

Changes in Serum Level of ALT, GGT and LDH in Human Immunodeficiency Mono-infected and HIV-HBV Co-Infected Patients

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Abstract

Liver markers indicate liver disorders that could be caused by drugs and infection such as hepatotropic viruses or its co-infection with HIV. Standard antiretroviral therapy (ART) consists of the combination of at least three antiretroviral (ARV) drugs to maximally suppress the HIV virus and stop the progression of HIV disease. This study was designed to determine the effect of antiretroviral therapy in co-infection of HIV with hepatitis B virus on serum ALT, GGT and LDH. Seventy six patients aged 16-65years including 50 HIV mono-infected and 26 HIV-HBV patients that have not initiated antiretroviral therapy were recruited from Federal Medical Centre, Owo-Nigeria. Viral serological study of the patients were carried out by ELIZA and Immunoblotting. LDH, GGT and ALT were determined biochemically by Spectrophotometry. The results obtained showed a significantly higher mean values of LDH, GGT and ALT in the HIV-mono-infected after drug administration (334.4 ± 11.2 , 24.9 ± 2.3 , and 42.4 ± 1.1) than the results (296.5 ± 7.4 ; 14.8 ± 1.4 and 27.8 ± 1.7) of the parameters obtained before the initiation of the drug with $p < 0.05$. There was a significantly higher mean values of LDH and ALT in the HIV patients co-infected with HBV before drug administration 440.3 ± 8.2 and 52.3 ± 0.5) than the results (288.3 ± 5.7 and 36.6 ± 0.6) of the parameters obtained after the initiation of the drug with $p < 0.05$. However, there was a significantly lower mean value of GGT in the HIV-HBV patients before drug administration than after the initiation of the antiretroviral drug ($p < 0.05$). This work revealed a significant increase in the values of LDH, GGT and ALT in the HIV-mono-infected patients after antiretroviral drug administration and in HIV-HBV patients before antiretroviral therapy. A significantly lower mean value of GGT in the HIV-HBV patients before drug administration than after the initiation of the antiretroviral drug. Evaluation of liver biomarkers in the treatment of HIV mono-infected and HIV-HBV patients is essential to avoid liver disorder and possible complications.

Keywords: ALT, γ GT,LDH, HIV, Coinfectio, HIV-HBV, Monoinfection

Introduction

Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) infections are common among HIV positive patients in our environment and rapid detection and investigation of these co-infections may attract better management to avoid complications such as liver cirrhosis, hepatocellular carcinoma, and thrombocytopenia (Obi et al., 2014).

Patients with HIV infection are treated with a combination of antiretroviral medications to control their HIV infection. Several of these drugs are associated with liver enzyme elevations (Bonacini et al., 2002). On the other hand, the use of HAART has resulted in profound and durable HIV suppression, so that progression to AIDS and the occurrence of opportunistic infections has declined in regions where HAART is readily available. As a corollary, several liver diseases (chiefly HCV or HBV infection) have become more important in terms of morbidity and mortality in patients with HIV infection (Bonacini et al., 2000).

Damage to the liver cell cytoplasmic membrane that may be caused by inflammation or leakage of cytoplasmic contents causes a relatively greater increase in the serum ALT than AST level (Wood et al., 2003). On the other hand, if the damage occurs in both the mitochondria and cytoplasmic membrane, there is a proportionally greater increase in both the serum AST and ALT levels. Hence, AST and ALT are referred to as the Markers of hepatocellular damage. (Havliir et al., 2003)

LDH is often measured in HIV patients as a non-specific marker for pneumonia due to *Pneumocystis jiroveci* (PCP). Elevated LDH in the setting of upper respiratory symptoms in an HIV patient suggests, but is not diagnostic for PCP. (Butt et al., 2002).

GGT is predominantly used as a diagnostic marker for liver disease in medicine, latent elevations in GGT are typically seen in patients with chronic viral hepatitis infections often taking 12 months or more to present. (Lim et al., 2007). Elevated serum GGT activity can be found in diseases of the liver, biliary system, and pancreas. In this respect, it is similar to alkaline phosphatase (ALP) in detecting disease of the biliary tract. This study was designed to examine the effect of antiretroviral therapy on the biochemical changes caused by co-infection of HIV with hepatitis B virus to provide useful information in the management of co-infection of HIV with hepatitis B virus.

Materials and Method

Study area

This study was carried out in Federal Medical Centre, Owo. Owo, is situated at the south-western Nigeria and is located at 7.1962 [latitude in decimal degrees], 5.58681 [longitude in decimal degrees] at an elevation/altitude of meters. The average elevation of Owo is 348 meters. The population of Owo is 276574.

