from the control subjects. Human immunodeficiency Virus does not always cause liver damage except in coinfection with hepatotropic virus or the antiretroviral drugs but AST is present in other cells other than liver cells like blood cells which are destroyed by HIV upon infection leading to the leakage of AST to the plasma[1]. The degree of severity of liver disorder in the immunosuppressed HIV patients co-infected with HBV or HCV was higher than in the HIV mono-infected patients and there was no significant difference in the degree of severity of hepatitis B or C in the immunosuppressed HIV patients co-infected with HBV compared with the HIV patients co-infected with HCV considering the alterations in the plasma level of the biochemical parameters. The pattern of the biochemical

parameters obtained in the HIV coinfected subjects with CD4+ T cell count below 150 cells per µL could also be associated by the reactivation of hepatitis B and C viruses as a result of immunodeficiency making it less possible for the patients to provide immunity that will prevent active viral hepatitis[12].

4.1. Conclusions

This work has been used to evaluate plasma ALT, AST and Total Bile Acids in monoinfected and coinfected HIV patients with CD4+ T cell count below 150 cells per µL. The result obtained showed :

a) A significantly higher mean value of plasma ALT,AST and Total Bile Acids in HIV patients co-infected with hepatitis B and C virus than the values obtained in the control subjects.
b) A significantly higher mean value of AST in the HIV mono-infected than the result obtained from the control subjects.
c) A significantly higher mean plasma value of ALT in HIV patients co-infected with hepatitis B and C virus compared with the HIV mono-infected patients.
d) The degree of severity of liver disorder in the immunosuppressed HIV patients co-infected with HBV or HCV was higher than in the HIV mono-infected patients
e) There was no significant difference in the degree of severity of hepatitis B or C in the immunosuppressed HIV patients co-infected with HBV compared with the HIV patients co-infected with HCV.

4.2. Recommendations

Newly diagnosed HIV patients with CD4+ T cell count below 150 cells per µL will benefit from the evaluation of their blood for liver damage using plasma ALT, AST, Total Bile Acids, hepatitis B and C viruses for early detection of liver related problems for appropriate management. Finally there was no significant difference in the degree of severity of hepatitis B or C or liver disorder in HIV patients co-infected with hepatitis B compared with those co-infected with hepatitis C virus in immunosuppressed state.

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