Sample size

Sample size was determined by the formula

$$N=4pq/l^2$$

Where,

N=sample size

P=prevalence of HIV co infection with hepatitis B and C in Nigeria.(4%=0.04)

$$Q=1-p (1-0.04=0.96)$$

l=permissible error (5% of p=0.05)

$$N=4 \times 0.04 \times 0.96 / 0.05 \times 0.05 = 61.44.$$

N=60 samples

Study Population

Patients confirmed to be seropositive for human immunodeficiency Virus (HIV) infection by Western blot technique were recruited for the study which include 38(50%) males; 38(50%) females; 50 HIV monoinfected, 26(33.3%) HIV-HBV coinfecting subjects aged 16 to 65 years.

After the baseline sampling the confirmed HIV positive patients were allowed to be placed on highly active antiretroviral therapy (HAART) regimes of the APIN PEPFAR program of the institution. The choice of drug combination administered to each patient will be within the jurisdiction of the clinician and all the patients were followed up for two months. Worthy of note is that different drug combination is administered to patients co-infected with either HBV or HCV.

Inclusion criteria

The test subjects included HIV monoinfected and co-infected HIV patients with HBV that are yet to initiate antiretroviral therapy were recruited and monitored before and after the administration of antiretroviral drugs. Both sexes were included within the age of 16 – 65 years.

Exclusion criteria

Patients who are seronegative to HIV regardless of whether they are anti-HCV or HBsAg seropositive were not studied as test. HIV patients that were under antiretroviral therapy were not included.

Sample Collection and Preparation

Five millilitre (5ml) volume of venous blood was obtained from each of the test subjects before and after the initiation of antiretroviral therapy into a plain bottle for the extraction of serum to be used for biochemical assay. Blood samples were collected by clean venepuncture from the antecubital fossa into the already labelled plain test tubes, without undue pressure on either the arm or the plunger of the syringe.

The samples were allowed to clot and were centrifuged at 3000 rpm to obtain the sera. The separated clear sera were transferred into sterile bottles and were used for the enzyme assay. When not used immediately, they were stored at -20°C and later used within five days.

Hepatitis B Testing (Using QuickProfile HBsAg test)

TEST PRINCIPLE:

QuickProfile HBsAg Test is a double antibody sandwich immunoassay. Colloidal gold conjugated anti- HBsAg antibody complexes are dry immobilized in the test device. When the sample is added, it migrates by capillary diffusion through the strip re-hydrating the gold conjugate complexes forming particles. These particles will continue to migrate along the strip until the Test Zone (T) where they are captured by anti-HBsAg antibodies immobilized there and a visible red line appears. If there is no HBsAg in sample, no red line will appear in the Test Zone (T). The gold conjugate complexes will migrate alone until they are captured in the Control Zone (C) by immobilized goat anti-mouse IgG antibody aggregating a red line, which indicates the validity of the test. (Schulster, 1981).

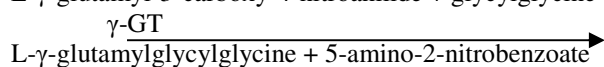
LABORATORY ANALYSIS

GAMMA GLUTAMYL TRANSFERASE

METHOD: ENZYMATIC METHOD (Using radox kit)

PRINCIPLE: The substrate L- γ -glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted by γ -GT in the sample to 5-amino-2-nitrobenzoate which can be measured at 405nm. (Szasz, 1969).

L- γ -glutamyl-3-carboxy-4-nitroanilide + glycylglycine



The coloured solution is measured using a spectrophotometer with a wavelength capability of 400 – 420nm.

CALCULATION

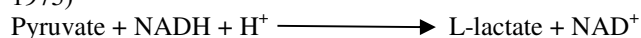
The GGT activity was calculated using the formula:

$$U/L = 1158 \times \Delta A_{405 \text{ nm/min}}$$

LACTATE DEHYDROGENASE

METHOD: ENZYMATIC METHOD (Using Agappe kit)

PRINCIPLE: Kinetic determination of lactate dehydrogenase according to the following reaction. (Wei Bhaar, 1975)



CALCULATION

$$\text{LDH-P activity (U/L)} = (\text{OD}/\Delta \text{min}) \times 16030.$$

3.6 3 ALANINE AMINOTRANSFERASE (SGPT)

METHOD: ENZYMATIC METHOD (Using Agappe kit)

PRINCIPLE

Kinetic determination of Alanine Aminotransferase (ALAT) according to the following reaction. (Expert panel on enzyme of the IFCC, 1976)



CALCULATION

$$\text{ALT activity (U/L)} = (\Delta \text{OD}/\text{min}) \times 1768$$

3.8 STATISTICAL ANALYSIS

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

Results

TABLE 4.1: INFLUENCE OF ANTIRETROVIRAL THERAPY ON SOME LIVER BIOMARKERS IN HIV MONOINFECTED SUBJECTS

Showing Mean \pm Standard error of mean, t-value, p- value and remark.

PARAMETERS	BASELINE (Mean \pm SEM)	AFTER DRUG ADMINISTRATION (Mean \pm SEM)	T-VALUE	P-VALUE	REMARK
LDH (U/L)	296.5 \pm 7.4	334.4 \pm 11.2	2.986970	0.002101	Significant
GGT (U/L)	14.8 \pm 1.4	24.9 \pm 2.3	3.407312	0.000617	Significant
ALT (U/L)	27.8 \pm 1.7	42.4 \pm 1.1	7.693382	0.00001	Significant

There was a significantly higher mean values of LDH, GGT and ALT in the HIV-mono-infected after drug administration (334.4 \pm 11.2, 24.9 \pm 2.3), and 42.4 \pm 1.1) than the results (296.5 \pm 7.4; 14.8 \pm 1.4 and 27.8 \pm 1.7) of the parameters obtained before the initiation of the drug with p <0.05.

4.2: INFLUENCE OF ANTIRETROVIRAL THERAPY ON SOME LIVER BIOMARKERS IN HIV - HBV COINFECTED SUBJECTS

Showing Mean \pm standard error of mean, t- value, p- value and remark.

PARAMETERS	BASELINE (Mean \pm SEM)	AFTER DRUG ADMINISTRATION (Mean \pm SEM)	T-VALUE	P-VALUE	REMARK
LDH (U/L)	440.3 \pm 8.2	288.3 \pm 5.7	-14.882078	<0.00001	Significant
GGT (U/L)	23.0 \pm 2.2	26.3 \pm 1.0	2.013984	0.024457	Significant
ALT (U/L)	52.3 \pm 0.5	36.6 \pm 0.6	-22.610385	<0.00001	Significant

There was a significantly higher mean values of LDH and ALT in the HIV patients co-infected with HBV before drug administration (440.3 \pm 8.2 and 52.3 \pm 0.5) than the results (288.3 \pm 5.7 and 36.6 \pm 0.6) of the parameters obtained after the initiation of the drug with $p < 0.05$. However, there was a significantly lower mean value of GGT in the HIV-HBV patients before drug administration than after the initiation of the antiretroviral drug ($p < 0.05$).

Discussion, Conclusion and Recommendation

Discussion

The significantly higher mean values of LDH, GGT and ALT in the HIV-mono-infected after drug administration than the results of the parameters obtained before the initiation of the drug could be due to the fact that some of the drugs could be haemolytic causing blood cells destruction probably causing increase in LDH as destruction of cells could be indicated by raised LDH (Butt *et al.*, 2002). In addition to this, increased concentration of liver biomarker such as GGT and ALT could be attributed to the fact that patients with HIV infection are treated with a combination of antiretroviral medications to control their HIV infection. Several of these drugs are associated with liver enzyme elevations. Drug-induced liver injury (DILI) is defined as the elevation of liver enzyme and/or bilirubin levels in association with the use of a medication or drug. The term "hepatotoxicity" may be misleading, especially in patients with HIV infection, because some of these events may not be directly caused by a toxic medication. Instead, acute viral hepatitis, reactivation of chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, and/or alcohol ingestion may all play a role in such events (Sulkowski *et al.*, 2000). Of note, patients often receive alternative and complementary medicines in association with HAART, and several of these have been associated with clear-cut DILI (Lewis and Strom, 2002).

There was a significantly higher mean value of LDH and ALT in the HIV patients co-infected with HBV before drug administration than the results of the parameters obtained after the initiation of the drug. This could be attributed to acute viral hepatitis, reactivation of chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, increased destruction of cells and/or alcohol ingestion suppression of the cause of the infection upon the initiation of antiretroviral drugs (Sulkowski *et al.*, 2000). However, there was a significantly lower mean value of GGT in the HIV-HBV patients before drug administration than after the initiation of the antiretroviral drug in HIV-HBV patients. This is consistent with the reports of Idoko *et al.*, (2014) on the impact of Hepatitis B Virus Infection on Human Immunodeficiency Virus Response to Antiretroviral Therapy in Nigeria.

Conclusion

This work showed a significant increase in the values of LDH, GGT and ALT in the HIV-mono-infected patients after antiretroviral drug administration and in HIV-HBV patients before antiretroviral therapy. A significantly lower mean value of GGT in the HIV-HBV patients before drug administration than after the initiation of the antiretroviral drug.

Recommendation

Evaluation of liver biomarkers in the treatment of HIV mono-infected and HIV-HBV patients is essential to avoid liver disorder and possible complications.

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